


# ISM 2024 Annual Meeting

## September 9-10, 2024

### Wohl Center, Bar Ilan University

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# General Information

**Meeting venue**
Wohl Center, Bar Ilan University, Ramat Gan

**Language**
The official language of the Meeting is English.

**Registration Desk**
The Registration Desk will be open at the Meeting venue throughout the Meeting days.

**Badges**
Please wear your name badge at all Meeting sessions and events.

**On-line Program and Abstracts**
The program and abstracts are also available on the ISM website:
https://www.ism.org.il/annualmeeting
To view an abstract, click on the presentation title.

**Poster Presentations**
Poster presentations are an important part of the scientific program and an invaluable opportunity for exchange of ideas and update on new research. The main opportunity for poster viewing and discussion is during the lunch and coffee breaks each day and authors are requested to stand by their poster to answer questions on the day of their presentation.

Posters will be on display on the following days:
1. Poster Session 1: Monday, 9 September
2. Poster Session 2: Tuesday, 10 September

The posters from each session will be on display all day with formal poster viewing, networking

All poster boards will be situated in the Poster Area.

Mounting of posters:
- Monday, September 9 at 09:00 AM
- Tuesday, September 10 at 09:15 AM

Removal of posters:
- Monday, September 9 by 05:30 PM
- Tuesday, September 10 at 05:45 PM

The organizers cannot be responsible for posters not removed by the above stated time.

Poster presenters are requested to refer to the scientific program for the board number on which they should display their poster.

**Data Projection**

Speakers are requested to bring their presentation on a USB flash drive to the A/V assistant in the session hall, at least an hour before the start of their session. The assistant will load the presentation onto the meeting computer.

**Best Poster Awards**

Poster awards will be presented at the Closing Ceremony on Tuesday, September 10. Prizes will be awarded only to prize recipients who are present at the closing session and collect their prize personally.

**Internet**

Internet at the Meeting venue is available free of charge in all public areas. Please visit the registration desk to receive the login details.

# Sponsors and Exhibitors

## Sponsors

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# Timetable

| | Monday, September 9 | | |
|---|---|---|---|
| | *Registration Area, Poster Area, Exhibition Area* | | |
| 8:30-9:00 | REGISTRATION, COFFEE BREAK, POSTER MOUNTING | | |
| | *Auditorium* | | |
| 9:00-9:15 | OPENING CEREMONY | | |
| | *Auditorium* | | |
| 9:15-10:15 | MECHANISMS OF BACTERIAL BIOFILM FORMATION AND FUNCTIONS | | |
| | *Poster Area, Exhibition Area* | | |
| 10:15-10:45 | COFFEE BREAK, POSTER VIEWING | | |
| | *Auditorium* | *Hall B* | *Hall C* |
| 10:45-12:15 | HOST-PATHOGEN INTERACTIONS I | MICROBIAL ECOLOGY AND EVOLUTION | FUNGAL BIOLOGY |
| | *Poster Area, Exhibition Area* | | |
| 12:15-12:45 | LUNCH BREAK | | |
| 12:45-14:00 | POSTER SESSION AND EXHIBITION | | |
| | *Auditorium* | *Hall B* | *Hall C* |
| 14:00-15:30 | CLINICAL VIROLOGY AND EPIDEMIOLOGY | COMPUTATIONAL BIOLOGY AND GENOMICS OF MICROBES | CLINICAL BACTERIOLOGY AND EPIDEMIOLOGY |
| | *Poster Area, Exhibition Area* | | |
| 15:30-16:00 | COFFEE BREAK, POSTER VIEWING | | |
| | *Auditorium* | *Hall B* | *Hall C* |
| 16:00-17:30 | VIRUSES OF EUKARYOTES | PLANT-MICROBE INTERACTIONS | THE MICROBIOME |

| | Tuesday, September 10 | | |
|---|---|---|---|
| | *Registration Area, Poster Area, Exhibition Area* | | |
| 8:45-9:15 | REGISTRATION, COFFEE BREAK, POSTER MOUNTING | | |
| | *Auditorium* | | |
| 9:15-10:15 | RAPID EVOLUTION OF PATHOGENS WITHIN PATIENTS | | |
| | *Poster Area, Exhibition Area* | | |
| 10:15-10:45 | COFFEE BREAK, POSTER VIEWING | | |
| | *Auditorium* | *Hall B* | *Hall C* |
| 10:45-12:15 | HOST-PATHOGEN INTERACTIONS II | APPLIED MICROBIOLOGY AND BIOTECHNOLOGY | BACTERIAL PHYSIOLOGY |
| | *Poster Area, Exhibition Area* | | |
| 12:15-12:45 | LUNCH BREAK | | |
| 12:45-14:00 | POSTER SESSION AND EXHIBITION | | |
| | *Auditorium* | | |
| 13:30-14:00 | ISM Annual Members' Assembly (for members only) | | |
| | *Auditorium* | *Hall B* | *Hall C* |
| 14:00-15:30 | MICROBIAL TOXINS | PARASITES | MICROBIAL SOCIETIES AND MICROBIAL INTERACTIONS |
| | *Poster Area, Exhibition Area* | | |
| 15:30-16:00 | COFFEE BREAK, POSTER VIEWING | | |
| | *Auditorium* | *Hall B* | *Hall C* |
| 16:00-17:30 | BACTERIOPHAGES | BENEFICIAL MICROBES | MICROBIAL GENETICS |
| | *Auditorium* | | |
| 17:30-17:45 | BEST POSTER PRIZE ANNOUNCEMENT AND CLOSING REMARKS | | |

# Agenda
# Monday, September 9

***08:30 - 09:00 REGISTRATION, COFFEE BREAK, POSTER MOUNTING,*** ***Poster Area, Exhibition Area, Registration Area***

**09:00 - 09:15 OPENING CEREMONY, Auditorium**

**Meital Gal Tanamy**; President of the Israel Society of Microbiology

**09:15 - 10:15 MECHANISMS OF BACTERIAL BIOFILM FORMATION AND FUNCTIONS, Auditorium**

Chair: Neta Sal-Man, *BGU*

**09:15 Mechanisms of bacterial biofilm formation and functions**

Knut Drescher[1]

*Biozentrum, University of Basel, Switzerland*

***10:15 - 10:45 COFFEE BREAK, POSTER VIEWING,*** ***Poster Area, Exhibition Area***

**10:45 - 12:15 HOST-PATHOGEN INTERACTIONS I, Auditorium**

Chairs: Shai Bel, *BIU* and Erez Mills, *HUJI*

**10:45 Elucidating stem cell innate immune responses**

Moshe Biton[1]

*Department of Immunology and Regenerative Biology, Weizmann Institute of Science, Israel*

**11:05 The frenemy within: on helminth regulation of the intestinal barrier**

Danielle Karo-Atar[1], Tamar Eliahu[1]

*Pharmacology and Clinical Biochemistry, Ben-Gurion University, Israel*

**11:25 Mycoviruses: harmless hitchhikers or backseat drivers of fungal disease?**

Neta Shlezinger[1], Vanda Lerer[1], John Adeoya[1], Marina Rocha[1], Hilla Hayby[1]

*The Koret School of Veterinary Medicine, The Hebrew University, Israel*

**11:40 The chicken reproductive tract microbiota and its roles**

Erez Mills[1]

*Department of Animal Sciences, The Hebrew University of Jerusalem, Israel*

**12:00 Virus entry is a major barrier for productive HCMV infection in monocytes**

Yaarit Kitsberg[1], Aharon Nachshon[1], Michal Schwartz[1], Noam Stern-Ginossar[1]

*Molecular genetics department, Weizmann Institute of Science, Israel*

**12:05 Fusolisin protease may regulate the tumor microenvironment**

Nicole Haj[1], Amjad Shehadeh[1], Viviana Scaiewicz[1], Reem Bsoul[1], Ofir Ventura[1], Perlie Admony[1], Karolina Świderska[2], Marcin Drag[2], Jan Potempa[3], Ofer Mandelboim[4], Gilad Bachrach[1]
[1]*The Institute of Dental Sciences, The Hebrew University-Hadassah School of Dental Medicine, Jerusalem*
[2]*Department of Chemical Biology and Bioimaging, Wroclaw University of Science and Technology, Poland*
[3]*Department of Microbiology, Faculty of Biochemistry, Biophysics and Biotechnology, Jagiellonian University*
[4]*Hebrew University-Hadassah Medical School, Institute for Medical Research Israel Canada (IMRIC), Jerusalem*

## 10:45 - 12:15 MICROBIAL ECOLOGY AND EVOLUTION, Hall B

Chairs: Debbie Lindell, *TIIT* and Dror Mintz, *ARO*

**10:45 Flagellar genes and cyclic-di-GMP modulate predation and biofilm formation in the bacterial predator *Bdellovibrio bacteriovorus***

Abhirup Mookherjee, Mohor Mitra, Rajesh Sathyamoorthy, Gal Sason, Polpass Arul Jose, Maria Martinenko, Ofra Matan, Shmuel Pietrokovski, Edouard Jurkevitch[1]
*Faculty of Agriculture, Food and Environment, The Hebrew University of Jerusalem*

**11:00 Pathogen contingency loci and the evolution of host specificity**

Hadas Hawlena[1], Ruth Rodríguez-Pastor, Nadav Knossow, Naama Shahar, Adam Z. Hasik, Daniel E. Deatherage, Ricardo Gutiérrez, Shimon Harrus, Luis Zaman, Richard E. Lenski, Jeffrey E. Barrick
*Mitrani Department of Desert Ecology, Ben-Gurion University of the Negev, Israel*

**11:20 Deconstructing the plant microbiome to discover plant protection traits**

Omri Finkel[1], Amal Mhalwes[1], Raphael Sapir[1]
*Plant and environmental Sciences, The Hebrew University of Jerusalem, Israel*

**11:40 Ecophysiological drivers and biogeochemical consequences of virus infection in marine diatoms**

Chana Kranzler[1]
*The Mina & Everard Goodman Faculty of Life Sciences, Bar-Ilan University, Israel*

**12:00 Biogels decelerate the sinking of organic particles to the ocean depths**

Uria Alcolombri[1], Alon Nissan[2], Jonasz Słomka[3], Roman Stocker[3]
[1]*Department of Plant and Environmental Sciences, The Hebrew University of Jerusalem, Israel*
[2]*The Robert H. Smith, Faculty of Agriculture, Food and Environment. Department of Soil and Water Sciences., The Hebrew University of Jerusalem, Israel*
[3]*Institute of Environmental Engineering, Department of Civil, Environmental and Geomatic Engineering, ETH Zurich, Switzerland*

**12:05 The Dead Sea sinkhole pools: a source of microbial diversity in an extreme environment**

Ke Li[1,2], Nadya Teutsch[2,3], Aia Oz[4,5], Revital Bookman[3], Itai Sharon[4,5], Sarit Avrani[1]
[1]*Department of Evolutionary and Environmental Biology and the Institute of Evolution,*

*University of Haifa, Israel*
[2]*Department of Geochemistry and Environmental Geology, The Geological Survey of Israel, Israel*
[3]*Department of Marine Geosciences, University of Haifa, Israel*
[4]*Department of Biotechnology, Migal - Galilee Research Institute, Israel*
[5]*Faculty of Sciences and Technology, Tel-Hai Academic College, Israel*

## 10:45 - 12:15 FUNGAL BIOLOGY, Hall C

Chairs: Nir Osherov, *TAU* and Oded Yarden, *HUJI*

**10:45 Exploring the diversity of antifungal drug tolerance**

Judith Berman[1]
*Molecular Microbiology & Biotechnology, Tel Aviv University*

**11:05 Belowground carbon transfer across mycorrhizal networks among trees: Facts, not fantasy**

Klein Tamir[1], Stav Livne-Luzon[1], Ido Rog[1]
*Plant and Environmental Sciences, Weizmann Institute of Science, Israel*

**11:25 Genome-wide CRISPRi exploration of plant-derived compounds for antifungal potential**

Ofri Levi[1]
*Biotechnology, MIGAL, ISRAEL*

**11:40 Cytokinin signaling and perception in fungi**

Gautam Anand, Rupali Gupta, Maya Bar[1]
*Dept. of Plant Pathology and Weed Research, ARO, Volcani Institute, Israel*

**12:00 Crossing of the border: deciphering fusarium oxysporum`s mammalian host adaptation and immune modulation strategies**

Marina Campos Rocha[1], Lin-Jun Ma[2], Neta Shlezinger[1]
[1]*Koret School of Veterinary Medicine, The Hebrew University of Jerusalem, Israel*
[2]*Biochemistry and Molecular Biology, University of Massachusetts Amherst, United States of America*

## *12:15 - 12:45 LUNCH BREAK, Exhibition Area*

## *12:15 - 12:45 POSTER SESSION I AND EXHIBITION Poster Area, Exhibition Area*

## 14:00 - 15:30 CLINICAL VIROLOGY AND EPIDEMIOLOGY, Auditorium

Chairs: Yonat Shemer-Avni, *BGU* and Yaniv Lustig, *SHEBA*

**14:00 Congenital cytomegalovirus: transmission and control at the maternal-fetal interface**

Dana Wolf[1]
*The Hebrew University Faculty of Medicine, Hadassah Hebrew University Medical Center, Israel*

**14:20 HPV in MSM with and without HIV**

Orna Mor[1,2], Roni Spector[2], Itzchak Levy[3,4], Maayan Itschaki[1]
[1]*Central Virology Laboratory, Ministry of Health, Israel*
[2]*School of Public Health, Faculty of Medicine, Tel Aviv University, Israel*
[3]*Infectious unit, Sheba Medical Center, Israel*
[4]*Faculty of Medicine, Tel Aviv University, Israel*

**14:40 SPRI - Navigating future pandemics with enhanced preparedness**

Yael Weiss-Ottolenghi[1]
*Sheba Pandemic Preparedness Research Institute, Sheba Medical Center, Tel Hashomer, Israel*

**14:55 How infected are we? Infection history to respiratory viruses during the Omicron outbreak in Israel**

Tomer Hertz[1], Hanna Oppenheimer, Shosh Zismanov, Lilach M. Friedman, Lior Nesher
[1]*Microbiology, Immunology and Genetics, Ben-Gurion University of the Negev, Israel*

**15:15 Molecular diagnostics of respiratory viruses in the community - a possible contribution to health policy management**

Ayelet Keren - Naus[1]
[1]*Clinical Virology lab, Soroka Medical Center, Israel*

**15:20 Is Human Rhinovirus (HRV) a pathogen that causes significant morbidity or an "innocent bystander" carried by hospitalized patients?**

Michal Mandelboim[1]
[1]*School of Public Health, Tel Aviv University, Israel*

## 14:00 - 15:30 COMPUTATIONAL BIOLOGY AND GENOMICS OF MICROBES, Hall B

Chairs: Eran Bosis, *BRAUDE* and Tal Pupko, *TAU*

**14:00 Navigating a fine balance: point-mutant cheater viruses disrupt the viral replication cycle**

Adi Stern[1]
[1]*School of Molecular Cell Biology & Biotechnology, Tel Aviv University, Israel*

**14:20 Escherichia coli adaptation under prolonged resource exhaustion is characterized by extreme parallelism and frequent historical contingency**

Shira Zion, Sophia Katz, Ruth Hershberg[1]
*The Ruth and Bruce Rappaport Faculty of Medicine, Technion - Israel Institute of Technology, Israel*

**14:40 A user-friendly web server for the analysis of large-scale microbial genomics data**

Yair Shimony[1], Oren Avram[2], Edo Dotan[1], Elya Wygoda[1], Noa Ecker[1], Naama Wagner[1], Gianna Durante[3], Jeff Chang[4], Tal Pupko[1]
[1]*The Shmunis School of Biomedicine and Cancer Research, Tel Aviv University, Israel*
[2]*UCLA Henry Samueli School of Engineering and Applied Science, University of California Los Angeles, California, USA*
[3]*Computer Science, Purdue University, Indiana, USA*
[4]*Department Of Botany And Plant Pathology, Oregon State University, Oregon, USA*

**14:55 Ancient origin of innate immune components in bacteria**

Tanita Wein[1], Philip Kranzusch[2], Rotem Sorek[3]
[1]*Department of Systems Immunology, Weizmann Institute of Science, Israel*
[2]*Department of Microbiology, Harvard Medical School, USA*
[3]*Department of Molecular Genetics, Weizmann Institute of Science, Israel*

**15:15 Insights into bacterial adaptation to hosts through massive-scale genomic analysis**

Ofir Segev[1], Alexander Geller[1], Cristina Bez[2], Peihan Wang[3], Menglu Zhang[3], Lusiné Nazaretyan[4], Fei Chen[3], Vittorio Venturi[2], Asaf Levy[1]
[1]*The Robert H. Smith Faculty of Agriculture, Food, and Environment, The Hebrew University of Jerusalem, Israel*
[2]*The Bacteriology Group, International Centre for Genetic Engineering and Biotechnology, Italy*
[3]*CAS Key Laboratory of Genome Sciences and Information, Beijing Institute of Genomics, Chinese Academy of Sciences and China National Center for Bioinformation, China*
[4]*Berlin Institute of Health, Charité-Universitätsmedizin Berlin, Germany*

**15:20 Plasmids fight back: anti-defense systems boost conjugation efficiency**

Bruria Samuel[1], David Burstein
*Department of The Shmunis School of Biomedicine and Cancer Research, Tel Aviv university, Israel*

## 14:00 - 15:30 CLINICAL BACTERIOLOGY AND EPIDEMIOLOGY, Hall C

Chair: Ronen Ben Ami, *TASMC*

**14:00 Fundamental insight into non-growing bacterial states for the tailoring of effective antibiotic treatments**

Adi Rotem, Nathalie Balaban[1]
[1]*Racah Institute, The Hebrew University, Israel*

**14:20 Genetic determinants underlying antimicrobial resistance (AMR) in wildlife in Israel**

Babatunde Beyioku[1], Roi Lapid[2], Roni King[3], Tomer Nissimyan[3], Prof Jacob Moran-Gilad[1]
[1]*Faculty of Health Sciences, Faculty of Health Sciences, Ben Gurion University of the Negev, Beer Sheva, Israel., Israel*
[2]*Faculty of Agriculture, The Robert H. Smith Faculty of Agriculture, The Hebrew University of Jerusalem, Rehovot, Israel, Israel*
[3]*Israel Nature and Parks Authority, Science & Conservation Division, Israel Nature and Parks Authority, Science & Conservation Division, Jerusalem, Israel, Israel*

**14:35 Bacterial physiology and antibiotic action**

Uri Alon[1]
[1]*Molecular Cell Biology, Weizmann Institute of Science, Israel*

**14:55 FT-IR in infection control: from local outbreak detection to national epidemiological surveillance**

Alona Keren-Paz[1], Mor Lurie-Weinberger[1], Nadya Rakovitsky[1], Moshe Bechor[1], Reut Efrati Epchtien[1], Yehuda Carmeli[1,2]
[1]*National Institute for Antibiotic Resistance and Infection Control, Ministry of Health, Israel*
[2]*Sackler School of Medicine, Tel Aviv University, Israel*

**15:10 The changing epidemiology of invasive group a streptococcus infections in Israel during 2019-2023 and the emergence of successful lineages**

Merav Ron[1], Shir Nesimian[2], Maya Davidovich-Cohen[2], Assaf Rokney[2]
[1]*1Public Health Laboratories –Jerusalem (PHL-J), Ministry of Health (MOH), Israel*
[2]*Public Health Laboratories –Jerusalem (PHL-J), Ministry of Health (MOH), Israel*

***15:30 - 16:00 COFFEE BREAK, POSTER VIEWING, Poster Area, Exhibition Area***

**16:00 - 17:30 VIRUSES OF EUKARYOTES, Auditorium**

Chairs: Ronit Sarid, *BIU* and Ran Taube, *BGU*

**16:00 SARS-CoV-2 nucleocapsid protein transfer from infected to uninfected cells induces antibody-mediated complement attack**

Alexander Rouvinski[1]
*Department of Microbiology and Molecular Genetics, Institute for Medical Research Israel-Canada, The Kuvin Center for the Study of Infectious and Tropical Diseases, The Hebrew University-Hadassah Medical School, The Hebrew University of Jerusalem, Israel*

**16:20 Numb-associated kinases are essential for Sandfly-borne Toscana virus entry**

Yarden Moalem, Rodolfo Katz, Anand G Subramaniam, Yehonathan Malis, Yakey Yaffe, Nofit Borenstein-Auerbach, Keshet Tadmor, Roey Raved, Ben M. Maoz, Ji Seung Yoo, Yaniv Lustig, Chen Luxenburg, Eran Perlson, Shirit Einav, Ella H. Sklan

**16:40 A cytomegalovirus encoded lncRNA blocks cell cycle progression**

Tal Fisher[1], Aharon Nachshon, Michal Schwartz, Noam Stern-Ginossar
[1]*Molecular genetics, Weizmann Institute, Israel*

**16:55 Elucidating the role of type-II interferon stimulated genes in the antiviral response to respiratory viruses**

Yiska Weisblum[1], Michael Tartell[2], Paul Bieniasz[2]
[1]*Department of Microbiology and Molecular Genetics and the Kuvin Center for the Study of Infectious and Tropical Diseases, IMRIC, Faculty of Medicine, The Hebrew University of Jerusalem, Israel*
[2]*Laboratory of Retrovirology, The Rockefeller University, USA*

**17:15 Discovery of an unrecognized nidovirus associated with granulomatous hepatitis in rainbow trout**

Sharon Karniely[1], Adi Faigenboim[2], Salsabeel Watted[1], Katia Lapin[1], Eduard Berenshtein[3], Avshalom Hurvitz[4], Arieli Bouznach[1], Asaf Berkowitz[5], Eran Bacharach[6], Avi Eldar[7]
[1]*Department of Viorology, Kimron Veterinary Institute, Israel*
[2]*Department of Plant Pathology, The Volcani Center, Israel*
[3]*EM Unit Research Core Facility, The Hebrew University of Jerusalem, Israel*
[4]*Upper Galilee, Dan Fish Farm, Israel*
[5]*Department of Poultry Diseases, Kimron Veterinary Institute, Israel*
[6]*The Shmunis School of Biomedicine and Cancer Research, Tel Aviv University, Israel*
[7]*Department of Virology, Kimron Veterinary Institute, Israel*

## 16:00 - 17:30 PLANT-MICROBE INTERACTIONS, Hall B

Chairs: Maggie Levy, *HUJI* and Maya Bar, *ARO*

**16:00** ***Pseudozyma aphidis* plays hide-and-seek with its host plant**

Maggie Levy[1], Shanee Alster[1], Avis Dafa-Berger[1], Neta Rotem[1]
*Department of Plant Pathology and Microbiology, The Robert H. Smith Faculty of Agriculture, Food and Environment, The Hebrew University of Jerusalem, Israel*

**16:20** **Unveiling the mechanisms determining tissue specificity in the plant pathogen Clavibacter michiganensis using experimental evolution**

Raj Kumar Verma, Veronica Roman-Reyna, Nathan Ben-Moche, Jonathan M Jacobs, Doron Teper[1]
*Plant Pathology and Weed Research, Agricultural Research Organization - Volcani Institute, Israel*

**16:40** **Ecological Implications of Introducing the Endophytic Bacterium Frateuria defendens on Melon Plant Health and Endophytic Community Dynamics**

Lilach Iasur Kruh[1]
*Department of Biotechnology Engineering, Braude College of Engineering, Israel*

**16:55** **Rhizosphere microbiome rewilding for enhancing crop productivity**

Elisa Korenblum[1]
*Plant Science Institute, The Volcani Center, Israel*

**17:15** **Rhizobial trickster: legume symbiont simultaneously fixes atmospheric nitrogen and denitrifiies nitrate, inducing high nodule biomass**

Moshe Alon[1], Omri Finkel
*Plant Science, Hebrew University, Israel*

**17:20** **Impact of soil type on plants productivity and assembly seed endophytic communities of Setaria viridis L.**

Kumar Uttam[1], Wout Yi Moe Oo[1], Max Kolton[1]
*The French Associates Institute for Dryland Agriculture and Biotechnology, Ben-Gurion University of the Negev, Israel*

## 16:00 - 17:30 THE MICROBIOME, Hall C

Chairs: Naama Geva-Zatorsky, *TIIT* and Nissan Yissachar, *BIU*

**16:00** **A role for the gut microbiome in the first 1000 of life**

Omry Koren[1]
*Azrieli Faculty of Medicine, Bar-Ilan University, Israel*

**16:20** **Enteric pathogens communication can be intercepted by microbiome-produced metabolites**

Neta Sal-Man[1]
*Microbiology and Immunology, Ben-Gurion University of the Negev, Israel*

**16:40 Diet affects gut microbe-host interactions by altering bacterial immune-modulatory functionality**

Noa Mandelbaum[1], Shaqed Carasso[1], Alon Kedem[1], Tamar Ziv[2], Tal Gefen[1], Naama Geva-Zatorsky[1,3]
[1]*Department of Cell Biology and Cancer Science, Rappaport Faculty of Medicine and Research Institute, Rappaport Technion Integrated Cancer Center (RTICC), Technion-Israel Institute of Technology, Israel*
[2]*Smoler Proteomics Center, Lokey Interdisciplinary Center for Life Sciences & Engineering, Technion-Israel Institute of Technology, Israel*
[3]*Humans and the Microbiome, CIFAR, Canada*

**16:55 Metaproteomic analysis of cancer immunotherapy modulation by the gut microbiome**

Lior Lobel[1]
*Faculty of Life Sciences, Bar-Ilan University, Israel*

**17:15 CoSMIC - A hybrid approach for large-scale, high-resolution microbial profiling of novel niches**

Knafo Maor[1], Shahar Rezenman[1], Tal Idan1[1], Michael Elgart[2], Shlomi Dagan[3], Ziv Reich[1], Ruti Kapon[1], Dagan Sade[1], Noam Shental[4]
[1]*Department of Biomolecular Sciences, Weizmann Institute of Science, Israel*
[2]*Metabolomic Discovery Unit, Metabolic Center, Sheba Medical Center, Israel*
[3]*Fisher Drug Discovery Resource Center, The Rockefeller University, USA*
[4]*Department of Mathematics and Computer Science, The Open University of Israel, Israel*

**17:20 Gut fermentability of edible architectures: Impact of insect chitin or amyloid-like fibrils on microbiota trajectories**

Gil Refael[1], Yizhaq Engelberg[2], Alon Romano[1], Gabriela Amiram[1], Eilon Barnea[2], Carmit Shani Levi[1], Sondra Turjeman[3], Meytal Landau[2,4], Omry Koren[3], Uri Lesmes[1]
[1]*Department of Biotechnology and Food Engineering, Technion - Israel Institute of Technology, Israel*
[2]*Department of Biology, Technion - Israel Institute of Technology, Israel*
[3]*Azrieli Faculty of Medicine, Bar Ilan University, Israel*
[4]*Centre for Structural Systems Biology (CSSB) and European Molecular Biology Laboratory (EMBL), German Synchrotron Research Centre (DESY), Germany*

# Agenda
# Tuesday, September 10

**08:45 - 09:15 REGISTRATION, COFFEE BREAK, POSTER MOUNTING,** ***Poster Area, Exhibition Area, Registration Area***

**09:15 - 10:15 RAPID EVOLUTION OF PATHOGENS WITHIN PATIENTS, Auditorium**

Chair: Dor Salomon, *TAU*

**09:15 Rapid evolution of pathogens within patients**

Jesse Shapiro[1]

*McGill University, Canada*

***10:15 - 10:45 COFFEE BREAK, POSTER VIEWING, Poster Area, Exhibition Area***

**10:45 - 12:15 HOST-PATHOGEN INTERACTIONS II, Auditorium**

Chairs: Roi Avraham, *WIS* and Ilan Rosenshine, *HUJI*

**10:45 Antibodies produced following monkeypox infection in humans effectively block monkeypox virus in a complement-dependent manner**

Natalia Freund[1]
*Faculty of Medicine, Tel Aviv University, Israel*

**11:05 Genomic features of bacterial adaptation to host, animals, and plants**

Asaf Levy[1], Ofir Segev[1], Alexander Geller[1]
*Plant pathology and microbiology, The Hebrew University of Jerusalem, Israel*

**11:25 Rare infection phenotypes and how to find them**

Camilla Ciolli Mattioli, Daniel Schraivogel, Roi Avraham
*Immunology and Regenerative Biology, Weizmann Institute of Science, Israel*

**11:40 Immune checkpoints pathways during acute viral infection**

Yotam Bar-On[1]
*Immunology, Technion, Israel*

**12:00 The role of host poly-LacNAc N-glycosylation in EPEC infection**

Klil Cohen Yaron[1], Yakov Socol[1], Noam Yedidi[1], Karthik Hullahalli[2], Matthew K Waldor[2], Ilan Rosenshine[1]
[1]*Department of Microbiology and Molecular Genetics, Institute of Medical Research Israel-Canada, Israel*
[2]*Department of Microbiology, Harvard Medical School, United States*

## 10:45 - 12:15 APPLIED MICROBIOLOGY AND BIOTECHNOLOGY, Hall B

Chairs: Gilad Bachrach, *HUJI* and Sima Yaron, *TIIT*

**10:45 Biotic-abiotic semiartificial cells for light-driven chiral molecule production**

Omer Yehezkeli[1]
*Faculty of Biotechnology & Food Engineering, Technion, Israel*

**11:05 Tackling fundamental challenges in biotechnology with computational models and AI**

Tamir Tuller[1]
*Biomedical Engineering, Ramot at Tel Aviv University LTD., Israel*

**11:25 Development of non-surgical sterilization/contraception methods by vaccinia virus based vaccine against sexual development hormones**

Elad Eliahoo[1], Ellen Daw[2], Daniel Barkan[2], Tal Raz[2]
[1]*Department of Virology, Kimron Veterinary Institute, Ministry of Agriculture and Rural Development, Israel*
[2]*Koret School of Veterinary Medicine, The Robert H. Smith Faculty of Agriculture, Food and Environment, Hebrew University in Jerusalem, Israel*

**11:40 Saliva based Diagnostics – A novel approach**

Omer Deutsch[1]
*Salignostics Ltd, Salignostics Ltd, Israel*

**12:00 Self-reporting porous silicon arrays for microbial infection diagnosis and their resistance**

Xin Jiang[1], Sagi Sphrits[2], Keren Boguslavsky[2], Gali Ron[1], Sarel Halachmi[2], Ester Segal[1]
[1]*Biotechnology and Food Engineering, Technion, Israel*
[2]*Department of Urology, Bnai Zion Center, Israel*

## 10:45 - 12:15 BACTERIAL PHYSIOLOGY, Hall C

Chairs: Daniel Dar, *WIS* and Eyal Gur, *BGU*

**10:45 The establishment of an intercellular nanotube bridge in bacteria**

Sigal Ben-Yehuda[1]
*Microbiology and Molecular Genetics, The Hebrew University of Jerusalem, Israel*

**11:05 Correlation and causation in the bacterial cell cycle**

Ariel Amir[1]
*Physics, Weizmann Institute, Israel*

**11:25 Quantitative characterization of the onset of bacterial swarm in Bacillus subtilis**

Ajesh Jose[1], Gil Ariel[2], Avraham Be'er[1]
[1]*Zuckerberg Institute for Water Research, Ben Gurion University of the Negev, Israel*
[2]*Department of Mathematics, Bar-Ilan University, Israel*

**11:40 L-methionine production in Escherichia coli: a pathway shift from trans- to direct sulfurylation**

Itamar Yadid[1]
*Biotechnology, Migal - Galilee Research Institute, Israel*

**12:00 Dissecting the phenotypic landscape of bacterial populations via imaging transcriptomics**

Zohar Persky[1], Daniel Dar
*Department of Plant & Environmental Sciences, Weizmann institute of science, Israel*

***12:15 - 12:45 LUNCH BREAK, Exhibition Area***

***12:45 - 14:00 POSTER SESSION II AND EXHIBITION Poster Area, Exhibition Area***

**13:30 - 14:00 ISM Annual Members' Assembly (for members only), Auditorium**

**14:00 - 15:30 MICROBIAL TOXINS, Auditorium**

Chairs: Asaf Levy, *HUJI* and Emanuel Hanski, *HUJI*

**14:00 Antimicrobial peptides (AMPs) as the next-generation antimicrobial drugs against bacterial and fungal pathogens**

Netanel Tzarum[1], Rina Fraenkel[1], Inbar Cahana[1], Noam Deouell[1], Shani Bruchim[1], Tomer Sivan[1], Asaf Levy[2], Neta Schlezinger[3]
[1]*Department of Biological Chemistry, Institute of Life Sciences, The Hebrew University of Jerusalem, Jerusalem*
[2]*Department of Plant Pathology and Microbiology, Faculty of Agriculture, Food, and Environment, The Hebrew University of Jerusalem, Rehovot*
[3]*Koret School of Veterinary Medicine, Faculty of Agriculture, Food, and Environment, The Hebrew University of Jerusalem, Rehovot*

**14:20 The impact of bacterial endoribonuclease toxins on multigenerational host-bacteria interactions**

Ilana Kolodkin-Gal[1], Omri Gilhar[2], Liat Rahamim-Ben Navi[1], Tsviya Olender[3], Hadar Ben Yoav[4]
[1]*Scojen Institute for Synthetic Biology, Reichman University, Israel*
[2]*Department of Plant and Environmental Sciences, Weizmann Institute of Science, Israel*
[3]*Department of Molecular Genetics, Weizmann Institute of Science, Israel*
[4]*Department of Biomedical Engineering, Ben-Gurion University of the Negev, Israel*

**14:40 Double trouble: a novel T6SS effector with two toxic domains**

Chaya-Mushka Fridman[1], Kinga Keppel[1], Eran Bosis[2], Dor Salomon[1]
[1]*Department of Clinical Microbiology and Immunology, Tel Aviv University, Israel*
[2]*Department of Biotechnology Engineering, Braude College of Engineering, Israel*

**14:55 Gamma-Mobile-Trio systems define a new class of mobile elements rich in bacterial defensive and offensive tools**

Tridib Mahata, Katarzyna Kanarek, Marimuthu Ragavan Rameshkumar, Eran Bosis[2], Udi Qimron, Dor Salomon
*Department of Biotechnology Engineering, Braude College of Engineering*

**15:15 Unveiling the Extracellular Contractile Injection Systems (eCIS) Target Specificity Determinants**

Nimrod Nachmias[1], Asaf Levy
*Faculty of agriculture, HUJI, Rehovot*

**15:20 Unmasking the metabolically active growth arrest induced by VapC in E. coli**

Irine Ronin[1], Adi Rotem[1], Orit Gefen[1], Liron Argaman[2], Hanah Margalit[2], Matthias Heinemann[3], Nathalie Balaban[4]
[1]*The Racah Institute of Physics, The Hebrew University in Jerusalem, Israel*
[2]*Microbiology department, The Hebrew University in Jerusalem, Israel*
[3]*Molecular Systems Biology, Groningen Biomolecular Sciences and Biotechnology Institute, Netherlands*
[4]*The Racah Institute of Physics, The Hebrew University in Jerusalem, Israel*

## 14:00 - 15:30 PARASITES, Hall B

Chair: Danielle Karo-Atar, *BGU*

**14:00 Targeting parasites with peptides: a focus on leishmania and trypanosoma treatment strategies**

Nir Qvit[1]
*Israel, Bar-Ilan University, Israel*

**14:20 Dynamic imaging: kinetics, morphologies and regulation of organelle biogenesis throughout plasmodium cell cycle**

Anat Florentin[1], Michal Shahar[1], Alia Qasem[1]
*Faculty of Medicine, The Hebrew University of Jerusalem, Israel*

**14:40 Synthetic antimicrobial peptides to combat drug resistance in the malaria parasite *Plasmodium falciparum***

Edo Kiper[1], Daniel Ben Hur[1], Daniel Alfandari[1], Naiem Ahmad Wani[1], Abel Cruz Camacho[1], Gal David Efrat[1], Mattia Morandi[2], Moshe Goldsmith[1], Ido Azuri[3], Ron Rotkopf[3], Roman Kamyshinsky[4], Ziv Porat[5], Irit Rosenhek-Goldian[4], Yechiel Shai[1], Neta Regev-Rudzki[1]
[1]*Biomolecular Sciences, Weizmann Institute of Science, Israel*
[2]*Institute of Organic Chemistry and Biochemistry, Czech Academy of Science, Czech Republic*
[3]*Bioinformatics Unit, Department of Life Sciences Core Facilities, Weizmann Institute of Science, Israel*
[4]*Chemical Research Support, Weizmann Institute of Science, Israel*
[5]*Department of Life Sciences Core Facilities, Weizmann Institute of Science, Israel*

**14:55 An important role of the SRP-independent targeting pathway in protein export in Plasmodium falciparum**

Karina Simantov Ron[1]
*Microbiology and Molecular Genetics, Hebrew University, Israel*

**15:15 UMSBP2 is a chromatin modeler protein that functions in regulation of gene expression and suppression of antigenic variation in trypanosomes**

Awakash Soni[1], Olga Klebanov-Akopyan[2], Esteban Erben[3], Inbar Plaschkes[4], Hadar Benyamini[4], Vera Mitesser[2], Amnon Harel[5], Katereena Yamin[6], Itay Onn[5], Joseph Shlomai[2]
[1]*Department of Microbiology and Molecular Genetics, The Hebrew University of Jerusalem, Israel*
[2]*Department of Microbiology and Molecular Genetics, The Hebrew University of Jerusalem, Israel*
[3]*Institute of Biotechnological Research, National University of San Martin, San Martın*
[4]*The Info-Core Bioinformatics Unit, The Hebrew University of Jerusalem, Israel*
[5]*Azrieli Faculty of Medicine, Bar-Ilan University, Israel*
[6]*Azrieli Faculty of Medicine, Bar-Ilan University, Isarel*

## 14:00 - 15:30 MICROBIAL SOCIETIES AND MICROBIAL INTERACTIONS, Hall C

Chairs: Yael Helman, *HUJI* and Einat Segev, *WIS*

**14:00 Reversion to metabolic autonomy underpins evolutionary rescue in a bacterial obligate mutualism**

Jonathan Friedman[1], Ignacio Melero-Jiménez[1], Yael Sorokin[1], Ami Merlin[1], Alejandro Couce[2]
[1]*Institute of Environmental Sciences, Hebrew University, Israel*
[2]*Centro de Biotecnología y Genómica de Planta, Universidad Politécnica de Madrid, Spain*

**14:20 Deep sea gas seeps are hotspots of microbial productivity and biotic interactions**

Maxim Rubin-Blum[1,2], Lina Ratinskaia[1,2], Stas Malavin[1,3]
[1]*Marine Biology, Israel Oceanographic and Limnological Research, Israel*
[2]*Department of Marine Biology, Leon H. Charney School of Marine Sciences, University of Haifa, Israel*
[3]*Department of Environmental Hydrology and Microbiology, Zuckerberg Institute for Water Research, The Jacob Blaustein Institutes for Desert Research, Ben-Gurion University of the Negev, Israel*

**14:40 The effect of phage peptide communication on bacterial social behavior**

Bat El Hagbi Lazar[1], Ronit Suissa[1], Meital Shema Mizrachi[1], Zohar Tik[1], Zoe Levi[1], Michael M. Meijler[1]
*Department of Chemistry, Ben Gurion University of the Negev, Israel*

**14:55 Bacterial physiology in an environmental context**

Einat Segev[1]
*Plant and Environmental Sciences, Weizmann Institute, Israel*

**15:15 Deciphering the identity of native molecular cargo transported via nanotubes among bacterial community members**

Saurabh Bhattacharya[1], Philipp Spät[2], Venkadesaperumal Gopu[1], Waner Yan[1], Manas Guria[1], Nir Dranitzky[1], Boris Macek[2], Ilan Rosenshine[1], Sigal Ben-Yehuda[1]
[1]*Department of Microbiology and Molecular Genetics, IMRIC, Faculty of Medicine, The Hebrew University of Jerusalem, Israel*
[2]*Department of Quantitative Proteomics, Interfaculty Institute for Cell Biology, University of Tübingen, Germany*

***15:30 - 16:00 COFFEE BREAK, POSTER VIEWING, Poster Area, Exhibition Area***

**16:00 - 17:30 BACTERIOPHAGES, Auditorium**

Chairs: Avigdor Eldar, *TAU* and Sarit Avrani, *UH*

**16:00 Predicting antibiotic resistance**

Roy Kishony[1]
*Biology, CS (secondary), biomedical Engineering (secondary), Technion- Israel Institute of Technology, Israel*

**16:20 The bully phage: A Shiga toxin-encoding phage suppresses host-sharing prophages**

Yael Litvak[1], Tamara Wellins, Rachel Hashuel, Maayan Rachimi, Eran Gutman, Reut Melki, Linoy Taktuk, Pe'era Pash Wasserzug, Karina Roitman
*Silberman Life Science Institute, The Hebrew University, Israel*

**16:40 A bacterial host factor confines phage localization for excluding the infected compartment through cell division**

Osher Pollak-Fiyaksel[1]
*Microbiology, Hebrew University of Jerusalem, Israel*

**16:55 Lysogeny in cyanobacteria**

Esther Cattan-Tsaushu, Yara Nwatha, Hoda Madi, Ke Li, Sarit Avrani[1]
*Department of Evolutionary and Environmental Biology, and the Institute of Evolution, University of Haifa*

**17:15 Biases in viral transmission modes can be explained by Bayesian inference**

Nitzan Aframian[1], Avigdor Eldar[1]
*Life Sciences, Tel Aviv University, Israel*

**17:20 Phages reconstitute NAD+ to counter bacterial immunity**

Ilya Osterman[1]
*The Department of Molecular Genetics, Weizmann Institute of Science, Israel*

## 16:00 - 17:30 BENEFICIAL MICROBES, Hall B

Chairs: Omry Koren, *BIU* and Elhanan Tzipilevich, *MIGAL*

**16:00 Functional plasticity in the gut microbiota-host interactions**

Naama Geva-Zatorsky[1]
*Civil and Environmental Engineering, Technion Institute of Technolohu, Israel*

**16:20 Systematic discovery of prokaryotic symbionts of ciliate protozoa in the rumen**

Elie Jami[1]
*Institute of Animal Science, Agricultural Research Organization (ARO) Volcani center, Israel*

**16:40 Harnessing plant-microbe interactions for sustainable agriculture: complementary approaches for identifying novel plant growth-promoting bacteria**

Alexander Evenko-Greenapple[1]
*Soil, water and environment, Volcani Research Center, Agricultural Research Organization, Israel*

**16:55 Auxin secretion by soil bacteria accelerated immune system maturation in young seedlings**

Elhanan Tzipilevich[1,2]
*Plant science, MIGAL - Galilee research institute, Israel*
*Biotechnology, Tel Hai Academic college, Israel*

**17:15 From harsh environments to sustainable agriculture: the biocontrol potential of BSC microalgae**

Dikla Eckstien, Bernard Ngeno, Nofet Margulis, Noga Maximov, Hagai Raanan

**17:20 Bacterial DNA inversion regions as predictors of response to melanoma immunotherapy**

Roni Keshet-David[1], Jia Zhang, Shaqed Carasso, Tal Gefen, Naama Geva-Zatorsky
*medicine, Technion, Israel*

## 16:00 - 17:30 MICROBIAL GENETICS, Hall C

Chair: Dan Bar Yaakov, *BGU*

**16:00 A phage lysis-lysogeny decision is guided by a toxin-antitoxin system of unique nature**

Avigdor Eldar[1], Regev Frenkel[1], Polina Guler[1]
*Shmunis school of biomedicine and cancer research, Tel-Aviv University, Israel*

**16:20 Studying small RNAs - the challenge**

Shoshy Altuvia[1], Itzhak Goloventzitz[1], Maya Elgrably-Weiss[1], Georg Jens[2]
[1]*Faculty of Medicine, The Hebrew University, Israel*
[2]*Faculty of Biology, University of Freiburg, Germany*

**16:40 Long-term impact: bacteriophage-driven DNA inversions shape bacteroides fragilis functionality**

Shaqed Carasso, Roni Keshet-David, Jia Zhang[1], Haitham Hajjo, Tal Gefen, Naama Geva-Zatorsky
*Medicine, Technion – Israel Institute of Technology, Israel*

**16:55 Small RNAs orchestrate bacterial-phage dynamics**

Sahar Melamed[1]
*Microbiology and Molecular Genetics, IMRIC, The Hebrew University of Jerusalem, Israel*

**17:15 Understanding the emergence of phenotypic heterogeneity in motility and virulence traits in Pseudomonas aeruginosa**

Knan Mijal Ztion Harris[1], Daniel Dar
*Plant and Environmental Sciences, Weizmann Institute, Israel*

**17:20 A-to-I mRNA editing enables bacteria to produce two protein versions from a single gene and affects bacterial growth**

Eyal Elias[1], Liron Didi[1], Daniel Arad[1], Ofir fargeon[1], Rinat Cohen-Pavon[1], Gil Sorek[2], Liron Levin[3], Dganit Melamed[4], Isaac Gifford[5], Jeffrey E. Barrick[5], Liam Aspit[1], Dan Bar-Yaacov[1]
[1]*The Shraga Segal department of Microbiology, Immunology, and Genetics, Ben-Gurion University of the Negev, Israel*
[2]*Department of Life Sciences, Ben-Gurion University of the Negev, Israel*
[3]*Bioinformatics Core Facility, Ben-Gurion University of the Negev, Israel*
[4]*The Smoler Protein Research Center, Technion Israel Institute of Technology, Israel*
[5]*Department of Molecular Biosciences, University of Texas, USA*

**17:30 - 17:45 BEST POSTER PRIZE ANNOUNCEMENT AND CLOSING REMARKS, Auditorium**

# Posters
# Poster Session I – Monday, September 9

## 12:45 - 14:00 POSTER SESSION I (Monday, September 9), Poster Area

**P-1** Harnessing the immunomodulatory effect of drugs for a desired immune response

**Shelly Hen-Avivi**[1], Noa Bossel Ben-Moshe[1], Roi Avraham[1]
*Department of Immunology and Regenerative Biology, Weizmann Institute of Science, Israel*

**P-2** A T6SS in the coral pathogen Vibrio coralliilyticus secretes an arsenal of anti-eukaryotic effectors and contributes to virulence

**Shir Mass**[1], Hadar Cohen[1], Motti Gerlic[1], Blake Ushijima[2], Julia C. Van Kessel[3], Eran Bosis[4], Dor Salomon[1]
[1]*Department of Clinical Microbiology and Immunology, School of Medicine, Faculty of Medical and Health Sciences, Tel Aviv University, Israel*
[2]*Department of Biology and Marine Biology, University of North Carolina Wilmington, USA*
[3]*Department of Biology, Indiana University, USA*
[4]*Department of Biotechnology Engineering, Braude College of Engineering, Israel*

**P-3** Impact of fusarium species composition and incidence on onion basal rot in northeastern Israel

**Ofir Degani**[1,2], Elhanan Dimant[2], Eliyahu Margalit[3]
[1]*Faculty of Sciences, Tel-Hai College, Israel*
[2]*Plant Sciences department, MIGAL—Galilee Research Institute, Israel*
[3]*Extension Service, Israel Ministry of Agriculture and Rural Development, Israel*

**P-4** Pipecolic acid secreted by malaria parasite promotes its growth

**Oren Sonia**[1]
*Biomolecular science, Weizmann Institute of Science, Israel*

**P-5** Influence of temperature on cyanobacteria-cyanophage interactions

**Laure Arsenieff**[1], Debbie Lindell[1]
*Faculty of Biology, Technion - Israel Institute of Technology, Israel*

**P-6** Tomato pith necrosis disease exhibits a synergistic interaction with Tomato Brown Rugose Fruit Virus and Pepino Mosaic Virus infections

**Evanson Ngugi**[1], Doron Teper[2], Aviv Dombrovsky[3]
[1]*Faculty of Agriculture, Food and Environment, Hebrew University of Jerusalem, Israel*
[2]*Department of Plant Pathology and Weed Research, Agricultural Research Organization, the Volcani Institute, Israel*
[3]*Department of Plant Pathology and Weed Research, Agricultural Research Organization, the Volcani Institute, Israel*

**P-7** Unveiling the role of hydrogen peroxide (H2O2) in algal-bacterial interactions

**Delia Narvaez-Barragan**[1], Einat Segev[1]
*Department of Plant and Environmental Sciences, Weizmann Institute of Science, Israel*

**P-8** Characterization of the B-cell antigen specific antibody repertoires in human tissues

**Lior Levy**[1], Rei Matsumoto[2,3], Joshua Gray[3], Yosuke Sakamoto[2,3], Hanna Oppenheimer[1], Lilach M. Friedman[1], Tomer Hertz[1,4], Donna L. Farber[2,3]
[1]*Department of Microbiology, Immunology and Genetics, The National Institute for Biotechnology in the Negev, Ben-Gurion University of the Negev, Israel*
[2]*Department of Surgery, Columbia University Irving Medical Center, USA*
[3]*Department of Microbiology and Immunology, Columbia University Irving Medical Center,*

*USA*
[4]*Vaccine and Infectious Disease Division, Fred Hutchinson Cancer Research Center, USA*

**P-9** Identifying polio antibody binding signatures are associated with protective poliovirus neutralization titers

**Mor Tetro**[1], Lilach M. Friedman[1], Merav Weil[2], Aharona Glatman-Freedman[3,4], Ravit Bassal[3,4], Lester M. Shulman[2,4], Javier Martin[5], Tomer Hertz[1,6]
[1]*Department of Microbiology, Immunology and Genetics, The National Institute for Biotechnology in the Negev, Ben-Gurion University of the Negev, Israel*
[2]*Central Virology Laboratory, Public Health Services, Sheba Medical Center, Israel*
[3]*Israel Center for Disease Control, Ministry of Health, Sheba Medical Center, Israel*
[4]*Department of Epidemiology and Preventive Medicine, School of Public Health, Faculty of Medical and Health Sciences, Tel Aviv University, Israel*
[5]*Division of Virology, National Institute for Biological Standards and Control, UK*
[6]*Vaccine and Infectious Disease Division, Fred Hutch Cancer Research Center, USA*

**P-10** Chryseobacterium rhizoplanae entomopathogenic interaction with the Spodoptera littoralis larvae

**Ronna Melamed**[1]
*Plant pathology and Microbiology, Hebrew University of Jerusalem, Israel*

**P-11** Probing host-drug-pathogen interactions to optimize treatment for staphylococcus aureus implant-associated infection

Oren Gordon[1,2], Merav Ordan, **Rotem Huminer**, Maria Abu Elasal, Timothy Read, Charles Peloquin, Nathan Archer, Lloyd Miller, Kimberly Davis, Jogarao Gobburu, Sanjay Jain
[1]*Infectious Diseases Unit, Department of Pediatrics, Faculty of Medicine, Hebrew University of Jerusalem, Hadassah Medical Center, Israel*
[2]*Goldyne Savad Institute of Gene Therapy, Faculty of Medicine, Hebrew University of Jerusalem, Hadassah Medical Center, Israel*

**P-12** When a Virus Meets an Algae: disentangling states of infection and resistance using single-cell transcriptomics

**Talia Shaler**[1], Ofir Raz[2], Ester Feldmesser[3], Daniella Schatz[3], Assaf Vardi[3]
[1]*Plant & Environmental Sciences, Weizmann Institute of Science, Israel*
[2]*Department of Computer Science and Applied Mathematics, Weizmann Institute of Science, Israel*
[3]*Bioinformatics Unit, Life Sciences Core Facilities, Weizmann Institute of Science, Israel*

**P-13** Glycan-preference characterization of the *Fusobacterium nucleatum* Fap2 lectin

**Ofir Rokach - Ventura**[1], Tamar Alon-Miamon[1], Lei Xia[2], Nicole Haj[1], Reem Bsoul[1], Viviana Scaiewicz[1], Perlie Admony[1], Shani Leviatan Ben-Arye[3], Vered Padler - Karavani[3], Oren Parnas[2], Gilad Bachrach[1]
[1]*The Hebrew University-Hadassah School of Dental Medicine, The Institute of Dental Sciences, Israel*
[2]*Department of Immunology and Cancer Research, Hebrew University-Hadassah Medical School, Institute for Medical Research Israel Canada (IMRIC), Israel*
[3]*The George S. Wise Faculty of Life Sciences, Tel Aviv University, Department of Cell Research and Immunology, The Shmunis School of Biomedicine and Cancer Research, Israel*

**P-14** Melanoma colonization by *Fusobacterium nucleatum*

**Reem Bsoul**[1], Ahmed Rishiq[2], Nicole Haj[1], Ofir Ventura[1], Viviana Scaiewicz[1], Ofer Mandelboim[2], Gilad Bachrach[1]
[1]*The Institute of Dental Sciences, The Hebrew University-Hadassah School of Dental*

*Medicine, Israel*
[2]*Immunology and Cancer Research, Institute for Medical Research Israel Canada (IMRIC), The Concern foundation laboratories at the Lautenberg Center for General and Tumor Immunology, Hebrew University-Hadassah Medical School, Israel*

**P-15** Using translation modulators to study Dengue virus translation regulation

**Rodolfo Katz**[1], Rina Rosin-Arbesfeld[1], Ella H. Sklan
*Faculty of Medicine, Tel Aviv University, Israel*

**P-16** Annual dynamics of cyanophage abundances and their infection in the Sargasso Sea

**Camelia Shopen Gochev**[1], Debbie Lindell[1]
*Faculty of Biology, Technion – Israel Institute of Technology, Israel*

**P-17** Host-driven selection, revealed by comparative analysis of xanthomonas type III secretion effectoromes, unveils novel recognized effectors

**Yao Xiao**, Shatrupa Ray, Saul Burdman, Doron Teper

**P-18** Impact of airborne algicidal bacteria on marine phytoplankton blooms

**Naama Lang-Yona**[1,2], J. Michel Flores[3], Tal Sharon Nir-Zadock[2], Inbal Nussbaum[2], Ilan Koren[3], Assaf Vardi[2]
[1]*Environmental, Water and Agricultural Engineering Unit, Technion - Israel Institute of Technology, Israel*
[2]*Department of Plant and Environmental Science, Weizmann Institute of Science, Israel*
[3]*Department of Earth and Planetary Sciences, Weizmann Institute of Science, Israel*

**P-19** The molecular mechanisms underlying acclimation to marine heat waves in diatom blooms

**Mai Sadeh**[1], Shifra Ben-Dror[2], Yael Fridmann-Sirkis[2], Gilgi Friedlander[3], Daniella Schatz[1], Asaf Vardi[1]
[1]*Department of Plant and Environmental Sciences, Weizmann Institute of Science, Israel*
[2]*Life Science Core Facilities, Weizmann Institute of Science, Rehovot, Israel, Weizmann Institute of Science, Israel*
[3]*The Nancy and Stephen Grand Israel National Center*

*for Personalized Medicine (G-INCPM), Weizmann Institute of Science, Israel*

**P-20** The origins of death at sea: Characterizing conserved cell death genes in distant algae

**Avia Mizrachi**[1], Mai Sadeh[1], Shifra Ben-Dor[2], Orly Dym[2], John K. Brunson[3,4], Andrew E. Allen[3,4], Daniella Schatz[1], Assaf Vardi[1]
[1]*Department of Plant and Environmental Sciences, Weizmann Institute of Science, Israel*
[2]*Department of Life Sciences Core Facilities, Weizmann Institute of Science, Israel*
[3]*Scripps Institution of Oceanography, University of California San Diego, USA*
[4]*Environmental Genomics, J. Craig Venter Institute, USA*

**P-21** Rhodopsin-carotenoid complexes in marine planktonic Asgard archaea

**Gali Tzlil**

**P-22** Variability in the abundance of a new clade of T7-like cyanophages with latitude in the North Pacific Ocean

**Elena Khavin**[1], Kira Kondratyeva[1], Debbie Lindell[1]
*Faculty of Biology, Technion - Israel Institute of Technology, Israel*

**P-23** The pathogenic potential of environmental vibrios in Israel

**Katarzyna Kanarek**[1], Kinga Keppel[1], Chaya Mushka Fridman[1], Dor Salomon[1]
*Department of Clinical Microbiology and Immunology, School of Medicine, Faculty of Medical and Health Sciences,, Tel Aviv University, Israel*

**P-24** Breaking the eco-evolutionary feedbacks leads to extinction in a bacterial mutualism

**Yael Baranovitch**, Ignacio Jose Melero-Jiménez, Jonathan Friedman

**P-25** Oxytactic bioconvection across bacteria species

**Oscar Gallardo**[1], Rinat Goren[1], Joel Stavans[1]
*Physics of Complex Systems, Weizmann Institute of Science, Israel*

**P-26** Evolution in microbial microcosms is highly parallel regardless of the presence of interacting species

**Nittay Meroz**[1], Tal Livny[1], Gal Toledano[1], Yael Sorokin[1], Nesli Tovi[1], Jonathan Friedman[1]
*Faculty of Agriculture, Hebrew University, Israel*

**P-27** Occurrence of under-the-radar antibiotic resistance bacteria and genes in anthropogenically affected produce

**Chagai Davidovich**[1,2], Kseniia Erokhina[1,2], Shlomo Blum[3], Eddie Cytryn[1]
[1]*Institute of Soil, Water and Environmental Sciences, Volcani Institute, Israel*
[2]*Robert H. Smith Faculty of Agriculture, Food and Environment, The Hebrew University of Jerusalem, Israel*
[3]*Dept. of Bacteriology, Kimron Veterinary Institute, Israel*

**P-28** Escherichia coli adaptation under prolonged resource exhaustion is characterized by extreme parallelism and frequent historical contingency

**Shira Zion**[1], Sophia Katz[1], Ruth Hershberg[1]
*Department of Medicine, Technion - Israel Institute of Technology, Israel*

**P-29** Annual dynamics of T7-like and T4-like cyanophages and their infection in the North Pacific Subtropical Gyre

**Svetlana Goldin**[1], Debbie Lindell[1]
*Faculty of Biology, Technion – Israel Institute of Technology, Israel*

**P-30** Evolution of cyanophages under different light conditions

**Yuval Aharon Shiran**, Debbie Lindell

**P-31** Adaptive loss-of-function mutation in B. subtilis' ThrC enzyme exposes metabolic bypass to isoleucine biosynthesis

**Shanni Hornstein**[1], Swati Sirotiya, Raul Mireles, Orr Giladi, Alexander Geller, Asaf Levy, Carolina Cano-Prieto[2], Pablo Cruz-Morales, Lianet Noda-García
[1]*Department of Plant Pathology and Microbiology. Faculty of Agriculture Food and Environment, Hebrew University of Jerusalem, Israel*
[2]*Novo Nordisk Foundation Center for Biosustainability, Danmarks Tekniske Universitet, Denmark*

**P-32** Investigating the activity of microbial populations in wetlands for the management of greenhouse gases emissions

**Julia Ghantous**[1], Raphy Zarecki
*Water Science, Tel-hai college, Israel*

**P-33** Balancing infection characteristics underlies cyanophage adaptation in complex environments

**Greenbaum Jonathan**[1], Debbie Lindell[1]
*Faculty of Biology, Technion - Israel Institute of Technology, Israel*

**P-34** Screening for a novel fungus in human shotgun metagenomics datasets

**Bareket Dassa**[1], Shifra Ben-Dor[1], Lena Fidel[1], Jarmila Kralova[2], Catalina Donic[2], Steffen Jung[2]
[1]*Department of Life Sciences Core Facilities, Weizmann Institute of Science, Israel*
[2]*Department of Immunology and Regenerative Biology, Weizmann Institute of Science, Israel*

**P-35** Identification of regulators vital for fungal adaptation and survival within the phagosomal environment

**John Adeyemi Adeoye**[1], Laura Fabre[2], Amelia Barber[2], Neta Shlezinger[3]
[1]*Korect School of Veterinary Medicine, Faculty of Agriculture, Food and Environment, The Hebrew University of Jerusalem, Israel*
[2]*Institute of Microbiology, Friedrich Schiller University, Germany*
[3]*Koret School of Veterinary Medicine,*

*Faculty of Agriculture, Food and Environment, The Hebrew University of Jerusalem, Israel*

**P-36** Lipid metabolism and transcriptional regulation in Botrytis cinerea under low temperature

**Gladys Karungari**[1,2], Ginat Raphael[2], Lavanya Gunamalai[2], Guy Schleyer[3], Tali Shalit[4], Oded Yarden[5], Carmit Ziv[2]
[1]*Department of Plant Pathology and Microbiology, The Hebrew Univeristy of Jerusalem, Israel*
[2]*Department of Postharvest Science, Agricultural Research Organization, Volcani Center, Israel*
[3]*Department of Plant and Environmental Sciences, Weizmann Institute of Science, Israel*
[4]*Life Sciences Core Facilities, Weizmann Institute of Science, Israel*
[5]*Department of Plant Pathology and Microbiology, The Hebrew University of Jerusalem, Israel*

**P-37** Wastewater-based surveillance of SARS-CoV-2 variants in Israel

**Neta Zuckerman**[1]
*Central Virology Laboratory, Israel Ministry of Health, Israel*

**P-38** Measles outbreak epidemiology in real-time: Development and implementation of a rapid strain classification test for measles

Efrat Bucris, Roberto Azar, Batya Mannasse, Hadar Assraf, Vardit Moshayoff, Tal Levin, Victoria Indenbaum, Yara Kanaaneh, Yaniv Lustig, **Oran Erster**[1]
*Central Virology Laboratory, Israel Ministry of Health, Israel*

**P-39** Exploring the dynamics and mechanisms of the HCV-induced epigenetic signature after cure

**Manar Hijaze**

**P-40** Uncovering single-cell inherited fitness strategies with Microcolony-seq

**Raya Faigenbaum-Romm**[1], Orit Gefen[1], Naama Katsowich-Nagar[2], Irine Ronin[1], Ilan Rosenshine[2], Nathalie Q. Balaban[1]
[1]*The Racah Institute of Physics, Edmond J. Safra Campus, The Hebrew University of Jerusalem, Israel*
[2]*Department of Microbiology and Molecular Genetics, Institute for Medical Research Israel-Canada, Faculty of Medicine, The Hebrew University of Jerusalem, Israel*

**P-41** Genomic diversity of carbapenem-resistant Acinetobacter baumannii (CRAB) reveals distinct virulence evolution

**Mor Lurie-Weinberger**[1], Alona Keren-Paz[1], Yehuda Carmeli[1,2]
[1]*National Institute for Antibiotic Resistance & Infection Control, TLVMC, Israel*
[2]*Medicine, Tel Aviv University, Israel*

**P-42** Elucidating the intracellular architecture of the Escherichia coli transcriptome and its driving mechanisms

**Roy Jacobson**[1], Zohar Persky, Daniel Dar
*Department of Plant and Environmental Sciences, Weizmann Institute of Science, Israel*

**P-43** Emergence and spread of ST913-IVa-t991 methicillin-resistant Staphylococcus aureus among pediatric populations in Israel.

**Moti Baum**[1], Einav Anuka[1], Maya Davidovich-Cohen[1], Assaf Rokney[1]
*Public Health Laboratories –Jerusalem (PHL-J), Public Health Services (PHS), Ministry of Health (MOH), Israel, Ministry of Health, Israel*

**P-44** WGS-based phylogeny of multidrug-resistant salmonella typhimurium in Israel

**Eugenia Yakunin**[1], Yosi Ilan Shorr[1]
*Public Health Laboratories Jerusalem, Public Health Division, Ministry of Health, Israel*

**P-45** HiSpoT a novel NGS-based method for simple, high-throughput genotyping of *Mycobacterium tuberculosis*

**Israel Nissan**[1], Ephraim Fass[2], Paul J. Freidlin[2], Gal Zizelski Valenci[2], Inna Kutikov[2], Mor Rubinstein[2], Yelena Losev[2], Hasia Kaidar-Shwartz[2], Zeev Dveyrin[2], Efrat Rorman[2]
[1]*The Laboratory for Poultry Diseases, Kimron Veterinary Institute, Ministry of Agriculture and Rural Development, Veterinary Services, Israel*
[2]*National Public Health Laboratory, Ministry of Health, Public Health Directorate, Israel*

**P-46** Genome-wide CRISPR-knockout screen reveals novel anti-viral host factors affecting respiratory viruses infections

**Randa Tissawak**[1]
*Microbiology and molecular genetics, The Hebrew University, Israel*

**P-47** Single-cell RNA-seq of the rare virosphere reveals the native hosts of giant viruses in the marine environment

**Amir Fromm**[1], Assaf Vardi[1]
*Department of Plant and Environmental Sciences, Weizmann Institute of Science, Israel*

**P-48** Quantification of Herpes simplex viruses recombination rates using an AI algorithm

**Maya Ralph-Altman**[1], Daniel Avhar[1], Yuval Altman[1], Enosh Tomer[1,2], Oren Kobiler[1]
[1]*Clinical Microbiology and Immunology, School of Medicine, Tel Aviv University, Israel*
[2]*The Central Virology Laboratory, Public Health Services, Ministry of Health, Sheba Medical Center, Tel-Hashomer, Israel*

**P-49** Structural insights into SARS-CoV-2 neutralization by anti-SARS-CoV-2 antibody isolated from severe convalescent donor

**Aakanksha Harit**[1], Michael Mordekovich[2], Lee S. Izhaki-Tavor[1], Natalia Freund[2], Moshe Dessau[1]
[1]*Azrieli Faculty of Medicine, Bar-Ilan University, Israel*
[2]*Faculty of Medical & Health Sciences, Tel Aviv University, Israel*

**P-50** The FDA-Approved Drug Amiloride is a Potent Inhibitor of Sandfly Virus Replication

**Yarden Moalem**[1], Prof. Ella Sklan[1]
*Microbiology and Clinical immunology, Faculty of Medical & Health Sciences, Tel Aviv university, Israel*

**P-51** Characterization of neutralization epitopes on the envelope protein of the Hepatitis C virus

**Manar Alkhateb**[1], Haneen Faris-Gadban[1], Noam Ben Shalom[2], Natalia T. Freund[2], Meital Gal-Tanamy
[1]*Molecular Virology Lab, Azrieli Faculty of Medicine in Galilee, Bar Ilan University, Israel*
[2]*Department of Microbiology and Immunology, Sackler School of Medicine, Tel Aviv University, Israel*

**P-52** Structural studies of Thrips Cyclophilin A, an Interactor of Tomatto Spotted Wilt Virus (TSWV) Glycoprotein N

**Adarsh Patel**[1], Marlonni Arajo[2], Anna Whitfield[2], Moshe Dessau[1]
[1]*Faculty of Medicine, Bar Ilan University, Israel*
[2]*Entomology and Plant Pathology, North Carolina State University, United Stated of America*

**P-53** Structural-evolution investigation of phenuiviridae envelop protein

**Suryakant Tiwari**[1], Hossin Bouz[1], Joel Alter[1], Moshe Dessau[1]
*Faculty of medicine, Bar-Ilan University, Israel*

**P-54** Interaction of the Kaposi's sarcoma-associated Herpesvirus vBcl-2 with the Cellular Protein KLHL-9

**Sapir Ohev**[1], Anastasia Gelgor Dontsov[1], Coral Haddad[1], Inna Kalt[1], Ronit Sarid[1]
*Life Sciences, bar ilan university, israel*

**P-55** Fate of the KSHV Tegument Proteins ORF45 and ORF52 During de novo Infection

**Hanan Abu-Hassan**[1], Dana Dünn-Kittenplon[1], Inna Kalt[1], Ronit Sarid[1]
*Life-science, Bar-Ilan University, Israel*

**P-56** Exploring wheat rhizosphere microbiome to enhance sustainable production

**Sharon Samuel**[1], David Zeevi[1], Avraham Levy[1]
*Plant and Environmental Sciences, Weizmann Institute of Science, Israel*

**P-57** Systematic exploration of plant-microbe antagonism using the unicellular algae *Chlamydomonas reinhardtii.*

**Tzila Vikniansky**[1], Elad Meilin, Naomie Alon, Dr. Michal Breker, Dr. Omri Finkel
*Department of Plant and Environmental Sciences, The Hebrew University of Jerusalem, Israel*

**P-58** Synergistic interactions in duckweed microbiome facilitate cobalamin production

**Dutta Rhishika**[1], Osnat Gillor[1], Inna Khozin-Goldberg[1]
*Zuckerberg Institute of Water Research, Ben Gurion University of the Negev, Israel*

**P-59** Establishing an experimental system to investigate sponge-microbe recognition mechanisms

**Chiara Conti**[1], Sofia Sizikov[1], Tzipora Peretz[1], Sagie Schif[2], Rinat Bar-Shalom[1], Laura Steindler[1]
[1]*Department of Marine Biology, Leon H. Charney School of Marine Sciences, University of Haifa, Israel*
[2]*Department of Biology, Faculty of Natural Sciences, University of Haifa, Israel*

**P-60** Reverse-engineering the structure of social networks via microbial strain-sharing

**Amiyaal Ilany**[1], Prameek Kannan[1], Omry Koren[2]
[1]*Faculty of Life Sciences, Bar-Ilan University, Israel*
[2]*Faculty of Medicine, Bar-Ilan University, Israel*

**P-61** Microbial dark matter sequences verification in amplicon sequencing and environmental metagenomics data

**Hana Barak**[1], Naomi Fuchs[2], Michal Liddor-Naim[2], Irit Nir[2], Alex Sivan[2], Ariel Kushmaro[2]
[1]*Department of Civil and Environmental Engineering, Ben Gurion University, Israel*
[2]*Avram and Stella Goldstein-Goren Department of Biotechnology Engineering, Ben Gurion University, Israel*

**P-62** Agricultural ecosystem restoration increases soil microbial diversity and reduces nitrogen loses

**Sweta Kumari**[1], Max Kolton[1], Orah Felicia Rein-Moshe[2]
[1]*The Jacob Blaustein Institute for Desert Research, Ben-Gurion University of the Negev, Israel*
[2]*Soil Erosion Research Station, State of Israel Ministry of Agriculture and Rural Development, Israel*

**P-63** Bacterial DNA inversions and functional plasticity during inflammation and encounter with bacteriophages

**Shaqed Carasso**[1], Rawan Zaatry[1], Haitham Hajjo[1], Dana Kadosh-Kariti[1], Nadav Ben-Assa[1], Rawi Naddaf[1], Noa Mandelbaum[1], Sigal Pressman[2], Yehuda Chowers[2], Tal Gefen[3], Kate Jeffrey[4], Juan Jofre[5], Michael Coyne[6], Laurie Comstock[6], Itai Sharon[7], Naama Geva-Zatorsky[3]
[1]*Medicine, Technion - Israel Institute of Technology, Israel*
[2]*Clinical Research Institute, Rambam Health Care Campus, Israel*
[3]*Medicine, Technion - Israel Institute of Technology, Israel*
[4]*Program in Immunology, Harvard Medical School, United States of America*
[5]*School of Biology, University of Barcelona, Spain*
[6]*Duchossois Family Institute and Department of Microbiology, The University of Chicago, United States of America*
[7]*Molecular and Computational Biosciences and Biotechnology, Migal Galilee Technology Center, Israel*

**P-64** Metaproteomic analysis of immunotherapy modulation by the microbiome

**Tal Nissenbaum**[1], Yochai Wolf[2], Lior Lobel[1]
[1]*The Goodman Faculty of Life Science, Bar-Ilan University, Israel*
[2]*The Ella Lemelbaum Institute for Immuno-oncology, Sheba Medical Center, Israel*

**P-65** The microbiome affects obesity-related metabolites in the process of aging

**Dana Binyamin**[1], Sondra Turjeman[1], Nofar Asulin[1], Ron Schweitzer[1,2], Omry Koren[1]
[1]*Azrieli Faculty of Medicine, Bar-Ilan University, Israel*
[2]*Galilee Research Institute, MIGAL, Israel*

**P-66** A novel method for microbial pathogenic cultures inactivation

**Dror Halachmi**[1], Mahmoud Abu-ghannam[1], Marina Feingold[1], Amal Shibli[1], Elad Bar-Cohva[1]
*Biotechnology Production, Merck Sigma-Aldrich Israel, Israel*

**P-67** Pipeline for rapidly evaluating activity and inferring mechanisms of action of prospective antifungal compounds

**Judith Kraut-Cohen**[1], Omer Frenkel[2], Shay Covo[3], Evgeniya Marcos-Hadad[3], Shmuel Carmeli[4], Eduard Belausov[5], Dror Minz[6], Eddie Cytryn[6]
[1]*Institute of Soil, Water and Environmental Sciences, Agricultural Research Organization, Volcani Center, Israel*
[2]*Department of Plant Pathology and Weed Research, Institute of Plant Protection, Agricultural Research Organization, Volcani Center, Israel*
[3]*Department of Plant Pathology and Microbiology, Robert H. Smith Faculty of Agriculture, Food and Environment, The Hebrew University, Israel*
[4]*Raymond and Beverly Sackler School of Chemistry, Faculty of Exact Sciences, Tel Aviv University, Israel*
[5]*Confocal Microscopy Unit, Agricultural Research Organization, Volcani Center, Israel*
[6]*Institute of Soil, Water and Environmental Sciences, Agricultural Research Organization, Volcani Center, Israel*

**P-68** Utilizing the beneficial bacterium B. subtilis to reduce chemical and biological corrosion.

**Avichay Nahami**[1,2], Yosi Shacham[1], Yuval Dorfan[2], Ilana Kolodkin-Gal[1]
[1]*Scojen institute for synthetic biology, Reichman, Israel*
[2]*Electrical engineering, HIT, Israel*

**P-69** Crustaceans as a source of new bistable rhodopsins for optogenetics

**Alina Pushkarev**[1], David Buhrke[1], Johannes Vierock[1], Peter Hegemann[1], Camille Brouillon[1,2], Sonja Kleinlogel[2], Moran Shalev Benami[3], Marjorie Lienard[4], Megan Porter[5]
[1]*Experimental Biophysics, Humboldt University of Berlin, Germany*
[2]*Institute of Physiology, University of Bern, Switzerland*
[3]*Department of Chemical and Structural Biology, Weizmann Institute of Science, Israel*
[4]*Department of Biology, University of Lund, Sweden*
[5]*Department of Biology, University of Hawai'i at Mānoa, United States*

**P-70** High CO2 levels increase reactive oxygen species (ROS) generation in cyanobacteria

**Myint Khin**[1]
*FAAB, Ben-Gurion University of the Negev, Israel*

**P-71** Secretion of Functional Interferon by the Type 3 Secretion System of Enteropathogenic Escherichia coli

**Ira Rostovsky**[1]
*Faculty of Health Sciences, Ben Gurion University of the Negev, Department of Microbiology and Immunology*

**P-72** The effect of storage temperature on salmonella survival and VBNC state on carrots

**Chani Bronshtein**[1,2], Tehila Behar[2], Lea Chuwer[2], Carmit Ziv[2], Danielle Duanis-Assaf[2]
[1]*Food and Environment, The Robert H. Smith Faculty of Agriculture, Israel*
[2]*Postharvest and Food Science, Volcani Center, Israel*

**P-73** Live microbiome profiling and PMA-qPCR monitoring of bacterial indicators for food safety and spoilage detection

**May Cohen Hakmon**[1], Keren Buhnik-Rosenblau[1], Hila Hanani[1], Hila Korach-Rechtman[1], Yechezkel Kashi[1]
*Biotechnology and Food Engineering, Technion, Israel*

**P-74** The root microbiome and mitigation of nitrous oxide (N2O) emissions from cereal fields

Ada Viterbo[1], **Omri Vardi-Perlstein**, Rona Ziskin, Dror Minz
*Soil, Water and Environmental Sciences, Agricultural Research Organization, Volcani Center, Israel*

**P-75** Combined Arachidonic acid, triclosan and fluoride treatment enhanced the anti-bacterial activity against Streptococcus mutans

**Avraham Melkam**[1,2], Ronit Vogt Sionov[1], Miriam Shalish[2], Doron Steinberg[1]
[1]*Faculty of Dental Medicine, Institute of Biomedical and Oral Research (IBOR), The Hebrew University of Jerusalem, Israel*
[2]*Department of Orthodontics, Faculty of Dental Medicine, Hadassah Medical Center, The Hebrew*

*University of Jerusalem, Israel*

**P-76** MinD-RNase E interplay controls localization of mRNAs to the *E. coli* cell poles

**Omer Goldberger**[1], Shanmugapriya Kannaiah[2], Nawsad Alam[3], Anat Nussbaum-Shochat[1], Ora Schueler-Furman[1], Tamar Geiger[4], Orna Amster-Choder[1]
[1]*Faculty of Medicine, The Hebrew University, Israel*
[2]*School of Medicine, Washington University, Missouri, USA*
[3]*Department of Biochemistry, University of Oxford, UK*
[4]*Department of Molecular Cell Biology, Weizmann Institute of Science, Israel*

**P-77** The Ecophysiology of bacterial rosettes

**Tamar Szoke**[1], Einat Segev[1]
*Plant and Environmental Sciences, Weizmann Institute of Science, Israel*

**P-78** The potential contribution of SAR11 to global warming via methyl-phosphonate biosynthesis

**Alhan Abu Hamoud**[1], Rinat Bar- Shalom[1], Tzipora Perez[1], Laura Steindler[1]
*Department of Marine Biology, Leon H. Charney School of Marine Sciences, University of Haifa, Israel*

**P-79** Does the precise mid-cell division ensure equal sharing between daughter cells?

**Sharanya Namboodiri**[1], Alexander Aranovich[1], Mario Feingold[1], Itzhak Fishov[2]
[1]*Department of Physics, Ben Gurion University of Negev, Israel*
[2]*Department of Life Sciences, Ben Gurion University of Negev, Israel*

**P-80** Investigating The Construction of The Bacterial Envelope

**Gideon Mamou**, Federico Corona[1], Ruth Cohen Khait[2], Nicholas Housden[3], Waldemar Vollmer, Colin Kleanthous
[1]*Centre for Bacterial Cell Biology, Newcastle University, UK*
[2]*The Department of Molecular and Computational Biosciences and Biotechnology, MIGAL, Israel*
[3]*Department of Biochemistry, University of Oxford, UK*

**P-81** Unifying principles of bactericidal versus bacteriostatic antibiotics

**Elizabeth Vaisbourd**[1], Anat Bren[1], David S. Glass[1], Yifan Yang[1], Avi Mayo[1], Uri Alon[1]
*Molecular Cell biology, Weizmann Institute of Science, Israel*

**P-82** Search, preparation and pesticidal activity of new functionally substituted monosaccharides for plant protection

**Valery Belakhov**[1], Irina Boikova[2]
[1]*Schulich Faculty of Chemistry, Technion – Israel Institute of Technology, Israel*

[2]*Microbiological Plant Protection Laboratory, All-Russian Institute of Plant Protection, Russia*

**P-83** Biofilm formation, interspecies interactions and niche adaptation play fundamental role in shaping dust microbiome structured communities

**Talal Salti**[1,2], Naama Lang Yona[1], Ilana Kolodkin-Gal[2]
[1]*Civil and Environmental Engineering, Technion, Israel*
[2]*Scojen Institute for Synthetic Biology, Reichman University, Israel*

**P-84** Abundant Sulfitobacter marine bacteria protect Emiliania huxleyi algae from pathogenic bacteria

**Roni Beiralas**[1], Noy Ozer, Einat Segev
*Plant and environmental sciences, Weizmann institute of science, Israel*

**P-85** The role of secondary metabolites in marine bacterial interactions in the context of an algal host

**Olesia Shlakhter**[1], Einat Segev[1]
*Department of Plants and Environmental Sciences, Weizmann Institute of Science, Israel*

**P-86** Visualizing division of labor and filament heterogeneity in filamentous multicellular cyanobacterium Nostoc sp. (Anabaena sp.) PCC7120

**Marharyta Skovorodka**[1], Daniel Dar[1]
*Plant and Environment, Weizmann Institute of Science, Israel*

**P-87** Systemic approach to unravel biofilm-amoeba arms-race

**Prem Anand Murugan**[1], Eva Zanditenas[2], Sergi Ankri[2], Ilana Kolodkin-Gal[1]
[1]*Scojen Institute of Synthetic biology, Reichman University, Israel*
[2]*Department of Molecular Microbiology, Ruth and Bruce Rappaport Faculty of Medicine, Technion, Technion, Israel*

**P-88** A Burkholderia cenocepacia-like environmental isolate rapidly kills the human fungal pathogen candida auris

**Jiawei Li**[1], Tingting Song[1], Marina Rocha[2], Neta Shlezinger[2], Jonathan Friedman[1]
[1]*The Institute of Environmental Sciences, The Hebrew University of Jerusalem, Israel*
[2]*Koret School of Veterinary Medicine, The Hebrew University of Jerusalem, Israel*

**P-89** Chemical crosstalk guides the interaction between the common soil bacteria pseudomonas chlororaphis and bacillus subtilis

**Zohar Tik**[1], Shaked Uzi-Gavrilov, Bat El Hagbi-Lazar, Orit Sivan[2], Michael M. Meijler[3]
[1]*Department of Chemistry, Ben-Gurion University of the Negev, Israel*
[2]*Department of Earth and Environmental Sciences, Ben-Gurion University of the Negev, Israel*
[3]*The National Institute for Biotechnology in the Negev, Ben-Gurion University of the Negev, Israel*

**P-90** Estimating horizontal gene transfer frequency of multidrug-resistant plasmids from wastewater

**Kseniia Erokhina**[1,2], Swapnil Kajale[1], Chagai Davidovich[1,2], Eddie Cytryn[1]
[1]*Institute of Soil, Water and Environmental Sciences, Volcani Institute, Israel*
[2]*Department of Agroecology and Plant Health, The Robert H. Smith Faculty of Agriculture, Food and Environment, The Hebrew University of Jerusalem, Israel*

**P-91** Phage therapy and pulsed blue light synergistic bactericidal effect over *Pseudomonas Aeruginosa* Preformed Biofilm

**Amit Rimon**[1], Jonathan Belin[1], Shunit Coppenhagen-Glazer[1], Lilach Gavish[2,3], Ronen Hazan[1]
[1]*Institute of Biomedical and Oral Research (IBOR), Faculty of Dental Medicine, The Hebrew University of Jerusalem, Israel*
[2]*3Institute for Research in Military Medicine (IRMM), Faculty of Medicine, The Hebrew University of Jerusalem, Israel*
[3]*The Department of Medical Neurobiology, Institute for Medical Research (IMRIC), Faculty of Medicine, The Hebrew University of Jerusalem, Israel*

**P-92** Structure-guided discovery of viral proteins that inhibit host immunity

**Erez Yirmiya**[1], Rotem Sorek[1]
*Department of Molecular Genetics, Weizmann Institute of Science, Israel*

**P-93** A novel phage-encoded RNA manipulates phage efficiency in bacteriophage Lambda

**Aviezer Silverman**[1], Reut Bruner[1], Raneem Nashef[1], Sahar Melamed[1]
*Department of Microbiology and Molecular Genetics, Hebrew University Of Jerusalem, Jerusalem*

**P-94** An archaeal CBASS system that eradicates a chronic viral infection

**Deepak Choudhary**[1], Uri Gophna[1], Himani Himani[1], Dana Vassover[1]
*George S. Wise Faculty of Life Sciences, Tel Aviv University, Israel*

**P-95** Diversification of molecular pattern recognition in bacterial NLR-like proteins

**Nathalie Bechon**[1], Nitzan Tal[1], Avigail Stokar-Avihail[1], Sarah Melamed[1], Gil Amitai[1], Rotem Sorek[1]
*Department of Molecular genetics, Weizmann Institute of Science, Israel*

**P-96** Identification and investigation of phage and host factors that regulate T4-like cyanophage genome expression

**Maor Sarah Berkovich**[1], Ran Tahan[1], Claudia Steglich[2], Debbie Lindell[1]
[1]*Faculty of Biology, Technion – Israel Institute of Technology, Israel*
[2]*Institute of Biology 3, Genetics & Experimental Bioinformatics, University of Freiburg, Germany*

**P-97** Combating Burkholderia cepacia complex infections by integrating different strategies in the development of phage treatments

**Ortal Yerushalmy**[1,2], Ron Browunshtine[1,2], Chani Rakov[1], Sivan Alkalay-Oren[1,2], Leron Khalifa[1,2], Amit Rimon1[1,2,3], Shunit Coppenhagen-Glazer[1,2], Daniel Gelman[2,3], Saima Aslam[4], John LiPuma[5], Ran Nir-Paz[2,6], Ronen Hazan[1,2]
[1]*Institute of Biomedical and Oral Research (IBOR), Hebrew University of Jerusalem, Israel*
[2]*IPTC, The Israeli Phage Therapy Center of Hadassah Medical Center and the Hebrew University, Israel*
[3]*Department of Military Medicine, Faculty of Medicine, The Hebrew University of Jerusalem, Israel*
[4]*Division of Infectious Diseases and Global Public Health, University of California San Diego, USA*
[5]*Department of Pediatrics, University of Michigan Medical School, USA*
[6]*Department of Clinical Microbiology and Infectious Diseases and the Faculty of Medicine, The Hebrew University of Jerusalem, Israel*

**P-98** A Burkholderia cenocepacia-like environmental isolate strongly inhibits the plant fungal pathogen Zymoseptoria tritici

**Tingting Song**[1], Yael Sorokin[1], Jonathan Friedman[1]
*Institute of Environmental Sciences, The Hebrew University of Jerusalem, Israel*

**P-99** Coordinated V-shaped structuring governs mature biofilm development in Lactobacillus plantarum during adaptation to acidic stress

**Athira Venugopal**[1], Ronit Vogt Sionov[1], Yulia Kroupitski[2], Moshe Shemesh[2]
[1]*Institute of Biomedical and Oral Research, The Hebrew University of Jerusalem, Israel*
[2]*Department of Food Sciences, Institute of Postharvest Technology and Food Sciences, Agricultural Research Organization, Israel*

**P-100** Unraveling the sensory mechanisms of bacterial defense systems via co-expression with phage genes

**Hadar Samra**, Ilya Osterman, Nitzan Tal, Jeremy Garb, Rotem Sorek

**P-101** A-to-I mRNA editing can control di sulfide bond formation in bacteria and the toxicity of the HokB toxin

**Liron Didi**[1], Liam Aspit[1], Dganit Melamed[2], Raz Zarivach[3], Orna Dahan[4], Yitzhak Pilpel[4], Dan Bar Yaacov[1]
[1]*Microbiology, Immunology and Genetics, Ben-Gurion University of the Negev, Israel*
[2]*The Smoler Protein Research Center, Technion Israel Institute of Technology, Israel*
[3]*Life Sciences, Ben-Gurion University of the Negev, Israel*
[4]*Molecular Genetics, Weizmann Institute of Science, Israe*

# Posters
# Poster Session I – Monday, September 9

## 12:45 - 14:00 POSTER SESSION II (Tuesday, September 10), Poster Area

**P-1** Profiling the systemic and gut anti-commensal antibody repertoire of children with Crohn's disease

**Tomer Zur**[1], Lilach M Friedman[1], Lior Levi[1], Oren Ledder[2], Dotan Yogev[2], Raffi Lev-Tzion[2], Esther Orlanski Meyer[2], Amit Assa[2], Shira Yuval Bar Asher[2], Asaf Azlay[2], Tasia Maslov[2], Dan Turner[2], Tomer Hertz[1]
[1]*Department of Microbiology, Immunology and Genetics, and The National Institute for Biotechnology in the Negev, Ben-Gurion University of the Negev, Israel*
[2]*Institute of Pediatric Gastroenterology and Nutrition, Shaare Zedek Medical Center, Israel*

**P-2** Endogenous ZAP affects flavivirus RNA interactome

Nguyen Phuong Khanh Le, Prince Pal Singh, **Uladzimir Karniychuk**[1]
*Veterinary Biosciences, The Ohio State University, USA*

**P-3** Proteome-wide dynamic mapping of organelle composition during enterovirus infection

**Yael Yair**[1], Ofir Atrakchi[2], Rachel Sharan[1], Tami Geiger[2], Orly Laufman[1]
[1]*Department of Molecular Genetics, Weizmann Institute of Science, Israel*
[2]*Department of Molecular Cell Biology, Weizmann Institute of Science, Israel*

**P-4** Generation and characterization of sars-cov-2 neutralizing antibodies associated with clinical outcomes from b-cell repertoires

**Pratik Das**[1], Michal Werbner[1], Modi Safra[2], Pazit Polak[2], Moshe Matan[3], Avi Peretz[4], Moshe Dessau[5], Gur Yaari[6], Meital Gal-Tanamy[1]
[1]*Molecular Virology Lab, Azrieli Faculty of Medicine, Bar Ilan University, Israel*
[2]*Bio-engineering, Faculty of Engineering, Bar Ilan University, Israel*
[3]*Clinical Microbiology Laboratory, Baruch Padeh Medical Center, Israel*
[4]*Azrieli Faculty of Medicine, Bar Ilan University, Israel*
[5]*The Laboratory of Structural Biology of Infectious Diseases, The Azrieli Faculty of Medicine, Bar Ilan University, Israel*
[6]*Bio-engineering, Faculty of Engineering, Bar Ilan University, Israel*

**P-5** Elucidating the relationship between virus infection and cell-cycle regulation in marine diatoms

**Linoy Bamshid**[1], Amit Tzur[1], Dina Spungin[1], Chana F. Kranzler[1]
*The Mina and Everard Goodman Faculty of Life Sciences, Bar-Ilan University, Israel*

**P-6** Virulence, resistance and coexistence: understanding host-virus infection dynamics in marine diatoms

**Ori Zucker**[1], Dina Spungin[1], Chana Kranzler[1]
*The Mina and Everard Goodman Faculty of Life Sciences, Bar-Ilan University, Israel*

**P-7** High titer against OC43 seasonal coronavirus enhances susceptibility to SARS-CoV-2 infections

**Hanna Oppenheimer**[1], Shosh Zismanov[1], Lilach M. Friedman[1], Lior Nesher[2], Tomer Hertz[2,3]
[1]*Department of Microbiology, Immunology and Genetics, Faculty of Health Sciences, Ben Gurion University, Israel*
[2]*Infectious Disease Institute, Soroka University Medical Center, and Faculty of Health Sciences, Ben Gurion University, Israel*
[3]*Vaccine and Infectious Disease Division, Fred Hutch Caner Research Center, Seattle*

**P-8** Characterization of the effect of diet on bacterial virulence

**Dor Braverman**[1], Neta Sal-Man[1]
*Microbiology and Immunology, Ben-Gurion University, Israel*

**P-9** Structural and functional characterization of the EspD protein of the T3SS of enteropathogenic Escherichia coli

**May Morhaim**[1]
*Microbiology, Ben Gurion University, Israel*

**P-10** ACE2 is a novel entry factor for HCV

**Samer Ayoub**[1], Moshe Dessau[2], Meital Gal-Tanamy[1]
[1]*Molecular Virology lab, The Azrieli Faculty of Medicine, Bar-Ilan University, Israel*
[2]*The Laboratory of Structural Biology of Infectious Diseases, The Azrieli Faculty of Medicine, Bar-Ilan University, Israel*

**P-11** Utilizing nutrient type compounds as anti-bacterial compounds: arginine and cysteine inhibit Salmonella survival in egg white

**Nir Ben-Porat**[1], Amital Ohayon[1], Tali Rosenberg[1], Abdulafiz Musa[2], Erik Petersen[2], Erez Mills[1]
[1]*Department of Animal Sciences, Robert H. Smith Faculty of Agriculture, Food, and Environment, The Hebrew University of Jerusalem, Israel*
[2]*Department of Health Sciences, College of Public Health, East Tennessee State University, United States*

**P-12** Deciphering the immune subsets in Salmonella Typhi human infection which underly disease outcome

**Noa Bossel Ben-Moshe**[1]
*Immunology and Regenerative Biology, Weizmann Institute of Science, Israel*

**P-13** Interactions of the chronic-infecting *Haloferax volcanii* pleomorphic virus 1 (HFPV-1) with its host

**Himani Singla**, Deepak kumar Choudhary, Israela Turgeman grott, Uri Gophna

**P-14** Characterizing the pathogen niche of Salmonella in the gut at early stages of disease

**Liron Levy-Efrati**[1], Noa Bossel Ben-Moshe[1], Roi Avraham[1]
*Department of Immunology and Regenerative Biology, Weizmann Institute of Science, Israel*

**P-15** Algal-bacterial host-pathogen interactions in the ocean

**Nitsan Albocher-Kedem**[1], Bareket Dassa[2], Assaf Vardi[1]
[1]*Plant and Environmental Sciences, Weizmann Institute of Science, Israel*
[2]*Life Sciences Core Facilities, Weizmann Institute of Science, Israel*

**P-16** Aerobic methane production in Nitrogen-depleted oceanic regions

**Jaime Abdiel Lazaro Garcia**[1], Rinat Bar Shalom, Alhan Abu Hamoud
*School of Marine Science, University of Haifa, Israel*

**P-17** Impact of biodegradable and non-biodegradable microplastic on soil microbial community composition and activity

**Lingyan (Ciel) Zhang**[1], Max Kolton[1]
*French Associates Institute for Agriculture and Biotechnology of Drylands, Ben Gurion University of the Negev, Israel*

**P-18** Exploring the influence of plant fiber on gut microbiome diversity

**Sarah Winkler**[1], Omer Lavy[1], Liron Levin[1], Stav Eyal[1], Anke Trautwein-Schult[2], Dörte Becher[2], Sarah Morais[1], Yitzhak Mizrahi[1]

[1]*Life Sciences, Ben Gurion University of the Negev, Israel*
[2]*Microbial Proteomics, University of Greifswald, Germany*

**P-19** Evolution and ecology of environmental microbiome in response to human interferences

**Guy Tadmor**[1], David Zeevi[1], Assaf Vardi[1]
*Plant and Environmental Science, Weizmann Institute of Science, Israel*

**P-20** Enhanced microbial succession for oil degradation in Arid region

**Kareem Neiroukh**

**P-21** Application of chromosomal barcoding to study the role of horizontal gene transfer in bacterial adaptation

**Elizaveta Miticheva**[1], Shimon Bershtein[1]
*Department of Life Sciences, Ben-Gurion University, Israel*

**P-22** Demystifying heterotrophic diazotrophy in streambeds

**Rand Lander**[1], Eyal Geisler[1], Carla Villamarin[1], Shai Arnon[1], Almog Gafni[1], Hagar Siebner[1], Edo Bar-Zeev[1]
*Zuckerberg Institute of Water Research, Ben-Gurion University of the Negev, Sde Boker, Israel*

**P-23** Pear flower and leaf microbiome dynamics during the naturally occurring spread of Erwinia amylovora

**Aia Oz**[1], Orly Mairesse[2,3], Shira Raikin[2], Hila Hanani[1], Hadar Mor[1], Mery Dafny Yelin[2], Itai Sharon[1,4]
[1]*Biotechnology, Migal – Galilee Research Institute, Israel*
[2]*Northern Agriculture Research & Development, Migal – Galilee Research Institute, Israel*
[3]*Sciences and Technology, Tel-Hai Academic College,, Israel*
[4]*Science and Technology, Tel-Hai Academic College, Israel*

**P-24** Metabolically-versatile Ca. Thiodiazotropha symbionts of the deep-sea lucinid clam Lucinoma kazani have the genetic potential to fix nitrogen

**Lina Ratinskaia**[1,2], Stas Malavin[1,3], Tal Zvi-Kedem[1,2], Simina Vintila[4], Manuel Kleiner[4], Maxim Rubin-Blum[1,2]
[1]*Department of Biology, National Institute of Oceanography, Israel Oceanographic and Limnological Research, Israel*
[2]*Department of Marine Biology, Leon H. Charney School of Marine Sciences, University of Haifa,, Israel*
[3]*Department of Environmental Hydrology and Microbiology, Zuckerberg Institute for Water Research, The Jacob Blaustein Institutes for Desert Research, Ben-Gurion University of the Negev, Israel*
[4]*Department of Plant and Microbial Biology, North Carolina State University, USA*

**P-25** Neutral dispersal and host-mediated selection shape bacterial metacommunity in galapagos marine iguanas

**Ido Grinshpan**[1], Omer Lavy[1], Alvah Zorea[1], Itai Amit[1], Liron Levin[1], Ori Furman[1], Daniel Somech[1], Otto X. cordero[2], Itzhak Mizrahi[1]
[1]*Life science, Ben Gurion University, Israel*
[2]*Climate, Environment & Life Science, MIT, USA*

**P-26** Diet Driven Selection of Microbial Communities and its functional-ecological outcomes

**Omer Lavy**[1], Sarah Whinkler[1], Sarah Morais[1], Liron Levin[1], Anke Trautwein-Schult[2], Dörte Becher[2], Itzik Mizrahi[3]

[1]*Department of Life sciencs, Ben Gurion university, Israel*
[2]*Department of Microbial Proteomics, University of Greifswald, Germany*
[3]*Department of Life sciences, Ben Gurion university, Israel*

**P-27** Diffusivity influences evolutionary interactions of bacteria and bacteriophages in space and time

**Christoph Velling**[1], Roy Kishony[1,2,3]
[1]*Faculty of Biology, Technion - Israel Institute of Technology, Israel*
[2]*Faculty of Computer Science, Technion - Israel Institute of Technology, Israel*
[3]*Faculty of Biomedical Engineering, Technion - Israel Institute of Technology, Israel*

**P-28** A systematic exploration shows that Bacillus subtilis has a readily accessible plastic metabolic network.

Shanni Horsntein, Swati Sirotiya, Raul Mireles, Alex Geller, Asaf Levi, **Lianet Noda-Garcia**

**P-29** Illuminating the secrets of eukaryotic microbial adaptation: differential gene expression in a naturally red-shifted pit lake under diverse light conditions

**Shai Fainsod**[1], Oded Beja[1]
*Biology, Technion - Israel Institute of Technology, Israel*

**P-30** The impact of micro-habitat fragmentation on microbial populations growth dynamics

**Nadav Kashtan**[1]
*Institute of Environmental sciences, The Herbrew University, Israel*

**P-31** Light-harvesting by antenna-containing xanthorhodopsins in cyanobacteria

**María del Carmen Marín Pérez**[1], Andrey Rozenberg[1], Keiichi Inoue[2], Oded Béjà[1]
[1]*Faculty of Biology, Technion-Israel Institute of Technology, Israel*
[2]*The Institute for Solid State Physics, University of Tokyo, Japan*

**P-32** Species sorting as the central paradigm modulating microbial assembly in a two-step biofilter with Ulva and periphyton

**Dzung Nguyen**, Matan Masasa, Ofer Ovadia, Lior Guttman

**P-33** Viral lysis vs. nutrient starvation: effect on prochlorococcus macromolecular structure and the microbial community

**Shira Givati**[1], Dikla Aharonovich[1], Natalia Belkin[2], Eyal Rahav[2], Qinglu Zeng[3], Daniel Sher[1]
[1]*Marine biology, Haifa University, Israel*
[2]*National Institute of Oceanography, Israel Oceanographic and Limnological Research, Israel*
[3]*Department of Ocean Science, Hong Kong University of Science and Technology, China*

**P-34** How can you live without Pds5? Finding new genes affecting the maintenance of sister chromatid cohesion.

**Sreejita Dutta**[1], Karan Choudhary, Nir Katz, Martin Kupiec
*Shmunis School of Biomedicine and Cancer Research, The George S. Wise Faculty ofLife Sciences, Tel Aviv University, Tel Aviv University, Israel*

**P-35** Aerial dissemination of pycnidiospores in rainless days: A case study of lasiodiplodia theobromae in avocado

**Satyendra Pratap Singh**[1], Alon Shomron[1], Ran Shulhani[2], Dani Shtienberg[2], Noam Alkan[1]
[1]*Department of Postharvest Science, Agricultural Research Organization, Volcani Center, Israel*

[2]*Department of Plant Pathology and Weed Science, Agricultural Research Organization, Volcani Center, Israel*

**P-36** Antifungal potential of CaEDTA against postharvest pathogen Botrytis cinerea.

**Durga Prasad**[1,2], Carmit Ziv[1]
[1]*Department of Postharvest Sciences, Agricultural Research Organization, Volcani Center, Israel*
[2]*The Robert H. Smith, Faculty of Agriculture, Food and Environment, The Hebrew University of Jerusalem, Israel*

**P-37** Bacterial-fungal crosstalk is defined by a fungal lactone mycotoxin and its degradation by a bacterial lactonase

Livnat Afriat-Jurnou[1,2], Keren Nudel, **Shlomit Dor**, Rami Cohen, Justin L. Eagan, Christina M. Hull, Nancy P. Keller, Dov Pruski
[1]*Department of Molecular and Computational Biosciences and Biotechnology, Migal-Galilee Research Institute, Israel*
[2]*Tel-Hai College, Faculty of Sciences and Technology, Upper Galilee*

**P-38** cVDPV3 outbreak in Israel (2021-2022) through enhanced environmental surveillance and mutation analysis: a genomic epidemiology study

**Merav Weil**[1], Itay Bar-Or[1], Hagar Morad-Eliyahu[1], Efrat Bucris[1], Leah Weiss[1], Rinat Vasserman[1], Ilana S Fratty[1,2], Ira Aguvaev[1], Zvi Cohen-Said[1], Rua Matar[1], Oran Erster[1], Lester M Shulman[1,3], Ruth Yishai[4], Lior Hecht-Sagie[5], Sharon Alroy-Preis[5], Ella Mendelson[1,3], Yaniv Lustig[1,3], Danit Sofer[1], Neta S. Zuckerman[1]
[1]*Central Virology Laboratory, Public Health Services, Ministry of Health, Israel*
[2]*The Israel Center for Disease Control, Israel Ministry of Health, Israel*
[3]*School of Public Health, Sackler Faculty of Medicine, Tel-Aviv University, Israel*
[4]*Department of Laboratories, Public Health Services, Ministry of Health, Israel*
[5]*Public Health Services, Ministry of Health, Israel*

**P-39** Identifying plasmid origin of transfer (oriT) utilizing a machine learning approach

**Ori Ben Shir**[1], Bruria Samuel[1], David Burstein[1]
*The Shmunis School of Biomedicine and Cancer Research, Tel Aviv University, Israel*

**P-40** Detecting positive epistasis in chronic infections of SARS-CoV-2

**Adi Ben Zvi**, Sheri Harari, Danielle Miller, Yael Maoz, Adi Stern

**P-41** The role of environmental factors in modulating the human breast milk microbiome composition

**Melody Kasher**[1], Shaqed Carasso[1], Tal-Avi Gefen[1], Naama Geva-Zatorsky[1], Kelsley Fehr[2,3], Meghan Azad[2,3], Geoffrey L Winsor[4], Jeffrey R Brook[5]
[1]*Department of Cell Biology and Cancer Science, Rappaport Faculty of Medicine, Technion, Israel*
[2]*Manitoba Interdisciplinary Lactation Centre (MILC), Children's Hospital Research Institute of Manitoba, Canada*
[3]*Department of Pediatrics and Child Health, University of Manitoba, Canada*
[4]*Department of Molecular Biology and Biochemistry, Simon Fraser University, Canada*
[5]*Department of Chemical Engineering and Applied Chemistry, Dalla Lana School of Public Health University of Toronto, Canada*

**P-42** Comparison of four diagnostic tests for helicobacter pylori infection

**Tsachi Tsadok Perets**[1,2], Rachel Gingold Belfer[3,4], Ram Dickman[3,4], Matan Siterman[3,4], Doron Boltin[3,4]
[1]*Gastroenterology Laboratory, Rabin Medical Center, Israel*

[2]*Digital Medical Technologies, Holon Institute of Technology, Israel*
[3]*Division of Gastroenterology, Rabin Medical Center, Israel*
[4]*School of Medicine, Tel Aviv University, Israel*

**P-43** Carbapenemase-producing Kluyvera in Israel - epidemiology of an evolving species.

**Gabrielle Levi**[1], Mor Lurie Weinberger[1], Alona Keren Paz[1], David Schwartz[1], Yehuda Carmeli[1,2]
[1]*National Institute for Antibiotic Resistance & Infection Control, Ministry of Health, Israel*
[2]*Sackler Faculty of Medicine, Tel Aviv University, Israel*

**P-44** Unraveling the mechanism of antibacterial action of chlorhexidine on enterococcus faecalis

**Nathanyel Sebbane**[1,2,3], Itzhak Abramovitz[4], Nurit Kot-Limon[5], Doron Steinberg[1]
[1]*Faculty of Dental Medicine, Biofilm Research Laboratory Institute of Biomedical and Oral Research (IBOR), Hebrew University-Hadassah, School of dental medicine, Israel*
[2]*Department of Endodontics, Faculty of Dental Medicine, Hebrew University-Hadassah, School of dental medicine, Jerusalem*
[3]*"Bina" Program, Faculty of Dental Medicine, Hebrew University-Hadassah, School of dental medicine, Jerusalem*
[4]*Head, Department of Endodontics, Faculty of Dental Medicine., Hebrew University-Hadassah, School of dental medicine, Israel*
[5]*Department of Endodontics, Faculty of Dental Medicine, Hebrew University-Hadassah, School of dental medicine, Israel*

**P-45** Pantoea ronii - a newly identified human opportunistic pathogen identified by whole genome sequencing

**Danielle Keidar Friedman**[1], Daniel Leshin-Carmel[2], Anka Tzur[3], Muriel Amsalem[3], Daria Tolkach[4], Tal Brosh-Nissimov[2], Nadav Sorek[3]
[1]*Emerging Infectious Disease Laboratory, Assuta Ashdod University Hospital, Israel*
[2]*Infectious Diseases Unit, Assuta Ashdod University Hospital, Israel*
[3]*Clinical Microbiology Laboratory, Assuta Ashdod University Hospital, Israel*
[4]*Emerging Infectious Disease Laboratory, Assuta Ashdod University Hospital, Israel*

**P-46** Diatom and virus succession within a dramatic spring bloom event in the northern red sea

**Jasmin Mellijor**[1], Yael Tennenbaum-Azulai[1], Dina Spungin[1], Yoav Avrahami[2,3], Assaf Vardi[4], Miguel Frada[2,3], Chana Kranzler[1]
[1]*The Mina and Everard Goodman Faculty of Life Sciences, Bar-Ilan University, Israel*
[2]*Department of Ecology, Evolution and Behavior, Silberman Institute of Life Sciences, The Hebrew University of Jerusalem, Israel*
[3],, *The Interuniversity Institute for Marine Sciences in Eilat, Israel*
[4]*Department of Plant and Environmental Sciences, Weizmann Institute of Science, Israel*

**P-47** The snakehead retrovirus promoter is versatile and generates maternally inherited transcripts in transgenic zebrafish

**Eran Bacharach**[1], Rachel Zamostiano[1], Odelia Pisanty[2], Reem Abu Rass[1], Avi Eldar[3], Marcelo Ehrlich[1], Yoav Gothilf[2]
[1]*The Shmunis School of Biomedicine and Cancer Research, The George S. Wise Faculty of Life Sciences, Tel Aviv University, Israel*
[2]*School of Neurobiology, Biochemistry and Biophysics, The George S. Wise Faculty of Life Sciences, Tel Aviv University, Israel*
[3]*Department of Virology, Kimron Veterinary Institute, Israel*

**P-48** Up regulation of the long non-coding RNA PURPL by Kaposi's sarcoma associated herpesvirus to suppress p53 activity

**Samia Showgan**, Guy Journo, Diana Meary, Vyacheslav Gurevich, Meir Shamay[1]
*Faculty of medicine, Bar Ilan University, Israel*

**P-49** KSHV genome harbors both constitutive and lytically induced enhancers

Nilabja Roy Chowdhury, Vyacheslav Gurevich, **Meir Shamay**[1]
*Azrieli Faculty of Medicine, Bar-Ilan University*

**P-50** Methyl-CpG-binding protein 2 as a Key Player in Kaposi`s sarcoma-associated herpesvirus Latency

**Ankita Awase**, Supriya Bhattacharya, Vyacheslav Gurevich[1], Prof. Meir Shamay
*Azrieli Faculty of Medicine, Daniella Lee Casper Laboratory in Viral Oncology, Bar-Ilan University, Israel*

**P-51** Systematic functional assay revealed constitutive as well as lytic enhancers within the KSHV genome

**Nilabja Roy Chowdhury**[1], Vyacheslav Gurevich[1], Meir Shamay[1]
*Azrieli Faculty of Medicine, Bar-Ilan University, Israel*

**P-52** The rhizosphere microbiome and exudome of Brachypodium spp. distributed along a steep climatic gradient

**Sheetal -**[1], Peter Reuveni[1], Sofia Arellano[1], Maor Matzrafi[1], Shilo Rosenwaser[2], Daniella Gat[1], Elisa Korenblum[1]
[1]*Plant Sciences Institute, Volcani Center (ARO), Israel*
[2]*Plant Sciences, Hebrew University of Jerusalem, Israel*

**P-53** Avocado root microbiome for regenerative agriculture

**Daniella Gat**[1,2], Adi Kushmaro Bier[3], Yael Bar-Noy
[1]*Plant Science, Agricultural Research Center (ARO) - Volcani Center, Israel*
[2]*The Robert H. Smith Institute of Plant Sciences and Genetics in Agriculture, The Hebrew University of Jerusalem, Israel*
[3]*Soil, Water and Environmental Science Institute, Agricultural Research Center (ARO) - Volcani Center, Israel*

**P-54** Identification of disease suppressive secondary metabolites producing bacteria

**Hildah Amutuhaire**

**P-55** The role of the gut microbiota in food allergies

**Nofar Asulin**[1]
*Azrieli Faculty of Medicine, Bar-Ilan university, Israel*

**P-56** The effect of the microbiome on lowering aggressive behavior in Drosophila melanogaster

**Rachel Levin**[1], Sondra Turjeman, Omry Koren
*The Azrieli Faculty of Medicine, Bar Ilan University, Israel*

**P-57** The neuro-immune effects of the gut microbiota at birth in a germ-free mouse model

**Soha Zgairy**[1]
*Azrieli Faculty of Medicine, Bar Ilan University, Israel*

**P-58** Resistance and resilience of desert soil microbial communities to prolonged heatwaves

**Leo Shteinberg**[1], Max Kolton[2]
[1]*High School, Kfar Silver Agricultural High School, Israel*
[2]*Department of French Associates Institute for Agriculture & Biotechnology of Drylands, The Jacob Blaustein Institute for Desert Research, Ben-Gurion University of the Negev, Israel*

**P-59** The effect of the gut microbiome on aggression

**Lelyan Moadi**

**P-60** Aerobic microbial methanogenesis and methane oxidation in aplysina aerophoba

**Martha Alejandra Duran Canche**[1], Chiara Conti[1], Jana Milucka[2], Sina Schorn[2], Laura Steindler[1]
[1]*Department of Marine Biology, Leon H. Charney School of Marine Sciences, University of Haifa, Israel*
[2]*De-part-ment of Biogeo-chem-istry, Max Planck Institute for Marine Microbiology, Germany*

**P-61** Microbial terroir across different Israeli vineyard soils

**Omri Bassevich**, Ayala Barkan, Yotam Zeit, Noam Reshef, Elisa Korenblum

**P-62** Etiology of postharvest decay of long-term stored carrots and the development of sustainable control measures

**Nadav Smila**[1,2]
*Department of Postharvest Science, Agricultural Research Organization, Volcani Institute, Israel*
*Department of Agroecology and Plant Health, the Robert H. Smith Faculty of Agriculture, Food and Environment, Hebrew University of Jerusalem, Israel*

**P-63** Bioinspired surfaces with anti-microbial properties for preventing fungal adhesion

**Hanan Abu-Hamad**[1], Elena Prudnikov[2], Iryna Polishchuk[2], Gali Ron[1], Boaz Pokroy[2], Ester Segal[1]
[1]*Department of Biotechnology and Food Engineering, Technion - Israel Institute of Technology, Israel*
[2]*Department of Materials Science and Engineering, Technion - Israel Institute of Technology, Israel*

**P-64** Developing a new protein delivery technique, using a bacterial secretion system

**May Tfilin Samuel**[1]
*Microbiology, Immunology and Genetics, Ben-Gurion University, Israel*

**P-65** Restricting bacterial bioreporter survival following field dispersal

**Liat Moscovici - Vald**[1]
*1Department of Plant & Environmental Sciences, Hebrew University of Jerusalem, Israel*

**P-66** Performance enhancement of bacterial bioreporters for the detection of trace explosives

**Etai Shpigel**[1], Benjamin Shemer[1], Tal Elad[1], Shilat Dadon-Simanowitz[1], Liat moscovici[1], Lidor David[2], Itay Levin[2], Lior Zimmerman[2], Shimshon Belkin[1]
[1]*Department of Plant & Environmental Sciences, The Hebrew University of Jerusalem,, Israel*
[2]*Enzymit, Enzymit Ltd., Israel*

**P-67** From raw ingredients to product- *Salmonella* survival during chocolate production

Tehila Behar, Zipora Tietel, **Danielle Duanis-Assaf**[1]
*Department of Food Science, Agricultural Research Organization (ARO), The Volcani Center, Israel*

**P-68** Isolation and characterization of novel methanol tolerant lipases from environmental bacteria for biodiesel production

**Aviv Fainstein**[1], Yoram Gerchman[1,2]
[1]*Department of Biology and Environment, Faculty of Natural Science, University of Haifa, Israel*
[2]*Department of Biology and Environment, Oranim College, Israel*

**P-69** Integrated use of electrochemical anaerobic reactors and genomic-based modeling for characterizing methanogenic activity in microbial communities exposed to BTEX contamination

**Jenny Yusim**[1,2,3], Raphy Zarecki[2], Shlomit Medina[2], Sari Mousa[3], Aviv Kaplan[1], Igal Gozlan[1], Mahdi Hassanin[3], Zeev Ronen[4], Katie Baransi-Karkaby[3], Dror Avisar[1], Isam Sabbah[3], Keren Yanuka-Golub[3], Shiri Freilich[2]
[1]*Water Research Center, Tel-Aviv University, Israel*
[2]*Newe-Ya'ar Research Center, Agricultural Research Organization, Ministry of Agriculture, Israel*
[3]*Institute of Applied Research, The Galilee Society, Israel*
[4]*Department of Environmental Hydrology and Microbiology, Ben-Gurion University of the Negev, Israel*

**P-70** Improvement in aqueous extractable caffeine content using solid-state fermentation was justified to be higher in green tea waste

**Bixia Qiu**[1,2], Avi Shpigelman[1], Yigal Yigal Achmon[1,2]
[1]*Department of Biotechnology and Food Engineering, Technion - Israel Institute of Technology, Israel*
[2]*Department of Biotechnology and Food Engineering, Guangdong Technion Israel Institute of Technology, GTIIT, China*

**P-71** Alcanivorax dominates the Hydrocarbon degrading community in Ex situ soil treatment.

Swati Gupta, Shlomit Medina, Shiri Freilich, **Zeev Ronen**[4]
*Zuckerberg institute for Water Research, Ben Gurion University of the Negev, Israel*

**P-72** Toxicity of smoke residues and their toxicity elimination using the human microbiome

**Tal Bar**[1], Karina Golberg[1], Robert Marks[1,2], Ariel Kushmaro[1,2]
[1]*Avram and Stella Goldstein-Goren Department of Biotechnology Engineering, Ben-Gurion University of the Negev, Israel*
[2]*The Ilse Katz Center for Nanoscale Science and Technology, Ben-Gurion University of the Negev, Israel*

**P-73** Metabolomics of anandamide-treated streptococcus mutans reveals mechanism of action

**Goldie Wolfson**[1], Ronit Vogt Sionov[1], Ori Shalev[2], Itzhack Polacheck[3], Maya Korem[3], Doron Steinberg[1]
[1]*Faculty of Dental Medicine, Institute of Biomedical and Oral Research (IBOR),The Hebrew University of Jerusalem, Israel*

[2]*Faculty of Medicine, Metabolomics Center, Core Research Facility, The Hebrew University of Jerusalem, Israel*
[3]*Department of Clinical Microbiology and Infectious Disease, Hadassah-Hebrew University Medical Center, Israel*

**P-74** Growth-phase-specific induction of viable but non-culturable state in Listeria innocua by a nature-based antimicrobial formulation

**Esther Mwangi**[1,2], Victor Rodov[1], Moshe Shemesh[1]
[1]*Department of Postharvest and Food Sciences, Agricultural Research Organization, The Volcani Institute, Israel*
[2]*Department of Biochemistry, Food Science and Nutrition, The Robert H. Smith Faculty of Agriculture, Food and Environment, Hebrew University of Jerusalem, Israel*

**P-75** Biofilm formation correlates with resistance to human serum in multidrug resistant clinical isolates of Klebsiella pneumoniae

**Hadas Fulman-Levy**[1], Polina Geva[1], Shiri Navon-Venezia[1,2]
[1]*Molecular Biology, Ariel University, Israel*
[2]*Adelson School of Medicine, Ariel University, Israel*

**P-76** Identification of Universal stress proteins in multidrug-resistant Klebsiella pneumoniae and their role in nutrient limitation

**Tsfia Kohen**[1], Polina Geva[1], Shiri Navon-Venezia[1,2]
[1]*Molecular Biology, Ariel University, Israel*
[2]*The Adelson School of Medicine, Ariel University, Israel*

**P-77** Repression by DNA looping: functional and structural analysis of the gram positive global transcriptional regulator ScoC

**Smadar Shulami**, Noam Hadad, Mnar Ghrayeb, Sergei Pomyalov, Yael Pazy, Liraz Chai, Yuval Shoham, Gil Shoham

**P-78** Single vesicle approaches for characterization of nucleic cargo in malaria parasite-derived extracellular vesicles

**Ewa Kozela**[1], Ekaterina Petrovich-Kopitman[2], Tali Dadosh[3], Abel Cruz Camacho[1], Yuval Berger[1], Yaara Shoham[1], Mattia Morandi[4], Neta Regev-Rudzki[1]
[1]*Biomolecular Sciences, Weizmann Institute of Science, Israel*
[2]*Flow Cytometry Unit, Weizmann Institute of Science, Israel*
[3]*Chemical Support Unit, Weizmann Institute of Science, Israel*
[4]*Institute of Organic Chemistry and Biochemistry, Czech Academy of Science, Czech Republic*

**P-79** Competitive interactions between culturable bacteria are highly non-additive

**Amichai Baichman-Kass**[1], Tingting Song[1], Jonathan Friedman[1]
*Plant Pathology and Microbiology, Hebrew University of Jerusalem, Israel*

**P-80** Multi-species biofilm characterization through ecological and evolutionary timescales using Raman spectroscopy

**Matvey Nikelshparg**[1], Adrian Lehvy[1], Shimon Bershtein[1]
*Department of Life Sciences, Ben-Gurion University of the Negev, Israel*

**P-81** Characterizing ecological and evolutionary dynamics in multiple-species biofilm using high-resolution barcodes

**Adrian Lehvy**[1], Matvey Nikelshparg[1], Shimon Bershtein[1]
*Life Sciences, Ben Gurion University, Israel*

**P-82** The ANMEsphere at the core of the cold seep microbial community

**Stas Malavin**[1,2], Maxim Rubin Blum[2]
[1]*Department of Desert Studies, Ben Gurion University of the Negev, Israel*
[2]*Department of Biology, Israel Oceanographic and Limnological Research, Israel*

**P-83** Insights into marine plasmid ecological dynamics: a global metagenomic perspective

**Lucy Androsiuk**[1,2], Shay Tal[2], Tal Shay[3]
[1]*Marine Biology and Biotechnology Program, Department of Life Sciences, Ben-Gurion University of the Negev, Eilat Campus, Israel*
[2]*National Center for Mariculture, Israel Oceanographic & Limnological Research, Israel*
[3]*Life Sciences, Ben-Gurion University of the Negev, Israel*

**P-84** Tangential Flow Filtration for Concentrating Marine Microbiota: Implications for Physiological Experiments and Microbial Composition

**Tzipora Peretz**

**P-85** The plastisphere: from ecosystem dynamics to pure cultures of "plastic-loving" microbes

**Matan Oren**[1]
*Molecular Biology Department, Ariel University, Israel*

**P-86** *Alcanivorax*, a genus of marine bacteria with varied tastes for hydrocarbons

**Aiswarya Kartha**[1], Keren Davidov, Sheli Itzhari, Matan Oren
*Department of Molecular Biology, Ariel University, Israel*

**P-87** Unveiling the mechanisms behind positive and negative interactions in marine microbial communities: a mathematical exploration of *Prochlorococcus* - heterotroph interactions

**Osnat Weissberg**[1], Daniel Sher[1], Mick Follows[2]
[1]*Leon H. Charney School of Marine Sciences, Marine biology, University of Haifa, Israel*
[2]*Department of Earth, Atmospheric and Planetary Sciences, MIT, United States*

**P-88** Solid-state NMR-based phage structural virology at atomic resolution

**Amir Goldbourt**[1], Orr Lusky[1], Yoav Shamir[1], Omry Morag[1]
*School of Chemistry, Tel Aviv University, Israel*

**P-89** The first gene in a T7-like cyanophage genome is critical for efficient infection and fitness

**Bashir Said**[1], Debbie Lindell
*Faculty of Biology, Technion – Israel Institute of Technology, Israel*

**P-90** Diversity and evolution of phage sponge proteins that cancel bacterial immune signaling

**Romi Hadary**

**P-91** Exploring cheater virus dynamics in a long-term evolution experiment of MS2 bacteriophage

**Arielle Kahn**[1], Moran Meir[1], Noam Harel[1], Uri Gophna[1], Adi Stern[1]
*Life science, Tel-Aviv University, Israel*

**P-92** Cyanobacteria lysogens evolution: phenotypic changes and their genetic background

**Esther Cattan- Tsaushu**[1], Israa Yasin[1], Sarit Avrani[1]
*Department of Evolutionary and Environmental Biology,The Institute of Evolution, Haifa University, Israel*

**P-93** Aspects of lysogeny in cyanobacteria

**Yara Nwatha**[1], Sarit Avrani[1], Esther Cattan-Tsaushu[1]
*Department of Evolutionary and Environmental Biology, and the Institute of Evolution, University of Haifa, Israel*

**P-94** Entrance to the lysogenic cycle in cyanobacteria: the factors that affect phage decision whether to enter a lytic or a lysogenic infection cycle

**Hoda Madi**[1], Sarit Avrani[1], Esther Cattan-Tsaushu[1]
*Department of Evolutionary and Environmental Biology, and the Institute of Evolution, University of Haifa, Israel*

**P-95** Modifying nitrous oxide emissions by manipulating bacterial communities in wheat roots and soil

**Rona Ziskin**[1], Ada Viterbo[1], Omri Vardi-Perlstein[1], Yitzhak Hadar[2], Roi Ben-David[3], Maya Ofek-Lalzar Maya Ofek-Lalzar[4], Shahar Baram[5], Dror Minz[1]
[1]*Institute of Soil, Water and Environmental Sciences, Volcani Institute, Israel*
[2]*Department of Plant Pathology and Microbiology, The Robert H. Smith Faculty of Agriculture, Food & Environment*

*The Hebrew University of Jerusalem, Israel*
[3]*Department of Vegetables and Field crops, Volcani Institute, Israel*
[4]*Bioinformatic Services Unit, University of Haifa, Israel*
[5]*Institute for Soil, Water and Environmental Sciences, Volcani Institute, Research teams-Newe Yaar, Israel*

**P-96** Selecting for short-term drought-tolerance inducing rhizobacteria via host mediated microbiome engineering.

**Israel (Wray) Ben Avraham (Hansen III)**

# Oral Presentations (Abstracts)

Plenary session:
MECHANISMS OF BACTERIAL BIOFILM FORMATION AND FUNCTIONS
(Monday, September 9, 2024 09:15)

Invited Lecture

14 Microbial societies and microbial interactions

Mechanisms of bacterial biofilm formation and functions

**Knut Drescher**

In nature, bacteria mostly live in surface-attached communities termed biofilms, in which cells are attached to each other through an extracellular matrix. In this presentation, I will first introduce microscopy and image processing techniques that enable us to monitor all individual cells in living biofilms. Based on these techniques, I will then show how we can quantify biofilm properties in space and time, which revealed new functions of biofilms. The specific biofilm function I will focus on in the presentation arises during the interaction with immune cells. I will show that for the human pathogen Vibrio cholerae, biofilm formation is not only a protective trait but also an aggressive trait to collectively predate different immune cells. We find that V. cholerae forms biofilms on the eukaryotic cell surface using an extracellular matrix comprising primarily mannose-sensitive hemagglutinin pili, toxin-coregulated pili, and the secreted colonization factor TcpF, which differs from the matrix composition of biofilms on other surfaces. These biofilms encase immune cells and establish a high local concentration of a secreted hemolysin to kill the immune cells before the biofilms disperse in a c-di-GMP-dependent manner. Together, these results uncover how bacteria employ biofilm formation as a multicellular strategy to invert the typical relationship between human immune cells as the hunters and bacteria as the hunted.

Parallel session:
HOST-PATHOGEN INTERACTIONS I (Monday, September 9, 2024 10:45)

Invited Lecture

01 Host-pathogen interactions

## Elucidating stem cell innate immune responses

**Moshe Biton**[1]
*Department of Immunology and Regenerative Biology, Weizmann Institute of Science, Israel*

In the small bowel, intestinal epithelial cells (IECs) sense and respond to microbial stimuli and noxious substances, provide crucial barrier function, and coordinate immune responses. Here, we interrogate early epithelial responses to an enteric intracellular pathogen by profiling individual IECs from the mouse small intestine. Utilizing GFP-labeled *Salmonella enterica*, we isolated invaded or bystander cells to distinguish inflammatory from invasion programs of specific epithelial cell subsets. In particular, we generated a *salmonella*-specific infection classifier containing an antimicrobial peptide (AMP) gene signature, including the *Reg3* gene family, enabling the identification of the *Salmonella enterica* infection landscape of IECs, with possible implications for inflammatory bowel disease (IBD) patients. Our findings show that *Salmonella enterica* targets differentiated Paneth, enterocytes and stem/progenitor cells at these early time points. As a response, a rapid intrinsic epithelial cellular remodeling to enterocyte and Paneth lineages, expressing AMP genes, to combat the intruder is initiated. Lastly, we uncovered an intestinal stem cell differentiation program via inflammasome activation to protect the crypt environment while eliminating infected stem cells from the overall stem cell pool. This novel Lgr5+ stem cell defense mechanism protects the gut epithelium from persistent bacterial infection while regenerating the tissue. We propose IEC remodeling as a novel immune response to handle different gut stresses mediated by Lgr5+-ISCs to maintain organizational principles of gut homeostasis and physiology.

Parallel session:
HOST-PATHOGEN INTERACTIONS I (Monday, September 9, 2024 10:45)

Invited Lecture

01 Host-pathogen interactions

## The frenemy within: on helminth regulation of the intestinal barrier

**Danielle Karo-Atar**[1], Tamar Eliahu[1]
*Pharmacology and Clinical Biochemistry, Ben-Gurion University, Israel*

Enteric helminths are a unique example of highly invasive yet truly tolerable parasites, promoting mechanism of host defense favoring tissue adaptation and repair over parasite expulsion. These intestinal worms form intimate connections with the intestinal epithelium, a frontline effector of barrier immunity and integrity. Nevertheless, understanding how helminths affect the function of epithelial cells - their closest contact - has remained limited. The epithelium is one of the most highly regenerative tissues in our body thanks to the self-renewal capacity of multipotent intestinal stem cells (ISC). In response to injury, the ISC compartment undergoes extensive transcriptional reprogramming to promote the rapid regeneration of the tissue and maintain barrier integrity. We have recently demonstrated how the enteric parasitic roundworm, Heligmosomoides polygyrus bakeri (Hpb), directly targets the ISC compartment to revert the epithelium into a highly regenerative yet immature fetal-like state that is critical for proper recovery of barrier integrity following damage. In addition, we and others have identified helminth-mediated suppression of expulsion-promoting secretory epithelial lineages (i.e. tuft and goblet cells). Taken together, our work supports the exciting concept that helminths promote the regenerative capacity of the intestine to subvert host resistance yet maintain tissue resilience. Therefore, in our lab we explore how helminth-mediated regulation of the intestinal epithelium coordinates the host response to infection.

Parallel session:
HOST-PATHOGEN INTERACTIONS I (Monday, September 9, 2024 10:45)

01 Host-pathogen interactions

## Mycoviruses: harmless hitchhikers or backseat drivers of fungal disease?

**Neta Shlezinger**[1], Vanda Lerer[1], John Adeoya[1], Marina Rocha[1], Hilla Hayby[1]
*The Koret School of Veterinary Medicine, The Hebrew University, Israel*

Fungal pathogens pose a significant threat to global health. As eukaryotes, they share considerable homology with their hosts, requiring the development of innovative, non-cross-reactive therapies. Aspergillus fumigatus accounts for approximately 65% of all invasive fungal infections in humans, with aspergillosis mortality rates approaching 50%. Mycoviruses, which are viruses that infect fungi, are well-known to transform fungal virulence in plants. However, despite their ubiquity and importance, their impact on fungal pathogenesis in mammals remains unknown. Using an A. fumigatus strain naturally infected with Aspergillus fumigatus polymycovirus-1 (AfuPmV-1), we found that the mycovirus bestows a significant survival benefit to the fungus under oxidative and heat stress and in the murine lung. Moreover, Afupmv-1 rewires stress responses in part by enhancing genetic stability through upregulation of mis6. Thus, Afupmv-1 modulates fungal fitness leading to heightened virulence and the progression of fungal diseases. Accordingly, mycoviruses play a significant role as "backseat drivers" in human fungal diseases, presenting important clinical implications. Therefore, they hold great promise as molecular targets for diagnostics and therapeutic interventions, opening up new avenues for potential treatment strategies.

Parallel session:
HOST-PATHOGEN INTERACTIONS I (Monday, September 9, 2024 10:45)

Invited Lecture

01 Host-pathogen interactions

## The chicken reproductive tract microbiota and its roles

**Erez Mills**[1]
*Department of Animal Sciences, The Hebrew University of Jerusalem, Israel*

An important mechanism for the vertical transmission of symbiotic bacteria from one generation to the next is the interaction with adults. However, in most reptiles as well as in poultry commercial operations, progeny hatch and grow in the absence of adults and the egg itself is the only connection between parents and their offspring. Thus, it is possible that the egg itself might act to vertically transmit bacterial symbionts. 16S rDNA sequencing shows a large overlap in the ASVs identified in chicken intestinal and reproductive tract samples. Furthermore, through direct culturing we show that live bacteria are found in the reproductive tract of hens. Interestingly, some phylogenetic groups could not be cultured specifically from the magnum portion of the reproductive tract, which is the part which secretes egg white. Finally, an in-vitro experiment shows these same phylogenetic groups do not survive incubation in egg white, while others do. To conclude, our results show that live bacteria can be found in the hen's reproductive tract. It remains unknown if they effect sperm motility and survival, insemination or egg formation. Our results also show that the magnum is a selective environment allowing the survival of specific phylogenetic groups. Further research is required to determine if survival in the magnum leads to integration into the forming egg and the colonization of the chick already in-ovo.

Parallel session:
HOST-PATHOGEN INTERACTIONS I (Monday, September 9, 2024 10:45)

01 Host-pathogen interactions

## Virus entry is a major barrier for productive HCMV infection in monocytes

**Yaarit Kitsberg**[1], Aharon Nachshon[1], Michal Schwartz[1], Noam Stern-Ginossar[1]
*Molecular genetics department, Weizmann Institute of Science, Israel*

Human Cytomegalovirus (HCMV) infection can result in either productive or non-productive infection, with the latter potentially leading to viral latency. Intriguingly, differentiation of monocytes, which are thought to support latent infection, renders them permissive for productive infection but the molecular mechanisms driving these differences are not fully understood. By measuring newly transcribed mRNA in monocytes and macrophages we demonstrate there is a major block in monocytes infection prior to viral gene expression. Next, by analyzing the transcriptomes of primary monocytes as well as two cell line monocytes, and their differentiated counterparts, we searched for changes which can explain this block in viral gene transcription. We show that multiple cell surface proteins involved in HCMV entry are not expressed and monocytes but are upregulated upon differentiation and correspondily, HCMV entry into monocytes is inefficient. Remarkably, overexpression of PDGFRα, a known receptor for HCMV that is not expressed on myeloid cells, enables productive infection in monocytes, demonstrating that monocytes can efficiently support productive infection given effecient entry of viral genomes. We find that this inefficient entry can be attributed to extremely low expression of several integrins, whose expression dramatically increases upon differentiation. By bypassing the entry barrier, we conduct a genetic screen allowing us to discover additional differentiation related processes that affect the ability of the virus to establish productive infection, namely the cell's proliferative status and the level of oxidative phosphorylation. Overall, our findings indicate that HCMV infection of monocytes is blocked prior to viral gene expression and that a major barrier for productive infection of monocytes is at the entry stage.

Parallel session:
HOST-PATHOGEN INTERACTIONS I (Monday, September 9, 2024 10:45)

10 Applied microbiology and biotechnology

## Fusolisin protease may regulate the tumor microenvironment

**Nicole haj**[1], Amjad Shehadeh[1], Viviana Scaiewicz[1], Reem Bsoul[1], Ofir Ventura[1], Perlie Admony[1], Karolina Świderska[2], Marcin Drag[2], Jan Potempa[3], Ofer Mandelboim[4], Gilad Bachrach[1]

[1]*The Institute of Dental Sciences, The Hebrew University-Hadassah School of Dental Medicine, Jerusalem*

[2]*Department of Chemical Biology and Bioimaging, Wroclaw University of Science and Technology, Poland*

[3]*Department of Microbiology, Faculty of Biochemistry, Biophysics and Biotechnology, Jagiellonian University*

[4]*Hebrew University-Hadassah Medical School, Institute for Medical Research Israel Canada (IMRIC), Jerusalem*

*Fusobacterium nucleatum* is an oral Gram-negative anaerobic bacterium known for its ability to translocate to and colonize various malignancies, including colon adenocarcinoma, breast cancer, and esophageal cancer. Elevated levels of *F. nucleatum* within tumor microenvironments have been correlated with adverse prognostic outcomes and increased resistance to chemotherapy. Therefore, it is critical to eliminate this bacterium in tumors.

Fusolisin is a verified functional serine protease of *F. nucleatum*. In our previous work, the growth of *F. nucleatum* in a complex medium was found to be inhibited by the serine protease inhibitors PMSF and AEBSF, indicating that serine protease activity is essential for fusobacteria growth. Therefore, fusolisin has been proposed as a potential target for eliminating *F. nucleatum*.

Recently, we generated a recombinant fusolisin (rfusolisin) that enables extensive research of this protease. Next, we tested whether fusolisin can affect the tumor microenvironment.

IFN-γ can act against cancer cells in multiple ways, including initiating apoptosis. In agreement, incubation of INF-γ with the colorectal cancer cell line RKO reduced cell viability. rfusolisin was found to inhibit this INF-γ effect on RKO cells by cleaving the cytokine.

We then developed small-molecule inhibitors of fusolisin, by utilizing a hybrid combinatorial substrate library (HyCoSul) of tetra peptides that uses 129 natural and unnatural amino acids mixtures. We observed that the growth of *F. nucleatum* ATCC 25586 was affected by the selected small molecule inhibitor.

We are now testing these inhibitors in the tumor microenvironment and expect them to reverse fusobacterial induced tumor exacerbation.

Parallel session:
MICROBIAL ECOLOGY AND EVOLUTION (Monday, September 9, 2024 10:45)

02 Microbial ecology and evolution

## Flagellar genes and cyclic-di-GMP modulate predation and biofilm formation in the bacterial predator *Bdellovibrio bacteriovorus*

Abhirup Mookherjee, Mohor Mitra, Rajesh Sathyamoorthy, Gal Sason, Polpass Arul Jose, Maria Martinenko, Ofra Matan, Shmuel Pietrokovski, **Edouard Jurkevitch**[1]
*Faculty of Agriculture, Food and Environment, The Hebrew University of Jerusalem*

*Bdellovibrio bacteriovorus*, is an obligate bacterial predator, thus unable to replicate without prey and consequently to form biofilms. However, spontaneous host-independent (H-I) variants grow axenically and can form biofilms. We screened 350 H-I mutants and found that single mutations in flagellar stator genes were sufficient to generate H-I strains, deficient in motility and able to adhere to surfaces but unable to develop biofilms. Genes associated with flagellar, prey-invasion, and secondary signal cyclic-di-GMP (CdG) functions were deregulated, and CdG cellular concentration changed significantly. Genetic complementation reversed the phenotype but functional complementation by paralogues was not observed. However, H-I growth and biofilm formation were obtained in the background of invasion pole-associated pilus mutants, with biofilm formation modulated by stator genes. Predation by all H-I strains was reduced in prey suspension but not in prey biofilms, compared to the parental strain. Lastly, we show that a massively expressed stand-alone CdG riboswitch is differentially expressed in H-I strains, that it controls motility and largely alters gene expression. Thus, stator and invasion pole-dependent CdG signaling controls the H-D and the H-I biofilm-forming phenotypes, with single mutations overriding requirements for a prey, enabling shifts from obligate to facultative predation, with potential consequences on community dynamics.

Parallel session:
MICROBIAL ECOLOGY AND EVOLUTION (Monday, September 9, 2024 10:45)

Invited Lecture

02 Microbial ecology and evolution

## Pathogen Contingency Loci and the Evolution of Host Specificity

**Hadas Hawlena**[1], Ruth Rodríguez-Pastor, Nadav Knossow, Naama Shahar, Adam Z. Hasik, Daniel E. Deatherage, Ricardo Gutiérrez, Shimon Harrus, Luis Zaman, Richard E. Lenski, Jeffrey E. Barrick
*Mitrani Department of Desert Ecology, Ben-Gurion University of the Negev, Israel*

Pathogens can evolve in various ways to better exploit their hosts on many scales, ranging from adaptations within an infection of an individual host to a series of infections spanning multiple host species. However, there is limited understanding of how pathogen genomes evolve within natural communities when exposed to diverse hosts. We examined how Bartonella bacteria that circulate in rodent communities in the Negev Desert dunes in Israel adapt to the different rodent species co-occurring there. We propagated 15 Bartonella populations through infections of either a single host species (Gerbillus andersoni or G. pyramidum) or alternating between the two. After 20 rodent passages, new strains with de novo mutations replaced the ancestor strain in most populations. Dominant evolutionary changes were linked to mutations in two mononucleotide simple sequence repeats (SSRs) causing frameshifts in the same adhesin gene. These mutations were found only in populations that had interacted with G. andersoni and altered the infection dynamics in this host. Similar SSRs in other genes are conserved and exhibit ON/OFF variation in Bartonella isolates from this region. Our findings suggest that SSR-based contingency loci play a significant role not only in generating antigenic variation to evade immune responses but also in mediating the evolution of host specificity.

Parallel session:
MICROBIAL ECOLOGY AND EVOLUTION (Monday, September 9, 2024 10:45)

Invited Lecture

02 Microbial ecology and evolution

## Deconstructing the plant microbiome to discover plant protection traits

**Omri Finkel**[1], Amal Mhalwes[1], Raphael Sapir[1]
*Plant and environmental Sciences, The Hebrew University of Jerusalem, Israel*

Plants rely on sophisticated defense and immunity mechanisms to protect themselves from pests and pathogens. It is now established, however, that the first layer of defense is provided to the plant by other organisms – the plant microbiota. The protection provided by the microbiota constitutes not just the plant's first line of defense, but possibly its most potent one, as disruptions to the microbiota render plants susceptible to otherwise asymptomatic infections.

To understand how this layer of defense is deployed, we applied a realistically complex and fully tractable plant-soil-microbiome microcosm. This system provides a platform for the discovery of novel plant-beneficial traits, which only emerge within a microbial community context. In order to identify which components of the plant microbiota are critical for plant defense, we deconstructed this microcosm top-down, removing different microbial groups from the community to examine their effect on the plant. Applying this method reveals a high redundancy in the microbial protection of plants. Taxonomically disparate microbial consortia have similarly beneficial effects. Further dissection of the microbiota however, started to reveal unique roles for different microbe. In previous work, we discovered the protective role of the genus Variovorax, which removed excess microbial auxins from the rhizosphere.

Here, we have applied a community deconstruction approach to understand which members of the root microbiota contribute most to its protection from two very different pathogens: The bacterial leaf pathogen Pseudomonas syringae and the necrotic fungal pathogen Botrytis cinerea. This approach revealed that each pathogen is controlled by different elements within the microbiota. While P. syringae is controlled mostly by the genus Bacillus (phylum Bacillota), B. cinerea is controlled mostly by members of the phylum Pseudomonodota. Using RNA-seq, we further show that the plant, defense-related genes in particular, responds very differently to the presence of Bacillota and Pseudomonodota in the root microbiota.

We conclude that microbial protection of plants is layered, and that labor is divided among different members of the community. These findings also demonstrate the power of microcosm deconstruction as a tool in unraveling the complexities of plant-microbiome interactions.

Parallel session:
MICROBIAL ECOLOGY AND EVOLUTION (Monday, September 9, 2024 10:45)

Invited Lecture

02 Microbial ecology and evolution

## Ecophysiological drivers and biogeochemical consequences of virus infection in marine diatoms

**Chana Kranzler**[1]
*The Mina & Everard Goodman Faculty of Life Sciences, Bar-Ilan University, Israel*

Diatoms are a widespread group of aquatic, photosynthetic microorganisms that contribute ~20% of global primary production. Marine viruses catalyze a range of ecological and biogeochemical processes, yet little is known about the specific viruses that infect diatoms. Using a combination of field- and lab- based approaches, we are exploring the factors that shape diatom host-virus interactions and subsequent biogeochemical cycling. In natural diatom assemblages, we identified distinct patterns of virus infection across diverse nutrient regimes and over spatial and temporal scales. Using the bloom-forming, centric diatom, *Chaetoceros tenuissimus*, we have identified and characterized a range of infection dynamics, highlighting how environment and host ecophysiology can shape virus life cycles in diatoms. In addition, using a combination of taxonomically distinct diatom viruses and marine bacterial isolates, we found that dissolved organic matter generated during virus infection differentially impacts downstream microbial processing. Together, these findings are advancing our understanding of the factors that contribute to virus infection in diatom communities and the role that viruses play in determining the fate of diatom carbon and associated elements in the ocean.

Parallel session:
MICROBIAL ECOLOGY AND EVOLUTION (Monday, September 9, 2024 10:45)

02 Microbial ecology and evolution

## Biogels decelerate the sinking of organic particles to the ocean depths

**Uria Alcolombri**[1], Alon Nissan[2], Jonasz Słomka[3], Roman Stocker[3]
[1]*Department of Plant and Environmental Sciences, The Hebrew University of Jerusalem,, Israel*
[2]*The Robert H. Smith, Faculty of Agriculture, Food and Environment. Department of Soil and Water Sciences., The Hebrew University of Jerusalem,, Israel*
[3]*Institute of Environmental Engineering, Department of Civil, Environmental and Geomatic Engineering,, ETH Zurich, Switzerland*

Understanding the vertical flux of carbon in the ocean is critical for comprehending Earth`s carbon cycle. This study investigates the role of biofilms on sinking particles, a key component of the biological gravitational pump that transfers photosynthetically fixed carbon from the ocean surface to its depths. Utilizing a vertical millifluidic column, we tracked individual particles and observed significant biofilm accumulation within just one day, leading to a reduction in their sinking speed by up to 45%. Through combined video analysis and mathematical modeling, we identified two primary mechanisms for this deceleration: increased drag from biofilm tendrils and enhanced buoyancy due to the biofilm’s low density. Our findings suggest that biofilms, pervasive in the ocean, can markedly slow down particle sinking, potentially attenuating the vertical carbon flux. This research offers new insights into the biophysical processes regulating particle descent and highlights the significant impact of biofilms on the oceanic carbon cycle. Understanding these interactions is essential for predicting how changes in oceanic biomass could affect carbon sequestration in marine environments.

Parallel session:
MICROBIAL ECOLOGY AND EVOLUTION (Monday, September 9, 2024 10:45)

02 Microbial ecology and evolution

## The Dead Sea sinkhole pools: a source of microbial diversity in an extreme environment

**Ke Li**[1,2], Nadya Teutsch[2,3], Aia Oz[4,5], Revital Bookman[3], Itai Sharon[4,5], Sarit Avrani[1]
[1]*Department of Evolutionary and Environmental Biology and the Institute of Evolution, University of Haifa, Israel*
[2]*Department of Geochemistry and Environmental Geology, The Geological Survey of Israel, Israel*
[3]*Department of Marine Geosciences, University of Haifa, Israel*
[4]*Department of Biotechnology, Migal - Galilee Research Institute, Israel*
[5]*Faculty of Sciences and Technology, Tel-Hai Academic College, Israel*

Continuous desiccation of the Dead Sea has created thousands of sinkholes along its shoreline, some of them filled with water. The volume, size, and salinity levels of sinkholes vary significantly, creating unique aquatic habitats that are forever changing. This special environment provides an ideal setup for studying microbial adaptation to a changing environment. In this study, we aim to characterize microbial life and examine the abiotic factors that affect the dynamics of microbial communities in the sinkhole environment. Therefore, we sampled seasonally 11 sinkholes near Ein Gedi for three years for chemical and metagenomic analyses. Water density and chemical composition of the pools vary greatly in the same site and for the same pools over time. Microbial communities in the sinkholes consist of various bacteria, archaea, and eukaryotic microorganisms, as well as DNA and RNA viruses. The most abundant archaeal group in the sinkholes is Halobacteriota, and the most abundant bacterial group is Proteobacteria. Abiotic factors such as density, chloride, and sulphate are found to have significant affection on the sinkhole microbes (P  0.05). Preliminary data also suggest that the distribution of cyanobacteria and cyanophages is limited by salinity levels. We were able to recover nearly 900 high-quality metagenomics assembled genomes (MAGs) that represent at least 217 species, 195 of which are new at the species-to-class levels. These results demonstrate that the sinkhole is a unique habitat containing various microbial communities affected by levels of salinity.

Parallel session:
FUNGAL BIOLOGY (Monday, September 9, 2024 10:45)

Invited Lecture

03 Fungal biology

## Exploring the diversity of antifungal drug tolerance

**Judith Berman**

Antifungal drug tolerance and resistance are distinct responses to drugs that inhibit fungal growth, with tolerance being a frequent feature and resistance being rare in clinical isolates of *Candida albicans*, the most common commensal and opportunistic fungal pathogen of humans. Intrinsic antifungal tolerance is a relatively stable, *strain-specific*, feature characterized by a proportion of the cells growing in drug concentrations that inhibit growth of the overall population. Strains with higher tolerance have a *larger subpopulation* of cells that can grow in the drug, in part by *overcoming drug-triggered cell cycle arrest* faster than cells with lower tolerance levels. Intrinsic tolerance levels are affected by growth and stress conditions in a strain-specific manner. The strain-specific differences in intrinsic tolerance are likely a function of the broad diversity of genetic backgrounds, affected by the many genes and pathways involved in drug response. We have embarked on a study of over 1500 independent *Candida albicans* isolates from human, animals and environments and have determined the genome sequence, drug and stress responses and other phenotypes to ask about how the diversity of physiological processes that affect drug responses in *C. albicans*.

Acquired antifungal drug tolerance appears rapidly, usually as a consequence of drug selection. *Hetero-tolerance* is a transiently stable type of acquired tolerance often conferred by genome changes such as aneuploidy or copy number variations that can be gained and lost rapidly. The prevailing hypothesis is that altered gene dosage on the affected genomic regions drive the acquired drug responses. Relative drug concentration has a dramatic effect on the evolution of survival strategies: Hetero-tolerant colonies arise frequently (~1/1000 cells within a single liquid passage) in cultures exposed to supra-MIC drug concentrations. Isolates with heterotolerance often carry one of several recurrent aneuploid chromosomes, alone or in combination with other chromosomes. By contrast, acquired resistance can be due to rare point mutations or to transiently stable changes in chromosome or other mechanisms of copy number variation, and appears only after multiple passages at sub-MIC drug concentrations. Furthermore, the recurrent aneuploidies that confer transient tolerance differ from those conferring transient resistance, highlighting the different mechanisms that distinguish tolerance and resistance.

Parallel session:
FUNGAL BIOLOGY (Monday, September 9, 2024 10:45)

Invited Lecture

03 Fungal biology

## Belowground carbon transfer across mycorrhizal networks among trees: Facts, not fantasy

**Klein Tamir**[1], Stav Livne-Luzon[1], Ido Rog[1]
*Plant and Environmental Sciences, Weizmann Institute of Science, Israel*

The mycorrhizal symbiosis between fungi and plants is among the oldest, ubiquitous and most important interactions in terrestrial life on Earth. Carbon (C) transfer across a common mycorrhizal network (CMN) was demonstrated over half a century ago in the lab, and later in the field. Recent years have seen ample progress in this research direction, including evidence for ecological significance of carbon transfer. Furthermore, specific cases where the architecture of mycorrhizal networks have been mapped and CMN-C transfer from mature trees to seedlings has been demonstrated have suggested that trees in forests are more connected than once thought. In a recent Perspective, Karst et al. (2023) offered a valuable critical review warning of over-interpretation and positive citation bias in CMN research. It concluded that while there is evidence for C movement among plants, the importance of CMNs remains unclear, as noted by others too. Here we argue that while some of these claims are justified, factual evidence about belowground C transfer across CMNs is solid and accumulating.

Parallel session:
FUNGAL BIOLOGY (Monday, September 9, 2024 10:45)

03 Fungal biology

## Genome-wide CRISPRi Exploration of Plant-derived Compounds for Antifungal Potential

**Ofri Levi**[1]
*Biotechnology, MIGAL, ISRAEL*

Fungi play crucial roles in ecosystems but also pose threats to agriculture and human health through fungal diseases and the evolution of antifungal resistance. This phenomenon, driven by extensive fungicide use, presents challenges in both agricultural and medical contexts. Resistance mechanisms involve genetic mutations and metabolic adaptations. To characterize fungal resistance mechanisms comprehensively, we developed a genome-wide CRISPRi-based approach. This method selectively inhibits genes to understand genetic factors and molecular pathways driving resistance, potentially informing future interventions in agriculture and medicine. Preliminary data utilizing ferulic acid (FA) validate the utility of this approach in identifying genes that enhance fungal resistance to plant-derived compounds. This research provides a comprehensive framework for addressing antifungal resistance, with significant implications for human health, agricultural sustainability, fungal biology, and environmental conservation.

Parallel session:
FUNGAL BIOLOGY (Monday, September 9, 2024 10:45)

Invited Lecture

03 Fungal biology

## Cytokinin signaling and perception in fungi

Gautam Anand, Rupali Gupta, **Maya Bar**[1]
*Dept. of Plant Pathology and Weed Research, ARO, Volcani Institute, Israel*

Cytokinin (CK) mediates plant immunity and disease resistance. Some phytopathogens have been reported to secrete CKs, and may manipulate host CK signaling during pathogenesis. We previously demonstrated that CK directly inhibits the growth, development, and virulence of fungal phytopathogens, by dis-regulating the cell cycle and reducing cytoskeleton organization and cellular trafficking in the fungus. Investigating the effects of CK on the biology of the phytopathogenic fungus Botrytis cinerea (Bc), we found that CK possesses a dual role in fungal biology. In a nutrient-rich environment, CK strongly inhibited Bc growth and de-regulated cytoskeleton organization. This effect diminished as nutrient availability decreased. In its second role, we showed that CK can promote glycolysis and energy consumption in Bc, both in vitro and in planta. In contrast with the inhibitory effects of CK on the fungal cell cycle and cellular trafficking, glycolysis and increased oxidation mediated by CK in Bc were stronger with waning nutrient availability. The metabolic effects of CK on the fungus likely reflect the role of plant CK during early fungal infection. In addition to the plant producing CK during its interaction with the pathogen for defense priming and pathogen growth inhibition, the pathogen likely exploits this increased CK to boost its metabolism and energy production, in preparation for the necrotrophic phase of the infection. We are currently engaged in deciphering the fungal CK perception and signaling pathways, identifying fungal components of CK internalization and sensing.

Parallel session:
FUNGAL BIOLOGY (Monday, September 9, 2024 10:45)

03 Fungal biology

## Crossing of the Border: Deciphering Fusarium oxysporum`s Mammalian Host Adaptation and Immune Modulation Strategies

**Marina Campos Rocha**[1], Lin-Jun Ma[2], Neta Shlezinger[1]
[1]*Koret School of Veterinary Medicine, The Hebrew University of Jerusalem, Israel*
[2]*Biochemistry and Molecular Biology, University of Massachusetts Amherst, United States of America*

Fusarium oxysporum is a cross-kingdom fungal pathogen that infects plants, animals, and humans. It exhibits host-specific interactions, leading to distinct immune responses and varying levels of virulence. This versatility highlights the wide range of infections that can be caused by F. oxysporum and its adaptability as a pathogen. However, a comprehensive comparative investigation of virulence and pathogenesis between plant- derived and human-derived isolates has not been undertaken. In this study, we investigate the strain-specific immune responses and virulence characteristics of three F. oxysporum strains: Fol4287 (NRRL 34936), derived from plants, and two clinical F. oxysporum strains, one originating from systemic infection (NRRL 32931) and another one from keratitis infection (NRRL 47514. Our results reveal that F. oxysporum isolates provoke diverse immune response profiles in macrophages. Notably, blood-derived isolate induce higher levels of pro-inflammatory cytokines compared to their plant and keratitis counterparts. The blood isolate also employs evasive strategies, demonstrating resistance to phagocytosis and causing increased cellular damage in macrophages. In a murine model, the blood isolate exhibits enhanced virulence, greater tissue damage, and increased fungal dissemination. Plant and keratitis isolates, on the other hand, trigger macrophage recruitment and elevate myeloperoxidase (MPO) activity, suggesting an augmented defense mechanism. Our study further explores the correlation between biofilm formation, ergosterol production, vesicle secretion, and antifungal resistance. The blood strain displays increased adhesion, resistance to azole drugs, elevated ergosterol production, and vesicle secretion. The upregulation of ergosterol biosynthesis genes indicates a regulatory mechanism underlying these traits. Moreover, the blood strain induces Caspase-1 activation, gasdermin-D cleavage, and the release of pro-inflammatory cytokines, signifying the induction of pyroptosis-mediated cell death. Our research also demonstrates that vesicles produced by the blood strain induce a similar immune response as observed when incubating this strain with macrophages. Finally, we provide evidence that vesicle-secreted proteins from F. oxysporum contribute to cellular damage and trigger an immune response. These findings shed light on the strain-specific interactions between F. oxysporum and the host immune system, highlighting the intricate mechanisms that underlie its pathogenesis and virulence.

Parallel session:
CLINICAL VIROLOGY AND EPIDEMIOLOGY (Monday, September 9, 2024 14:00)

Invited Lecture

04 Clinical virology and epidemiology

## Congenital cytomegalovirus: transmission and control at the maternal-fetal interface

**Dana Wolf**[1]
*The Hebrew University Faculty of Medicine, Hadassah Hebrew University Medical Center, Israel*

Congenital cytomegalovirus infection (cCMV) is the most common intrauterine infection worldwide. Approximately 20% of congenitally-infected infants suffer from neurodevelopmental disabilities, and/or sensorineural hearing loss, which can manifest at birth or develop later during childhood. The rate of transplacental transmission is higher following primary vs. nonprimary (i.e., recurrent) maternal infection, highlighting the role of maternal immunity in fetal protection. Despite the global health burden of cCMV, there is no available vaccine to prevent cCMV, and no effective antenatal biomarkers of cCMV severity have been established for clinical use. Our current understanding of the maternal immune responses modulating human CMV transplacental transmission and of the pathogenesis of cCMV brain injury is largely limited by the absence of animal models for human cCMV.

Addressing these gaps, we have developed a unique experimental system of ex vivo viral infection in native human placental tissues, recapitulating the authentic events of viral infection and immune responses at the maternal-fetal interface. Further combining these models with studies in patients' clinical amniotic fluid samples, linked to well-defined clinical data, we have revealed previously unknown immune correlates of protection, and discovered novel prenatal biomarkers which can predict the severity of disease in the fetus and the newborn.

The findings could guide personalized treatment of CMV-infected fetuses and newborns, advancing prenatal and neonatal care. The identified correlates of protection, combined with data derived from our recently implemented cCMV universal newborn screening, will inform the development of vaccine against CMV.

Parallel session:
CLINICAL VIROLOGY AND EPIDEMIOLOGY (Monday, September 9, 2024 14:00)

Invited Lecture

04 Clinical virology and epidemiology

# HPV in MSM with and without HIV

**Orna Mor**[1,2], Roni Spector[2], Itzchak Levy[3,4], Maayan Itschaki[1]
[1]*Central Virology Laboratory, Ministry of Health, Israel*
[2]*School of Public Health, Faculty of Medicine, Tel Aviv University, Israel*
[3]*Infectious unit, Sheba Medical Center, Israel*
[4]*Faculty of Medicine, Tel Aviv University, Israel*

Background:

Human papillomavirus (HPV) is one of the most common sexually transmitted pathogens, responsible of several different diseases ranging from benign common warts to invasive carcinoma at a variety of anatomical sites. New molecular assays capable of distinguishing between 28 different HPV genotypes including the high-risk and low-risk HPV have been introduced. Recent European studies have demonstrated increasing incidence of both anal and oral cancers in HIV-positive patients, especially in men who have sex with men (MSM), when compared with HIV-negative controls. However, the prevalence of HPV infection, genotype diversity and determinants of HPV infection in MSM with and without HIV-1 has not been previously assessed in Israel.

Methods:

A total of 299 MSM 18 years old, 78 HIV-negative and 221 HIV-positive participants were recruited. Anal and oral swabs were collected and used for HPV genotyping with the Allplex™ HPV28 Detection kit (Seegene, Hylabs) following the manufacturer`s instructions.

Results:

Overall prevalence of any HPV in anal samples was 86% and 54% in HIV+ and HIV- individuals, respectively. In HIV+MSM the prevalence of any HPV in anal samples was significantly higher compared to oral swabs (84% vs 4%). No correlation between anti HPV vaccination and detection of HPV was identified although in general those with HIV who were not vaccinated were more likely to be HPV positives for one of the viruses included those included in the Gardasil vaccines. Main risk factors for HPV infection (13 hrHPV genotypes) were being HIV+ and making drugs other than cannabis. Younger HIV+MSM were significantly more likely to be positive for anal HPV.

Conclusions:

Anal HPV infection was common (50%) in both MSM HIV+ and HIV- although the incidence in HIV+ was significantly higher. Unexpectedly, no correlation was found between anti HIV vaccination and HPV positivity. A follow-up study on those with anal and oral hrHPV should be initiated and cytology as well as High Resolution Anoscopy (HRA) and/or examination of the oral cavity should be provided for hrHPV positive cases.

Parallel session:
CLINICAL VIROLOGY AND EPIDEMIOLOGY (Monday, September 9, 2024 14:00)

04 Clinical virology and epidemiology

## SPRI - Navigating Future Pandemics with Enhanced Preparedness

**Yael Weiss-Ottolenghi**[1]
*Sheba Pandemic Preparedness Research Institute, Sheba Medical Center, Tel Hashomer, Israel*

During the COVID-19 pandemic, the Sheba Health Care Workers (HCW) cohort study was launched, enrolling over 9,000 healthcare workers who provided crucial blood samples and data. This ongoing study has yielded significant insights into immune responses to infection and vaccination, real-world vaccine effectiveness, and the differences between healthy and vulnerable populations. These findings have had a substantial impact on both national and global health policies.

Despite being in a post-COVID-19 era, continuous waves of infection reveal the ongoing vulnerability of certain groups, such as the elderly and immunocompromised, highlighting the urgent need for new treatments against SARS-CoV-2, including monoclonal antibodies (mAbs).

Based on Sheba experience during the COVID-19 pandemic, we recognized the importance of preparing for future pandemics. Consequently, the Sheba Pandemic Preparedness Research Institute (SPRI) was established.

SPRI`s vision is to foster collaborative, multidisciplinary research that promptly translates basic science into clinical solutions, enabling rapid deployment of biological countermeasures, during future pandemics. The institute collaborates with the US National Institutes of Health (NIH), leveraging innovative technologies to accelerate research translation.

To further enhance future pandemic preparedness, SPRI has established various cohorts to study: zoonotic exposures (e.g., avian influenza), travelers (e.g., Dengue, WNV), Hajj pilgrims, and antimicrobial resistance, monitoring immune responses to these potential pandemic threats and developing therapeutics for them.

It is anticipated that research and developments at SPRI will pave the way for enhanced preparedness in navigating future pandemics.

Parallel session:
CLINICAL VIROLOGY AND EPIDEMIOLOGY (Monday, September 9, 2024 14:00)

Invited Lecture

04 Clinical virology and epidemiology

## How infected are we? Infection history to respiratory viruses during the Omicron outbreak in Israel

**Tomer Hertz**, Hanna Oppenheimer, Shosh Zismanov, Lilach M. Friedman, Lior Nesher

The SARS-CoV-2 pandemic highlighted the importance of asymptomatic infections to disease spreading and in shaping immune memory. While healthy individuals are continuously infected the prevalence of symptomatic and asymptomatic infections has not been well studied. Some individuals report multiple symptomatic infections annually, while others report no symptomatic infections. However, it is unclear whether these individuals are truly protected from infections, or simply harbor multiple asymptomatic infections. To address these questions, we conducted a clinical study following 110 individuals longitudinally across 9 months, with weekly collection of swabs and symptom questionnaires and monthly blood samples. To quantify infection history, we developed a multiplex-PCR panel that included 18 common human respiratory viral infections and herpes viruses.

During the first year of the trial (9/2021-7/2022), we recruited 51 individuals aged 24-65, of which 48 completed the study. We used our PCR panel to test 1578 swabs collected in year 1 resulting in 28,404 PCR tests. The most common infection detected was SARS-CoV-2 (n=54). The second most common infection was HRV and surprisingly, the third most common were seasonal coronaviruses (n=27). Two individuals remained PCR negative to all 14 respiratory viral infections throughout the study. There were 4 individuals that were infected 5 times, and another 3 that were infected 4 times each. Importantly 70% of the infections were asymptomatic.

Our data highlight the role of asymptomatic infections in shaping our immune history to respiratory viruses. They also identify a gradient of susceptibility to viral infections in healthy adults.

Parallel session:
CLINICAL VIROLOGY AND EPIDEMIOLOGY (Monday, September 9, 2024 14:00)

04 Clinical virology and epidemiology

## Molecular diagnostics of respiratory viruses in the community - a possible contribution to health policy management

**Ayelet Keren - Naus**

Viruses and bacteria can both cause respiratory disease with similar symptoms, there is no ability to differentiate between them based on a clinic alone. Laboratory tests performed for the community provide diagnostics for bacterial pathogens only. Detection of respiratory viruses in the community can prevent unnecessary administration of antibiotics and providing appropriate early treatment reduces morbidity, referring to the ER, and hospitalizations. Between October 2022 to March 2023, a pilot was carried out to diagnose respiratory viruses of patients in the community. The aim was to test the contribution of early diagnosis to the well-being of patients. Once a week, 93 random community samples of patients with respiratory illness which sent to SARS-CoV-2 diagnosis, were simultaneously tested for the following viruses: Influenza A, Influenza B, Human metapneumovirus, Parainfluenza1-4, Adenovirus A-F, Rhinovirus A-C, and Respiratory syncytial virus A/B. Results were assimilated in the medical files and were available to the community caregivers in the same day. During the study period, Statistic reports of the various respiratory viruses in the hospitalized patients as well as SARS-CoV-2 were available to clinicians to raise awareness of pathogens circulating in the population.

Our results, show that positive samples for each virus in the community reflects the results obtained among hospitalized patients over the age of 18. Total viral infection ranged from 7.1% to 32.2%. Samples positive for influenza A reached up to 10.7%. Together with Covid, viral infection in the community was between 51.1% - 83.9%.

The results emphasize the importance of real-time monitoring for patients in the community.

Parallel session:
CLINICAL VIROLOGY AND EPIDEMIOLOGY (Monday, September 9, 2024 14:00)

04 Clinical virology and epidemiology

# Is Human Rhinovirus (HRV) a pathogen that causes significant morbidity or an "innocent bystander" carried by hospitalized patients?

**Michal Mandelboim**[1]
*School of Public Health, Tel Aviv University, Israel*

Background: Data on epidemiological and clinical characteristics of HRV infections in hospitalized children and adults is limited.

Objectives: To assess clinical characteristics among HRV-positive patients hospitalized during 2019-2022, in an Israeli tertiary medical center.

Methods: Clinical characteristics of nasopharyngeal HRV-positive patients with cycle threshold (CT)≤32 were obtained from medical files and analyzed in relation to age subgroups and comorbidities, samples with CT≤25 underwent sequencing.

Results: Overall, 883 patients were enrolled and divided into the following age groups: 0-1 (201, 22.8%), 1-3 (152, 17.2%), 3-5 (56, 6.3%), 5-18 (86, 9.7%), 18-40 (52, 5.9%), 40-65 (101, 11.4%), 65 (235, 26.6%). Comorbidity rates were low, and increased with age. Clinical presentation in younger age groups featured bronchiolitis, stridor and asthma exacerbation, while older age groups were more likely to present with pneumonia, COPD exacerbation and bronchitis. ICU admissions and mechanical ventilation were more frequent among younger age groups, while older age groups required more oxygen support.

Of the 128 sequenced samples, HRV-A was the predominant lineage (68.8%), followed by HRV-C (30.5%) and HRV-B (1.0%). A wide range of serotypes were detected, fairly evenly distributed among clinical characteristics, with no one serotype associated with a particular outcome. Serotypes associated with various clinical characteristics differed among children and adults.

Conclusions: Comorbidities and clinical presentation of hospitalized HRV-positive patients varied among different age groups. Severe morbidity, as reflected in higher rates of ICU admissions, was more common in children. Serotype distribution was different in children compared with adults. We suggest that HRV infection is a significant cause of hospitalization in both children and adults.

Parallel session:
COMPUTATIONAL BIOLOGY AND GENOMICS OF MICROBES
(Monday, September 9, 2024 14:00)

Invited Lecture

05 Computational biology and genomics of microbes

## Navigating a fine balance: point-mutant cheater viruses disrupt the viral replication cycle

**Adi Stern**

Cheater viruses, alternatively denoted as defective interfering viruses, cannot replicate on their own yet replicate faster than the wild type (WT) when the two viruses coinfect the same cell. Cheaters must possess two genetic features: a defect, which leads to their inability to infect cells on their own, and a selective advantage over WT during co-infection. Previously, we have discovered two point-mutant cheaters of the MS2 bacteriophage. Here, we set out to discover the possible repertoire of cheater MS2 viruses by performing experimental evolution at a very high multiplicity of infection (MOI). Our results revealed a third cheater that arose in eight of eight biological replicas. This finding led to two interesting observations: first, all three discovered cheaters reside in very close proximity on the genome. This region encodes for multiple functions: overlapping reading frames as well as overlapping RNA structures critical for transitions from one stage of the replication cycle to another. Each of the three cheaters disrupt the fine balance necessary for phage replication, in different ways that create a defect + advantage. These added mutations tend to alter other stages of the viral replication cycle, complementing the disruptions created by the original cheater mutation. Overall, our findings highlight the key features necessary for the creation and maintenance of a point mutant cheater virus.

Parallel session:
COMPUTATIONAL BIOLOGY AND GENOMICS OF MICROBES
(Monday, September 9, 2024 14:00)

Invited Lecture

05 Computational biology and genomics of microbes

## Escherichia coli adaptation under prolonged resource exhaustion is characterized by extreme parallelism and frequent historical contingency

Shira Zion, Sophia Katz, **Ruth Hershberg**[1]
*The Ruth and Bruce Rappaport Faculty of Medicine, Technion - Israel Institute of Technology, Israel*

Like many other non-sporulating bacterial species, Escherichia coli is able to survive prolonged periods of resource exhaustion, by entering a state of growth called long-term stationary phase (LTSP). In July 2015, we initiated a set of evolutionary experiments aimed at characterizing the dynamics of E. coli adaptation under LTSP. In these experiments populations of E. coli were allowed to initially grow on fresh rich media, but were not provided with any new external growth resources since their establishment. Utilizing whole genome sequencing data obtained for hundreds of clones sampled at 12 time points spanning the first six years of these experiments, we found that E. coli continuously adapts genetically, through the highly convergent accumulation of mutations. Upon entry into LTSP, long-lasting lineages are established and this lineage structure is in itself convergent, with similar lineages arising across independently evolving populations. The high parallelism with which adaptations occur under LTSP, combined with the LTSP populations` lineage structure, enable us to screen for pairs of loci displaying a significant association in the occurrence of mutations, suggestive of a historical contingency. We find that such associations are highly frequent and that a third of convergently mutated loci are involved in at least one such association. By establishing new LTSP experiments, starting from populations in which cells are marked with unique barcodes, we are able to examine our lineage structure at far higher resolution and show that adaptation is even more convergent than is possible to observe using whole genome sequencing.

Parallel session:
COMPUTATIONAL BIOLOGY AND GENOMICS OF MICROBES
(Monday, September 9, 2024 14:00)

05 Computational biology and genomics of microbes

## A user-friendly web server for the analysis of large-scale microbial genomics data

**Yair Shimony**[1], Oren Avram[2], Edo Dotan[1], Elya Wygoda[1], Noa Ecker[1], Naama Wagner[1], Gianna Durante[3], Jeff Chang[4], Tal Pupko[1]
[1]*The Shmunis School of Biomedicine and Cancer Research, Tel Aviv University, Israel*
[2]*UCLA Henry Samueli School of Engineering and Applied Science, University of California Los Angeles, California, USA*
[3]*Computer Science, Purdue University, Indiana, USA*
[4]*Department Of Botany And Plant Pathology, Oregon State University, Oregon, USA*

Large-scale analysis of bacterial genomic datasets contributes to the comprehensive characterization of complex microbial dynamics within a microbiome and among different bacterial strains. The study of large-scale bacterial evolutionary dynamics poses many challenges. These include data-mining steps, such as gene annotation, orthologs detection, sequence alignment and phylogeny reconstruction. These steps require the use of multiple bioinformatics tools and ad-hoc programming scripts, making the entire process cumbersome, tedious, and error-prone due to manual handling. This motivated the development of the M1CR0B1AL1Z3R web server, a ‘one-stop shop’ for conducting comparative microbial genomics analysis via a simple graphical user interface.

Here we present an ongoing effort towards releasing M1CR0B1AL1Z3R v2.0. The new release will include additional comparative genomics outputs, an improved graphical interface, and accuracy and efficiency improvements. Some of the novel features to be implemented are: (i) genomes completeness analysis (ii) extracting putative open reading frames (iii) comparative genomics analysis such as GC content, ANI measures, and codon bias between genomes and between genes; (iv) extracting orthologous groups and assigning them to known KEGG Orthology groups; (v) analyzing gene presence–absence patterns and identifying orphan genes; (vi) reconstructing a phylogenetic tree based on the core genome of the input dataset.

M1CR0B1AL1Z3R facilitates the analysis of dozens of bacterial genomes using advanced techniques, with the click of a button. M1CR0B1AL1Z3R is freely available at https://microbializer.tau.ac.il/.

Parallel session:
COMPUTATIONAL BIOLOGY AND GENOMICS OF MICROBES
(Monday, September 9, 2024 14:00)

Invited Lecture

05 Computational biology and genomics of microbes

## Ancient origin of innate immune components in bacteria

**Tanita Wein**[1], Philip Kranzusch[2], Rotem Sorek[3]
[1]*Department of Systems Immunology, Weizmann Institute of Science, Israel*
[2]*Department of Microbiology, Harvard Medical School, USA*
[3]*Department of Molecular Genetics, Weizmann Institute of Science, Israel*

Caspase recruitment domains (CARDs) and pyrin domains are facilitators of inflammatory cell death. Upon pathogen recognition, CARD domains activate caspases, which, in turn, cleave and activate gasdermin pore-forming proteins to induce pyroptotic cell death. We show that CARD domains are present in bacterial anti-phage defense systems, where they are essential for protease-mediated activation of bacterial gasdermins leading to cell death. We further show that multiple bacterial anti-phage defense systems utilize CARD domains to activate a variety of cell death effectors. We find that these systems are triggered by an immune evasion protein that phages use to overcome the bacterial defense system RexAB, demonstrating that phage proteins inhibiting one defense system can activate another. Our results suggest that CARD domains are an ancient component of innate immune systems conserved from bacteria to humans, and that CARD-dependent activation of gasdermins is conserved in organisms across the tree of life.

05 Computational biology and genomics of microbes

## Insights into Bacterial Adaptation to Hosts through Massive-Scale Genomic Analysis

**Ofir Segev**[1], Alexander Geller[1], Cristina Bez[2], Peihan Wang[3], Menglu Zhang[3], Lusiné Nazaretyan[4], Fei Chen[3], Vittorio Venturi[2], Asaf Levy[1]
[1]*The Robert H. Smith Faculty of Agriculture, Food, and Environment, The Hebrew University of Jerusalem, Israel*
[2]*The Bacteriology Group, International Centre for Genetic Engineering and Biotechnology, Italy*
[3]*CAS Key Laboratory of Genome Sciences and Information, Beijing Institute of Genomics, Chinese Academy of Sciences and China National Center for Bioinformation, China*
[4]*Berlin Institute of Health, Charité-Universitätsmedizin Berlin, Germany*

In this study, we employed a computational approach to systematically identify and characterize host-associated genes by comparing bacterial genomes isolated from host and non-host environments. Our hypothesis posited that genes enriched in host-associated bacteria are likely involved in host adaptation.

We conducted an analysis of 72,000 high-quality, distinct bacterial isolate genomes using five comprehensive statistical enrichment tests. Our findings revealed over 2000 protein domains enriched in host-associated bacteria across various genera. Encouragingly, these domains include well-known host-associated functions, such as nitrogen fixation, virulence factors, and iron chelators. Moreover, our analysis identified numerous poorly annotated domains with potential host-association functions.

We leveraged the same dataset to compare animal-associated bacteria with their plant-associated counterparts. This analysis enabled us to identify protein domains exclusively associated with adaptation to specific host kingdoms. For example, we discovered enrichment of plant-associated protein domains in Chryseobacterium and Flavobacterium involved in xylan degradation, a crucial process targeting the abundant material found in plant cell walls.

To validate our computational predictions, we conducted experimental assays on selected protein domains lacking clear annotation. Knockout mutations were performed in genes containing enriched host-associated protein domains in various plant-associated bacteria. The mutated strains exhibited a substantial reduction, up to a 10,000-fold decrease, in their ability to colonize rice roots.

Our analysis has generated a robust database of thousands of protein domains awaiting further study, which serves as a gold mine for scientists interested in exploring and characterizing novel functions related to host interactions.

Parallel session:
COMPUTATIONAL BIOLOGY AND GENOMICS OF MICROBES
(Monday, September 9, 2024 14:00)

05 Computational biology and genomics of microbes

## Plasmids Fight Back: Anti-Defense Systems Boost Conjugation Efficiency

**Bruria Samuel**[1], David Burstein
*Department of The Shmunis School of Biomedicine and Cancer Research, Tel Aviv university, Israel*

Plasmids are an important source of antibiotic-resistance genes that mobilize horizontally between bacteria, including numerous human pathogens. Despite various bacterial defense mechanisms, such as CRISPR-Cas, restriction-modification systems, and SOS-response genes, that prevent the invasion of mobile elements, plasmids efficiently and robustly overcome these barriers during conjugation. Here, we show that the leading region of plasmids, the first to enter recipient cells, is a hotspot for an extensive repertoire of anti-defense systems, encoding anti-CRISPR, anti-restriction, anti-SOS, and other counter-defense proteins. We further identified in the leading region a prevalence of promoters known to allow expression from ssDNA, potentially facilitating rapid protection against bacterial immunity during the early stages of plasmid invasion. Our experimental results corroborate the importance of anti-defense gene localization in the leading region for efficient conjugation. These analyses suggest that focusing on the leading region of plasmids could lead to the discovery of diverse anti-defense genes. Combined, our findings reveal a new facet of plasmid dissemination and provide theoretical foundations for developing efficient conjugative delivery systems for natural microbial communities.

Parallel session:
CLINICAL BACTERIOLOGY AND EPIDEMIOLOGY (Monday, September 9, 2024 14:00)

Invited Lecture

06 Clinical bacteriology and epidemiology

## Fundamental insight into non-growing bacterial states for the tailoring of effective antibiotic treatments

Adi Rotem, **Nathalie Balaban**[1]
*Racah Institute, The Hebrew University, Israel*

When bacteria enter a growth-arrested state, they may persist under extensive antibiotic treatments without acquiring resistance. The phenomenon of antibiotic persistence has been associated with treatment failure. However, despite many years of intense research, no clear consensus on its mechanism has emerged. Here we demonstrate that persistence by growth-arrest may originate from two fundamentally different modes: either from a regulated growth-arrest, leading to a protected cellular state, or from a dysregulated, disrupted, growth-arrest. Using modelling and experimental approaches, we characterize each persister type. Importantly, we find treatments that preferentially target each mode. This understanding resolves previous conflicting results and presents a general framework for distinguishing between regulated and disrupted growth-arrest which should be broadly relevant for the description of cells under stress.

Parallel session:
CLINICAL BACTERIOLOGY AND EPIDEMIOLOGY (Monday, September 9, 2024 14:00)

06 Clinical bacteriology and epidemiology

# Genetic determinants underlying antimicrobial resistance (AMR) in wildlife in Israel.

**Babatunde Beyioku**[1], Roi Lapid[2], Roni King[3], Tomer Nissimyan[3], Prof Jacob Moran-Gilad[1]

[1]*Faculty of Health Sciences, Faculty of Health Sciences, Ben Gurion University of the Negev, Beer Sheva, Israel., Israel*

[2]*Faculty of Agriculture, The Robert H. Smith Faculty of Agriculture, The Hebrew University of Jerusalem, Rehovot, Israel, Israel*

[3]*Israel Nature and Parks Authority, Science & Conservation Division, Israel Nature and Parks Authority, Science & Conservation Division, Jerusalem, Israel, Israel*

Background: Antimicrobial resistance (AMR) is a major global health challenge. The One Health approach to AMR recognizes the interconnectivity between human, animal, and environmental health and is central to mitigation efforts. Wildlife species may be exposed to AMR via human anthropogenic pollution and predation on livestock carcasses, thus increasing the potential for wildlife species to act as reservoirs of clinically relevant antibiotic resistance genes (ARGs). However, data on AMR in wildlife are scarce, especially in Israel.

Methods: In total, 430 samples (nasal=215, rectal=134 and cloacal=81) samples were collected via swabs from 215 animals in which concurrent nasal and rectal/cloacal samples were collected from each animal. The animals sampled were wild boars (n=90), golden jackals (n=44), griffon vultures (n=68) and cattle egrets (n=13). The animals were sampled across Israel in collaboration with the Israel National and Parks Authority (INPA). Samples were extracted using the Qiagen PowerSoil Kit and subject to real-time PCR for the detection of the ARGs: blaCTX-M15, blaSHV, qnrS, and Sul1.

Results: The prevalence of different ARGs per wildlife species and sample types are summarized in the Figure and Tables below:

Conclusions: Wildlife species in Israel harbor a high rate of clinically relevant ARGs, suggesting wildlife has an important role in the propagation of ARGs across One Health. The mechanism of acquisition of ARGs in wildlife in Israel should be further studied.

Parallel session:
CLINICAL BACTERIOLOGY AND EPIDEMIOLOGY (Monday, September 9, 2024 14:00)

Invited Lecture

06 Clinical bacteriology and epidemiology

## Bacterial physiology and antibiotic action

**Uri Alon**

Antibiotic effectiveness depends on many factors. While many mechanistic details of antibiotic action are known, the connection between death rate and bacterial physiology is poorly understood. A common observation is that death rate in antibiotics rises linearly with growth rate; however, it remains unclear how other factors, such as environmental conditions and whole-cell physiological properties, affect bactericidal activity. To address this, we developed a high-throughput assay to precisely measure antibiotic-mediated death. We found that death rate is linear in growth rate, but the slope depends on environmental conditions. Growth under stress lowers death rate compared to non-stressed environments with similar growth rate. To understand stress's role, we developed a mathematical model of bacterial death based on resource allocation that includes a newly defined stress-response sector; we identify this sector using RNA-seq. Our model accurately predicts death rate and minimal inhibitory concentration across a wide range of growth conditions. This death-growth model improves our understanding of bacterial physiology and suggests conditions that may improve antibiotic efficacy.

Parallel session:
CLINICAL BACTERIOLOGY AND EPIDEMIOLOGY (Monday, September 9, 2024 14:00)

06 Clinical bacteriology and epidemiology

## FT-IR in Infection Control: from Local Outbreak Detection to National Epidemiological Surveillance

**Alona Keren-Paz**[1], Mor Lurie-Weinberger[1], Nadya Rakovitsky[1], Moshe Bechor[1], Reut Efrati Epchtien[1], Yehuda Carmeli[1,2]
[1]*National Institute for Antibiotic Resistance and Infection Control, Ministry of Health, Israel*
[2]*Sackler School of Medicine, Tel Aviv University, Israel*

Fourier Transform Infrared Spectroscopy (FT-IR) has emerged as a powerful analytical technique in microbiology and infection control, enabling rapid and precise phenotypic characterization of bacteria using their unique spectral fingerprints. The National Institute for Antimicrobial Resistance and Infection Control directs and coordinates national infection control and prevention efforts, employing FT-IR at multiple scales.

Firstly, FT-IR facilitates the investigation of potential bacterial outbreaks in medical institutions through rapid profiling of microbial isolates, supporting real-time infection control interventions. Secondly, bacterial isolates collected from various medical institutions are routinely analyzed using FT-IR, with their spectra archived in a national repository. This repository serves as a comprehensive tool for nationwide surveillance of circulating bacterial strains. Integrated with bioinformatic analysis of bacterial genomes, it enables monitoring of national epidemiology, revealing dissemination patterns of antibiotic-resistant genes and nationwide clonal dynamics of pathogens such as carbapenem-resistant Enterobacterales (CPE), carbapenem-resistant Acinetobacter baumannii (CRAB), and Pseudomonas aeruginosa.

Lastly, efforts are underway to detect emerging high-risk sequence types of critical pathogens through routine epidemiological surveillance. A protocol leveraging the AI capabilities of FT-IR software is being developed to facilitate the detection of such newly emerging clones.

FT-IR is invaluable for both local infection control and for shaping national public health strategies, significantly contributing to proactive surveillance and rapid response to emerging infectious threats.

Parallel session:
CLINICAL BACTERIOLOGY AND EPIDEMIOLOGY (Monday, September 9, 2024 14:00)

06 Clinical bacteriology and epidemiology

## The Changing epidemiology of Invasive Group A Streptococcus infections in Israel during 2019-2023 and the emergence of successful lineages

**Merav Ron**[1], Shir Nesimian[2], Maya Davidovich-Cohen[2], Assaf Rokney[2]
[1]*1Public Health Laboratories –Jerusalem (PHL-J), Ministry of Health (MOH), Israel*
[2]*Public Health Laboratories –Jerusalem (PHL-J), Ministry of Health (MOH), Israel*

*Streptococcus pyogenes* is a major cause of community-acquired and nosocomial invasive infections linked with morbidity and mortality worldwide. Laboratory surveillance of invasive GAS (iGAS) cases is performed at the national reference center with focus on molecular epidemiology, genomic analysis and virulence profiling of emerging types.

During 2019-2023, a total of 3,236 iGAS isolates were analyzed at the reference center. The most common source of iGAS was blood (53.4%), followed by wounds (28.5%). Following an initial decrease in incidence in the beginning of the COVID-19 pandemic, a striking increase was observed. Yearly incidence rates per 100,000 population during 2019-2023 were 6.8, 5.3, 6.1, 7.4 and 8.4.

Molecular analysis revealed 142 *emm* types with the emergence and outbreak of previously rarely observed lineages. We have characterized several dominant and emerging outbreak strains of *S. pyogenes*; *emm*77.0 (16.3% of 2021 cases) *emm*1.0 (19.4% of 2022 cases), and *emm*104.0 (21.4% of 2023 cases). Strains of *emm*77.0 and *emm*104.0 were more prevalent in the elderly male group whereas *emm*1.0 was prevalent in both children and elderly. WGS analysis of geographically dispersed samples revealed that *emm*77.0 and *emm*104.0 were clonal. The emerging types presented various genetic profiles contributing to antimicrobial resistance (*tet*M, *dfr*G) and virulence (*spe*A, *spe*C, *spe*G, *spe*K, *spe*L, *spe*M). The rapid expansion and epidemiological dynamics of the outbreak clones may be due to COVID-19-related restrictions influencing the population immunity or driven by genomic adaptation of the bacterial strains. Genomic analysis and laboratory surveillance are a significant key in enhancing our understanding of iGAS epidemiology.

Parallel session:
VIRUSES OF EUKARYOTES (Monday, September 9, 2024 16:00)

Invited Lecture

07 Viruses of eukaryotes

## SARS-CoV-2 nucleocapsid protein transfer from infected to uninfected cells induces antibody-mediated complement attack

**Alexander Rouvinski**[1]
*Department of Microbiology and Molecular Genetics, Institute for Medical Research Israel-Canada, The Kuvin Center for the Study of Infectious and Tropical Diseases, The Hebrew University-Hadassah Medical School, The Hebrew University of Jerusalem, Israel*

SARS-CoV-2 infection triggers strong antibody response toward Nucleocapsid-Protein (NP), suggesting extracellular presence beyond its intra-virion RNA binding. Interestingly, NP was found to decorate infected and proximal uninfected cell-surfaces. Here, we propose a new mechanism through which extracellular NP on uninfected cells contributes to COVID-19 pathogenicity. We show that NP binds to cell-surface sulfated linear-glycosaminoglycans on SARS-CoV-2 infected and also on SARS-CoV-2 uninfected, non-susceptible cells laying in proximity. Importantly, subsequent binding of anti-NP-IgG, present in sera of recovered patrients, generated clusters that triggered C3b complement deposition by the classical pathway. Heparin analogs enoxaparin and pentosan-polysulfate outcompeted NP-binding, and thereby rescued cells from anti-NP IgG-mediated complement deposition. Our findings unveil how extracellular NP may exacerbate COVID-19 tissue damage through overwhelmed immune response targeting not only infected but also uninfected cells, and suggest leads for preventative therapy.

Parallel session:
VIRUSES OF EUKARYOTES (Monday, September 9, 2024 16:00)

Invited Lecture

07 Viruses of eukaryotes

## Numb-associated kinases are essential for Sandfly-borne Toscana virus entry.

Yarden Moalem, Rodolfo Katz, Anand G Subramaniam, Yehonathan Malis, Yakey Yaffe, Nofit Borenstein-Auerbach, Keshet Tadmor, Roey Raved, Ben M. Maoz, Ji Seung Yoo, Yaniv Lustig, Chen Luxenburg, Eran Perlson, Shirit Einav,
**Ella H. Sklan.**

Sandfly-borne Toscana virus (TOSV) is an emerging Phlebovirus currently prevalent in southwestern Europe and Northern Africa. Infection with TOSV is typically asymptomatic or results in mild febrile illness. However, TOSV can invade the nervous system and cause meningitis, encephalitis, and other neurological complications. In some endemic regions, TOSV ranks among the top three causes of summer meningitis. Despite its clinical significance, the viral and host factors regulating TOSV infection are still unknown. Here, we investigated the early stages of TOSV infection. Our findings highlight adaptor-associated kinase 1 (AAK1) and, to a lesser extent, cyclin G-associated kinase (GAK), members of the Numb-associated kinases family of Ser/Thr kinases and regulators of the host clathrin-associated adaptor proteins (AP)-2 as host factors regulating viral infection. Notably, FDA-approved multi-kinase inhibitors Sunitinib and Gefitinib, targeting these kinases, demonstrated significant inhibition of TOSV infection. Mechanistically, Sunitinib was found to impede viral entry and AP2 phosphorylation, a process that could be rescued by exogenous AP2M1 transfection. These results underscore AAK1 and GAK as promising host targets for potential interventions against Phleboviruses.

07 Viruses of eukaryotes

## A cytomegalovirus encoded lncRNA blocks cell cycle progression

**Tal Fisher**, Aharon Nachshon, Michal Schwartz, Noam Stern-Ginossar

Human cytomegalovirus (HCMV) is a prototypical herpesvirus which infects the majority of the population. HCMV encodes four lncRNAs, whose functions are still poorly understood. lncRNA RNA2.7 is the most abundant viral transcript, accumulating in the cytosol and amazingly accounting for 13% of the non-ribosomal RNA in infected cells.

Using a deletion mutant virus lacking RNA2.7, we characterized RBPs specifically bound to this lncRNA. We performed genome-wide measurements and showed that through RBP interactions, RNA2.7 alters mRNA stability to affect gene expression. We further show RNA2.7 is critical for viral propagation specifically in cycling cells. This is caused by inhibition of the G1-S cell-cycle transition and involves sequestered RBPs. These results support a mechanism by which RNA2.7 sequesters RBPs to change gene expression and drive a cell-cycle arrest.

Overall, our results elucidate the enigmatic function of RNA2.7 and demonstrate how a viral lncRNA can disrupt cellular processes to promote viral propagation.

Parallel session:
VIRUSES OF EUKARYOTES (Monday, September 9, 2024 16:00)

Invited Lecture

07 Viruses of eukaryotes

## Elucidating the role of type-II interferon stimulated genes in the antiviral response to respiratory viruses

**Yiska Weisblum**[1], Michael Tartell[2], Paul Bieniasz[2]
[1]*Department of Microbiology and Molecular Genetics and the Kuvin Center for the Study of Infectious and Tropical Diseases, IMRIC, Faculty of Medicine, The Hebrew University of Jerusalem, Israel*
[2]*Laboratory of Retrovirology, The Rockefeller University, USA*

Respiratory viruses pose a significant threat to global health, emphasizing the need for a deeper understanding of host-virus interactions. Innate immunity mediated by interferons (IFNs) serves as an initial barrier against viral replication, with some IFN-stimulated genes (ISGs) encoding potent antiviral proteins. While type-I ISGs have been extensively studied, the role of type-II ISGs remains underexplored, despite ubiquitous expression of IFNγ receptors. Using RNAseq, we identified type-I and -II ISGs in lung epithelial cells, and assessed their impact on viral replication via an ISG-targeted CRISPR KO screen. Cells were treated with IFN and then infected with four respiratory viruses: respiratory syncytial virus, influenza A virus, human coronavirus OC43, and adenovirus. In our screen of 365 ISGs, we identified 55 candidate antiviral effectors. Notably, 27 type-I ISGs and 35 type-II ISGs exhibited antiviral activity, while 7 effectors were inhibitory following treatment with either type of IFN. Most antiviral proteins targeted one virus, but some were active against multiple viruses. Notably, ring finger protein 213 (RNF213) emerged as an IFNγ-induced antiviral protein with activity against all RNA viruses examined. Mechanistic studies identified the specific RNF213 domains and activities crucial for its antiviral function, and revealed that alterations within these domains impact its subcellular localization.

Our study offers insights into the complementary roles of type-I and type-II IFNs in antiviral immunity and the intricate mechanisms combating respiratory pathogens. Further investigation into the activities and functions of RNF213 and other newly identified ISGs could uncover novel therapeutic strategies against respiratory viral infections.

Parallel session:
VIRUSES OF EUKARYOTES (Monday, September 9, 2024 16:00)

07 Viruses of eukaryotes

## Discovery of an unrecognized nidovirus associated with granulomatous hepatitis in rainbow trout

**Sharon Karniely**[1], Adi Faigenboim[2], Salsabeel Watted[1], Katia Lapin[1], Eduard Berenshtein[3], Avshalom Hurvitz[4], Arieli Bouznach[1], Asaf Berkowitz[5], Eran Bacharach[6], Avi Eldar[7]

[1]*Department of Viorology, Kimron Veterinary Institute, Israel*
[2]*Department of Plant Pathology, The Volcani Center, Israel*
[3]*EM Unit Research Core Facility, The Hebrew University of Jerusalem, Israel*
[4]*Upper Galilee, Dan Fish Farm, Israel*
[5]*Department of Poultry Diseases, Kimron Veterinary Institute, Israel*
[6]*The Shmunis School of Biomedicine and Cancer Research, Tel Aviv University, Israel*
[7]*Department of Virology, Kimron Veterinary Institute, Israel*

Rainbow trout (Oncorhynchus mykiss) is the principal species of inland-farmed fish in the Western hemisphere. Recently, we diagnosed in farmed rainbow trout in Israel a disease in which the hallmark is granulomatous-like hepatitis. No microbial agents could be isolated from lesions. Still, unbiased high throughput sequencing and bioinformatics analyses revealed the presence of a novel piscine nidovirus that we named `Trout Granulomatous Virus` (TGV). TGV genome (28,767 nucleotides long) is predicted to encode non-structural (1a and 1ab) and structural (S, M, and N) proteins that resemble proteins of other known piscine nidoviruses. High loads of TGV transcripts were detected by quantitative RT-PCR in diseased fish and were visualized in hepatic granulomatous sites by fluorescence in situ hybridization. Transmission electron microscopy (TEM) revealed coronavirus-like particles in hepatic lesions. These findings support an association of TGV with hepatic lesions. A detailed analysis of additional organs from diseased fish revealed the presence of TGV RNA in kidney, spleen, heart, gills and intestine suggesting wide tissue tropism of the virus. Viral RNA was also detected in diseased fish stool and in water from pools, which contained diseased fish, which may point to possible routes of viral transmission. There are no indications that the virus is zoonotic. The identification and detection of TGV provide means to control TGV spread in trout populations.

Parallel session:
PLANT-MICROBE INTERACTIONS (Monday, September 9, 2024 16:00)

Invited Lecture

08 Plant-microbe interactions

## *Pseudozyma aphidis* plays hide-and-seek with its host plant

**Maggie Levy**[1], Shanee Alster[1], Avis Dafa-Berger[1], Neta Rotem[1]
*Department of Plant Pathology and Microbiology, The Robert H. Smith Faculty of Agriculture, Food and Environment, The Hebrew University of Jerusalem, Israel*

Beneficial microorganisms need to overcome the plant defense system to establish on or within plant tissues. Like pathogens, beneficial microbes can manipulate a plant's immunity pathways, first by suppressing and hiding to establish on the host and then by inducing resistance to protect the plant. We demonstrated that although *Pseudozyma aphidis* can activate microbe-associated molecular pattern (MAMP)-associated genes, it does not activate MAMP-triggered callose deposition and can, moreover, suppress such deposition triggered by Flg22 or chitin. While MAMP's associated gene activation by *P. aphidis* was not dependent on Salicylic acid, Jasmonic acid, or Ethylene signalling, suppression of MAMP-triggered callose deposition required the Salicylic acid and Jasmonic acid signaling factors JAR1-1 and E3 ubiquitin ligase COI1 yet did not rely on EIN2, NPR1, or the transcription factor JIN1/MYC2. Later on, P. aphidis or its secreted metabolites prime plants' defense machinery via local and systemic induction of PR genes in a manner independent of SA, JA, and NPR1. Finally, *P. aphidis* fully or partially reconstituted PR1 and PDF1.2 expression in SA related mutants, but not in a JA related mutant, after *B. cinerea* infection, suggesting that *P. aphidis* can bypass the SA/NPR1, but not JA, pathway to activate PR genes. Thus, either partial gene activation is sufficient to induce resistance, or the resistance can be directed through other pathogen-resistance genes or pathways as well.

Parallel session:
PLANT-MICROBE INTERACTIONS (Monday, September 9, 2024 16:00)

Invited Lecture

08 Plant-microbe interactions

## Unveiling the mechanisms determining tissue specificity in the plant pathogen Clavibacter michiganensis using experimental evolution

Raj Kumar Verma, Veronica Roman-Reyna, Nathan Ben-Moche, Jonathan M Jacobs, **Doron Teper**[1]
*Plant Pathology and Weed Research, Agricultural Research Organization - Volcani Institute, Israel*

The plant pathogenic bacterium Clavibacter michiganensis (Cm) is a systemic vascular pathogen that thrives in both the host xylem vessels and intracellular apoplast tissues during different stages of the disease cycle. To identify traits and loci associated with adaptation to each of these two host microenvironments, we conducted tissue-specific experimental evolution, adapting twenty independent clone lineages to either vascular or apoplastic lifestyles through repeated passaging in tomato stems or leaves. Evolved Cm populations after fifteen passages were characterized for aggression in the host and virulence-associated traits. These characterizations demonstrated clear differential associations of virulence-associated traits to adapted tissue. The majority of vascular-adapted clones displayed enhanced biofilm formation, reduced cellulase activity, reduced exopolysaccharides (EPS) production, and attenuated virulence on tomato compared to the parent clone. On the other hand, apoplast-adapted clones displayed reduced biofilm formation and enhanced EPS production while maintaining their aggression on tomato. To identify loci associated with tissue specificity, we determined the complete genome sequence of all plant-evolved clones and used comparative genomics to identify genome alterations. Interestingly, six out of the ten vascular-adapted clones harbored two independent mutations in a putative Cro/CI-type transcriptional regulator, suggesting that this regulator may play a role in vascular association. These experiments provide new insights into the tissue adaptation of plant pathogenic bacteria and suggest a role for cellulase activity and biofilm formation in tissue specificity.

Parallel session:
PLANT-MICROBE INTERACTIONS (Monday, September 9, 2024 16:00)

08 Plant-microbe interactions

## Ecological Implications of Introducing the Endophytic Bacterium Frateuria defendens on Melon Plant Health and Endophytic Community Dynamics

**Lilach Iasur Kruh**[1]
*Department of Biotechnology Engineering, Braude College of Engineering, Israel*

The use of bacterial biocontrol agents in agriculture has gained attention as a sustainable approach to combat plant diseases. However, the introduction of exogenous bacteria into plant systems raises concerns about their potential impact on the native endophytic community and overall plant health. This study explores the ecological consequences of introducing the endophytic bacterium Frateuria defendens, a potential biocontrol agent against Mollicute diseases, into melon plants.

Thirteen F. defendens isolates were obtained from Hyalesthes obsoletus, an insect vector transmitting phytoplasma (mollicute phytopathogen), and screened for their ability to secrete antimicrobial substances and colonize melon plants. Only one isolate, designated FD, successfully colonized the melon shoot and sap. The presence of FD enhanced the sap`s antimicrobial activity against the model Mollicute bacterium, suggesting a potential biocontrol mechanism.

Notably, the introduction of F. defendens and temporal changes in sample collection induced distinct shifts in the structure and diversity of the endophytic microbial populations within the melon phyllosphere. The successful colonization of melon plants by the FD isolate highlights its potential as a biocontrol agent. However, this study also underscores the importance of thoroughly understanding the ecological consequences of introducing exogenous endophytic bacteria into plant systems.

This research illuminates the complex interplay among plant hosts, their native microbiota, and introduced biocontrol agents, providing valuable insights for the development of sustainable agricultural practices.

Parallel session:
PLANT-MICROBE INTERACTIONS (Monday, September 9, 2024 16:00)

Invited Lecture

08 Plant-microbe interactions

## Rhizosphere microbiome rewilding for enhancing crop productivity

**Elisa Korenblum**

A diverse rhizosphere microbiome is essential for sustaining ecosystem services and protecting against plant pathogens, thus rhizosphere microbiome rewilding (that is, restoring key members of the diverse plant microbiota lost through domestication and intensive agriculture practices, such as overuse of pesticides and fertilizers) could enhance the sustainability of food production in a changing climate. We study plant-microbiome interactions with the goal of reintroducing beneficial microbes to restore healthy soil. For instance, by comparing the rhizosphere microbiome of diploid wild ancestors of modern bananas, M. acuminata (AA), M. balbisiana (BB) and triploid cultivars that derived from genome hybridizations into autotriploid cultivars (AAA) and allotriploid cultivars (AAB and ABB) grown in field conditions, we observed that B-genome affects rhizosphere microbiome associations. We also examined the microbial community on the roots of avocado trees treated with different regimes of cover-crop growth for improving soil health by diversifying the soil microbiome. The objective of this study is to identify microbial populations linked to productivity in avocado trees and soil quality. Moreover, we use culturomics and bacterial chemotyping for developing a multi-host microbial synthetic community (OmniSynCom) from the rhizosphere of different wild plant populations for a comprehensive understanding of the chemical interactions of natural microbial communities. One approach to enhancing crop growth and yield in a changing climate is to integrate beneficial microbiomes, which have co-evolved with wild plants in harsh environments (such as arid or semi-arid zones), into agricultural practices.

Parallel session:
PLANT-MICROBE INTERACTIONS (Monday, September 9, 2024 16:00)

08 Plant-microbe interactions

## Rhizobial trickster: legume symbiont simultaneously fixes atmospheric nitrogen and denitrifiies nitrate, inducing high nodule biomass

**Moshe Alon**, Omri Finkel

Legumes have a symbiotic relationship with nitrogen-fixing bacteria housed in specialized root structures called nodules. The size of these nodules is negatively correlated with the availability of soil nitrate. However, the identity and functional capabilities of the symbiotic bacteria are rarely studied in the context of the regulation of nodule size in response to variations in the availability of soil nitrate. We grew *Medicago polymorpha*, an annual legume, with four different bacterial strains, isolated from nodules of plants grown in four different soil types, and measured their nodule biomass in response to both high and low availability of soil nitrate. Under low nitrate availability, one out of the four strains showed significantly increased nodule biomass compared to the others. Using comparative genomics, we found that only the strain that produced higher nodule biomass possessed the full denitrification metabolic pathway, with the ability to reduce nitrate to nitrous oxide gas. By reducing available nitrate to gas form, this strain might have effectively reduced the availability of nitrate as a nitrogen source for the plant, inducing higher nodule biomass construction. Thus, this strain may gain increased fitness due to the larger than necessary nodules, by both providing the plant with atmospheric nitrogen and reducing the availability of nitrate. This may eventually decrease the fitness of the plant, as nodule construction and maintenance are energetically costly. Furthermore, since nitrous oxide is a potent greenhouse gas, our results have implications for our understanding of the role of agriculture in contributing to global climate change.

Parallel session:
PLANT-MICROBE INTERACTIONS (Monday, September 9, 2024 16:00)

08 Plant-microbe interactions

## Impact of soil type on plants productivity and assembly seed endophytic communities of Setaria viridis L.

**Kumar Uttam**[1], Wout Yi Moe Oo[1], Max Kolton[1]
*The French Associates Institute for Dryland Agriculture and Biotechnology, Ben-Gurion University of the Negev, Israel*

Plant-associated microorganisms are vital for nutrient uptake, disease suppression, and stress resistance. Nevertheless, the role of the soil microbial community in the assembly of the seed microbiome is poorly understood. Additionally, whether stochastic or deterministic processes govern seed microbiome community assembly is poorly characterized. Therefore, a better understanding of plant-bacteria interactions paves a path for improving plant resistance and resilience to environmental perturbations and mitigating yield decline. We hypothesized that plants would equip seeds with similar functional microbial diversity despite being grown in soils with different biochemical properties. Thus, the assembly of seed microbial communities is controlled by deterministic processes. To support this hypothesis, a model plant, Setaria viridis, was grown in soils with contrasting microbial richness (Negev and PotMix), and plant development was recorded throughout the growing season. Seed endophytic community composition was assessed at the end of the growing season using 16S amplicon sequencing. S. viridis grown in PotMix soil developed significantly higher below- and above-ground biomass than plants grown in Negev soil. Although significant differences in microbial community diversity and composition were recorded between bulk soil communities, these differences were not observed in the plant compartment. Moreover, up to 40% of seed endophytes comprised putative diazotrophs. The results show that plants equipped seeds with almost identical endophytic communities regardless of soil bacterial composition, indicating that deterministic processes govern the assembly process.

Parallel session:
THE MICROBIOME (Monday, September 9, 2024 16:00)

Invited Lecture

09 The microbiome

## A role for the gut microbiome in the first 1000 of life

**Omry Koren**[1]
*Azrieli Faculty of Medicine, Bar-Ilan University, Israel*

During gestation the female body undergoes hormonal, metabolic, and immunological changes such as an increase in body fat early in pregnancy followed by reduced insulin sensitivity later in gestation. Pregnancy progression is also associated with dramatic alterations in the composition of the gut microbiota. In the gut, a decrease in diversity is observed as pregnancy progresses which is accompanied by an increase in "between sample" diversity and an increase in the relative abundance of Proteobacteria and opportunistic pathogens. We've demonstrated that some of these changes are mediated by progesterone. In addition, germfree mice inoculated with gut microbiota from pregnant women presented metabolic changes mirroring those of the pregnant women.

We are now at the point of trying to understand whether pregnancy associated microbiota alterations are required for a healthy pregnancy and whether pregnancy complications such as gestational diabetes are associated with dysbiosis? and if the microbiome is associated with pregnancy complications can it be used for early prediction and prevention?

Parallel session:
THE MICROBIOME (Monday, September 9, 2024 16:00)

Invited Lecture

09 The microbiome

## Enteric pathogens communication can be intercepted by microbiome-produced metabolites

**Neta Sal-Man**[1]
*Microbiology and Immunology, Ben-Gurion University of the Negev, Israel*

Reported numbers of diarrheal samples exhibiting co-infections or multiple infections, with two or more infectious agents, are rising, likely due to advances in bacterial diagnostic techniques. Bacterial species detected in these samples include Vibrio cholerae (V. cholerae) and enteropathogenic Escherichia coli (EPEC), which infect the small intestine and are associated with high mortality rates. It has previously been reported that EPEC exhibit enhanced virulence in the presence of V. cholerae owing to their ability to sense and respond to elevated concentrations of cholera autoinducer 1 (CAI-1), which is the primary quorum-sensing (QS) molecule produced by V. cholerae. We examined this interspecies bacterial communication in the presence of indole, a major microbiome-derived metabolite found at high concentrations in the human gut. Interestingly, we discovered that although indole did not affect bacterial growth or CAI-1 production, it impaired the ability of EPEC to enhance its virulence activity in response to the presence of V. cholerae. Furthermore, the co-culture of EPEC and V. cholerae in the presence of B. thetaiotaomicron, an indole-producing commensal bacteria, ablated the enhancement of EPEC virulence. Together, these results suggest that microbiome compositions or diets that influence indole gut concentrations may differentially impact the virulence of pathogens and their ability to sense and respond to competing bacteria.

Parallel session:
THE MICROBIOME (Monday, September 9, 2024 16:00)

09 The microbiome

## Diet affects gut microbe-host interactions by altering bacterial immune-modulatory functionality

**Noa Mandelbaum**[1], Shaqed Carasso[1], Alon Kedem[1], Tamar Ziv[2], Tal Gefen[1], Naama Geva-Zatorsky[1,3]

[1]*Department of Cell Biology and Cancer Science, Rappaport Faculty of Medicine and Research Institute, Rappaport Technion Integrated Cancer Center (RTICC), Technion-Israel Institute of Technology, Israel*

[2]*Smoler Proteomics Center, Lokey Interdisciplinary Center for Life Sciences & Engineering, Technion-Israel Institute of Technology, Israel*

[3]*Humans and the Microbiome, CIFAR, Canada*

Gut bacteria reside in a dynamic environment that changes according to nutrition, health states, diurnal cycles, and more. To date, most studies correlating between the gut microbiota and the host focused on bacterial composition rather than functionality (e.g., immune-modulation activity). Gut bacteria are known to affect the host immune system. Moreover, we showed that even different strains of the same species can affect the immune system in distinct ways, sometimes with contradicting effects (e.g., anti- and pro-inflammatory effects). Therefore, studying the functionality of specific bacterial strains of interest, under different conditions, is crucial. Host genetics, health state and diet were shown to affect microbial composition, however, their effects on bacterial functionality are mostly overlooked. We hypothesize that gut bacterial immune-modulatory functionality can be altered in response to environmental conditions such as bacterial growth conditions and the host diet. Our data demonstrate alterations in the orientation of phase variable promoters of Bacteroides thetaiotaomicron VPI 5482 strain, under different diets in humans and under different growth conditions in vitro (e.g., different carbon sources). Furthermore, B. theta grown in vitro under these distinct conditions exerted different effects on the host immune system. Our results, emphasize the functional plasticity of B. theta when exposed to different carbon sources and to different dietary components. In conclusion, studying the effects of specific dietary components on the immune-modulatory functionalities of key members of the gut microbiota will allow tailored dietary recommendations to patients based on their microbiome composition, targeting their immune system, based on their health conditions.

Parallel session:
THE MICROBIOME (Monday, September 9, 2024 16:00)

Invited Lecture

09 The microbiome

## Metaproteomic Analysis of Cancer Immunotherapy Modulation by the Gut Microbiome

**Lior Lobel**[1]
*Faculty of Life Sciences, Bar-Ilan University, Israel*

The gut microbiome is critical in modulating health and physiology, including its influence on the response to cancer immunotherapy. However, previous metagenomic sequencing studies provided limited insights into the microbiome`s activity. We employed a metaproteomic approach to identify differentially expressed proteins and metabolic pathways in stool samples from immune checkpoint blockade (ICB) responders and non-responders to gain deeper insights into the molecular mechanisms.

Stool samples were collected from melanoma patients undergoing ICB combination therapy at Sheba Medical Center in Israel. DNA and protein extraction was performed, and shotgun DNA sequencing generated a comprehensive cohort-specific protein database for metaproteomic analysis. Tryptic peptides derived from protein fractions were labeled with tandem mass tags (TMT) for multiplexed LC-MS3 analysis. Quantitative analysis was conducted using fragpipe, and statistical analyses were performed with MSstatsTMT in R.

Our findings revealed hundreds of differentially abundant bacterial proteins and tens of host proteins in responder versus non-responder stool samples. We identified distinct bacterial metabolic pathway expression patterns between the microbiomes of responders and non-responders, one of which is glycolysis. Glycolysis-related enzyme enrichment in non-responders' microbiomes is intriguing biologically but also represents the strength of our approach, as metagenomic analyses will fail to identify such an enrichment due to the ubiquitous nature of glycolysis genes. Ongoing efforts involve validating differentially abundant metabolites using LC-MS/MS, and assessing their role in mediating anti-tumor immunity by disrupting relevant bacterial pathways in orthotopic tumor mouse models.

Parallel session:
THE MICROBIOME (Monday, September 9, 2024 16:00)

09 The microbiome

## CoSMIC - A hybrid approach for large-scale, high-resolution microbial profiling of novel niches

**Knafo Maor**[1], Shahar Rezenman[1], Tal Idan1[1], Michael Elgart[2], Shlomi Dagan[3], Ziv Reich[1], Ruti Kapon[1], Dagan Sade[1], Noam Shental[4]
[1]*Department of Biomolecular Sciences, Weizmann Institute of Science, Israel*
[2]*Metabolomic Discovery Unit, Metabolic Center, Sheba Medical Center, Israel*
[3]*Fisher Drug Discovery Resource Center, The Rockefeller University, USA*
[4]*Department of Mathematics and Computer Science, The Open University of Israel, Israel*

Microbiome studies have become integral to a wide range of biological research from ecology to oncology, mainly relying on short-read sequencing of variable regions along the 16S rRNA gene. However, the comprehensiveness of 16S rRNA studies has been persistently challenged due to a lack of primer universality and primer biases, causing differences between a study`s results and the underlying bacterial community. Moreover, relying on a small part of the gene often provides low resolution, hampering downstream taxonomy-based analysis and the ability to harmonize results from studies performed using different variable regions.

Here, we introduce a framework called `Comprehensive Small Ribosomal Subunit Mapping and Identification of Communities` (CoSMIC), effectively addressing these challenges. CoSMIC begins with long-read full-length sequencing of the 16S rRNA gene, using generic Locked Nucleic Acid primers over pooled samples. This step augments the 16S rRNA gene reference database with novel niche-specific gene sequences. Subsequently, CoSMIC optimizes a set of primer pairs targeting multiple non-consecutive variable regions along the gene, followed by standard short-read sequencing of each sample. Data from the different regions are seamlessly integrated using the SMURF framework, thus alleviating primer-based biases and providing resolution at the scale of the full 16S rRNA gene. We first estimated CoSMIC`s performance using a mock community aiming to detect all full-length 16S rRNA sequences. CoSMIC correctly identified more full-length 16S rRNA genes with significantly higher specificity than standard methods. It dramatically increased the resolution of detected sequences while avoiding the inclusion of incorrect sequences. We also evaluated CoSMIC across plant, root, soil, and marine sponge samples, yielding higher profiling accuracy and unprecedented resolution compared to standard methods while detecting ~40,000 novel full-length 16S rRNA gene sequences.

CoSMIC is well-suited for profiling unexplored habitats, offering full-length 16S rRNA resolution at a fraction of the cost of per-sample long-read sequencing. The flexibility in primer selection allows researchers to optimize their experimental designs. The full-length 16S rRNA sequences detected in CoSMIC`s initial step can be uploaded to 16S rRNA databases, enabling future researchers to conduct even more cost-effective profiling studies in these niches.

09 The microbiome

## Gut fermentability of edible architectures: Impact of insect chitin or amyloid-like fibrils on microbiota trajectories

**Gil Refael**[1], Yizhaq Engelberg[2], Alon Romano[1], Gabriela Amiram[1], Eilon Barnea[2], Carmit Shani Levi[1], Sondra Turjeman[3], Meytal Landau[2,4], Omry Koren[3], Uri Lesmes[1]
[1]*Department of Biotechnology and Food Engineering, Technion - Israel Institute of Technology, Israel*
[2]*Department of Biology, Technion - Israel Institute of Technology, Israel*
[3]*Azrieli Faculty of Medicine, Bar Ilan University, Israel*
[4]*Centre for Structural Systems Biology (CSSB) and European Molecular Biology Laboratory (EMBL), German Synchrotron Research Centre (DESY), Germany*

Current food innovations are confronted by the need to elucidate their digestive fate and impact on health. To this end, novel food ingredients such as chitin or amyloid-like fibrils (AF), prove to be slowly digestible and could be fermented by the gut microbiota. This work will integrate two relevant studies: one into chitin from edible crickets (Refael et al, 2023) and another into protein amyloids (Refael et al, 2024); both have explored their digestibility and colonic fermentability.

First, we show chitin isolated from edible crickets has a prebiotic effect like fructo-oligosaccharides based on in-vitro human colonic fermentations (n=10) coupled to metagenomic, QIIME2 and PCoA analyses. In fact, this chitin fraction was found to induce enrichment of symbiotic Bacteroidaceae and low Firmicutes: Bacteroidetes (F/B) ratio. Second, we demonstrate amyloids of β-lactoglobulin (BLG) or ovalbumin (OVA) can be fabricated (affirmed by DLS, TEM and ThT assay) and have attenuated protein gastric and intestinal digestibility, and low levels of bioaccessible peptides (confirmed by LC-MS/MS). Concomitantly, anaerobic human fecal fermentations (n=5) show AF architectures also support low F/B ratio. Further, alpha-diversity scores were significantly lower (p0.003) for OVA (121±29, n=13) in comparison to OVA-AF (161±27, n=17). Beta-diversity analysis shows OVA fermentation shifts ecosystem trajectories compared to OVA-AF (p0.05) and ANCOM-BC analysis shows specific symbionts of the gut are significantly conserved in the presence of OVA-AF (e.g., Roseburia).

Overall, these studies provide evidence that novel food ingredients can exhibit attenuated digestibility in the upper GI and support diverting gut microbiota trajectories towards favorable ones.

Parallel session:
HOST-PATHOGEN INTERACTIONS II (Tuesday, September 10, 2024 10:45)

Invited Lecture

01 Host-pathogen interactions

## Antibodies Produced Following Monkeypox Infection in Humans Effectively Block Monkeypox Virus in a Complement-Dependent Manner

**Natalia Freund**[1]
*Faculty of Medicine, Tel Aviv University, Israel*

Monkeypox virus (Mpox) is a transmissible, zoonotic virus, genetically similar to the deadly Variola virus. The 2022 Mpox outbreak was the largest recorded outside Africa, affecting nearly 90,000 individuals across more than 100 non-endemic countries. This change in viral spread highlights the possible global health threat posed by Mpox. As mass Smallpox vaccination campaign ceased over 5 decades ago, most of the world's population is currently not protected from pox viruses, further emphasizing the necessity of new management measures. Currently, no monoclonal antibodies (mAbs) against Mpox exist and little is known about how to elicit them. Mpox resides as mature and enveloped virions, and depending on it, distinct proteins are displayed on the viral surface. We expressed Mpox antigens from both forms and used these antigens to screen sera of 11 Mpox recoverees diagnosed in Israel during May-June 2022 at 6 weeks and 40 weeks post infection while comparing to sera response from MVA-vaccinees. Furthermore, we used the Mpox recombinant antigens to single cell sort and sequence the B cell repertoire from the human donors. We then isolated 22 Mpox-specific mAbs. Six mAbs exhibited impressive neutralization against Mpox, as well as the related pox viruses, in a complement-dependent fashion. Combinations of different mAbs targeting both viral forms demonstrated synergistic effects. This study can be the basis for a novel universal pox vaccine development and for new anti-pox mAb-based treatment regimens.

Parallel session:
HOST-PATHOGEN INTERACTIONS II (Tuesday, September 10, 2024 10:45)

Invited Lecture

01 Host-pathogen interactions

## Genomic features of bacterial adaptation to host, animals, and plants

**Asaf Levy**[1], Ofir Segev[1], Alexander Geller[1]
*Plant pathology and microbiology, The Hebrew University of Jerusalem, Israel*

Microbes colonize diverse ecological niches, including those within multicellular organisms. Host adaptation is facilitated by a multitude of genes, which remain largely unexplored. Here, we systematically identified and characterized host-associated genes by comparing bacterial genomes isolated from hosts (animals or plants) and non-host environments. We analyzed 72,000 bacterial isolate genomes from nine phyla and revealed over 2,000 protein domains and 14,000 Alphafold protein clusters that are enriched in host-, animal-, and plant-associated bacteria at the genus or family level. These domains and proteins include well-known functions, such as biological nitrogen fixation, virulence factors, and iron chelators. Moreover, we identified numerous poorly annotated domains and proteins with potential host-association functions. To validate our predictions, we conducted colonization assays to study selected host-associated protein domains lacking annotation. Knockout mutations were performed in genes containing enriched protein domains in various plant-associated bacteria. The mutated strains exhibited a substantial reduction in their ability to colonize rice roots. We generated a database of thousands of proteins and domains awaiting further study, which can serve to study novel functions related to host-microbe interactions at the gene level.

Parallel session:
HOST-PATHOGEN INTERACTIONS II (Tuesday, September 10, 2024 10:45)

01 Host-pathogen interactions

## Rare infection phenotypes and how to find them

**Camilla Ciolli Mattioli**, Daniel Schraivogel, Roi Avraham

Over the course of host-pathogen evolution, both players in this tug-of-war have set out strategies to win over the other, by adapting to challenging environments on the pathogen side, and by detecting and eradicating the intruder on the host side. Following infection with intracellular pathogens, macrophages can meet different fates, where pathogen clearance and host cell death are the epitomes at the opposite ends of the spectrum. Intermediate outcomes are also possible, where the pathogen can persist inside the host, or form a replicative niche. What distinguishes these different outcomes and which molecular mechanisms mediate them, is still an open question, where the answer could pave the way to the discovery of new potential drug targets, especially in light of the increasing emergence of antibiotic-resistant pathogens.

Here, thanks to the newly established high-speed image-enabled cell sorting (ICS) technology that allows image-based sorting at high speed, we study at a genome-wide level the genetic determinants that would favour two of the phenotypes that manifest in the infection model of Salmonella and macrophages: a persister-prone state where Salmonella is unable to replicate however persists inside the host cell, and a more successful one where Salmonella forms a replicative niche inside the host. Given the robust and high-throughput set-up that ICS allows, we are able to precisely sort cells infected with one single bacterium (persister-like) versus many (replicative-like), and follow-up for the identification of enriched bacterial and host mutants able to derive such phenotypes.

Parallel session:
HOST-PATHOGEN INTERACTIONS II (Tuesday, September 10, 2024 10:45)

Invited Lecture

01 Host-pathogen interactions

## Immune checkpoints pathways during acute viral infection

**Yotam Bar-On**[1]
*Immunology, Technion, Israel*

Immune checkpoints play a pivotal role in regulating the immune system activity. These checkpoints encompass molecules and pathways that act as inhibitory regulators on the immune system. The seminal findings that tumor cells can exploit immune checkpoints to impede anti-tumor immunity has transformed cancer immunotherapy and immune checkpoint blockade has emerged as a highly successful approach in restoring anti-tumor immune functions and treating cancer patients. However, our understanding of the involvement of immune checkpoints in viral infections is comparatively limited. In our quest to dissect the role of immune checkpoint receptor during acute viral infection we demonstrated the inhibitory receptor LAG-3 can recognize influenza virus-infected epithelial cells. We further show that this recognition is mediated by the direct binding of LAG-3 to influenza virus hemagglutinin. Moreover, we uncovered the mechanism of LAG-3-hemagglutinin interaction and the possible effect of LAG-3-hemagglutinin interaction on the antiviral immune response and provide a thorough evaluation of the role played by LAG-3 in the context of acute viral infections and on the antiviral T cell responses.

Parallel session:
HOST-PATHOGEN INTERACTIONS II

01 Host-pathogen interactions

## The role of host poly-LacNAc N-glycosylation in EPEC infection.

**Klil Cohen Yaron**[1], Yakov Socol[1], Noam Yedidi[1], Karthik Hullahalli[2], Matthew K Waldor[2], Ilan Rosenshine[1]
[1]*Department of Microbiology and Molecular Genetics, Institute of Medical Research Israel-Canada, Israel*
[2]*Department of Microbiology, Harvard Medical School, United States*

Enteropathogenic Escherichia coli (EPEC) is a gram-negative bacterium responsible for widespread outbreaks of diarrhea globally, particularly fatal infantile diarrhea in developing countries. EPEC infects the small intestine through a sequence of three key steps: (i) initial adherence to host epithelial cells facilitated by the type 4 bundle-forming pilus (BFP), (ii) injection of effector proteins via the type three secretion system, and (iii) establishment of close attachment to the host cell, mediated by the interaction between the surface-exposed domain of the effector protein Tir and an outer membrane protein on the bacterium known as intimin. To identify human host factors crucial for EPEC infection, we conducted two distinct CRISPR screens using two cell type and gRNA libraries. The first screen targeted the final step of EPEC infection—the Tir-intimin interaction—while the second screen focused on identifying genes involved in the cytotoxic effects induced by EPEC. Both screens highlighted genes encoding enzymes required for synthesizing poly-N-acetyllactosamine (Poly-LacNAc). To validate these finding I KO the MGAT1 gene encoding for the first enzyme in this pathay. The KO cells, lacking poly-LacNAc, were significantly immune to EPEC infection. Reciprocal genetic analysis demonstrated the necessity of LacNAc for BFP-mediated adherence. Interestingly, in the absence of BFP, the type 1 fimbriae became the dominant factor facilitating EPEC adherence to host cells. In addition to its role in attachment, our research revealed that poly-LacNAc also influences protein translocation, a finding that warrants deeper investigation. These discoveries hold promise for developing effective therapies against EPEC and potentially other enteropathogenic bacteria.

Parallel session:
APPLIED MICROBIOLOGY AND BIOTECHNOLOGY (Tuesday, September 10, 2024 10:45)

Invited Lecture

## Biotic-Abiotic Semiartificial Cells for Light-Driven Chiral Molecule Production

**Omer Yehezkeli**[1]
*Faculty of Biotechnology & Food Engineering, Technion, Israel*

Utilizing bacteria to produce pharmaceutical compounds or fuel is a highly desirable goal for greener and sustainable industry. In the last few decades, many biotechnological tools have been developed to address the most challenging issues humanity is facing, such as climate change and disease prevention and treatment. While these engineered biological components have led to significant progress, the development of additional tools could lead to another leap forward.

Here, we present a new concept for a self-sustained biotic-abiotic cyborg bacterium, which comprises a biological system with an add-on—an inorganic nano-based organelle. In the designed whole-cell biohybrid system, we exploit the unique structure of Stable Protein 1 (SP1) for the biosynthesis of various size-constrained inorganic nanomaterials within living systems. These nanomaterials are optically and electronically active nanoparticles (NPs) that can be used for various catalytic or photocatalytic processes.

Specifically, the talk will focus on the biosynthesis of CdS NPs that are stabilized by a pre-designed SP1 variant at ambient conditions. The resulting hybrid allows a size-controlled crystalline nano element formation. The developed biohybrids were employed for NADPH photo-regeneration, or photo-electro-regeneration which in turn activates the imine reductase (IRED) enzyme. The latter enables enhanced generation of chiral amine molecules that can be used as precursors in the pharmaceutical industry. This concept was further extended to a fully integrated photocatalytic NADPH regeneration system in a living bacterium, where the photoactive NPs were used for solar-driven enzymatic cascade activation.

Parallel session:
APPLIED MICROBIOLOGY AND BIOTECHNOLOGY (Tuesday, September 10, 2024 10:45)

Invited Lecture

10 Applied microbiology and biotechnology

## Tackling fundamental challenges in biotechnology with computational models and AI

**Tamir Tuller**

A large proportion of intracellular biological process and phenomena are mediated by many subtle interactions between the genetic material and various macromolecular machines. These interactions can`t be efficiently predicted and designed without the help of computational models, algorithms, and AI.

I will briefly review the computational pipeline we are developing for tackling various fundamental challenges in biotechnology such as gene expression optimization, genetic stability, and biosafety using this approach. The presentation will include various examples from fields such as food tech, biosensors, and the recombinant proteins industry.

Parallel session:
APPLIED MICROBIOLOGY AND BIOTECHNOLOGY (Tuesday, September 10, 2024 10:45)

10 Applied microbiology and biotechnology

## Development of non-surgical sterilization/contraception methods by vaccinia virus based vaccine against sexual development hormones

**Elad Eliahoo**[1], Ellen Daw[2], Daniel Barkan[2], Tal Raz[2]
[1]*Department of Virology, Kimron Veterinary Institute,*

*Ministry of Agriculture and Rural Development, Israel*
[2]*Koret School of Veterinary Medicine,*

*The Robert H. Smith Faculty of Agriculture, Food and Environment, Hebrew University in Jerusalem, Israel*

Over-population of straying animals is an increasing problem in modern cities that can lead to public and health hazardous, as well as the danger of transferring zoonotic diseases to humans. The current approved method, Trap-Neuter-Return (TNR), is a surgical sterilization/castration that requires catching the animal, involving anesthesia and the presence of trained professional stuff and keeping the animals under supervision until releasing them. Hence, there is a need for alternative non-surgical methods for neutering of straying animals, one that will be cheap and easy to conduct. Our approach is based on vaccinating the straying animals against key sex hormones such as GnRH - the “master hormone of reproduction”. For this purpose, we used Modified Vaccinia Ankara (MVA) strain of Vaccinia Virus, a poxvirus that had been used to vaccinate humans against the Smallpox Virus and, as a vector, wild animals against Rabies. MVA strain is an attenuated version of Vaccinia Virus that cannot multiply in mammalians cells and is used as a safe vector to induce strong immune response against different antigens. Here, we have tested recombinants MVA that express sex hormones in different combinations to induce strong and lasting immune response against them in mice. Our results present a strong immune response to the vaccine against the tested hormones. In addition, we observed the effect of the vaccine in changes in the vaginal cytology and the histology of the female gonads and in the male sperm count and viability. Moreover, our fertility tests had indicated that the recombinant MVA vaccines had impaired the male mice to in pregnant female mice. Hence, MVA based immune-sterilization/contraception has the potential for the development of a novel, safe, cheap and effective non-surgical method to control stray animals` populations.

Parallel session:
APPLIED MICROBIOLOGY AND BIOTECHNOLOGY (Tuesday, September 10, 2024 10:45)

10 Applied microbiology and biotechnology

## Saliva based Diagnostics – A novel approach

**Omer Deutsch**[1]
*Salignostics Ltd, Salignostics Ltd, Israel*

Developing a sensitive, reliable, and user-friendly saliva-based diagnostic technology for home use poses a significant challenge. Success in this endeavor could dramatically enhance telemedicine access for patients outside major healthcare facilities. To address this challenge, we have designed an innovative platform consisting of three main components: a metered saliva collection device with absorbent properties, a sample preparation unit that processes saliva to enhance biomarker detectability, and a detector that provides colorimetric visualization of the target biomarker. Each component is independently modifiable, allowing for customization based on specific biomarker requirements to achieve optimal sensitivity.

Our platform has been successfully utilized for the detection of proteins, as well as RNA and DNA-based assays. To demonstrate its effectiveness, we developed a saliva-based β-hCG test for pregnancy detection. This assay is able to detect low concentrations of the hormone in saliva. In our experiments, the detection limit was below 1 mIU/mL, significantly lower than the 10-20 mIU/mL standard in urine tests. This test has received a CE approval for home use, and was successfully launched in Europe (UK and Sweeden) and in Israel. Additionally, this platform has been adapted for the detection of COVID-19 and Strep A.

We have also developed a PCR-based version of this platform, where the colorimetric detector is replaced with a collection tube for PCR analysis, the sample preparation unit is modified for long-term room temperature preservation, and the collection device is adapted for both nasal swabs and saliva samples. During the COVID-19 pandemic, this system demonstrated comparable sensitivity to traditional PCR assays performed immediately.

In summary, our platform is user-friendly, sensitive, and reliable, and has been well-received by home users. In my presentation, I will provide a detailed overview of the platform's components, clinical validation performance of the main current applications along with usability pilots.

Parallel session:
APPLIED MICROBIOLOGY AND BIOTECHNOLOGY (Tuesday, September 10, 2024 10:45)

10 Applied microbiology and biotechnology

## Self-reporting porous silicon arrays for microbial infection diagnosis and their resistance

**Xin Jiang**[1], Sagi Sphrits[2], Keren Boguslavsky[2], Gali Ron[1], Sarel Halachmi[2], Ester Segal[1]
[1]*Biotechnology and Food Engineering, Technion, Israel*
[2]*Department of Urology, Bnai Zion Center, Israel*

The rise of drug-resistant microorganisms poses a significant global health threat (1-2). Rapid detection, identification and antimicrobial susceptibility testing (AST) are crucial yet unmet clinical challenges in treating microbial infections. Current clinical workflows for antimicrobial therapy are multi-step, culture-dependent, and labor-intensive, often taking several days to provide results, thereby delaying effective treatment. To provide same-day result, we have developed an integrated point-of-care assay which relies on optical monitoring, via Phase-Shift Reflectometric Interference Measurements (PRISM), of microbial behaviors on miniaturized silicon photonic chips. PRISM enables simultaneous diagnosis of microbial infections, differentiation between bacterial and fungal infections, and rapid AST for clinically relevant species, with a total assay time of (1 – 6 hours) (3-4), as illustrated in Figure 1. Preliminary experiemts on clinical urine specimens with pure Escherichia coli (E. coli) infection via PRISM present high specificity (~ 95%) for bacteriuria determination and provide antimicrobial susceptibility profile with 90% accuracy within 90 minutes. These results are compared to standard microbiological laboratory analysis including microbial culture for infection screening and identification and Vitek 2 for AST, respectively. Additionally, on-going double-blinded tests show similarly promising results by significantly shortened the assay time ( 90 minutes vs. 2 days) for E. coli infection. The PRISM assay shows great potential for integration into healthcare systems, particularly for diagnosing urinary tract infections. Moreover, it can be adapted for other infections, such as life-threatening sepsis, facilitating patient-tailored therapy and improving therapeutic outcomes.

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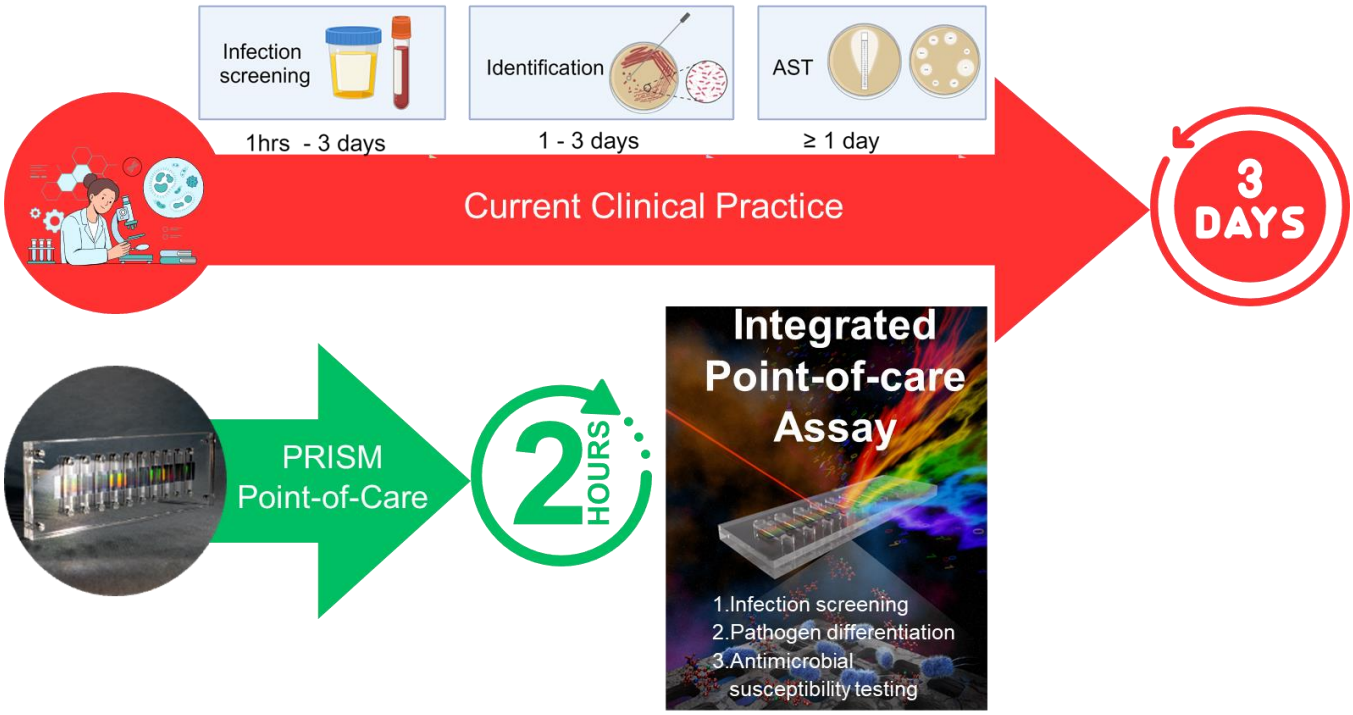


Figure 1: Schematic demonstration of the integrated point-of-care PRISM assay, capable of rapidly detecting microbial infections and their antimicrobial drug susceptibility profile by monitoring microbial behavior on silicon diffraction gratings

Parallel session:
BACTERIAL PHYSIOLOGY (Tuesday, September 10, 2024 10:45)

Invited Lecture

11 Bacterial physiology

## The Establishment of an Intercellular Nanotube Bridge in Bacteria

**Sigal Ben-Yehuda**[1]
*Microbiology and Molecular Genetics, The Hebrew University of Jerusalem, Israel*

We have previously identified a mode of bacterial communication mediated by nanotubes that bridge neighboring cells. Utilizing Bacillus subtilis as a primary model organism, we demonstrated that nanotubes serve as conduits for intercellular trade of cytoplasmic molecules, antibiotic resistance proteins, toxic proteins, and even non-conjugative plasmids, thus playing a central role in bacterial physiology and population dynamics. Despite the documented prevalence of bacterial nanotubes across various bacterial species using electron microscopy methodologies, their visualization through live imaging has posed challenges, and real-time establishment of bacterial cell-to-cell connection has not been recorded. We developed a methodology to visualize nanotube dynamics in real time. We found that nanotubes are projected from sites of lipid accumulation and subsequently engage in a dynamic search process, scanning the surface of nearby bacteria for a period of a few minutes, during which lytic enzymes are deposited over the potential recipient surface to rapture the cell wall. Once a breach is formed, the nanotube paves its way to reach the recipient membrane, establishing a cell-to-cell membranous bridge. I will present the first visualization of an intercellular bridging process in bacteria.

Parallel session:
BACTERIAL PHYSIOLOGY (Tuesday, September 10, 2024 10:45)

Invited Lecture

11 Bacterial physiology

## Correlation and causation in the bacterial cell cycle

**Ariel Amir**[1]
*Physics, Weizmann Institute, Israel*

How cells regulate the timing of cell division and the initiation of new rounds of DNA replication is a longstanding problem. In recent years, new models of cell size homeostasis have been proposed for bacteria, yeast and mammalian cells. A common method to test such models has been to compare the correlations predicted by them to those experimentally measured (e.g., the correlation of cell size at birth and division or the generation time). However, distinct models may predict identical sets of correlation coefficients. I will show that using conditional independence tests (considering the correlation of a pair of variables conditioned on a third) provides new insights into the problem. We find that for *E. coli* replication is tightly coupled to division in slow growth conditions while in fast growth conditions the division event is influenced by additional processes.

Parallel session:
BACTERIAL PHYSIOLOGY (Tuesday, September 10, 2024 10:45)

11 Bacterial physiology

## Quantitative characterization of the onset of bacterial swarm in Bacillus subtilis

**Ajesh Jose**[1], Gil Ariel[2], Avraham Be'er[1]
[1]*Zuckerberg Institute for Water Research, Ben Gurion University of the Negev, Israel*
[2]*Department of Mathematics, Bar-Ilan University, Israel*

Bacterial swarming is a complex biological phenomenon where thousands of cells collectively move and migrate on the surface. Unlike planktonic cells, swarming bacteria develop elongated bodies, increased number of flagella, and a distinct gene expression profile, all regulated by intricate intra- and inter- cellular networks. These genetic and phenotypic adaptations have profound physical implications, yet most of the research has predominantly focused on active regimes, where the cells are already transitioned to the swarming stage exhibiting vigorous motion. This study aims to quantify and elucidate the initial stages of swarming in Bacillus subtilis - from individual cells to the transition into collective swarming state. We show that various biophysical factors such as cell quantity, surfactant secretion, number of flagella and flagellar activity, critically influence the initiation of swarming behavior. Employing different mutants, we specifically pinpoint the roles of specific cellular processes and their associated physical mechanisms in the buildup to collective motion. Our findings intend to bridge the gap in understanding the fundamental trigger of swarming, providing insights into the mechanism that governs this complex behavior.

Parallel session:
BACTERIAL PHYSIOLOGY (Tuesday, September 10, 2024 10:45)

Invited Lecture

11 Bacterial physiology

## L-Methionine Production in Escherichia coli: A Pathway Shift from Trans- to Direct Sulfurylation

**Itamar Yadid**[1]
*Biotechnology, Migal - Galilee Research Institute, Israel*

Methionine is an essential amino acid for vertebrates and a key metabolite across all organisms, it also serves diverse roles in food, feed, and pharmaceutical applications. While bacterial fermentation is commonly employed for amino acid and metabolite production, achieving high yields of L-methionine biosynthesis remains a challenging task due to stringent cellular regulation. Escherichia coli relies on a trans-sulfurylation pathway, whereas other bacteria utilize the more parsimonious direct-sulfurylation route to incorporate sulfur into methionine. We replaced E. coli's tightly regulated trans-sulfurylation pathway with the more universally employed direct-sulfurylation pathway found in other bacteria. By developing an auxotrophic E. coli strain (MG1655) through simultaneous deletion of the genes encoding Homoserine O-succinyltransferase (metA) and Cystathionine gamma-synthase (metB) and introducing the genes encoding Homoserine O-acetyltransferase (metX) and O-acetyl-L-homoserine sulfhydrylase (metY) from specific bacterial sources, we demonstrated enhanced methionine production via the direct-sulfurylation pathway. Complementation of the genetically modified E. coli with metX/metY from Cyclobacterium marinum or Deinococcus geothermalis, along with deletion of the global repressor metJ and overexpression of the transporter YjeH, led to increase of up to 126 and 160-fold in methionine production relative to the wild-type strain, respectively. Furthermore, we utilized genetic libraries constructed from the metX/metY genes originating from bacterial strains that failed to complement the methionine auxotroph. Through enrichment cycles on methionine-depleted media, we successfully isolated methionine-producing strains and identified specific amino acids responsible for this change. Our findings offer a novel strategy for investigating methionine biosynthesis and provide a robust platform for augmenting L-methionine production through fermentation processes.

Parallel session:
BACTERIAL PHYSIOLOGY (Tuesday, September 10, 2024 10:45)

11 Bacterial physiology

## Dissecting the phenotypic landscape of bacterial populations via imaging transcriptomics

**Zohar Persky**[1], Daniel Dar
*Department of Plant & Environmental Sciences, Weizmann institute of science, Israel*

Clonal bacterial populations display phenotypic differences at the individual cell level, even when grown in homogenous environments. This functional heterogeneity is primarily driven by cell-cell variability in gene expression, a parameter that can be tuned by bacteria to produce specialized subpopulations. The resulting phenotypic variation allows bacteria to survive complex perturbations via preadapted cells (e.g., bet-hedging) and to establish cooperative interactions (e.g., division of labor). This phenomenon, which has been implicated in pathogenesis and detected in diverse microbial species, has been highly challenging to characterize systematically due to the technical complexity of profiling gene expression in individual bacteria. Here, we present a novel transcriptomics method based on massively multiplexed mRNA Fluorescent In situ Hybridization (mRNA-FISH), capable of measuring thousands of genes across multiple conditions at single-cell and even molecule resolution. We applied this new approach (called par2FISH) to Escherichia coli, profiling hundreds of thousands of cells collected from various population growth stages. By analyzing cell-cell expression variability for approximately 2,400 genes, we uncovered novel metabolic and auxiliary pathways that are specifically upregulated in subparts of the population in a growth-phase-dependent manner. Our results indicate that par2FISH can effectively capture the single-cell phenotypic landscape of bacteria with unprecedented sensitivity and scope, providing the means to understand how microbes manage their gene expression strategies from the individual level to the collective.

Parallel session:
MICROBIAL TOXINS (Tuesday, September 10, 2024 14:00)

Invited Lecture

12 Microbial toxins

## Antimicrobial Peptides (AMPs) as the Next-Generation Antimicrobial Drugs Against Bacterial and Fungal Pathogens

**Netanel Tzarum**[1], Rina Fraenkel[1], Inbar Cahana[1], Noam Deouell[1], Shani Bruchim[1], Tomer Sivan[1], Asaf Levy[2], Neta Schlezinger[3]
[1]*Department of Biological Chemistry, Institute of Life Sciences,, The Hebrew University of Jerusalem, Jerusalem*
[2]*Department of Plant Pathology and Microbiology, Faculty of Agriculture, Food, and Environment, The Hebrew University of Jerusalem, Rehovot*
[3]*Koret School of Veterinary Medicine, Faculty of Agriculture, Food, and Environment, The Hebrew University of Jerusalem, Rehovot*

Antimicrobial-resistant bacterial and fungal pathogens caused more than 2.5 million deaths in 2019, with the forecast of a yearly 10 million deaths globally by 2050. New strategies to fend off pathogenic microbes are critically needed, as only a few antimicrobials have been successfully developed in recent years. To this aim, our collaborators at the Levy lab have developed a computational approach and discovered novel toxin domains of Polymorphic toxins (PTs) and the cognate immunity genes (PIMs) that can neutralize the toxin activities. The toxicity of nine novel toxins (PT1-9) that cause bacterial or yeast cell death was validated. In collaboration with the Shlezinger lab, we demonstrate, for several PTs, potent antifungal activity against several fungal pathogens included in the top priority list of WHO.

To investigate the novel PTs enzymatic activity, we expressed three novel PTs mutants and demonstrated using biochemistry studies that two toxins have efficient metallo-DNAse activity. The crystal structures of two novel toxin-immunity protein complexes shed light on the enzymatic mechanism of the PTs and the PIM's neutralization activity. Next, we have developed a protocol for the large-scale production of wild-type PTs, an essential step for biochemical and functional studies and the applicability of the PTs as antimicrobial drugs. A proof-of-concept study using PT9 indicated the feasibility of this method for high-yield AMP expression. Therefore, our work provides a detailed understanding of the novel toxins` activity and lays the foundation for developing innovative antibacterial and antifungal treatments.

Parallel session:
MICROBIAL TOXINS (Tuesday, September 10, 2024 14:00)

Invited Lecture

12 Microbial toxins

## The impact of bacterial endoribonuclease toxins on multigenerational host-bacteria interactions

**Ilana Kolodkin-Gal**[1], Omri Gilhar[2], Liat Rahamim-Ben Navi[1], Tsviya Olender[3], Hadar Ben Yoav[4]
[1]*Scojen Institute for Synthetic Biology, Reichman University, Israel*
[2]*Department of Plant and Environmental Sciences, Weizmann Institute of Science, Israel*
[3]*Department of Molecular Genetics, Weizmann Institute of Science, Israel*
[4]*Department of Biomedical Engineering, Ben-Gurion University of the Negev, Israel*

Epigenetics is generally defined as stable alterations in gene expression potentials that arise during development and cell proliferation or alterations in DNA function without alterations in DNA sequence. This narrow definition is now extended to the alteration of DNA as well as regulatory proteins without nucleotide sequence variance. These hereditary changes allow adaptation to transmit the data contained to the next generation, often allowing complex organisms a rapid path for adaptation.

While studied extensively in the context of human diseases, it is now becoming evident that any phenotype results from dynamic interactions between the genotype, epigenome, and environment. Our results show a regulatory role for the toxin-anti-toxin system of NdoA/NdoI during plant-*Bacillus subtilis* multigenerational interactions, representing a simplified system of microbe-plant symbiosis. Using the RNA silencing approach, we now expand this system to understand the roles of the homolog toxin-antitoxin system MazF/MazE during GI colonization by *Escherichia coli*. We hypothesize that host attachment involves the induction of a confined set of hereditary reversible changes in gene expression that allow host colonizers to advance over non-colonizing competitors of the same species. At the same time, it maintains the overall plasticity of gene expression and rapid adaptation.

Parallel session:
MICROBIAL TOXINS (Tuesday, September 10, 2024 14:00)

12 Microbial toxins

## Double trouble: a novel T6SS effector with two toxic domains

**Chaya-Mushka Fridman**[1], Kinga Keppel[1], Eran Bosis[2], Dor Salomon[1]
[1]*Department of Clinical Microbiology and Immunology, Tel Aviv University, Israel*
[2]*Department of Biotechnology Engineering, Braude College of Engineering, Israel*

Many Gram-negative bacteria employ the type VI secretion system (T6SS), a macromolecular protein delivery apparatus, to outcompete rival bacteria. The T6SS fires a missile-like structure decorated with toxic proteins, called effectors, into neighboring cells, where these effectors target conserved processes or structures. Known effectors comprise either a single toxic domain or multiple domains including an N-terminal T6SS-loading domain and a C-terminal toxic domain. Despite the identification of various T6SS effectors and toxic domains, many remain unknown. Here, we investigated the T6SS of the human pathogen *Aeromonas jandaei* and identified a novel effector, Ate1. Remarkably, we found that Ate1 has two distinct toxic domains: one located at the C-terminus and the other at the N-terminus. The genes flanking Ate1 encode cognate immunity proteins, one for each toxic domain. We showed that both toxic domains target the periplasmic compartment where each can be neutralized by its cognate immunity protein. We further demonstrated the ability to replace the two toxic domains with other, non-T6SS-related domains and retain T6SS-mediated secretion of the chimeric proteins. Our findings reveal the first example of a T6SS effector containing two independent toxic domains with distinct immunity proteins, thus illuminating novel concepts governing the diversity and complexity of T6SS-mediated interbacterial competitions.

Parallel session:
MICROBIAL TOXINS (Tuesday, September 10, 2024 14:00)

Invited Lecture

12 Microbial toxins

## Gamma-Mobile-Trio systems define a new class of mobile elements rich in bacterial defensive and offensive tools

Tridib Mahata, Katarzyna Kanarek, Marimuthu Ragavan Rameshkumar, **Eran Bosis**[2],
Udi Qimron, Dor Salomon
*Department of Biotechnology Engineering, Braude College of Engineering*

Conflicts between bacteria and their rivals led to an evolutionary arms race and the development of bacterial immune systems. Although diverse immunity mechanisms were recently identified, many remain unknown, and their dissemination within bacteria is poorly understood. Here, we describe a widespread genetic element, defined by the presence of the Gamma-Mobile-Trio (GMT) proteins, that serves as a bacterial survival kit. We show that GMT containing genomic islands are active mobile elements with cargo comprising various anti phage defense systems, in addition to antibacterial type VI secretion system (T6SS) effectors and antibiotic resistance genes. We identify four new anti-phage defense systems encoded within GMT islands. A thorough investigation of one system reveals that it is triggered by a phage capsid protein to induce cell dormancy. Our findings underscore the need to broaden the concept of `defense islands` to include also antibacterial offensive tools, such as T6SS effectors, as they share the same mobile elements as defensive tools for dissemination.

Parallel session:
MICROBIAL TOXINS (Tuesday, September 10, 2024 14:00)

12 Microbial toxins

## Unveiling the Extracellular Contractile Injection Systems (eCIS) Target Specificity Determinants

**Nimrod Nachmias**[1], Asaf Levy
*Faculty of agriculture, HUJI, Rehovot*

Extracellular contractile injection systems (eCIS) represent a diverse family of bacteriophage tail-derived protein toxin delivery complexes in bacteria and archaea. eCIS are vital for microbial interaction with hosts and they use tail fiber for target cell binding. Here, we conducted a comprehensive exploration of eCIS tail fiber genes, providing insights into their remarkable diversity, target cells, functional adaptations, and intriguing evolutionary dynamics. We developed a computational method to identify 1,169 eCIS tail fiber proteins from natural microbes that carry four new domains responsible for tail fiber attachment to eCIS baseplates, thereby significantly expanding the repertoire of known tail fiber genes. Importantly, we revealed a surprisingly high diversity of target receptor binding modules located at the C termini of tail fibers, suggesting direct eCIS roles in adhesion to different eukaryotes and bacteria. We experiemntally showed that the tail fiber from Paenibacillus sp. URHA0014 can bind to and direct eCIS injection into THP1 cells. This study enhances our understanding of eCIS target specificity determinants and suggests new eCIS target cells, with implications for medicine, agriculture, and biotechnology.

Parallel session:
MICROBIAL TOXINS (Tuesday, September 10, 2024 14:00)

12 Microbial toxins

## Unmasking the metabolically active growth arrest induced by VapC in E. coli

**Irine Ronin**[1], Adi Rotem[1], Orit Gefen[1], Liron Argaman[2], Hanah Margalit[2], Matthias Heinemann[3], Nathalie Balaban[4]
[1]*The Racah Institute of Physics, The Hebrew University in Jeruslaem, Israel*
[2]*Microbiology department, The Hebrew University in Jerusalem, Israel*
[3]*Molecular Systems Biology, Groningen Biomolecular Sciences and Biotechnology Institute, Netherlands*
[4]*The Racah Institute of Physics, The Hebrew University in Jerusalem, Israel*

Growth arrest protects bacteria from many stressful environments. Toxin-antitoxin (TA) modules are widespread in bacteria and have been associated with stress response, dormancy, and antibiotic tolerance. Mutations in the VapBC toxin-antitoxin module were repeatedly observed in the evolution of E.coli cultures under intermittent antibiotic exposure. In this study, we focus on the induction of growth arrest in E. coli by VapC, a conserved toxin found in gram positive and gram negative bacteria. We show that the VapC toxin is more stable than the VapB antitoxin, and the accumulation of the former causes a non-lethal growth arrest. Despite their inability to divide, VapC-induced growth arrested bacteria are metabolically active, and are able to respond to an induction signal and produce fluorescent proteins. This metabolically active state depletes nutrients in the medium, preventing the growth of competitors. Comparative studies of the proteome and RNA-seq analyses between growth arrest at stationary phase and VapC-induced growth arrest revealed widely different regulatory mechanisms. Further proteomics and transcriptomics analyses identified possible active metabolic mechanisms, which may serve as an “Achilles heel” for targeting bacteria during antibiotic treatment. Our findings may provide a framework for designing new drugs and treatments against bacterial tolerance.

Parallel session:
PARASITES (Tuesday, September 10, 2024 14:00)

Invited Lecture

13 Parasites

## Targeting Parasites with Peptides: A Focus on Leishmania and Trypanosoma Treatment Strategies

**Nir Qvit**[1]
*Israel, Bar-Ilan University, Israel*

Parasitic diseases caused by Leishmania and Trypanosoma species pose significant health challenges globally, leading to severe morbidity and mortality. Conventional treatments often have limitations including toxicity, resistance, and inadequate efficacy. Recent advancements in peptide-based therapies offer a promising alternative. Peptides, due to their high specificity and ability to target unique parasite antigens, can be designed to inhibit key biological processes essential for parasite survival and proliferation.

Herein we present the development and application of peptide-based treatments targeting Leishmania and Trypanosoma. For Leishmania, peptides have been engineered to interfere with essential enzymes critical for parasite viability. Similarly, in Trypanosoma, peptides targeting critical enzymes disrupt vital functions and essential mechanisms.

Our research highlights the potential of these peptides to overcome current treatment limitations, offering advantages such as reduced side effects and targeted action. Preclinical studies demonstrate promising results, including enhanced efficacy and reduced toxicity compared to conventional drugs.

Overall, peptide-based therapies represent a cutting-edge approach to combatting parasitic diseases, potentially leading to more effective and safer treatments for Leishmania and Trypanosoma infections.

Parallel session:
PARASITES (Tuesday, September 10, 2024 14:00)

Invited Lecture

13 Parasites

## Dynamic Imaging: Kinetics, Morphologies and Regulation of Organelle Biogenesis Throughout Plasmodium Cell Cycle

**Anat Florentin**[1], Michal Shahar[1], Alia Qasem[1]
*Faculty of Medicine, The Hebrew University of Jerusalem, Israel*

The apicoplast of Plasmodium falciparum undertakes unique morphologies throughout the parasite's cell cycle. The details and the regulation of these sequential physical changes, collectively referred to as organelle biogenesis, are unknown. Here, we report the development of a dynamic imaging platform that enables us to follow subcellular structures in high resolution throughout the 48 hours replication cycle of a live parasite within Red Blood Cells. We generated transgenic parasites in which both the apicoplast and the nucleus are fluorescently tagged, enabling us to dissect the coordinated development of these two organelles. Using this approach, we revealed a new and highly-detailed sequence of morphological events that proceed organelle division and is tightly correlated with nuclear replication. Next, we developed an analytical pipeline using various computational tools to measure changes in organelles' length, volume and counts, enabling us to determine the kinetics and orchestration of these processes. We discovered that although the apicoplast and nuclear dynamics are tightly correlated in space and time, the two processes can occur independently of each other. We therefore tested the hypothesis that apicoplast biogenesis is regulated by an organelle intrinsic mechanism, involving replication of organelle genome. Using qPCR, we determined the basal ploidy of the apicoplast chromosome, and to measure its replication rate over time. Finally, we used a pharmaceutical approach to test whether DNA replication, transcription or translation in the organelle, but not in the nucleus, play a role in organelle biogenesis. Collectively, these results reveal new organelle biology and unique regulatory mechanisms.

13 Parasites

## Synthetic antimicrobial peptides to combat drug resistance in the malaria parasite *Plasmodium falciparum*

**Edo Kiper**[1], Daniel Ben Hur[1], Daniel Alfandari[1], Naiem Ahmad Wani[1], Abel Cruz Camacho[1], Gal David Efrat[1], Mattia Morandi[2], Moshe Goldsmith[1], Ido Azuri[3], Ron Rotkopf[3], Roman Kamyshinsky[4], Ziv Porat[5], Irit Rosenhek-Goldian[4], Yechiel Shai[1], Neta Regev-Rudzki[1]

[1]*Biomolecular Sciences, Weizmann Institute of Science, Israel*
[2]*Institute of Organic Chemistry and Biochemistry, Czech Academy of Science, Czech Republic*
[3]*Bioinformatics Unit, Department of Life Sciences Core Facilities, Weizmann Institute of Science, Israel*
[4]*Chemical Research Support, Weizmann Institute of Science, Israel*
[5]*Department of Life Sciences Core Facilities, Weizmann Institute of Science, Israel*

Malaria is a deadly infectious disease caused by the *Plasmodium falciparum* (*Pf*) parasite, the most virulent *Plasmodium* species. The lack of an effective vaccine and the emergence of drug-resistant parasites increase the challenge of eradicating malaria worldwide. Synthetic antimicrobial peptides (AMPs) offer a therapeutic avenue against infectious agents with a reduced risk of drug resistance. However, their application in targeting the malaria parasite remains underutilized. Here, we identified two synthetic AMPs capable of significantly inhibiting *Pf* growth. Remarkably, these AMPs exclusively interact and damage red blood cells (RBCs) infected with *Pf* parasites (*Pf*-iRBCs), leaving naïve human RBCs unharmed. To elucidate the selective mode of action (MoA) of synthetic AMPs, we labeled the peptide with a fluorophore to measure their binding affinity to *Pf*-iRBCs as well as monitor their subcellular localization. We discovered that active AMPs exhibit strong binding to *Pf*-iRBCs, reaching peak affinity during the late developmental stages of *Pf*. Moreover, AMPs effectively cross the host membrane, causing extensive damage to the *Pf*-iRBCs' membrane and cytoskeleton. We subsequently aimed to investigate the AMPs' MoA further using large unilamellar vesicles. We identified the cholesterol depletion from *Pf*-iRBCs as the facilitator of the selective interaction of AMPs to *Pf*-iRBCs.

Overall, our study not only demonstrates the power of these resistance-reducing synthetic AMPs as novel antimalarial drugs but also identifies cholesterol as a major component of the infected cell membrane, opening avenues for further therapeutic development in malaria treatment.

PARASITES (Tuesday, September 10, 2024 14:00)

Invited Lecture

13 Parasites

## An important role of the SRP-independent targeting pathway in protein export in Plasmodium falciparum

**Karina Simantov Ron**

The malaria parasite *Plasmodium falciparum* (P. falciparum) extensively modifies the host red blood cell through the export of numerous parasitic proteins in a complex multistep process. This is an essential process, not only required for invasion and survival but also important for the pathogenicity and virulence of the parasite. Protein import into the ER is the first step in the export pathway, however, little is known about the mechanism of protein import into the ER for proteins lacking definite export elements, including the major virulence factor, PfEMP1. Here we identified and explored the role of a novel SRP-independent targeting (SND) pathway for ER targeting and import of export proteins. The main components of this pathway, PfSnd2 and PfSnd3 are well conserved, and function as ER membrane proteins. We found that the knockdown of PfSnd2 disrupts the export and surface expression of the virulent PfEMP1 and several other export proteins. Furthermore, we investigated and unveiled some of the structural elements important for targeting proteins to the SND pathway for ER import. Altogether, our studies highlight the involvement and role of an alternative ER targeting pathway as the first step in the export of proteins essential for the pathogenicity of the parasite.

Parallel session:
PARASITES (Tuesday, September 10, 2024 14:00)

13 Parasites

## UMSBP2 is a chromatin modeler protein that functions in regulation of gene expression and suppression of antigenic variation in trypanosomes

**Awakash Soni**[1], Olga Klebanov-Akopyan[2], Esteban Erben[3], Inbar Plaschkes[4], Hadar Benyamini[4], Vera Mitesser[2], Amnon Harel[5], Katereena Yamin[6], Itay Onn[5], Joseph Shlomai[2]

[1]*Department of Microbiology and Molecular Genetics, The Hebrew University of Jerusalem, Israel*

[2]*Department of Microbiology and Molecular Genetics, The Hebrew University of Jerusalem, Israel*

[3]*Institute of Biotechnological Research, National University of San Martin, San Martın*

[4]*The Info-Core Bioinformatics Unit, The Hebrew University of Jerusalem, Israel*

[5]*Azrieli Faculty of Medicine, Bar-Ilan University, Israel*

[6]*Azrieli Faculty of Medicine, Bar-Ilan University, Isarel*

Packaging and condensation of genomes in the chromatin structure limit their accessibility to the transcription and replication machineries, while chromatin uncoiling by chromatin remodeling complexes renders the genome accessible to these activities. We studied the Universal Minicircle Sequence binding proteins (UMSBPs) which are CCHC-type zinc-finger proteins that bind the single-stranded G-rich UMS sequence, conserved at the replication origins of the mitochondrial DNA (kDNA) of trypanosomatids. Trypanosoma brucei nuclear UMSBP2, previously reported to function in the replication and segregation of kDNA, has been recently shown to colocalize with telomeres and is essential for chromosomal end protection. Here, we report that UMSBP2 interacts selectively with core histones, and linker histone H1 results in the remodeling of T. brucei chromatin. DNA remodeling by UMSBP2 is mediated via protein-protein interactions and is independent of its previously described DNA binding activity. UMSBP2 silencing, results in a higher nucleosomal density profile than in cells expressing TbUMSBP2 and this phenotype could be reversed by supplementing the depleted cells with UMSBP2. Depletion of UMSBP2 also results in telomeres depletion from the nuclear periphery to the nucleoplasm. UMSBP2 also has the capacity to remodel chromatin structure in higher eukaryotes and bacteria. RNA-seq analyses revealed that UMSBP2 knockdown affects multiple gene expression in parasite but the most significant affects observed in derepression of multiple subtelomeric variant surface glycoproteins (VSG) genes in T. brucei. These observations suggest that UMSBP2 is a chromatin remodeler that functions in the regulation of gene expression and plays an essential role in the control of antigenic variation in T. brucei.

Parallel session:
MICROBIAL SOCIETIES AND MICROBIAL INTERACTIONS
(Tuesday, September 10, 2024 14:00)

Invited Lecture

14 Microbial societies and microbial interactions

## Reversion to metabolic autonomy underpins evolutionary rescue in a bacterial obligate mutualism

**Jonathan Friedman**[1], Ignacio Melero-Jiménez[1], Yael Sorokin[1], Ami Merlin[1], Alejandro Couce[2]
[1]*Institute of Environmental Sciences, Hebrew University, Israel*
[2]*Centro de Biotecnología y Genómica de Planta, Universidad Politécnica de Madrid, Spain*

Populations facing lethal environmental change can avoid extinction by undergoing rapid genetic adaptation, a phenomenon termed evolutionary rescue. Despite much theoretical and empirical research, evolutionary rescue in the context of communities remains poorly understood, especially in mutualistic ones, where survival is constrained by the less adaptable partner. Here, we explored empirically the likelihood, population dynamics, and genetic mechanisms underpinning evolutionary rescue in an obligate mutualism mediated by reciprocal amino acid exchange in Escherichia coli. We observed that 80% of the communities avoided extinction when exposed to two different types of lethal stresses. Of note, only one of the partners survived in all these cases. Genetic and phenotypic analyses show that the survivor reverted to autonomy by metabolically bypassing the auxotrophy, with little evidence of specific adaptation to the stressors. Crucially, we found that the mutualistic partners were more sensitive to both stresses than prototrophs, so that reversion to autonomy was sufficient to alleviate stress below lethal levels. We observed this increased sensitivity across several other stresses, suggesting that this may be a general property of obligate mutualisms mediated by amino acid exchange. Our results reveal that evolutionary rescue critically depends on the specific genetic and physiological details of the interacting partners, adding rich layers of complexity to the endeavor of predicting microbial community resilience under intense environmental deterioration.

Parallel session:
MICROBIAL SOCIETIES AND MICROBIAL INTERACTIONS
(Tuesday, September 10, 2024 14:00)

14 Microbial societies and microbial interactions

## Deep sea gas seeps are hotspots of microbial productivity and biotic interactions

**Maxim Rubin-Blum**[1,2], Lina Ratinskaia[1,2], Stas Malavin[1,3]
[1]*Marine Biology, Israel Oceanographic and Limnological Research, Israel*
[2]*Department of Marine Biology, Leon H. Charney School of Marine Sciences, University of Haifa, Israel*
[3]*Department of Environmental Hydrology and Microbiology, Zuckerberg Institute for Water Research, The Jacob Blaustein Institutes for Desert Research, Ben-Gurion University of the Negev, Israel*

Discharge of gas-rich fluids from the seabed yields unique biodiversity hotspots in the often-barren deep sea. Microbes play a crucial role in this habitat, mitigating the emissions of the greenhouse gas methane and producing vast biomass through chemosynthesis. We focus on the taxonomic and functional diversity of microbes that thrive near gas-rich brine pools at the water depth of 1150 m, 60 km offshore Israel, asking how different species cooperate in these hotspots of productivity and biotic interactions. Omics shows that in brines and adjacent sediments, where methane and sulfide concentrations were in the millimolar range, microbes cooperate to catalyze carbon cycling. Dominant specialists fix carbon and oxidize methane, whereas diverse archaea and bacteria ferment macromolecules, playing a key role in necromass turnover. Cooperation between organotrophs (Bacteroidota), sulfate reducers (Desulfobacterota), and thiotrophs such as the giant sulfur bacteria Thiomargarita fuel carbon cycling within animal burrows at seep chemotones, that is, the transition zones between chemosynthetic and background habitats. Metabolic handoffs play a key role not only for the free-living communities but also in chemosynthetic symbioses with invertebrates, as thiotrophic, methylotrophic and organotrophic microbes co-occur in host cells, fixing carbon, recycling organics and sharing common goods, such as cobalamin (B12). Studying this unique deep-sea habitat provides an exciting opportunity to explore microbial functions and interactions in an extreme environment. It opens a door for multidisciplinary collaborative studies in various related fields.

Parallel session:
MICROBIAL SOCIETIES AND MICROBIAL INTERACTIONS
(Tuesday, September 10, 2024 14:00)

14 Microbial societies and microbial interactions

## The effect of phage peptide communication on bacterial social behavior

**Bat El Hagbi Lazar**[1], Ronit Suissa[1], Meital Shema Mizrachi[1], Zohar Tik[1], Zoe Levi[1], Michael M. Meijler[1]
*Department of Chemistry, Ben Gurion University of the Negev, Israel*

Quorum sensing (QS), a cell-to-cell communication mechanism, allows bacteria to synchronize gene expression within a population. As a result, the bacteria can act as a group in response to changes in cell population density. Both Gram-positive and Gram-negative bacteria use this kind of regulation by production and detection of molecules called autoinducers. Bacteriophages are the most abundant and diverse biological entity on Earth and they can profoundly affect their bacterial host. This interspecies interaction can play an important role in shaping the structure of microbial communities, and have a profound effects on biological processes. Despite their importance, little is known on the interaction between most phages and bacterial communities and their general structure of infection. In this work, we evaluated the effects of a peptide encoded by a phage that infects Gram-positive bacteria and its effect on the host social behavior, while also testing the metabolic profile to gain a comprehensive understanding of the underlying mechanisms involved. Preliminary results indicate that in the presence of the peptide, there is a disruption in biofilm morphology as well as inhibition in biofilm formation of the bacteria. Our results provide exciting evidence for recognition of the peptide by the QS systems of the bacteria and therefore imply phage-host chemical interactions.

Parallel session:
MICROBIAL SOCIETIES AND MICROBIAL INTERACTIONS
(Tuesday, September 10, 2024 14:00)

Invited Lecture

14 Microbial societies and microbial interactions

## Bacterial physiology in an environmental context

**Einat Segev**

Microorganisms have evolved in the cold, nutrient-poor ocean for millions of years. Yet, laboratory studies of marine bacteria often use conditions vastly different from their natural habitats. To better understand microbial physiology and their environmental roles, we study marine bacteria under conditions that mirror their natural environment.

Beyond environmental conditions such as temperature and nutrient regimes, many marine bacteria are influenced by interactions with their algal hosts. Algal-bacterial interactions include competition, cooperation, and communication, which significantly impacts both algae, bacteria, and their environment. In marine ecosystems, these interactions shape nutrient fluxes and biogenic material burial.

My talk will zoom into our recent discoveries about bacterial physiology in the context of algal hosts, focusing on the bacterial transition from starvation to growth. I will present our findings on how algal cues shorten bacterial lag phases and trigger bacterial extracellular matrix production.

Parallel session:
MICROBIAL SOCIETIES AND MICROBIAL INTERACTIONS
(Tuesday, September 10, 2024 14:00)

14 Microbial societies and microbial interactions

## Deciphering the identity of native molecular cargo transported via nanotubes among bacterial community members

**Saurabh Bhattacharya**[1], Philipp Spät[2], Venkadesaperumal Gopu[1], Waner Yan[1], Manas Guria[1], Nir Dranitzky[1], Boris Macek[2], Ilan Rosenshine[1], Sigal Ben-Yehuda[1]
[1]*Department of Microbiology and Molecular Genetics, IMRIC, Faculty of Medicine, The Hebrew University of Jerusalem, Israel*
[2]*Department of Quantitative Proteomics, Interfaculty Institute for Cell Biology, University of Tübingen, Germany*

Bacterial survival in natural niches is majorly dictated by interactions among the community comprising species. A predominant mechanism of contact-dependent communication is mediated by membranous bridges, termed nanotubes, connecting neighboring bacteria of the same and different species. Nanotubes, previously discovered in our laboratory, serve as conduits for the exchange of cytoplasmic molecules such as amino acids, proteins, and even non-conjugative plasmids. However, the extent and the identity of the nanotube mediated-trafficked proteome remained elusive. We aim to explore the potential nanotube mediated protein trade between the two model bacteria Bacillus subtilis (Bs) and Escherichia coli (Ec). Accordingly, following the co-culture of the two species, Ec was separated and isolated from Bs by immuno-separation, and subjected to a quantitative mass spectrometry analysis, tailored to delineate Ec from Bs peptides. A co-culture of Bs and Ec mutants, deficient in nanotube formation, was incubated in parallel to ascertain nanotube exclusive cargo. Remarkably, a repertoire of more than one hundred Bs proteins were monitored to be transferred to Ec, potentially via nanotubes. Intriguingly, a substantial proportion of the detected proteins are defined as proteases and peptidases, hinting at novel function of the nanotube cargo in scavenging peptides and amino acids from the neighboring species. Further validation of putative cargo candidates is currently in progress. As far as we know, global analysis of protein spread among members of a bacterial community has not been previously reported; as such, our findings could transform our view of bacterial interactions within multi-species communities in nature.

Parallel session:
BACTERIOPHAGES (Tuesday, September 10, 2024 16:00)

Invited Lecture

15 Bacteriophages

## Predicting Antibiotic Resistance

**Roy Kishony**[1]
*Biology, CS (secondary), biomedical Engineering (secondary), Technion- Israel Institute of Technology, Israel*

Antibiotic resistance is growing as a major public health concern. Predicting antibiotic resistance and the evolutionary paths leading to resistance is key for our ability to control the spread of drug resistant pathogens. I will describe a series of experimental-computational methodologies for following and identifying recurrent patterns in the evolution of antibiotic resistance in the lab and in the clinic. Combined with machine-learning approaches applied to electronic patient records, these tools can lead to predictive diagnostics of antibiotic resistance and personalized treatments of microbial infections.

Parallel session:
BACTERIOPHAGES (Tuesday, September 10, 2024 16:00)

Invited Lecture

15 Bacteriophages

## The bully phage: A Shiga toxin-encoding phage suppresses host-sharing prophages

**Yael Litvak**[1], Tamara Wellins, Rachel Hashuel, Maayan Rachimi, Eran Gutman, Reut Melki, Linoy Taktuk, Pe'era Pash Wasserzug, Karina Roitman
*Silberman Life Science Institute, The Hebrew University, Israel*

Gut bacteria frequently encode multiple prophages, yet only a subset or a singular prophage produce virions upon induction. We show that a Shiga toxin-producing prophage inhibits virion production and DNA replication of another host-sharing prophage and represses its induction during intestinal colonization. We demonstrate that the shiga toxin phage lysogenizes various commensal E. coli strains in the gut during infection. In these lysogens, the Shiga toxin phage was induced by DNA-damaging agents; however, it repressed the replication of co-hosted prophages that were active in the parental strain. We further found that the mechanism of phage-phage cross-inhibition is common among Shiga toxin-encodying phages and in other temperate E. coli phages. Our work presents a novel mechanism that governs interactions between host-sharing temperate phages and plays a significant role in regulating polylysogeny and virulence.Top of Form

Parallel session:
BACTERIOPHAGES (Tuesday, September 10, 2024 16:00)

15 Bacteriophages

## A bacterial host factor confines phage localization for excluding the infected compartment through cell division

**Osher Pollak-Fiyaksel**[1]
*Microbiology, Hebrew University of Jerusalem, Israel*

Viruses frequently induce the formation of specialized subcellular compartments to facilitate their replication and assembly. Yet, here we uncover a “host-derived” confinement mechanism, compartmentalizing bacteriophage (phage) production to enable phage caging through cell division. By employing the bacterium Bacillus subtilis and its lytic phages, we identified YjbH, highly conserved among Gram-positive bacteria, as a host factor that limits plaque expansion. YjbH was found to directly bind the penetrating phage genome via its Helix-Turn-Helix DNA-binding domain and to accumulate into a focus at the site of DNA injection. We further revealed that YjbH constricts the synthesis of phage components, including DNA and capsid proteins, to a specific subcellular locale. Consequently, the division machinery is recruited to produce adjacent septations, often asymmetric, effectively trapping and excluding the infected compartment. This newly discovered “exclude and survive” defense mechanism may represent a prevalent strategy employed by the host to contain viral spread.

Parallel session:
BACTERIOPHAGES (Tuesday, September 10, 2024 16:00)

Invited Lecture

15 Bacteriophages

## Lysogeny in cyanobacteria

Esther Cattan-Tsaushu, Yara Nwatha, Hoda Madi, Ke Li, **Sarit Avrani**[1]
*Department of Evolutionary and Environmental Biology, and the Institute of Evolution, University of Haifa*

Cyanobacteria, a globally widespread phylum of photosynthetic bacteria, can form harmful blooms in freshwater ecosystems, which impact water quality and represent a significant economic, ecological and public health challenge. Cyanophages, which are viruses that can infect and kill cyanobacteria, are likely important regulators of cyanobacterial blooms dynamics. Cyanophages, similarly to other phages, can have different life cycles: lytic or lysogenic. Lytic phages replicate within their host and then lyse and kill the host cell to release the phage progeny to the surrounding environment. By contrast, lysogenic (temperate) phages have the ability to integrate their genome into the host's genome and stay (mostly) dormant as a prophage that replicates alongside the host, until a biotic or abiotic signal leads to the induction of the prophage to enter a lytic cycle. Studies examining phage-host interactions have traditionally centered on antagonistic interactions, which typically involve lytic infections. However, more recent findings indicate that interactions between phages and their hosts frequently exhibit a spectrum of infection dynamics, spanning from lysis to persistent lysogeny for long periods. The dramatic differences between infections that result in host death (lytic) compared to those in which the host survives with an inserted prophage (lysogenic) strongly influence the ecology and evolution of both the phage and host. Here, I will share our recent findings of evidence for the existence of lysogeny in cyanobacteria in lab experiments and in the environment, as well as physiological characterization of lysogeny-related elements in cyanophages.

Parallel session:
BACTERIOPHAGES (Tuesday, September 10, 2024 16:00)

15 Bacteriophages

## Biases in viral transmission modes can be explained by Bayesian inference

**Nitzan Aframian**[1], Avigdor Eldar[1]
*Life Sciences, Tel Aviv University, Israel*

Some viruses transition between vertical and horizontal modes of transfer. Temperate phages, for example, choose between lysis (horizontal transmission) and lysogeny (vertical transmission), both upon infection and from the dormant, prophage, state. The optimal decision under given conditions depends on the prospective fitness of each mode, regardless of history. However, it is widely observed that newly infecting phages are heavily biased towards lysis, and established prophages towards lysogeny. Here, we analytically show that these opposite biases can be explained by differences in information regarding the composition of the surrounding bacterial population. Phages that have just entered a sensitive host, have effectively sampled a sensitive host from the bacterial population. From the phage point of view, this should increase the probability that the population is rich in sensitive bacteria, and in turn increase the expected benefit of lysis. Prophages, on the other hand, are more likely to be surrounded by other lysogens. We theoretically demonstrate that this process of Bayesian inference can explain biases in lysis\lysogeny decisions that are observed in experiments and simulations, and that it is not redundant with other sources of information (e.g. communication). This framework may also help explain life-cycle transition rates of other mobile genetic elements, and of viruses infecting more complex organisms.

Parallel session:
BACTERIOPHAGES (Tuesday, September 10, 2024 16:00)

15 Bacteriophages

## Phages reconstitute NAD+ to counter bacterial immunity

**Ilya Osterman**[1]
*The Department of Molecular Genetics, Weizmann Institute of Science, Israel*

Bacteria defend against phage infection via a variety of antiphage defense systems. Many defense systems were recently shown to deplete cellular nicotinamide adenine dinucleotide (NAD+) in response to infection, by breaking NAD+ to ADP-ribose (ADPR) and nicotinamide. It was demonstrated that NAD+ depletion during infection deprives the phage from this essential molecule and impedes phage replication. Here we show that a substantial fraction of phages possess enzymatic pathways allowing reconstitution of NAD+ from its degradation products in infected cells. We describe NAD+ reconstitution pathway 1 (NARP1), a two-step pathway in which one enzyme phosphorylates ADPR to generate ADPR-pyrophosphate (ADPR-PP), and the second enzyme conjugates ADPR-PP and nicotinamide to generate NAD+. Phages encoding the NARP1 pathway can overcome a diverse set of defense systems, including Thoeris, DSR1, DSR2, SIR2-HerA, and SEFIR, all of which deplete NAD+ as part of their defensive mechanism. Phylogenetic analyses show that NARP1 is primarily encoded on phage genomes, suggesting a phage-specific function in countering bacterial defenses. A second pathway, NARP2, allows phages to overcome bacterial defenses by building NAD+ via metabolites different than ADPR-PP. Our findings report a unique immune evasion strategy where viruses rebuild molecules depleted by defense systems, thus overcoming host immunity.

Parallel session:
BENEFICIAL MICROBES (Tuesday, September 10, 2024 16:00)

Invited Lecture

16 Beneficial microbes

## Functional plasticity in the gut microbiota-host interactions

**Naama Geva-Zatorsky**

In recent years, there has been a scientific awakening to the impact of the gut microbiota on host physiology. It is currently clear that the microbiota has profound effects on host physiology, yet studies on their causal effects, and functional molecules at play are still in their infancy. Microbiome studies, in the past two decades, have mainly focused on bacterial taxonomy with some functional attributes. Our major interest is to characterize the molecular mechanisms underlying gut microbe-host interactions. We are interested in host-related environmental factors that affect bacterial functionality and bacterial functional plasticity, and their implications on the host immune system. To this end, we are characterizing bacterial mechanisms, which can enable bacterial functional plasticity in response to environmental cues such as host physiology, and their potential implications on host health.

Parallel session:
BENEFICIAL MICROBES (Tuesday, September 10, 2024 16:00)

Invited Lecture

16 Beneficial microbes

## Systematic discovery of prokaryotic symbionts of ciliate protozoa in the rumen

**Elie Jami**[1]
*Institute of Animal Science, Agricultural Research Organization (ARO) Volcani center, Israel*

Microbial cross-domain interactions are considered of high impact for the eco-evolutionary relationship of microbial eukaryotes and prokaryotes. The ciliate protozoa community residing in the rumen harbors a remarkable species diversity and represents up to 50% of the ruminal microbial biomass. Similar to other protists inhabiting diverse environments, rumen protozoa are presumed to engage in interactions with their prokaryotic neighbors. However, despite numerous conjectures, no stable interaction has been conclusively demonstrated thus far. Here we reveal on a community scale numerous novel symbiotic interactions between rumen protozoa and bacteria. Our systematic approach of the establishment of a symbiont selective DNA enrichment, enabled the discovery of several bacterial genomes that are specifically associated with protozoa and spann across different phylogenetic lineages and exhibiting hallmark features indicative of obligatory symbiosis. These features encompass a reduced genome size, low GC content and an absence of genes crucial for essential cellular processes such as energy production and amino acid biosynthesis. Interestingly, some of the genomes identified are close relatives to known bacterial symbionts of various protists found in diverse environments. Our findings shed light on the intricate web of symbiotic relationships within the rumen ecosystem, offering new insights into the yet unexplored interactions between microbial eukaryotes and their bacterial neighbors.

Parallel session:
BENEFICIAL MICROBES (Tuesday, September 10, 2024 16:00)

16 Beneficial microbes

## Harnessing Plant-Microbe Interactions for Sustainable Agriculture: Complementary Approaches for Identifying Novel Plant Growth-Promoting Bacteria

**Alexander Evenko-Greenapple**[1]
*Soil, water and environment, Volcani Research Center, Agricultural Research Organization, Israel*

Bacterial biostimulants are a sustainable alternative to agrochemicals, enhancing plant growth and mitigating abiotic stress through complex mechanisms. This study aims to isolate and identify novel biostimulants from the rhizosphere of various tomato species, including wild-local relatives and agriculturally important varieties. We utilized culture-dependent (plate screening) to isolate bacteria from root samples and test them in planta, and culture-independent methods (NGS) to analyze root community composition. Combining these methods revealed several promising bacterial biostimulants that improved plant growth and stress resilience. These were chosen based on their effects on plant phenotype rather than traditional microbiological assays, which often poorly correlate with field performance. Additionally, we employed Host-Mediated Microbiome Enrichment (HMME) by selecting the rhizosphere linked to beneficial plant phenotypes. This method enriches bacterial populations advantageous for plant growth, resulting in a plant phenotypically associated microbial community for further testing. In this approach, we aimed to refine microbial populations in the tomato rhizosphere, searching for connections between rhizosphere community and plant performance. The effectiveness of biostimulants was assessed by measuring their impact on shoot dry weight under saline stress, short drought and ambient conditions. Out of 250 screened, 21 isolates demonstrated significant growth promotion compared to the control (E. coli), with the top isolate achieving between 30% and 60% improvement under stress and ambient conditions, respectively. This study emphasizes the importance of complementary techniques in identifying new agriculturally relevant isolates, as well as their potential for sustainable agriculture in the face of rapidly salinizing soils and climate change.

Parallel session:
BENEFICIAL MICROBES (Tuesday, September 10, 2024 16:00)

Invited Lecture

16 Beneficial microbes

## Auxin secretion by soil bacteria accelerated immune system maturation in young seedlings

**Elhanan Tzipilevich**[1,2]
*Plant science, MIGAL - Galilee research institute, Israel*
*Biotechnology, Tel Hai Academic college, Israel*

Root immunity is essential for protecting the plant from pathogens and managing proper interactions with the root microbiota. The immune system is routinely explored in mature roots and leaves. However, how the immune system develops after seed germination and whether the soil microbiota affects the development are largely unknown. We have found that the immune system is gradually maturing during the first days of root outgrowth after germination, and accordingly, attenuation of pathogen colonization is impaired in very young seedlings compared to older ones. Further, we have found that the interaction of seedlings with specific soil bacteria accelerates the maturation of plant immunity. Specifically, auxin secretion by bacteria served as a developmental signal to trigger immune system ontogeny and pathogen inhibition.

Parallel session:
BENEFICIAL MICROBES (Tuesday, September 10, 2024 16:00)

16 Beneficial microbes

## From Harsh Environments to Sustainable Agriculture: The Biocontrol Potential of BSC Microalgae

Dikla Eckstien, Bernard Ngeno, Nofet Margulis, Noga Maximov, **Hagai Raanan**

The use of microorganisms as biocontrol agents against soilborne plant pathogens offers a promising alternative to chemical pesticides, yet their effectiveness under field conditions remains limited. This study investigates the potential of highly resilient microalgae from harsh environments, such as Biological Soil Crusts (BSCs), as sustainable biocontrol agents. We isolated and identified fifty-nine cyanobacteria and green algae and assessed their antifungal activity against eight representative soilborne pathogens through dual-culture plate assays and toxicity tests.

Results demonstrated significant inhibitory effects on fungal growth by many microalgae strains, with variations observed among different strains and pathogen species. Notably, some strains even promoted fungal growth, underscoring the complexity of microalgae-pathogen interactions. Among the microalgae, the green algal genus *Desmodesmus*, especially *Desmodesmus subspicatus*, exhibited superior antifungal efficacy.

To better understand the impact of environmental conditions on the antifungal activity of microalgae, further research examined how various environmental factors influence the antimicrobial properties of representative BSC microalgae. This comprehensive assessment revealed that the antifungal activity of microalgae, such as *Leptolyngbya ohadii* and *Chlorella ohadii*, is strongly affected by environmental conditions. These findings underscore the potential of BSC microalgae as effective biocontrol agents in dryland ecosystems and highlight the crucial role of environmental conditions in developing sustainable biocontrol strategies against soilborne pathogens.

Parallel session:
BENEFICIAL MICROBES (Tuesday, September 10, 2024 16:00)

16 Beneficial microbes

## Bacterial DNA inversion regions as predictors of response to melanoma immunotherapy

**Roni Keshet-David**[1], Jia Zhang, Shaqed Carasso, Tal Gefen, Naama Geva-Zatorsky
*medicine, Technion, Israel*

The aim of this study was to identify the bacterial DNA inversions that are differentially inverted between responders and non-responders to melanoma immunotherpy, and subsequently to test the potential immunomodulatory effects of the genes influenced by the inversions.

We analyzed publicly available databases fecal metagenomes of melanoma patients' prior to immunotherapy treatment. Using computational tools we identified genomic phase variable regions that can differentiate between responders and non-responders to melanoma immunotherapy.

Using qPCR we can use the metagenomic finding to create a fast, cheap and avilable biomarker for response that can serve as a prognostic marker in the future.

restricting this region to a specific DNA inversion configuration where one of the identified genes is consistutively expressed results in reduction of biofilm formation. Furthermore, splenocytes interacting with bacteria with the "locked" configuration compared to WT decreased secretion of pro-inflammatory cytokines IL-6 and TNF-alpha.

Conclusion: we used bacterial DNA inversions as biomarkers for response to immunotherapy to melanoma and are studying its potential for future use in cancer patients management.

Parallel session:
MICROBIAL GENETICS (Tuesday, September 10, 2024 16:00)

Invited Lecture

17 Microbial genetics

## A phage lysis-lysogeny decision is guided by a toxin-antitoxin system of unique nature

**Avigdor Eldar**[1], Regev Frenkel[1], Polina Guler[1]
*Shmunis school of biomedicine and cancer research, Tel-Aviv University, Israel*

Lysis-Lysogeny decision of many Bacillus phages is mediated by a communication system named arbitrium. Recently, we have identified that arbitrium-based lysis-lysogeny decision of Bacillus subtilis phage phi3T is mediated by its interaction with a toxin-antitoxin system of the MazEF family. Here, I will briefly describe this interaction and present further insights into the activity of the B. subtilis MazEF system. While this system is highly homologous to other well-studied MazEF system, its mode of action in the cell is dramatically different than that observed for other toxin-antitoxin systems. This suggests a needed extension for the definition of such systems and their role in bacterial physiology.

Parallel session:
MICROBIAL GENETICS (Tuesday, September 10, 2024 16:00)

Invited Lecture

17 Microbial genetics

## Studying small RNAs - the challenge

**Shoshy Altuvia**[1], Itzhak Goloventzitz[1], Maya Elgrably-Weiss[1], Georg Jens[2]
[1]*Faculty of Medicine, The Hebrew University, Israel*
[2]*Faculty of Biology, University of Freiburg, Germany*

The investigation of small RNAs (sRNAs) has evolved significantly over the years. Initially, the primary challenge in sRNA research was determining how and where to identify these molecules. Numerous methods have been developed to pinpoint sRNA-encoding genes. However, in recent years, it has become evident that the more pressing challenge is understanding their specific functions. Traditionally, examining the roles of sRNAs has been a labor-intensive and time-consuming process, often requiring repeated overexpression screenings of each sRNA. This is especially relevant when exploring the impact of sRNAs in host-pathogen interactions.

In this study, we present genetic and physiological screenings for functional sRNAs utilizing a plasmid library that expresses 893 short RNA fragments derived from *Salmonella* cultured under infection-related conditions. To enable the expression of this library, the RNA fragments were inserted downstream of an ectopic inducible promoter, followed by a transcription termination signal. When macrophages were infected with *Salmonella* containing the library, we identified functional sRNAs that either inhibited or promoted the invasion and/or intracellular survival of *Salmonella* during the infection process. I will share the story of one or two of these sRNAs that were discovered through selection in macrophages.

Parallel session:
MICROBIAL GENETICS (Tuesday, September 10, 2024 16:00)

17 Microbial genetics

## Long-term impact: bacteriophage-driven DNA inversions shape bacteroides fragilis functionality

Shaqed Carasso, Roni Keshet-David, **Jia Zhang**[1], Haitham Hajjo,
Tal Gefen, Naama Geva-Zatorsky
*Medicine, Technion – Israel Institute of Technology, Israel*

This study aimed to explore the long-term and dynamic interactions between Bacteroides-associated bacteriophage Barc2635 and Bacteroides fragilis. We focused on the bacteriophage effects on genomic DNA inversions which can lead to molecular phase variation and functional plasticity of the common gut resident B.fragilis. To this end, we analyzed DNA inversions of various genomic regions, with a focus on outersurface polysaccharides and a type 1 restriction-modification (T1RM) system.

Germ free mice were monocolonized with B.fragilis alone or B.fragilis with Barc2635 for maximum 8 weeks. Fecal samples were collected to measure the population of bacteria and bacteriophage. DNA sequencing was performed to analyse the genomic inversions and mutational analysis in B.fragilis.

Fluctuations on the number of B.fragilis and Barc2635 in the first week were observed, after which a dynamic equilibrium was reached. Shifts in the orientation of multiple DNA promoter regions within B.fragilis were observed for its own adaptation and to the presence of Barc2635 bacteriophage. These phase variations influence the functional plasticity of B.fragilis such as the production of anti-inflammatory polysaccharide A. Notably, polysaccharide G responded most rapidly to Barc2635. Additionally, shifts in the ratio of different combinations of the B.fragilis T1RM were also observed. These inversions lead to an increase in the diversity of the microbial population.

These findings suggest that bacteriophages can coexist with the gut microbiota in the host and are able to induce microbial genomic alterations leading to alterations in microbial functional plasticity. These alterations can potentially modulate the host immune response in a dynamic and reversible manner.

Parallel session:
MICROBIAL GENETICS (Tuesday, September 10, 2024 16:00)

Invited Lecture

17 Microbial genetics

## Small RNAs Orchestrate Bacterial-Phage Dynamics

**Sahar Melamed**[1]
*Microbiology and Molecular Genetics, IMRIC, The Hebrew University of Jerusalem, Israel*

Understanding the intricate mechanisms by which bacteria adapt to their natural environment is crucial for elucidating their survival strategies. Bacteria face numerous challenges when interacting with bacteriophages (phages). Phage biology research has led to countless discoveries regarding almost every cellular process and revealing unpredicted mechanisms. One mechanism bacteria use to adapt to changes in their environment is facilitated by small RNAs (sRNAs), which regulate gene expression post-transcriptionally. While sRNAs were investigated for their roles in various cellular processes and under a range of conditions, the impact of post-transcriptional regulation on bacterial interactions with phages remains understudied.

Here, through the combination of high-throughput methodologies with small-scale experimental approaches, we uncover the involvement of sRNAs in the phage infection cycle. We employed RIL-seq (RNA Interaction by Ligation and sequencing), a global in-vivo identification method for sRNA-RNA interactions, to unveil intricate networks of sRNA-RNA interactions in *Escherichia coli* during lambda phage infection. Analysis of the RIL-seq data with RNA-seq data obtained under the same conditions provides valuable insights into the biological pathways important for the interactions between bacteria and phages. Specifically, we shed light on the involvement and importance of specific sRNAs under phage infection conditions. We characterize a novel phage-encoded sRNA that regulates key genes in E. coli and aids the infection cycle. This research demonstrates the application of deep sequencing methodologies to unravel the significant role of sRNAs in bacterial communication with their surrounding environment, further complicating the already complex interactions between bacteria and phages.

Parallel session:
MICROBIAL GENETICS (Tuesday, September 10, 2024 16:00)

17 Microbial genetics

## Understanding the emergence of phenotypic heterogeneity in motility and virulence traits in Pseudomonas aeruginosa

**Knan Mijal Ztion Harris**[1], Daniel Dar
*Plant and Environmental Sciences, Weizmann Institute, Israel*

Clonal bacterial populations exhibit remarkable phenotypic heterogeneity at the single-cell level, a phenomenon that can be genetically regulated to enhance bacterial survival and cooperation. Recent advances in imaging-based transcriptomics now allow high-throughput mapping of the phenotypic landscape by identifying genes specifically expressed in subpopulations. However, elucidating the mechanisms driving the emergence of these subpopulations and their broader implications remains challenging. In this project, I aim to develop a generalizable method to systematically identify the genes involved in subpopulation formation and determine the full transcriptomic differences between subpopulations. To achieve this, I am developing a Fluorescence-Activated Cell Sorting (FACS) approach based on mRNA Fluorescent In Situ Hybridization (FISH) that can be applied to isogenic or transposon-mutagenized populations. As an initial application, I will apply this FISH-FACS method to the opportunistic pathogen Pseudomonas aeruginosa, conducting an in-depth exploration of its motile and hypervirulent subpopulations using high-throughput genetics and transcriptomics.

Parallel session:
MICROBIAL GENETICS (Tuesday, September 10, 2024 16:00)

17 Microbial genetics

## A-to-I mRNA editing enables bacteria to produce two protein versions from a single gene and affects bacterial growth

**Eyal Elias**[1], Liron Didi[1], Daniel Arad[1], Ofir fargeon[1], Rinat Cohen-Pavon[1], Gil Sorek[2], Liron Levin[3], Dganit Melamed[4], Isaac Gifford[5], Jeffrey E. Barrick[5], Liam Aspit[1], Dan Bar-Yaacov[1]

[1]*The Shraga Segal department of Microbiology, Immunology, and Genetics, Ben-Gurion University of the Negev, Israel*

[2]*Department of Life Sciences, Ben-Gurion University of the Negev, Israel*

[3]*Bioinformatics Core Facility, Ben-Gurion University of the Negev, Israel*

[4]*The Smoler Protein Research Center, Technion Israel Institute of Technology, Israel*

[5]*Department of Molecular Biosciences, University of Texas, USA*

Adenosine-to-inosine (A-to-I) mRNA editing can affect the sequence and function of translated proteins because the ribosome reads inosine (I) as guanosine (G). Previously, we discovered that A-to-I mRNA editing occurs in Escherichia coli. However, the prevalence of A-to-I mRNA editing across bacteria and its biological significance are still unknown.

Here, we show that A-to-I mRNA editing occurs in hundreds of genes across dozens of bacterial species. We find that editing events occur within and require a conserved sequence motif. Notably, most editing events (80%) are predicted to recode protein sequences. Indeed, by mass spectrometry, we show for the first time that A-to-I mRNA editing enables bacteria to produce two versions of a protein from a single gene. Finally, we find that perturbing mRNA editing in Acinetobacter baylyi affects bacterial growth, especially at high temperatures.

Overall, our work reveals that A-to-I mRNA editing is widespread and can impact bacterial physiology, likely by modulating protein function.

# Poster Presentations (Abstracts)

POSTER SESSION I (Monday, September 9) (Monday, September 9, 2024 12:45)

Poster presentation

01 Host-pathogen interactions

## Harnessing the immunomodulatory effect of drugs for a desired immune response

**Shelly Hen-Avivi**[1], Noa Bossel Ben-Moshe[1], Roi Avraham[1]
*Department of Immunology and Regenerative Biology, Weizmann Institute of Science, Israel*

The global rise of antibiotic-resistant bacteria exceeds the rate of new antibiotic discovery and leads to an urgent need for alternative therapeutic strategies. Host-directed therapies (HDT) hold the promise to optimize the immune response towards a more favorable infection outcome to the host. Surprisingly, recent evidence suggest that certain antibiotics also impact host immune processes, with either beneficial or deleterious effect that is independent of direct pathogen control. In this study, we aim to systematically screen a large number of approved therapeutic drugs, including all classes of antibiotics as well as commonly used anti-viral, anti-fungal, and anti-parasite drugs. The screen is conducted using human peripheral blood mononuclear cells (PBMCs), which enable high-throughput testing of drug effects in the context of an intact immune system and in an animal-free model. As an output, we measured changes in the secretion of 12 cytokines. We tested the immunomodulatory effect of a large panel of drugs on naive PBMCs as well as stimulated PBMCs in order to check if immunomodulatory effects are dependent on cells activation. Our data suggest that known drugs with immunomodulatory effects tested strongly in the assay, validating our approach. In addition, our screen suggests new robust hits for drugs with strong immunomodulatory effect. We plan to address the following questions for the hits from the screen: Under which conditions does the drug affect the host cells? Which cellular pathways are affected, and what are the immunomodulatory consequences of drugs beyond pathogen clearance?

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Poster presentation

01 Host-pathogen interactions

## A T6SS in the coral pathogen Vibrio coralliilyticus secretes an arsenal of anti-eukaryotic effectors and contributes to virulence

**Shir Mass**[1], Hadar Cohen[1], Motti Gerlic[1], Blake Ushijima[2], Julia C. Van Kessel[3], Eran Bosis[4], Dor Salomon[1]

[1]*Department of Clinical Microbiology and Immunology, School of Medicine, Faculty of Medical and Health Sciences, Tel Aviv University, Israel*

[2]*Department of Biology and Marine Biology, University of North Carolina Wilmington, USA*

[3]*Department of Biology, Indiana University, USA*

[4]*Department of Biotechnology Engineering, Braude College of Engineering, Israel*

Vibrio coralliilyticus (Vcor) is a pathogen of coral and shellfish, leading to devastating economic and ecological consequences worldwide. Although rising ocean temperatures correlate with increased Vcor pathogenicity, the specific molecular mechanisms and determinants contributing to virulence remain poorly understood. Here, we systematically analyzed the type VI secretion system (T6SS), a contact-dependent toxin delivery apparatus, in Vcor. We identified two omnipresent T6SSs that are activated at temperatures in which Vcor becomes virulent; T6SS1 is an antibacterial system mediating interbacterial competition, whereas T6SS2 mediates anti-eukaryotic toxicity and contributes to mortality during infection of an aquatic model organism, Artemia salina. Using comparative proteomics, we identified the T6SS1 and T6SS2 toxin arsenals of three Vcor strains with distinct disease etiologies. Remarkably, T6SS2 secretes at least nine novel anti-eukaryotic toxins comprising core and accessory repertoires. We propose that T6SSs differently contribute to Vcor's virulence: T6SS2 plays a direct role by targeting the host, while T6SS1 plays an indirect role by eliminating competitors.

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Poster presentation

01 Host-pathogen interactions

## Impact of Fusarium Species Composition and Incidence on Onion Basal Rot in Northeastern Israel

**Ofir Degani**[1,2], Elhanan Dimant[2], Eliyahu Margalit[3]
[1]*Faculty of Sciences, Tel-Hai College, Israel*
[2]*Plant Sciences department, MIGAL—Galilee Research Institute, Israel*
[3]*Extension Service, Israel Ministry of Agriculture and Rural Development, Israel*

Fusarium basal rot (FBR) places a significant limitation on Allium production worldwide. The damage caused by the disease can be observed throughout the entire crop cycle. This research aimed to further our understanding of the impact of FBR on the cultivation of onions (Allium cepa) in northeast Israel. It focused on studying the composition and incidence of Fusarium species involved in disease outbursts in two representative fields, one in Galilee (Hula Valley) and the second in the Golan Heights, where the disease incidences reached 8%. Using colony morphology, microscopic taxonomic keys, and molecular methods, a new, unreported Neocosmospora (previously Fusarium solani) species complex (SC, mostly N. falciformis) was discovered as a wildly spread member of the Fusarium pathobiome community. This species complex appeared more generalist in its nature since it was found in all three onion cultivars' samples. It was also less virulent in seed germination (42–52% higher sprout biomass, p  0.05) and bulb pathogenicity tests (41–45% less necrotic) than Fusarium acutatum. Whereas the Galilee yellow Orlando (Riverside) onion cultivar bulbs sampled were colonized by Neocosmospora SC (70%) and two other, less abundant species, F. oxysporum f. sp. cepae and F. acutatum (15% each), the Golan Heights field's Fusarium community showed host specificity. In the Golan Heights field, F. oxysporum f. sp. cepae inhabited the red Ha2 onion cultivar bulbs, whereas F. acutatum colonized the yellow Ha1 cultivar (40% and 50% prevalence along with Neocosmospora SC). A better understanding of the complexity of this disease caused by different Fusarium species and with a divergence in host susceptibility and virulence is critical for developing disease management strategies. Since each Fusarium species reacts differently to pest control treatments, changes in the species composition may require specifically adapted management solutions.

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Poster presentation

01 Host-pathogen interactions

## Pipecolic acid secreted by malaria parasite promotes its growth

**Oren Sonia**[1]
*Biomolecular science, Weizmann Institute of Science, Israel*

Plasmodium falciparum (Pf) stands as the predominant cause of malaria, resulting in more than 500,000 reported deaths annually. Patients with cerebral malaria, a severe form of malaria disease, exhibit elevated plasma L-pipecolic acid (PA) concentrations compared to those with mild malaria. The exact role of PA in Pf infection and its biosynthesis within the parasite remains elusive. Here we investigated the role of secreted PA in Pf biology. Using LC/MS metabolomics we found that the malaria parasite while growing inside its host human Red Blood Cell (RBC) secrets PA during a distinct blood stage, the Trophozoites.

We next demonstrated that pretreatment of the host naïve RBCs with PA, significantly enhances parasitic growth. To investigate the impact of PA on its primary host, RBCs, we extended our analysis to measure the biophysical alterations in the pretreated naïve RBCs using atomic force microscopy. Surprisingly, we found that PA modify the mechanical properties of the membrane of the host cells, turning it softer compared to the non-treated cells. Overall, our findings reveal that secretion of PA primes naïve RBCs for parasite invasion by inducing mechanical changes in membrane stiffness, suggesting PA secreted metabolite as a potential regulator in the communication dynamics during the parasite-host interaction.

POSTER SESSION I (Monday, September 9) (Monday, September 9, 2024 12:45)

Poster presentation

01 Host-pathogen interactions

## Influence of temperature on cyanobacteria-cyanophage interactions

**Laure Arsenieff**[1], Debbie Lindell[1]
*Faculty of Biology, Technion - Israel Institute of Technology, Israel*

Over recent decades, studies have demonstrated the effect of temperature on virus-phytoplankton interactions. Variations of this abiotic parameter can dramatically alter the outcomes of their interplay, involving changes in host susceptibility, infection properties or even in viral-life strategy. Elucidating how temperature is involved in the control of host-virus interactions is thus of prime importance. In marine systems, cyanobacteria of the genera Prochlorococcus and Synechococcus are substantial contributors to primary production. The cyanophages that infect them are considered to be main drivers in their mortality, diversity and evolution. Despite the ecological importance of cyanophages, their responses to temperature have not been examined to date. Here we report on the interplay between cyanobacteria and their phages as a result of changes in this environmental variable, with experiments performed at 17°C, 21°C and 26°C. Infection dynamic experiments showed that temperature impacted the viral cycle of a cyanophage, with a latent period twice as long at the lowest temperature compared to the highest temperature. We further investigated the influence of temperature on the host-range of cyanophages. We observed distinct changes in infection patterns, with several Synechococcus-cyanophage systems displaying shifts in resistance/sensitivity when grown at different temperatures. Interestingly, temperature did not affect the Prochlorococcus-cyanophage interactions tested. Our study reveals that temperature can affect virus-host systems at different levels and that the effects vary between interactions. Our findings raise questions on the mechanisms involved in the resistance/sensitivity shifts and how temperature influences them.

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Poster presentation

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# Tomato pith necrosis disease exhibits a synergistic interaction with Tomato Brown Rugose Fruit Virus and Pepino Mosaic Virus infections

**Evanson Ngugi**[1], Doron Teper[2], Aviv Dombrovsky[3]
[1]*Faculty of Agriculture, Food and Environment, Hebrew University of Jerusalem, Israel*
[2]*Department of Plant Pathology and Weed Research, Agricultural Research Organization, the Volcani Institute, Israel*
[3]*Department of Plant Pathology and Weed Research, Agricultural Research Organization, the Volcani Institute, Israel*

Pith necrosis disease, caused by species of Pseudomonas and Pectobacterium is among the diseases affecting tomato production. Symptoms on infected plants include yellowing of young leaves, top-wilting and stem splitting. Stems display hollowing symptoms and are associated with profuse adventitious roots. Upon lengthwise cutting of the affected stem, a dark discoloration in the pith is observed.

Over the few years, the emergence of Tomato Brown Rugose Fruit Virus (ToBRFV) has significantly increased its prevalence in tomato greenhouses, causing a dreadful impact on the tomato industry in Israel. Concurrently with the emergence of ToBRFV, there has been a notable increase in pith necrosis in greenhouse tomatoes. We hypothesized that ToBRFV might facilitate opportunistic infections by other pathogens, hence could be the cause of the increased pith necrosis incidences. To test this, pith necrosis-symptomatic tomato plant samples were collected and tested for the presence of ToBRFV and Pepino Mosaic Virus (PepMV). Samples also underwent bacterial isolation and identification through RpoB sequencing.

Most samples were positive for either ToBRFV, PepMV or both and contained high inoculum of bacteria from diverse lineages, among which were the already reported as causal agents of pith necrosis. These inlude Xanthomanas perforans, Pseudomonas mediterranea, P. capsici and P. viridiflava. Inoculating tomato plants with the bacteria alone resulted in localized lesions that failed to expand beyond the area of inoculation while some showed no symptoms at all. Co-inoculation of the bacterial isolates with PepMV and ToBRFV, however, led to expanded necrotic lesions along the inner stem. Data collected supports that tomato viral infection increases susceptibility to pith necrosis caused by opportunistic pathogens.

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Poster presentation

01 Host-pathogen interactions

## Unveiling the Role of Hydrogen Peroxide (H2O2) in Algal-Bacterial Interactions

**Delia Narvaez-Barragan**[1], Einat Segev[1]
*Department of Plant and Environmental Sciences, Weizmann Institute of Science, Israel*

Hydrogen peroxide (H2O2) naturally occurs in the ocean, produced by both abiotic and biotic processes, and acts as a signaling molecule in many organisms. Previous studies reported H2O2 production in the algae Emiliania huxleyi in laboratory cultures. In the environment, E. huxleyi forms dense blooms that harbor a multitude of heterotrophic bacteria, at times dominated by Roseobacters. This is particularly interesting in the context of H2O2 metabolism because bacterial degradation of algal metabolites might also produce H2O2. Despite the centrality of H2O2 in various cellular functions, its influences on algal blooms and algal-bacterial interactions remain unclear.

Here, using an environmentally relevant model system that consists of the alga E. huxleyi and the Roseobacter bacterium Phaeobacter inhibens, we study the role of H2O2 in algal-bacterial interactions. Our results show that H2O2 is detected throughout the interaction between algae and bacteria. An increase in H2O2 levels is observed during the bacterial-induced algal death, pointing to its possible involvement in algal demise. Additionally, manipulating the H2O2 content in algal-bacterial co-cultures impacts the fate of the interaction. We found that P. inhibens produces H2O2 in pure cultures, with increased production when mimicking algal host conditions. Using genetic manipulation, we identified bacterial genes involved in H2O2 metabolism and demonstrated their contribution to algal demise. Finally, we uncover a previously overlooked link between H2O2 and betaine metabolism. Bacteria exposed to betaine, a well-known algal metabolite, produce more H2O2. Modification of betaine levels impacts the algal-bacterial interaction. Betaine might function as a signaling molecule, driving bacterial H2O2 production and triggering algal demise. Uncovering the role of H2O2 in algal-bacterial interactions will allow us to better understand the H2O2 ecological functions in marine environments and its influence on microbial dynamics.

POSTER SESSION I (Monday, September 9) (Monday, September 9, 2024 12:45)

Poster presentation

01 Host-pathogen interactions

## Characterization of the B-cell antigen specific antibody repertoires in human tissues

**Lior Levy**[1], Rei Matsumoto[2,3], Joshua Gray[3], Yosuke Sakamoto[2,3], Hanna Oppenheimer[1], Lilach M. Friedman[1], Tomer Hertz[1,4], Donna L. Farber[2,3]

[1]*Department of Microbiology, Immunology and Genetics, The National Institute for Biotechnology in the Negev, Ben-Gurion University of the Negev, Israel*

[2]*Department of Surgery, Columbia University Irving Medical Center, USA*

[3]*Department of Microbiology and Immunology, Columbia University Irving Medical Center, USA*

[4]*Vaccine and Infectious Disease Division, Fred Hutchinson Cancer Research Center, USA*

Memory B cells can be found not only in the serum and lymphoid organs, but also in non-lymphoid tissues. Tissue-resident memory B cells (BRMs) are strategically positioned to provide immediate protection against pathogens at mucosal and barrier sites. Here we aim to elucidate the dynamics of antigen-specific B cell responses in various tissues from human organ donors.

BRMs from 6 lymphoid and non-lymphoid tissues, were stimulated in vitro to differentiate to antibody-secreting plasma cells. Utilizing antigen microarrays encompassing a wide range of antigens from childhood vaccines, human herpes viruses, common respiratory viruses, as well as commensal bacteria and fungi, we profiled secreted IgG and IgA and compared it to the serum IgG and IgA repertoires.

BRMs from lung and lung-associated lymph nodes (LLNs) from 1-3 years old children were biased towards respiratory pathogens, contrasting with the predominantly vaccine-specific antibodies found circulating in the serum. These findings may underscore the localized nature of B cell responses within the respiratory system during early development.

We also profiled BRMs in 23 adult donors across 6 tissues: blood, mesenteric lymph nodes, bone marrow, LLN, lung, and spleen. Preliminary results unveiled significant differences in antigen-specific repertoires across tissues, revealing both tissue-specific and shared specificities across multiple sites. Responses to childhood vaccines and viruses were overall much more tissue specific than responses to commensal bacteria and fungi. This exploration sheds light on the intricate interplay between tissue-specific immunity and systemic antibody responses, providing valuable insights into the mechanisms of immune surveillance and defense across human tissues.

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Poster presentation

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# Identifying polio antibody binding signatures are associated with protective poliovirus neutralization titers

**Mor Tetro**[1], Lilach M. Friedman[1], Merav Weil[2], Aharona Glatman-Freedman[3,4], Ravit Bassal[3,4], Lester M. Shulman[2,4], Javier Martin[5], Tomer Hertz[1,6]

[1]*Department of Microbiology, Immunology and Genetics, The National Institute for Biotechnology in the Negev, Ben-Gurion University of the Negev, Israel*

[2]*Central Virology Laboratory, Public Health Services, Sheba Medical Center, Israel*

[3]*Israel Center for Disease Control, Ministry of Health, Sheba Medical Center, Israel*

[4]*Department of Epidemiology and Preventive Medicine, School of Public Health, Faculty of Medical and Health Sciences, Tel Aviv University, Israel*

[5]*Division of Virology, National Institute for Biological Standards and Control, UK*

[6]*Vaccine and Infectious Disease Division, Fred Hutch Cancer Research Center, USA*

The global effort to eradicate polio through vaccination has dramatically reduced disease incidence. However, challenges persist, including Vaccine-Derived Polio Viruses (VDPVs) outbreaks and the need for high vaccination coverage post-eradication. Currently, protection following vaccination is assessed using a neutralization assay, which requires live polioviruses, is restricted by the WHO to prevent VDPV outbreaks. To address this, we developed PAM – a novel Polio Antigen Microarray antibody binding assay that measures antibody profiles against a range of polio antigens without using live polioviruses. The PAM assay includes inactivated polio viral antigens, virus-like particles (VLPs), and synthetic peptides from capsid proteins of 7 strains representing all three polio serotypes.

Using the PAM assay, we characterized the IgG and IgA poliovirus antibody repertoires in serum samples from 223 polio-vaccinated Israeli adults with known neutralization titers, including 81 individuals with neutralizing titers ≤ 8 against one or more serotypes. Significant associations were found between antibody binding to specific antigens and neutralization titers. For instance, IgG and IgA responses to a type 3 oral polio vaccine (OPV) strain antigen correlated with neutralization against all three serotypes (p0.05). While antibody responses to viral antigens were linked with protection, IgG and IgA responses to specific clusters of linear peptides showed associations with both protection and susceptibility. A logistic regression model identified multivariate antibody signatures correlated with protection from type 1 and type 3 polioviruses. This study highlights the potential of the PAM assay for predicting protection from polioviruses without using live viruses, aiding the global polio eradication effort.

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## Chryseobacterium rhizoplanae entomopathogenic interaction with the Spodoptera littoralis larvae

**Ronna Melamed**[1]
*Plant pathology and Microbiology, Hebrew University of Jerusalem, Israel*

Spodoptora littoralis (also called cotton leaf worm), a harmful polyphagous moth responsible for damage to various crops, developed resistance to pesticides over time posing challenges in its control. The aim of this research is to explore potential toxins for biological pest management, with a focus on the entomopathogenic properties of Chryseobacterium rhizoplanae, an environmental bacterium isolated from corn and known for its resistance to a wide range of antibiotics.

In the experiments conducted, Chryseobacterium rhizoplanae was injected to the 5th stage larvae of S.litoralis, resulting mortality rates ranging from 70-90%. To investigate the virulence mechanism of the bacterium against the moth, both in-vivo and in vitro evolution experiments for C.rhizoplanae were performed. In the in-vivo evolution, the bacterium was injected into the larvae`s hemolymph and isolated 24-hours post injection, then the isolated bacterium was injected to the next larvae for 10 consecutive times. Simultaneously, in-vitro evolution involved transferring bacteria from the overnight growth medium to fresh medium continuously for over three months. Decrease in mortality rates was observed while injecting both the in-vivo and in-vitro evolved strains compared to the ancestral strain. This decrease in virulence might attribute to the loss of virulence factors during evolution.

Furthermore, in-vivo growth of the bacteria was observed, indicating an adaptation and specialization to the host environment. These findings provide insights into the intricate interactions between C.rhizoplanae and S.littoralis larvae, offering potential candidate for biological pest control.

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Poster presentation

01 Host-pathogen interactions

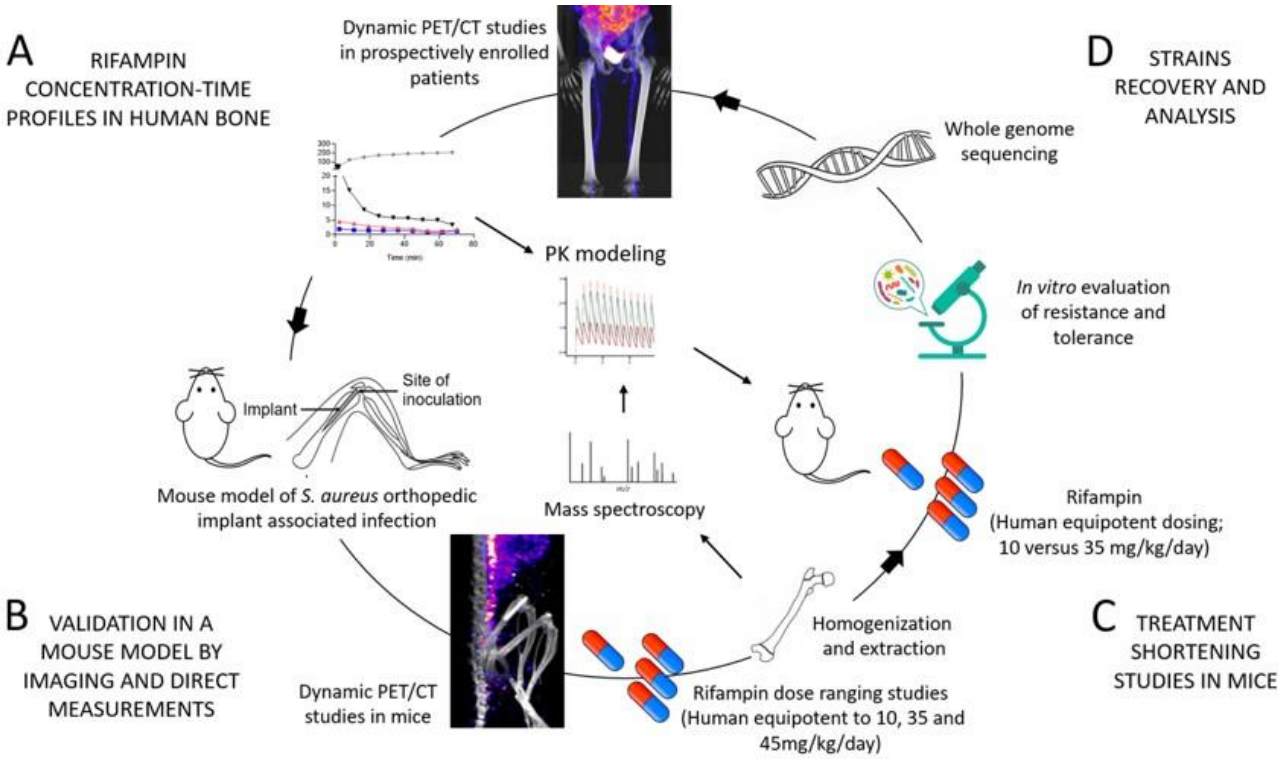
## Probing Host-Drug-Pathogen Interactions to Optimize Treatment for Staphylococcus aureus Implant-Associated Infection

Oren Gordon[1,2], Merav Ordan, **Rotem Huminer**, Maria Abu Elasal, Timothy Read, Charles Peloquin, Nathan Archer, Lloyd Miller, Kimberly Davis, Jogarao Gobburu, Sanjay Jain

[1]*Infectious Diseases Unit, Department of Pediatrics, Faculty of Medicine, Hebrew University of Jerusalem, Hadassah Medical Center, Israel*

[2]*Goldyne Savad Institute of Gene Therapy, Faculty of Medicine, Hebrew University of Jerusalem, Hadassah Medical Center, Israel*

The action of current treatments on persistent bacterial infections is not well-understood, resulting in high rates of recurrence. Multiple host-drug-pathogen interactions govern antimicrobial activity at the site of infection, but these are poorly defined. Consequently, current clinical practices heavily rely on in vitro bacterial antibiotic susceptibility, and on generic population-based drug pharmacokinetics. Staphylococcus aureus orthopedic implant-associated infection is a devastating surgical complication. Current regimens include combination with rifampin which has dose-dependent bactericidal activity. However, treatment regimens have not been optimized owing to lack of data on bacterial dynamics and antibiotic pharmacokinetics at the infected site. We employed a non-invasive holistic approach using dynamic 11C-rifampin positron emission tomography (PET) to image patients with S. aureus bone infection (n=3) or controls (n=12). The area under the concentration-curve bone to plasma ratio was 0.14, indicating lower bone penetration than previously thought. PET-based pharmacokinetic modeling predicted rifampin bone concentrations and facilitated studies in mice. High-dose rifampin was required to achieve adequate bone concentrations, enhanced bacterial killing and shortened the duration of therapy. Combination therapy with standard dose rifampin, however, was associated with treatment failure due to slow-growing, rifampin resistant bacteria. These strains developed tolerance to different antibiotics associated with mutations in the citric acid cycle gene sdhB. High-dose rifampin ameliorated antibiotic resistance (0% vs. 38%; P=0.04), mitigated bone remodeling (P0.01) and reduced selection of mutations in genes related to bacterial resistance and persistence. High-dose rifampin has optimal bone exposure that results in favorable bacterial dynamics and improves treatment for S. aureus orthopedic implant infection.


A
RIFAMPIN CONCENTRATION-TIME PROFILES IN HUMAN BONE
Dynamic PET/CT studies in prospectively enrolled patients
PK modeling
Mass spectroscopy
Mouse model of S. aureus orthopedic implant associated infection
Implant
Site of inoculation
B
VALIDATION IN A MOUSE MODEL BY IMAGING AND DIRECT MEASUREMENTS
Dynamic PET/CT studies in mice
C
TREATMENT SHORTENING STUDIES IN MICE
Rifampin dose ranging studies (Human equipotent to 10, 35 and 45mg/kg/day)
Homogenization and extraction
Rifampin (Human equipotent dosing; 10 versus 35 mg/kg/day)
D
STRAINS RECOVERY AND ANALYSIS
Whole genome sequencing
In vitro evaluation of resistance and tolerance

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Poster presentation

01 Host-pathogen interactions

## When a Virus Meets an Algae: disentangling states of infection and resistance using single-cell transcriptomics

**Talia Shaler**[1], Ofir Raz[2], Ester Feldmesser[3], Daniella Schatz[3], Assaf Vardi[3]
[1]*Plant & Environmental Sciences, Weizmann Institute of Science, Israel*
[2]*Department of Computer Science and Applied Mathematics, Weizmann Institute of Science, Israel*
[3]*Bioinformatics Unit, Life Sciences Core Facilities, Weizmann Institute of Science, Israel*

Phytoplankton blooms are transient events of exceptionally high primary productivity that play key roles in marine biogeochemical cycles. These blooms are terminated by lytic viruses and yet reoccur annually, suggesting some resistant cells survive the infection. Despite the ecological importance of this host-virus arms race, antiviral mechanisms are understudied in phytoplankton. We use a model system of the ubiquitous bloom-forming algae Emiliania huxleyi and its giant virus, E. huxleyi virus (EhV), to study these interactions and resistance mechanisms. Currently, we lack sensitive tools to detect virus-resistant alga cells, which hinders our ability to track host-virus dynamics and assess its ecological significance in the marine environment. Here, we analyze the transcriptomic dynamics during a viral infection time series that results in the emergence of resistant subpopulations. We use a highly sensitive approach of single-cell RNA sequencing to map the population`s phenotypic heterogeneity and discern between cells in different states as reflected by their gene expression profiles. By disentangling cell states in a heterogeneous population, we aim to identify rare phenotypes of resistant cells and understand how they emerge in the population. Then, by linking transcriptomic fingerprints to phenotypic outcomes, we wish to study host-virus interactions in natural algal blooms. Additionally, we investigate the functional roles of specific genes that are expressed in resistant cells and share homology to conserved immunity systems in bacteria, plants, and humans, such as Toll-interleukin-1 receptor domains.

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Poster presentation

01 Host-pathogen interactions

## Glycan-preference characterization of the *Fusobacterium nucleatum* Fap2 lectin

**Ofir Rokach - Ventura**[1], Tamar Alon-Miamon[1], Lei Xia[2], Nicole Haj[1], Reem Bsoul[1], Viviana Scaiewicz[1], Perlie Admony[1], Shani Leviatan Ben-Arye[3], Vered Padler - Karavani[3], Oren Parnas[2], Gilad Bachrach[1]

[1]*The Hebrew University-Hadassah School of Dental Medicine, The Institute of Dental Sciences, Israel*

[2]*Department of Immunology and Cancer Research, Hebrew University-Hadassah Medical School, Institute for Medical Research Israel Canada (IMRIC), Israel*

[3]*The George S. Wise Faculty of Life Sciences, Tel Aviv University, Department of Cell Research and Immunology, The Shmunis School of Biomedicine and Cancer Research, Israel*

Presence of the *Fusobacterium nucleatum* in several cancers is linked to poor treatment outcome. *F. nucleatum* is presumed to colonize specific tumors via its lectin, Fap2. This study aims to identify the tumor glycans recognized by Fap2.

Previous research in colon and breast cancer showed a correlation between attachment of *F. nucleatum* and high levels of tumor-displayed D-galactose-β (1-3)-N-acetyl-D-galactosamine (Gal-GalNAc).

Here, a genome-wide mutant library was generated in the Panc02 pancreatic cancer cell line and sorted by the mutant's ability to bind the Gal-GalNAc lectin Peanut agglutinin (PNA). Our results indicate that genes crucial for PNA binding are not necessarily essential for attachment of *F. nucleatum* ATCC23726 (726). For example, mutations in the enzyme C1GALT1, mediating the addition of galactose to the GalNAc structure, along with its chaperone COSMC, resulted in loss of PNA attachment but an elevated binding of 726. This increase may be attributed to an elevated presentation of GalNAc on the cell membrane. Differences in glycan display were observed between the pancreatic Panc02 and breast AT3 tumor cell lines. Gal-GalNAc was identified as the primary glycan for Fap2 binding in Panc02, while GalNAc was the main glycan ligand in AT3. In both cell lines, flow cytometry revealed that presence of sialic acid obstructs the binding of 726 to the cancer cells. This impediment was subsequently validated in vivo. Specifically, in Panc02, it was revealed that ST3GAL2 is the primary sialyltransferase responsible for impeding the binding of 726.

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Poster presentation

01 Host-pathogen interactions

## Melanoma colonization by *Fusobacterium nucleatum*

**Reem Bsoul**[1], Ahmed Rishiq[2], Nicole Haj[1], Ofir Ventura[1], Viviana Scaiewicz[1], Ofer Mandelboim[2], Gilad Bachrach[1]

[1]*The Institute of Dental Sciences, The Hebrew University-Hadassah School of Dental Medicine, Israel*

[2]*Immunology and Cancer Research, Institute for Medical Research Israel Canada (IMRIC), The Concern foundation laboratories at the Lautenberg Center for General and Tumor Immunology, Hebrew University-Hadassah Medical School, Israel*

*Fusobacterium nucleatum* is an oral anaerobic bacterium found to be enriched in tumors including colon, esophageal, pancreatic and breast cancers. *F. nucleatum* inhibits anti-tumor immune responses by binding to CEACAM1 and TIGIT, reduces responsiveness to chemotherapy, and increases tumor malignancy and metastasis. The presence of *F. nucleatum* in melanoma skin cancer has been proposed. However, the mechanisms mediating its colonization remain unknown. CEACAM1 was previously shown to play a role in *F. nucleatum* docking to gingival cells, while Gal-GalNAc was proposed to mediate the attachment of *F. nucleatum* to multiple tumors.

Here, flow cytometry (FACS) was employed to assess the adhesion of *F. nucleatum* to various melanoma cell lines, including a CEACAM1-knockout line generated using CRISPR-CAS9. Additionally, levels of Gal-GalNAc and CEACAM1 in melanoma, were analyzed by fluorescent microscopy of melanoma tissue microarrays (TMAs), and by analysis of melanoma RNA sequencing data in the Tumor Cancer Genome Atlas (TCGA) database.

Our preliminary results suggest that high level of Gal-GalNAc rather than CEACAM1 mediates fusobacterial adhesion to melanoma cells.

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Poster presentation

01 Host-pathogen interactions

## Using translation modulators to study Dengue virus translation regulation

**Rodolfo Katz**[1], Rina Rosin-Arbesfeld[1], Ella H. Sklan
*Faculty of Medicine, Tel Aviv University, Israel*

Dengue virus (DENV) infections are rapidly becoming a significant global health problem. DENV is a single-stranded positive-sense RNA virus of the Flaviviridae family that can cause life-threatening diseases. Currently, there are no effective antivirals, and the newly approved vaccines cannot be distributed quickly enough to contain the current outbreaks. A hallmark of all viruses is their inability to encode ribosomes and translation machinery components, necessitating the hijacking of the host machinery for their translation. To better understand how Flaviviruses exploit host cell translation machinery and to identify druggable vulnerabilities, we examined the effect of a panel of translation regulators on DENV and the related Zika virus (ZIKV) infections in human and mosquito cell lines. DENV and ZIKV primarily employ a cap-dependent mechanism for translation initiation. However, previous studies have shown that both viruses can also initiate translation using a cap-independent internal ribosomal entry site. Consequently, inhibition of translation initiation did not significantly affect DENV or ZIKV translation or viral RNA accumulation. In contrast, inhibition of translation elongation had a pronounced effect on DENV translation and viral RNA production. Using various translation elongation modulators, we identified the peptidyl transferase reaction during the translation elongation step as the most vulnerable stage. FDA-approved inhibitors of this stage, homoharringtonine, and bruceantin, significantly inhibited viral replication in cell lines and monocyte-derived primary human macrophages, suggesting that targeting translation elongation might be a viable anti-flaviviral strategy.

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Poster presentation

02 Microbial ecology and evolution

## Annual dynamics of cyanophage abundances and their infection in the Sargasso Sea

**Camelia Shopen Gochev**[1], Debbie Lindell[1]
*Faculty of Biology, Technion – Israel Institute of Technology, Israel*

Viruses are the most abundant entities in the oceans and affect bacterial distribution. This study investigates the annual dynamics of cyanophages, viruses infecting cyanobacteria. Focusing on the Bermuda Atlantic Time-series station, monthly samplings over two years unveil fluctuations in cyanophage abundances and infection of the dominant cyanobacterium, *Prochlorococcus.* As known for this region, we observed distinct seasonal patterns in cyanobacterial abundances. *Prochlorococcus* peaked in late summer deep in the photic zone and *Synechococcus* reached its peak in spring at the surface. The T7-like cyanophages dominated the cyanophage community, different to other regions studied thus far, outnumbering T4-like cyanophages by two-fold on average. Abundances of T4-like and T7-like cyanophages peaked in fall with maximum abundances at 80-100 meters, and were consistent for both years. Infection of *Prochlorococcus* was generally greater at depth than at the surface throughout the year. Infection levels were highest by far in fall, deep in the photic zone. Interestingly, seasonal infection patterns were different for the two cyanophage groups, with infection by T7-like cyanophages greater than by T4-like cyanophages in most months. T4-like cyanophage infection was low at the surface (0-2.5%), with maximal infection in subsurface waters in summer and fall (3.5-5%). Infection of *Prochlorococcus* by T7-like cyanophages was relatively low in surface waters (0-4%), reaching 20-26% in the fall at depth. These findings suggest seasonal differences in the impact of cyanophages on their cyanobacterial hosts. This further suggests that the seasonality of these two cyanophage groups is attributed to distinct responses to biotic and abiotic seasonal changes.

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02 Microbial ecology and evolution

## Host-driven selection, revealed by comparative analysis of xanthomonas type III secretion effectoromes, unveils novel recognized effectors

**Yao Xiao**, Shatrupa Ray, Saul Burdman, Doron Teper

Xanthomonas species are specialized plant pathogens, often exhibiting a narrow host range. They rely on the translocation of effector proteins through the type III secretion system to colonize their respective hosts. The effector arsenal varies among Xanthomonas spp., typically displaying species-specific compositions. This species-specific effector composition, collectively termed the effectorome, is thought to influence host specialization. We determined the plant host-derived effectoromes of more than 300 deposited genomes of Xanthomonas species associated with either Solanaceae or Brassicaceae hosts. Comparative analyses revealed clear species-specific effectorome signatures. However, Solanaceae or Brassicaceae host-associated effectorome signatures were not detected. Nevertheless, host biases in the presence or absence of specific effector classes were observed. To assess whether host-associated effector absence results from selective pressures, we introduced effectors unique to Solanaceae pathogens to Xanthomonas campestris pv. campestris (Xcc), and effectors unique to Brassicaceae pathogens to Xanthomonas euvesicatoria pv. euvesicatoria (Xeue), and evaluated if these introductions hindered virulence on their respective hosts. Introducing the effector XopI into Xcc reduced virulence on white cabbage leaves without affecting localized or systemic colonization. Introducing the XopAC or XopJ5 effectors into Xeue reduced virulence and colonization on tomato but not on pepper. Additionally, XopAC and XopJ5 induced a hypersensitive response on tomato leaves when delivered by Xeue or through Agrobacterium-mediated transient expression, confirming recognition in tomato. This study demonstrates the role of host-derived selection in establishing species-specific effectoromes, identifying XopAC and XopJ5 as recognized effectors in tomato.

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02 Microbial ecology and evolution

## Impact of airborne algicidal bacteria on marine phytoplankton blooms

**Naama Lang-Yona**[1,2], J. Michel Flores[3], Tal Sharon Nir-Zadock[2], Inbal Nussbaum[2], Ilan Koren[3], Assaf Vardi[2]

[1]*Environmental, Water and Agricultural Engineering Unit, Technion - Israel Institute of Technology, Israel*

[2]*Department of Plant and Environmental Science, Weizmann Institute of Science, Israel*

[3]*Department of Earth and Planetary Sciences, Weizmann Institute of Science, Israel*

Ocean microbes are involved in global processes such as nutrient and carbon cycling. Recent studies indicated diverse modes of algal-bacterial interactions, including mutualism and pathogenicity, which have a substantial impact on ecology and oceanic carbon sequestration, and hence on climate. However, the airborne dispersal and pathogenicity of bacteria in the marine ecosystem remained elusive. Here we isolated an airborne algicidal bacterium, *Roseovarius nubinhibens*, emitted to the atmosphere as primary marine aerosol (also referred as sea spray aerosols) and collected above a coccolithophore bloom in the North Atlantic Ocean. The aerosolized bacteria retained infective properties and induced lysis of *Gephyrocapsa huxleyi* cultures. This suggests that the transport of marine bacteria through the atmosphere can effectively spread infection agents over vast oceanic regions, highlighting its significance in regulating cell fate in algal blooms.

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02 Microbial ecology and evolution

# The Molecular Mechanisms Underlying Acclimation to Marine Heat Waves in Diatom Blooms

**Mai Sadeh**[1], Shifra Ben-Dror[2], Yael Fridmann-Sirkis[2], Gilgi Friedlander[3], Daniella Schatz[1], Asaf Vardi[1]

[1]*Department of Plant and Environmental Sciences, Weizmann Institute of Science, Israel*

[2]*Life Science Core Facilities, Weizmann Institute of Science, Rehovot, Israel, Weizmann Institute of Science, Israel*

[3]*The Nancy and Stephen Grand Israel National Center*

*for Personalized Medicine (G-INCPM), Weizmann Institute of Science, Israel*

Marine heatwaves (MHW) are periodic anomalies in sea surface temperature. According to recent reports, their intensity, duration and frequency are increasing in the past 50 years due to climate changes. MHW have various effects on macroflora and fauna, however little is known on their impact on microbial life and specifically on algal blooms. We plan to investigate the molecular and biochemical mechanisms involved in MHW response of algal blooms, specifically, diatoms- a key phytoplankton group responsible for 40% of photosynthesis in the ocean. Our preliminary results show that after exposure to MHW treatment of 33°C for 72 hours, half of the Phaeodactylum tricornutum population dies but then recovers completely after the HW. Interestingly, Cell death peaks three days after returning to ambient conditions. We conducted time-course proteomics analysis of this experimental setup and revealed key proteins crucial to the thermotolerance response such as heat-shock factors and metacaspases(MC). MC were suggested to be involved in cell-fate regulation in response to stress, though their specific role remained unclear. Using CRISPR-cas9, we generated triple-mutants of 3 metacaspase genes, which exhibited increased sensitivity to HW treatment. Proteomics analysis showed that each MC has a different response and dynamic during the HW. Our goal is to eventually develop heat-stress related protein markers. We have also investigated the role of PtMC5 in-vitro to validate the biochemical involvement in protein aggregates clearance during heat-stress similarly shown in yeast and plant metacaspase. In addition, we studied natural populations from the Red Sea and investigated their response to MHW in a mesocosm setup. We aim to understand the strategies employed by marine diatoms to cope with MHW and their ecological impact on diatom blooms.

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02 Microbial ecology and evolution

# The origins of death at sea: Characterizing conserved cell death genes in distant algae

**Avia Mizrachi**[1], Mai Sadeh[1], Shifra Ben-Dor[2], Orly Dym[2], John K. Brunson[3,4], Andrew E. Allen[3,4], Daniella Schatz[1], Assaf Vardi[1]

[1]*Department of Plant and Environmental Sciences, Weizmann Institute of Science, Israel*

[2]*Department of Life Sciences Core Facilities, Weizmann Institute of Science, Israel*

[3]*Scripps Institution of Oceanography, University of California San Diego, USA*

[4]*Environmental Genomics, J. Craig Venter Institute, USA*

Phytoplankton drive global primary production and form vast blooms in aquatic ecosystems. Bloom demise and phytoplankton rapid turnover fuel microbial life and are suggested to involve programmed cell death (PCD) induced by diverse environmental stressors. However, the PCD molecular components in algae, and protists in general, remain elusive. Here, we reveal novel PCD and stress resilience genes conserved across distant algal lineages. Previously, we showed that early oxidation in the chloroplast predicts subsequent cell death in isogenic subpopulations that emerge following H2O2 treatment in the diatom Phaeodactylum tricornutum. Here, we performed transcriptome analysis of sorted sensitive and resilient cells to discover genes linked to their contrasting fates. By cross-comparison with a large-scale mutant screen in the green alga Chlamydomonas reinhardtii, we identified functionally relevant conserved PCD gene candidates, including the cysteine protease cathepsin X/Z (CPX). CPX mutants in P. tricornutum CPX1 and C. reinhardtii CEP12 exhibited profound resilience to oxidative stress, supporting a conserved function in algal PCD. P. tricornutum cpx1 mutants, generated using CRISPR-Cas9, also exhibited resilience to the diatom-derived toxin cyanogen bromide. Phylogenetic and predictive structural analyses show that CPX is highly conserved in eukaryotes, and algae exhibit strong structural similarity to human cathepsin CTSZ, a protein linked to various diseases. CPX is expressed by diverse algae across the oceans and during toxic Pseudo-nitzschia blooms, supporting its ecological importance. Elucidating PCD components in algae sheds light on the evolutionary origin of PCD in unicellular organisms and on the cellular strategies employed by the population to cope with stressful conditions.

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02 Microbial ecology and evolution

## Rhodopsin-carotenoid complexes in marine planktonic Asgard archaea

**Gali Tzlil**

Microbial rhodopsins are retinal-bound, light-activated membranal proteins found in diverse aquatic microbes. In the ocean’s photic zone, 50% of microbes harbor a rhodopsin gene, potentially enabling their phototropic lifestyle. We recently reported on the widespread binding of carotene antennas to microbial rhodopsins, suggesting a significant impact on rhodopsin-mediated phototrophy.

Thus far, we characterized novel antennas-rhodopsin complexes from a bacterial origin. In this project, we screen for novel antennas-rhodopsin complexes from diverse microbial origins. Surprisingly, we found antennas-rhodopsin complexes originating from marine planktonic Asgard Archaea. Furthermore, we demonstrate that this rhodopsin can bind diverse hydroxylated carotenoids (e.g., lutein, diatoxanthin and fucoxanthin) and transfer energy. Our results indicate that the use of light-harvesting antennas in microbial rhodopsins is potentially utilized by open ocean Asgard Archaea to support phototrophic lifestyle.

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02 Microbial ecology and evolution

# Variability in the abundance of a new clade of T7-like cyanophages with latitude in the North Pacific Ocean

**Elena Khavin**[1], Kira Kondratyeva[1], Debbie Lindell[1]
*Faculty of Biology, Technion - Israel Institute of Technology, Israel*

The T7-like cyanophage group is one of two main families of viruses infecting picocyanobacteria from the genera *Prochlorococcus* and *Synechococcus*. They are double-stranded DNA lytic viruses, morphologically similar to the T7 phage of *Escherichia coli*. For a long time two clades of T7-like cyanophages were known, clades A and B. Recently, a new group, clade C, was discovered. However, little is known about the distribution and infection patterns of this new clade. Here we investigated the relative abundance and infection level of clade C in the North Pacific Ocean compared with the two previously known clades. We used metagenomic and virome data along transects in three cruises that sailed from the North Pacific Subtropical Gyre (NPSG) through a transition zone towards the Subpolar Gyre. We found that the fraction of reads recruiting to clade C decreased from subtropical to subpolar latitudes in some transects. In several samples, clade C was the dominant T7-like cyanophage. More reads were recruited to clade C in surface waters than at the deep chlorophyll maximum (DCM) in the NPSG and the Subpolar Gyre. In contrast, in the transition zone clade C had similar relative abundance in both surface and DCM samples. This study provides insight into latitude-dependent variability of the relative abundance of the three known clades of T7-like cyanophages in the North Pacific Ocean and indicates that different clades within a single cyanophage family respond different to environmental gradients.

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02 Microbial ecology and evolution

## The pathogenic potential of environmental vibrios in Israel

**Katarzyna Kanarek**[1], Kinga Keppel[1], Chaya Mushka Fridman[1], Dor Salomon[1]
*Department of Clinical Microbiology and Immunology, School of Medicine, Faculty of Medical and Health Sciences,, Tel Aviv University, Israel*

The rise in ocean water temperatures is strongly correlated with the emergence of gram-negative pathogenic bacteria of the genus Vibrio and their associated diseases, collectively named vibriosis. Vibrios, which impact both human health and aquaculture food production, are known for their ability to horizontally share genetic information leading to the emergence of new pathogenic strains. Although no major Vibrio outbreak was reported in Israel in the past two decades, a slow yet steady increase in vibriosis has been reported by the Israeli Ministry of Health since 2006. In this work, we set out to monitor the pathogenic potential of environmental vibrios in Israel and assess traits that may contribute to virulence or to the emergence of new pathogenic strains. We sequenced the genomes of 23 vibrios isolated from Eilat, Tel Aviv, and Ma`agan Michael coastal waters during the summer of 2023. Genome analyses revealed diverse Vibrio species harboring known toxin secretion systems and diverse toxin repertoires. Several strains were able to kill bone marrow-derived macrophages, and few were also able to exert toxic activity towards the aquatic animal model, Artemia salina. In addition, we found that few of the isolated strains are resistant to antibiotics commonly used to treat vibriosis. Lastly, we found potent antibacterial activity of some isolates, as determined by interbacterial competition assays, associated with the type VI secretion system (T6SS). Together, our findings reveal the clear pathogenic potential of environmental vibrios in Israel to both humans and aquaculture. These findings underscore the need to continue monitoring the spread of antibiotic resistance and virulence traits in the local Vibrio populations.

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02 Microbial ecology and evolution

## Breaking the eco-evolutionary feedbacks leads to extinction in a bacterial mutualism

**Yael Baranovitch**, Ignacio Jose Melero-Jiménez, Jonathan Friedman

Coevolution is a key force structuring how ecological communities form and function. Specifically, eco-evolutionary feedbacks (EEFs), consisting of reciprocal adaptations between interacting species, are thought to be a key mechanism driving coevolution within communities. EEFs have been shown to be crucial in antagonistic interactions, where they lead to evolutionary arms races. However, the effect of EFFs on other types of interactions is poorly understood. In particular, it is unclear whether EFFs reinforce or degrade mutualistic interactions, which are widespread interactions shaping ecological communities. To address this knowledge gap, I developed an experimental system that allows evolving one species in the presence of another species that is maintained in its ancestral state, preventing any EEFs. Using this system, I (co)evolved a pair of Escherichia coli strains that exchange essential amino acids and form an obligate mutualism. Surprisingly, preventing EFFs destabilized our system, and both species survived only when they coevolved. The survival was driven by the evolution of one of the species that evolved to higher density, reducing its amino acid requirements for growth and thereby supporting its partner`s survival. These findings reveal a case in which the evolution of one species plays a key role in preventing the extinction of its partner, underscoring the intricate dynamics of coevolution within microbial communities.

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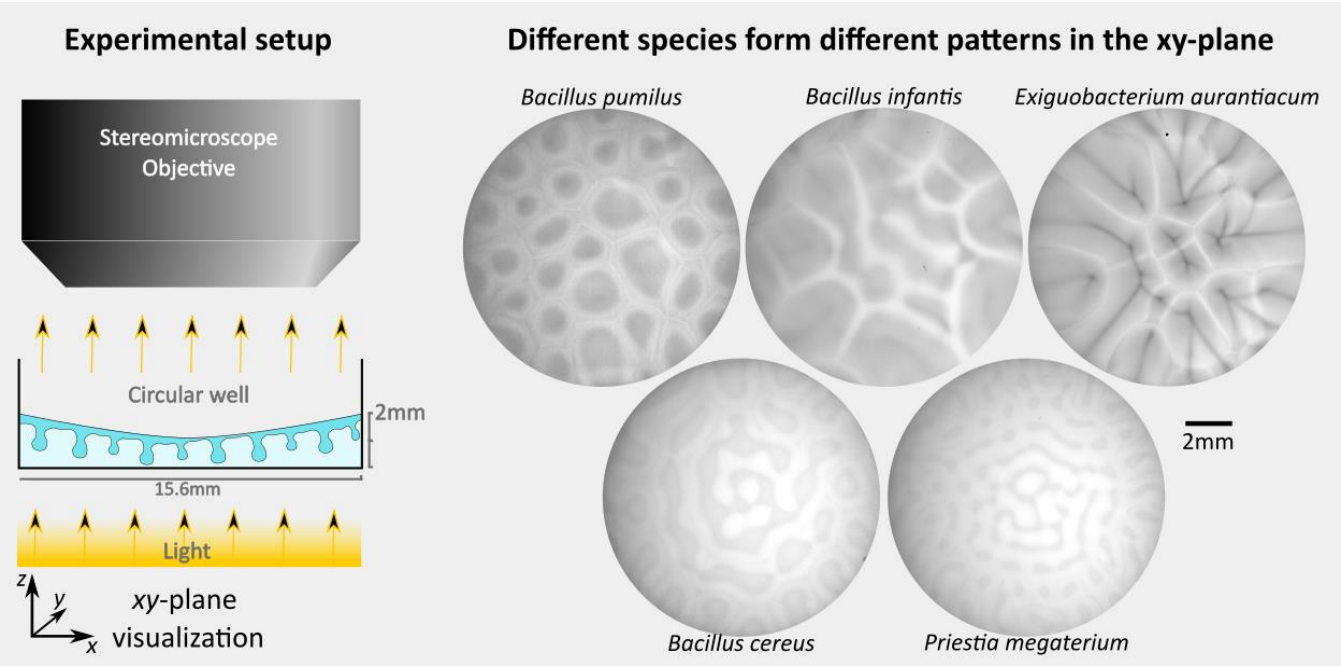
Poster presentation

02 Microbial ecology and evolution

# Oxytactic bioconvection across bacteria species

**Oscar Gallardo**[1], Rinat Goren[1], Joel Stavans[1]
*Physics of Complex Systems, Weizmann Institute of Science, Israel*

Bioconvection is a collective motion exhibited by different microorganisms, such as oxytactic bacteria. It consists of persistent macroscopic hydrodynamic flows that travel up and down the bulk liquid. The flows auto-organize in well-defined geometrical patterns with a spatial structure of high and low bacterial density regions. Different species form different characteristic patterns, but despite understanding the cause of bioconvection, it is unknown what determines the development of the different patterns. We tackle this question by performing different characterizations of bacterial properties and behavior by timelapse microscopy and other techniques on bacteria isolates from the Cuatro Cienegas Basin, an ancient oligotrophic oasis in the Mexican desert that serves as a natural evolutionary laboratory. We conclude that some characteristics of the motility of each species are important parameters for pattern morphology during bioconvection, and discuss the ecological impact of bioconvection in nature.



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02 Microbial ecology and evolution

## Evolution in microbial microcosms is highly parallel regardless of the presence of interacting species

**Nittay Meroz**[1], Tal Livny[1], Gal Toledano[1], Yael Sorokin[1], Nesli Tovi[1], Jonathan Friedman[1]
*Faculty of Agriculture, Hebrew University, Israel*

Evolution often follows similar trajectories in replicated populations, suggesting that it may be predictable. However, populations are naturally embedded in multispecies communities and the extent to which evolution is contingent on the specific species interacting with the focal population is still largely unexplored. Here, we study adaptations in strains of 11 different species experimentally evolved both in isolation and in various pairwise co-cultures. While partner-specific effects are detectable, evolution was mostly shared between strains evolved with different partners; similar changes occurred in strains' growth abilities, in community properties, and in about half of the repeatedly mutated genes. This pattern persisted even in species pre-adapted to the abiotic conditions. These findings indicate that evolution may not always depend strongly on the biotic environment, making predictions regarding coevolutionary dynamics less challenging than previously thought.

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02 Microbial ecology and evolution

## Occurrence of under-the-radar antibiotic resistance bacteria and genes in anthropogenically affected produce

**Chagai Davidovich**[1,2], Kseniia Erokhina[1,2], Shlomo Blum[3], Eddie Cytryn[1]
[1]*Institute of Soil, Water and Environmental Sciences, Volcani Institute, Israel*
[2]*Robert H. Smith Faculty of Agriculture, Food and Environment, The Hebrew University of Jerusalem, Israel*
[3]*Dept. of Bacteriology, Kimron Veterinary Institute, Israel*

Treated-wastewater (TWW) irrigation and manure amendment are critical for sustainable agriculture in water- and nutrient-stressed regions. However, these practices can potentially disseminate pathogens and antimicrobial resistance (AMR) determinants to crops, which can be transmitted through the food chain to humans. Previous studies indicate that pathogen and AMR indicators from wastewater and manure survive poorly in the environment, suggesting that ecological barriers prevent their dissemination. However, a recent study in our lab demonstrated that these elements can persist at below detection levels in low quality TWW-irrigated soil, and potentially proliferate under favorable conditions. Here, we explored this "under the radar" phenomenon in TWW-irrigated and poultry litter-amended lettuce, using an enrichment platform that resembles gut-like conditions and a myriad of molecular and cultivation-based tools. Enrichment uncovered clinically-important multidrug resistant pathogen indicators and a diverse array of antibiotic resistance genes (ARGs) in the anthropogenically treated lettuce that were not detected with direct analyses nor with enriched freshwater-irrigated samples. Selected resistant E.coli isolates were capable of horizontally transferring plasmids with multiple ARGs to a susceptible strain. Overall, our study sheds light on the hidden risk of "under the radar" AMR determinants in anthropogenically affected agro-environments, providing a platform that can be adopted to improve risk assessment models in the future.

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02 Microbial ecology and evolution

## Escherichia coli adaptation under prolonged resource exhaustion is characterized by extreme parallelism and frequent historical contingency

**Shira Zion**[1], Sophia Katz[1], Ruth Hershberg[1]
*Department of Medicine, Technion - Israel Institute of Technology, Israel*

Like many other non-sporulating bacterial species, Escherichia coli is able to survive prolonged periods of resource exhaustion, by entering a state of growth called long-term stationary phase (LTSP). In July 2015, we initiated a set of evolutionary experiments aimed at characterizing the dynamics of E. coli adaptation under LTSP. In these experiments populations of E. coli were allowed to initially grow on fresh rich media, but were not provided with any new external growth resources since their establishment. Utilizing whole genome sequencing data obtained for hundreds of clones sampled at 12 time points spanning the first six years of these experiments, we reveal several novel aspects of the dynamics of adaptation. First, we show that E. coli continuously adapts genetically, up to six years under resource exhaustion, through the highly convergent accumulation of mutations. We further show that upon entry into LTSP, long-lasting lineages are established. This lineage structure is in itself convergent, with similar lineages arising across independently evolving populations. The high parallelism with which adaptations occur under LTSP, combined with the LTSP populations` lineage structure, enable us to screen for pairs of loci displaying a significant association in the occurrence of mutations, suggestive of a historical contingency. We find that such associations are highly frequent and that a third of convergently mutated loci are involved in at least one such association. Combined our results demonstrate that LTSP adaptation is characterized by remarkably high parallelism and frequent historical contingency.

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Poster presentation

02 Microbial ecology and evolution

## Annual dynamics of T7-like and T4-like cyanophages and their infection in the North Pacific Subtropical Gyre

**Svetlana Goldin**[1], Debbie Lindell[1]
*Faculty of Biology, Technion – Israel Institute of Technology, Israel*

The North Pacific Subtropical Gyre (NPSG) is an example of an oligotrophic, open ocean ecosystem, characterized by low seasonal variability. The photoautotrophic microbial community in this region is dominated by cyanobacteria from the genus Prochlorococcus. Despite the wealth of information about the cyanobacterial community in the NPSG, the dynamics of the cyanophages remains understudied. Our aim is to investigate abundances of two major cyanophage families: T4-like cyanomyoviruses and T7-like cyanopodoviruses, and infection over the annual cycle. Towards this end we analyzed depth profiles sampled over the photic zone for two years. Clear and reproducible depth distribution patterns were found. The abundances of both cyanophage families, Prochlorococcus, and the number of infected Prochlorococcus cells were low at the surface and increased with depth to form a subsurface peak. In nearly all depth profiles there was a switch in abundance of cyanophages with depth: T4-likes were more abundant at the surface and T7-likes deeper in the water column, suggesting different responses of the two cyanophage groups to environmental factors. Seasonal variability in the abundances and infection of cyanophages was observed, with significantly more cyanophages and infected Prochlorococcus in Jun-Dec than in Jan-May. Particularly high cyanophage abundances and infection rates were found in the fall. In contrast, Prochlorococcus displayed only weak seasonal fluctuations throughout the year. These findings suggest that other factors besides host availability alone regulate cyanophage abundance and distribution. Furthermore, the strong seasonal changes despite the low seasonality suggests that cyanophages are sensitive to relatively small changes in environmental conditions.

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Poster presentation

02 Microbial ecology and evolution

## Evolution of cyanophages under different light conditions

**Yuval Aharon Shiran**, Debbie Lindell

Marine cyanobacteria from the genera Prochlorococcus and Synechococcus are very abundant, widespread and contribute much to primary production in the ocean. The cyanobacteria serve as hosts to cyanophages, marine viruses that infect them. Environmental conditions such as light intensity can affect the phage infection cycle. However, it remains unclear whether cyanophages undergo adaptation to different light intensities on an evolutionary scale, and what genetic changes are responsible. Moreover, we don’t know whether acquisition of auxiliary metabolic genes, such as the photosynthetic gene psbA, contributes to this adaptation. Here, we investigate whether cyanophages can adapt to light intensity, and aim to determine the genotypic consequences of such adaptations. We simulated an evolutionary process using semi-batch cultures of 3 Syn9 strains (Syn9 WT, mutant with partial deletion of psbA gene, and a mutant with whole deletion of psbA gene) at two light intensities. The evolutionary experiment showed a significant change in plaque size and host lysis between the evolved and ancestral strains. This indicates higher fitness of the evolved populations. No reacquisition of the gene psbA was detected at the end of the experiment. However, sequencing of evolved populations showed mutations in a total of 29 genes. Six of the genes had mutations that were unique for high light evolved populations, and one gene had mutations only in low light evolved populations. Furthermore, the majority of mutations were nonsynonymous for each light and strain treatment. These findings suggest that the cyanophage mutations are not due to genetic drift and result from adaptation.

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Poster presentation

02 Microbial ecology and evolution

## Adaptive loss-of-function mutation in B. subtilis' ThrC enzyme exposes metabolic bypass to isoleucine biosynthesis

**Shanni Hornstein**[1], Swati Sirotiya, Raul Mireles, Orr Giladi, Alexander Geller, Asaf Levy, Carolina Cano-Prieto[2], Pablo Cruz-Morales, Lianet Noda-García
[1]*Department of Plant Pathology and Microbiology. Faculty of Agriculture Food and Environment, Hebrew University of Jerusalem, Israel*
[2]*Novo Nordisk Foundation Center for Biosustainability, Danmarks Tekniske Universitet, Denmark*

Loss-of-function (LoF) mutations play a crucial role in evolution, as they tend to be the preferable adaptive solution in many cases. These mutations contribute to fitness by reducing the activity of a gene or eliminating it altogether (null mutation). This study showcases a specific LoF mutation in the ThrC enzyme (threonine synthase, E.C. 4.2.3.1) in Bacillus subtilis, resulting in rerouting the L-isoleucine (Ile) biosynthetic pathway. In Bacillus, Ile biosynthesis is carried out by the ilvA gene, encoding a threonine dehydratase (E.C. 4.3.1.19). It converts L-threonine to 2-oxobutanoate, an indispensable metabolite in this pathway. In our laboratory, we evolved seven B. subtilis strains that, despite lacking the ilvA gene, can synthesize this amino acid and grow half as fast as the wild-type strain. Comparative genomic analysis revealed four of those seven strains to have the same mutation: T55M mutation in ThrC. Before, it was observed that B. subtilis ThrC has a weak IlvA function. Here, we tested if the T55M mutant had increased this activity, compensating for the missing ilvA gene. We found, however, that ThrC_T55M lost its IlvA function, contradicting our initial hypothesis. Then, with metabolomics, we showed that the ΔilvA+ThrC_T55M strain depletes the O-acetyl-L-serine metabolite by about 5-fold compared to the wild type. This shows that Ile biosynthesis could occur via this intermediate, reusing part of the Cysteine and Methionine biosynthetic pathways. Thus, our results shed light on a new metabolic bypass and microbial adaptation through LoF mutations in central metabolic pathways in B. subtilis.

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Poster presentation

02 Microbial ecology and evolution

## Investigating the activity of microbial populations in wetlands for the management of greenhouse gases emissions

**Julia Ghantous**[1], Raphy Zarecki
*Water Science, Tel-hai college, Israel*

Wetlands are critical in the global carbon cycle by acting as significant carbon reservoirs and regulating carbon fluxes between the atmosphere and terrestrial systems. Microbial communities have a central role in organic matter breakdown, resulting in methane production by archaeal methanogens, a significant greenhouse gas (GHG) contributing to climate change. The primary objective of this research is to investigate the microbial ecology of wetlands and its role in GHG emissions, especially methane. Specifically, the study aims to compare GHG emission profiles across different wetland sites and examine the influence of microbial community composition on emissions. This work involves environmental monitoring of two natural wetland reserves (Ein Afek and Ein Nimfit, Israel) and one constructed wetland (Kibutz Hardoof, Israel). Soil and water samples were collected for monitoring microbial dynamics and physiochemical parameters, including high-throughput amplicon sequencing of the 16S ribosomal RNA gene. Sequencing results show that the microbial community composition varied among the sites. Methanogenesis and nitrogen-fixing bacteria are prevalent in Ein Nimfit, while fermentative bacteria dominate in Ein Afek. In contrast, carbohydrate degradation processes are commonly observed in Kibutz Hardoof. This variation in community composity corresponds to the variation on the emission profiles that was detected in an ongoing microcosm experiment, simulating wetland profile depths. Additionally, Methane production was selectively effected by the addition of organic matter, with variations in the effect of different metabolic additives on different source communities. The investigation of various wetland sites and microbial activities provides insights into potential strategies for managing emissions.

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Poster presentation

02 Microbial ecology and evolution

## Balancing infection characteristics underlies cyanophage adaptation in complex environments

**Greenbaum Jonathan**[1], Debbie Lindell[1]
*Faculty of Biology, Technion - Israel Institute of Technology, Israel*

Marine cyanobacteria are abundant primary producers, responsible for roughly a quarter of oceanic carbon fixation. Cyanobacteria-infecting bacteriophages (cyanophages) have massive ecological significance, as they affect the abundance and diversity of the cyanobacterial community. Cyanophages depend entirely on their cyanobacterial hosts for propagation. Therefore, the composition of the cyanobacterial community has the potential to determine the ability of cyanophages to persist, and their evolutionary trajectory. Here, we aim to examine the effect of the cyanobacterial community, consisting of both susceptible and an adsorbable resistant cyanobacteria, on cyanophage evolution. We found that six out of twelve evolved cyanophage populations decreased time to cell lysis compared to the ancestral phage when infecting the original susceptible host. Three of these populations evolved in the presence of a resistant strain. The shorter lysis time indicates a change in at least one infection characteristic, resulting in increased phage fitness. Surprisingly, we found that attachment to both the susceptible and resistant cyanobacterial strains decreased in evolved populations. We hypothesize that this allows cyanophages to limit the amount of futile infections while still maintaining high levels of progeny production. Genomic analysis revealed point mutations in a putative holin in three populations with increased fitness. This mutation has the potential to shorten the phage latent period, hypothesized to be beneficial when a susceptible host is abundant. Our findings suggest that, in order for cyanophages to gain a competitive advantage in complex environments where an adsorbable resistant cyanobacterium is abundant, a balance is maintained between different infection characteristics.

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Poster presentation

03 Fungal biology

## Screening for a novel fungus in human shotgun metagenomics datasets

**Bareket Dassa**[1], Shifra Ben-Dor[1], Lena Fidel[1], Jarmila Kralova[2], Catalina Donic[2], Steffen Jung[2]

[1]*Department of Life Sciences Core Facilities, Weizmann Institute of Science, Israel*

[2]*Department of Immunology and Regenerative Biology, Weizmann Institute of Science, Israel*

The mycobiota are a critical part of the human microbiome, but host–fungal interactions remain incompletely understood. Recently, a novel fungal commensal of the Kazachstania genus was isolated from laboratory mouse intestines, and by serendipity was shown to prevent the colonization of Candida albicans in immunosuppressed mice. Phylogenetic analysis of the 26S rDNA and whole-genome sequence of the new fungus placed it within the Saccharomycetaceae clade. A bioinformatics screen was designed to identify fungal species, specifically C. albicans and the newly identified Kazachtania weizmannii in thousands of public human metagenomes, using nucleotide markers that are both unique to the fungus and distinct from other fungi and bacteria. Although most metagenomes have been generated to detect prokaryotic microbiota, our screen yielded evidence that K. weizmannii is also part of human intestinal and vaginal microbiomes. This reported competitive fungal commensalism could have potential therapeutic value for mitigating C. albicans-mediated diseases.

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Poster presentation

03 Fungal biology

## Identification of regulators vital for fungal adaptation and survival within the phagosomal environment

**John Adeyemi Adeoye**[1], Laura Fabre[2], Amelia Barber[2], Neta Shlezinger[3]
[1]*Korect School of Veterinary Medicine, Faculty of Agriculture, Food and Environment, The Hebrew University of Jerusalem, Israel*
[2]*Institute of Microbiology, Friedrich Schiller University, Germany*
[3]*Koret School of Veterinary Medicine,*

*Faculty of Agriculture, Food and Environment, The Hebrew University of Jerusalem, Israel*

The primary antifungal mechanism in neutrophils involves the induction of genetically regulated cell death (RCD) through NADPH oxidase (NOX) mediated oxidative burst upon phagocytosis of A. fumigatus conidia. Interestingly, NOX deficient neutrophils exhibit significant residual conidiacidal activity and can still induce fungal RCD, Strongly suggesting the presence of non-oxidative RCD-inducing anti-fungal effectors in neutrophils. A. fumigatus can adapt to survive within the hostile neutrophil phagosome by resisting RCD. This is crucial for fungal virulence, pathogenicity, and adaptation to host environment. With the molecular processes involved in the oxidative and non-oxidative induction of fungal RCD in phagocytosed conidia largely unknown, our study explores the molecular processes governing the survival and adaptation of A. fumigatus conidia within the phagosomal environment of neutrophils. We used directed experimental evolution to select A. fumigatus variants evolved to withstand oxidative and non-oxidative neutrophil challenge within the murine environment. Two independent lines were serially passaged in wild-type and NOX-deficient mice, both with C57BL/6 background. Whole genome sequencing was performed on evolved strains and their ancestral counterparts. Surprisingly, evolved strains from both WT and NOX deficient conditions exhibited an increased phagocytosis rate and heightened neutrophil death. Specific single nucleotide polymorphisms affecting regulatory sequences of Kynureninase-1 and an iron-sulfur cluster assembly gene were identified in strains evolved in NOX sufficiency. The genetic changes observed in strains evolved under these NOX conditions suggest an adaptive strategy that involves modulation of the pathogen’s metabolism to interfere with host immune response and stress regulation under a combination of iron deprivation and oxidative stress. Our results uncover molecular survival mechanisms of A. fumigatus within lung neutrophils, further advancing the understanding of host-pathogen interactions.

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Poster presentation

03 Fungal biology

# Lipid metabolism and transcriptional regulation in Botrytis cinerea under low temperature

**Gladys Karungari**[1,2], Ginat Raphael[2], Lavanya Gunamalai[2], Guy Schleyer[3], Tali Shalit[4], Oded Yarden[5], Carmit Ziv[2]

[1]*Department of Plant Pathology and Microbiology, The Hebrew Univeristy of Jerusalem, Israel*

[2]*Department of Postharvest Science, Agricultural Research Organization, Volcani Center, Israel*

[3]*Department of Plant and Environmental Sciences, Weizmann Institute of Science, Israel*

[4]*Life Sciences Core Facilities, Weizmann Institute of Science, Israel*

[5]*Department of Plant Pathology and Microbiology, The Hebrew University of Jerusalem, Israel*

Despite cold storage, Botrytis cinerea causes significant postharvest losses in many stored fruits and vegetables. This pathogen`s diverse infection strategies and a broad host range makes it difficult to control. Lipid metabolism and remodeling are key strategies for fungi to cope with temperature stresses. We performed an integrated lipidomic and transcriptomic time course analysis to explore the metabolic changes of lipids in B. cinerea after cold shift from 22ºC to 5ºC. In the transcriptome analysis of B. cinerea after cold exposure, a total of 33 lipid-related differentially expressed genes were annotated and classified as belonging to various lipid metabolism pathways. Genes involved in glycerolipid and glycerophospholipid metabolism, fatty acid degradation, and ether lipid metabolism were upregulated between 2 and 4 hours after cold stress, while genes involved in fatty acid biosynthesis and unsaturated fatty acid biosynthesis were downregulated after 2 hours. Most genes involved in sterol and sphingolipid metabolism were downregulated at 2 hours, with some upregulated from 8 to 48 hours. Lipidomic data revealed significant changes in lipid composition starting 24 hours after the cold shift, with increases in glycerolipids and glycerophospholipids. The most abundant lipid classes, glycerophosphocholine (PC), triacylglycerols (TG), and glycerophosphoethanolamine (PE), showed significant increases at 24 hours. These changes in lipid composition align with the upregulation of genes involved in glycerolipid and glycerophospholipid metabolism early after cold exposure, indicating a coordinated response at the genetic and lipidomic levels to adapt to cold stress, which may be involved in the fungus' ability to proliferate at low temperatures.

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Poster presentation

04 Clinical virology and epidemiology

## Wastewater-based surveillance of SARS-CoV-2 variants in Israel

**Neta Zuckerman**[1]
*Central Virology Laboratory, Israel Ministry of Health, Israel*

Wastewater surveillance of SARS-CoV-2 has emerged as a promising approach for monitoring the presence of the virus, its quantities and variant distribution, offering advantages over clinical sampling in terms of population catchment size and bypassing the need for direct individual testing. Here, we present the surveillance and characterization of SARS-CoV-2 variants and identification of novel mutations in Israeli wastewater since the beginning of the pandemic, using SARS-CoV-2 whole genome sequencing and a designated pipeline for mutation analysis. Variants identified in wastewater correlated with those found in clinical samples, both overall and in specific locations. This highlights the importance of wastewater surveillance for public health, as it reflects clinical findings and proves crucial in situations where clinical samples are unavailable.

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Poster presentation

04 Clinical virology and epidemiology

## Measles outbreak epidemiology in real-time: Development and implementation of a rapid strain classification test for measles

Efrat Bucris, Roberto Azar, Batya Mannasse, Hadar Assraf, Vardit Moshayoff, Tal Levin, Victoria Indenbaum, Yara Kanaaneh, Yaniv Lustig, **Oran Erster**[1]
*Central Virology Laboratory, Israel Ministry of Health, Israel*

In this study, we developed multiplex Real-time PCR assays that distinguish between different measles strains, and used them for rapid classification of clinical samples. Israel is considered a measles-eliminated country, due to high vaccine coverage. However, recent global surges in measles outbreaks led to concurrent cases increase in Israel (40 diagnosed cases in 2023-2024). In order to improve the measles diagnostic capacity, we improved the standard measles-rubella test by incorporating a vaccine-specific reaction, so that the sample is immediately identified as WT measles or vaccine-originated. Additionally, we developed a secondary assay that differentiates between measles strains B3 and D8, and the vaccine.

All three reactions demonstrated high specificity, identifying only their respective target. Their analytical sensitivity was less than 20 target copies per sample. Testing of 92 samples from 2018-2019 showed that 89 samples were of D8 strain and 3 originated from the vaccine. The 40 samples from 2023-2024 were partitioned between B3 (18), D8 (17) and Vac (5) strains. This suggests that unlike the 2018 outbreak, recent cases originated from multiple sources, including vaccine-derived morbidity cases. Testing of more recent samples is currently underway.

Implementation of the new assays provided important information within a short time, helping public health authorities to conduct efficient epidemiological investigation, thereby eliminating the need for unnecessary measures.

These results demonstrate the usefulness of the new assay in providing rapid epidemiological information that otherwise requires PCR followed by sequencing, which is far more time-and resource-consuming, especially during outbreaks, and is less amendable for high-throughput.

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Poster presentation

04 Clinical virology and epidemiology

## Exploring the dynamics and mechanisms of the HCV-induced epigenetic signature after cure

**Manar Hijaze**

Background: Hepatitis C Virus (HCV) is a leading cause of chronic liver disease and cirrhosis. Cirrhotic patients face a 4% annual risk of developing hepatocellular carcinoma (HCC), the third most deadly cancer globally. Our study identified that HCV infection induces an "epigenetic signature" in hepatocytes, promoting oncogenic gene expression and cancerous phenotypes. Notably, these epigenetic changes persist even after virus eradication by direct-acting antivirals (DAAs), suggesting a "hit and run" mechanism. We hypothesize that DAA treatment regime influence this persistent epigenetic signature.

Methods: To explore our hypothesis, we treated HCV-infected cells with various DAAs for differing durations and analyzed the epigenetic signature dynamics at multiple points post-cure.

Results: Our results revealed distinct epigenetic dynamics based on the DAA used. Various combinations and concentrations of DAAs exhibit a range of epigenetic signature persistence correlating with the duration of treatment until HCV cure. Studying of the dynamics of epigenetic signature reveals that HCV-induced alterations are initially erased following cure but re-emerge a few weeks later. We identified and used specific cell surface markers whose expression correlates with the epigenetic signature to sort the epigenetic signature bearing cells. We observed that a small subset of hepatocytes bearing the epigenetic signature expands over time. Long-term treatment prevents the reappearance of the epigenetic signature post-cure.

Conclusion: These findings suggest that DAA treatment regime plays a crucial role in the persistence of oncogenic epigenetic signatures after HCV cure. Understanding these mechanisms will aid in developing strategies to mitigate the risk of HCC in patients post-HCV eradication.

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Poster presentation

05 Computational biology and genomics of microbes

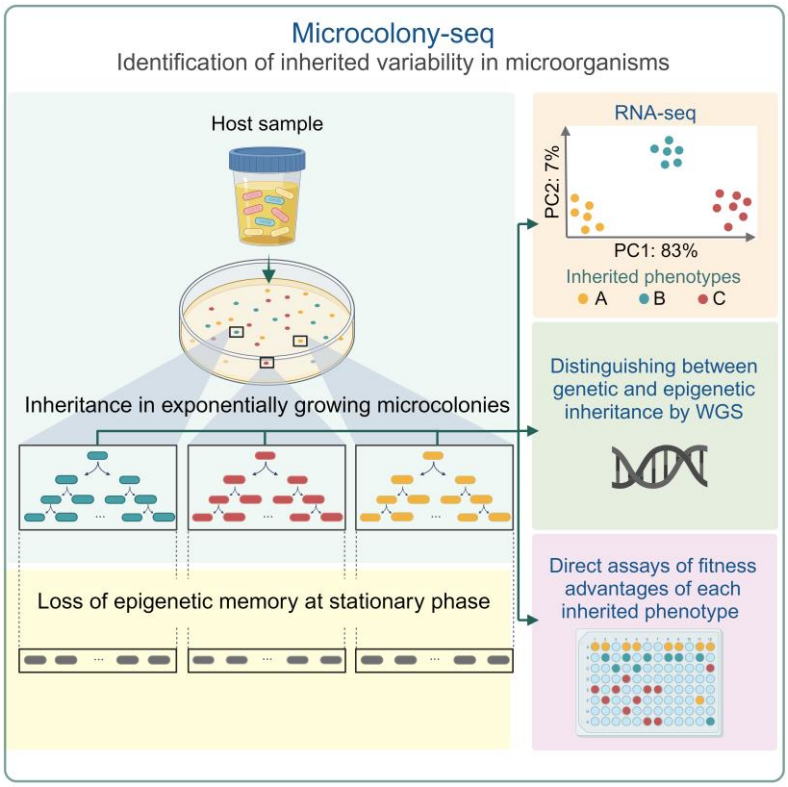
# Uncovering single-cell inherited fitness strategies with Microcolony-seq

**Raya Faigenbaum-Romm**[1], Orit Gefen[1], Naama Katsowich-Nagar[2], Irine Ronin[1], Ilan Rosenshine[2], Nathalie Q. Balaban[1]

[1]*The Racah Institute of Physics, Edmond J. Safra Campus, The Hebrew University of Jerusalem, Israel*

[2]*Department of Microbiology and Molecular Genetics, Institute for Medical Research Israel-Canada, Faculty of Medicine, The Hebrew University of Jerusalem, Israel*

From multicellular eukaryotes to bacteria, phenotypic heterogeneity plays a central role in fundamental processes such as development and stress response. Recent single-cell RNA-seq technologies have been developed to detect heterogeneity in bacteria, yet the distinction between heritable and transient, and between genetic and epigenetic variabilities, remains a challenge. Here, we introduce Microcolony-seq, a high-throughput method focusing on single-cell inherited heterogeneity. Applied to human pathogens, Microcolony-seq has uncovered a wealth of inherited phenotypes. Notably, epigenetically inherited states have been identified in a human urine infection sample, shedding light on the bacterial physiology within the host. By combining whole-genome and RNA-sequencing on microcolonies, Microcolony-seq differentiates between genetic and epigenetic variabilities. The method provides a quantitative characterization of co-existing subpopulations and allows detection of their respective fitness advantages. Microcolony-seq offers a platform for systematic detection of inherited heterogeneity in microbes, revealing differentiated subpopulations and pointing to strategies for infection mitigation.



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Poster presentation

05 Computational biology and genomics of microbes

## Genomic diversity of carbapenem-resistant Acinetobacter baumannii (CRAB) reveals distinct virulence evolution

**Mor Lurie-Weinberger**[1], Alona Keren-Paz[1], Yehuda Carmeli[1,2]
[1]*National Institute for Antibiotic Resistance & Infection Control, TLVMC, Israel*
[2]*Medicine, Tel Aviv University, Israel*

We examined virulence factor (VF) evolution in carbapenem-resistant Acinetobacter baumannii (CRAB) and the association with antibiotic resistance genes (ARGs). Using WGS, we assessed 136 VFs among 246 CRAB isolates belonging to ST2 and ST3; 49 VFs were universally present, 61 were ubiquitous and 26 occurred sporadically. The distribution of the 26 sporadic VF genes differed between ST2 and ST3: the distribution was homogenous in ST3 but heterogeneous in ST2. ST2 strains clustered into 8 distinct evolutionary clades with intra-clade homogeneity and high inter-clade heterogeneity. ARGs did not correlate with VFs. Homogeneity in ST2, and ST3 uniformity suggest a distant evolutionary segregation of VFs at the root of ST and clade divergence. VF content reflects remote events resulting in minimal variation between isolates belonging to the same ST/clade, while ARG composition reflects more recent events, leading to between-isolate variation.

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Poster presentation

05 Computational biology and genomics of microbes

## Elucidating the intracellular architecture of the Escherichia coli transcriptome and its driving mechanisms

**Roy Jacobson**[1], Zohar Persky, Daniel Dar
*Department of Plant and Environmental Sciences, Weizmann Institute of Science, Israel*

The physical distribution of mRNAs within a cell can shape post-transcriptional regulation and protein-protein interactions by creating distinct subcellular environments. It has recently been established that bacterial mRNAs can preferentially localize to different parts of the cell, including the plasma membrane, the nucleoid, or the cell poles. However, this behavior has so far been studied in detail only for a small number of genes, and most of the observed localization patterns do not have a described mechanism or function. A major challenge toward understanding this basic aspect of bacterial gene expression is the difficulty of studying subcellular RNA localization at both high-throughput and resolution. Here, we leverage advancement in massively multiplexed mRNA Fluorescent In situ Hybridization (mRNA-FISH) to measure the intracellular distributions of ~2,400 Escherichia coli genes at molecule resolution. This first-of-its-kind data provides a near comprehensive view of the spatial architecture of the E. coli transcriptome. Using data-driven methods, we characterize the different patterns that transcripts take and their driving mechanisms to gain insights into the mechanisms and implications of this behavior.

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Poster presentation

06 Clinical bacteriology and epidemiology

## Emergence and spread of ST913-IVa-t991 methicillin-resistant Staphylococcus aureus among pediatric populations in Israel.

**Moti Baum**[1], Einav Anuka[1], Maya Davidovich-Cohen[1], Assaf Rokney[1]
*Public Health Laboratories –Jerusalem (PHL-J), Public Health Services (PHS), Ministry of Health (MOH), Israel, Ministry of Health, Israel*

Methicillin resistant Staphylococcus aureus (MRSA) is a major cause of skin and soft tissue infections (SSTIs) in community and healthcare settings globally. The focus of this study is a genomic and epidemiological analysis of MRSA ST913-Iva-t991, a globally rare type sporadically reported in the Middle East, which is on the rise in Israel.

Over the last decade, the ST913-IVa-t991 CA-MRSA lineage has emerged and disseminated as a major clone circulating among pediatric populations in Israel. spa type t991 has emerged over the course of the study and increased dramatically among SSTI patients, from 5 cases (4%) in 2012 to 159 cases (27%) in 2020. Most SSTI cases (70%) were pediatric patients who reside in Arab and Ultraorthodox Jewish localities. Selected isolates were tested by broth microdilution and several antimicrobial resistance profiles, which could be associated with patient residence and demographic background were detected. wgMLST phylogeny demonstrated diversification among the lineage. Virulence profiles included the exfoliative toxin A (eta) gene, and diversity was observed in genes associated with adhesion. Global contextualization based on genomic analysis indicates that the geographical distribution is limited to the Middle East. These findings suggest complex evolutionary dynamics and warrants further surveillance for local circulation, transmission and global exportation.

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06 Clinical bacteriology and epidemiology

## WGS-based phylogeny of multidrug-resistant salmonella typhimurium in israel

**Eugenia Yakunin**[1], Yosi Ilan Shorr[1]
*Public Health Laboratories Jerusalem, Public Health Division, Ministry of Health, Israel*

Salmonella enterica serovar Typhimurium is a leading global cause of non-typhoidal salmonellosis associated with multidrug resistance and a broad host range. In Israel, S. Typhimurium caused 5%-10% of yearly human salmonellosis cases between 2013 and 2023, and was detected in poultry and livestock. We used genomic methods to study the population structure of S.Typhimurium in Israel, with focus on lineages that present multidrug resistance, global contextualization, and molecular mechanisms of antimicrobial resistance (AMR).

The National Salmonella Reference Center assessed by broth microdilution the susceptibility of 507 S.Typhimurium strains collected in 2016-2022 from diverse sources. Multidrug resistance (MDR) was detected in 201 (40%) of strains. A sample of 59 MDR strains with 20 unique profiles were further analyzed by whole-genome sequencing (WGS).

WGS revealed diverse lineages among the Israeli S.Typhimurium population, in which the predominant sequence types were the globally disseminated ST-376 (36%), ST-34 (32%) and ST-19 (24%). The most common MDR profile (ST-376) included closely related human, bovine and poultry isolates that carried the antibiotic resistance genes (ARG) blaTEM-1A, floR, sul2, and tet(B). Third-generation cephalosporin resistance was observed among ST-34 and ST-19 and associated with blaCTX-M and blaSHV genes. Resistance to first-line antimicrobials—ampicillin (59%), ciprofloxacin (18%), and folate pathway inhibitor combinations (9%)—was associated with the blaTEM, qnr, and dfrA genes. Various plasmids were detected, including IncFIB(S), IncFIIB(S), IncQ and IncX1.

This is the first study to provide insight to the population structure of S.Typhimurium in Israel that highlights phylogeny and the genetic basis of AMR.

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Poster presentation

06 Clinical bacteriology and epidemiology

# HiSpoT a novel NGS-based method for simple, high-throughput genotyping of *Mycobacterium tuberculosis*

**Israel Nissan**[1], Ephraim Fass[2], Paul J. Freidlin[2], Gal Zizelski Valenci[2], Inna Kutikov[2], Mor Rubinstein[2], Yelena Losev[2], Hasia Kaidar-Shwartz[2], Zeev Dveyrin[2], Efrat Rorman[2]

[1]*The Laboratory for Poultry Diseases, Kimron Veterinary Institute, Ministry of Agriculture and Rural Development, Veterinary Services, Israel*

[2]*National Public Health Laboratory, Ministry of Health, Public Health Directorate, Israel*

**Background:**

Spoligotyping serves as a gold standard for *M. tuberculosis* molecular epidemiology, enabling separation of *M. tuberculosis* isolates into different biogeographic lineages. Spoligotyping deciphers the polymorphisms in the chromosomal CRISPR/DR locus. Nevertheless, method application is based on 1990s technologies, that are labor demanding, require highly skilled technicians, and the results are manually decoded. In this study we demonstrate the development of HiSpoT, a novel NGS based method for simple, one-tube, high throughput genotyping of M. tuberculosis.

**Methods:**

Amplicon libraries compatible with the Illumina platforms, were produced by innovative primers design and meticulous PCR calibration. Spacer sequences from *M. tuberculosis* template DNA were amplified followed by addition of barcodes and Illumina flow-cell adapters, in a single PCR tube (microplate well). Ninety-six barcoded samples were pooled and purified. Sequencing was performed using MiSeq's V2 nano kit. For bioinformatics analysis Python based software was employed for automatic, precise and rapid spoligotyping and lineage determination. We named this method HiSpoT.

**Results:**

For method validation, we tested *M. tuberculosis* isolates in Israel from years 2020-2022. Comparative analysis of spoligotypes obtained by HiSpoT and whole genome sequencing showed excellent lineage match. The HiSpoT method required less than a day for library preparation and ~17 hours of MiSeq run.

**Conclusions:**

HiSpoT is a cost effective, high-throughput simple spoligotyping method that reduces time to results. This powerful single tube method for NGS library preparation, can be adjusted to multiple utilizations in other microorganisms as well as for metagenomics applications.

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Poster presentation

07 Viruses of eukaryotes

## Genome-wide CRISPR-Knockout Screen Reveals Novel Anti-viral Host Factors Affecting Respiratory Viruses Infections

**Randa Tissawak**[1]
*Microbiology and molecular genetics, The Hebrew University, Israel*

Respiratory syncytial virus (RSV) poses global health risks, especially in infants, elderly, and immunocompromised populations. Hence, exploring novel therapeutic approaches is crucial to address challenges such as broad-spectrum protection, evolving viral variants, and the specific needs of immunocompromised individuals. One promising strategy involves utilization of natural host antiviral defense mechanisms. Thus, our goal is to detect novel interferon-dependent host antiviral factors, aiming to identify more potent antiviral strategies to target RSV. We performed a genome-wide CRISPR-KO screen in lung epithelial cells. Cells that were highly susceptible to infection despite interferons (IFN) treatment were analyzed and genes involved in antiviral mechanisms were identified. Interestingly, LATS2, AMOTL2, and NF2 that were among the highest-ranking genes, are all components of the Hippo pathway. Notably, we discovered that these genes are post-transcriptionally regulated by IFN and are not IFN-stimulated genes (ISGs), and as such, were not previously identified through conventional ISG-targeted screens. highlighting the uniqueness of our genome-wide screening methodology in identifying new antiviral components. To validate the significance of these components in restricting RSV infection, we knockedout (KO) the identified genes in A549 cells. Subsequently, treated them with IFN and infected them with RSV. Cells with the KO exhibited increased susceptibility to RSV infection. Finally, we investigated whether additional respiratory viruses are susceptible to the same antiviral action of the Hippo pathway. These studies will reveal the mechanisms underlying the involvement of the Hippo pathway in the host immune response against respiratory viruses, providing new opportunities for developing host-based antiviral strategies.

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07 Viruses of eukaryotes

## Single-cell RNA-seq of the rare virosphere reveals the native hosts of giant viruses in the marine environment

**Amir Fromm**[1], Assaf Vardi[1]
*Department of Plant and Environmental Sciences, Weizmann Institute of Science, Israel*

Giant viruses are globally distributed in marine ecosystems and play a significant role as evolutionary drivers of eukaryotic plankton and regulators of global biogeochemical cycles. Although recent metagenomic studies have significantly expanded the repertoire of known marine giant viruses, most of these viruses have unknown hosts, which hinders our ability to understand their lifecycle and assess their ecological importance. In this study, we aim to discover the native hosts of giant viruses using a novel, ultrasensitive, single-cell metatranscriptomic approach. By applying this approach to natural plankton communities and utilizing specific gene markers for giant viruses, We detected hundreds of single cells from multiple host lineages infected by diverse giant viruses. These cells included members of the algal groups Chrysophycae and Prymnesiophycae and a rare infected population of the under-studied class Katablepharidaceae. Katablepharids were infected by a strain of giant virus from a recently defined lineage (Imitervirales-07). Detection of infected Katablepharids cells enabled subsequent tracking of a high prevalence of cell-fate regulation genes in the virus that were highly expressed during infection. We further examined this host-virus dynamics in a temporal resolution and suggested that this giant virus controls the rise and demise of its host population. Our results demonstrate how single-cell metatranscriptomics is a powerful approach to linking viruses with their authentic host and studying their ecological significance in a culture-independent approach in the marine environment.

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## Quantification of Herpes simplex viruses recombination rates using an AI algorithm

**Maya Ralph-Altman**[1], Daniel Avhar[1], Yuval Altman[1],
Enosh Tomer[1,2], Oren Kobiler[1]
[1]*Clinical Microbiology and Immunology, School of Medicine, Tel Aviv University, Israel*
[2]*The Central Virology Laboratory, Public Health Services, Ministry of Health, Sheba Medical Center, Tel-Hashomer, Israel*

Herpesviruses are a common group of large double stranded DNA viruses. Herpes simplex virus-1 is an abundant human pathogen that also serves as a model for replication for this group of viruses. Recombination is a major driving force of evolution of herpesviruses. Both intragenomic and intergenomic (between coinfecting viruses) recombination were observed both in vivo and in vitro. However, recombination is thought to be tightly associated with replication, thus unique mechanisms involved in only in viral recombination were hard detect. Here, we developed a relatively robust and sensitive assay for detection and quantification of viral recombination. The assay is based on triple fluorescent genes incorporated into the viral genomes to identify homologous and non-homologous recombination events between co-infecting viruses. To obtain unbiased quantification of the recombination rates, we used a machine learning algorithm that classifies viral plaques according to their colors. This setting allowed us to test several perturbations of the infection process and measure the recombination rates from thousands of plaques from each condition. The rates of non-homologous recombination levels were too low and noisy to detect significant changes resulting from the different treatments, whereas the homologous recombination rates were high and robust, allowing for the detection of significant changes following the different perturbations.

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## Structural insights into SARS-CoV-2 neutralization by anti-SARS-CoV-2 antibody isolated from severe convalescent donor

**Aakanksha Harit**[1], Michael Mordekovich[2], Lee S. Izhaki-Tavor[1], Natalia Freund[2], Moshe Dessau[1]
[1]*Azrieli Faculty of Medicine, Bar-Ilan University, Israel*
[2]*Faculty of Medical & Health Sciences, Tel Aviv University, Israel*

The COVID-19 pandemic, caused by the newly emerged SARS-CoV-2, posed serious threat to public health and global economy. The interplay between SARS-CoV-2, neutralizing antibodies and immune cells contributes to pathogenesis and protective immunity against the disease. The spike (S) protein of SARS-CoV-2, a class I trimeric membrane fusion protein, mediates the viral entry into the host cell through its receptor-binding domain (RBD). Therefore, understanding the recognition mechanisms of potentially neutralizing monoclonal antibodies (mAbs) against the receptor-binding domain (RBD) becomes very essential for novel developments in SARS-CoV-2 biology and immunology. Recent studies have demonstrated multi-clonal neutralizing responses against SARS-CoV-2 by antibodies isolated from donors with severe COVID-19 manifestations. In the present study, we report the crystal structure of Fab fragment of TAU-1109, a potent neutralizing antibody isolated from severe convalescent donor, in complex with the receptor-binding domain (RBD) of wild-type SARS-CoV-2 at 2.56 Å resolution. Interestingly, the high molecular resolution structure provides insights of a unique binding epitope for this anti-SARS-CoV-2 neutralizing antibody. Hence, our study demonstrates additional mechanistic understanding of antibody neutralization of SARS-CoV-2 and paves the way towards in SARS-CoV-2 immunology.

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## The FDA-Approved Drug Amiloride is a Potent Inhibitor of Sandfly Virus Replication

**Yarden Moalem**[1], Prof. Ella Sklan[1]
*Microbiology and Clinical immunology, Faculty of Medical & Health Sciences, Tel Aviv university, Israel*

Amiloride, an FDA-approved ion channel inhibitor clinically used as a diuretic for treating high blood pressure, has been found to inhibit the replication of several positive-strand RNA viruses. Mechanistically, amiloride binds to conserved structured elements within the 5′-end viral RNA of coronaviruses and/or acts as a competitive inhibitor of nucleoside triphosphates (NTPs) and $Mg^{2+}$ in coxsackievirus B3, affecting both VPg uridylation and RNA elongation.

Here, we present preliminary results suggesting that amiloride and its derivatives are potent inhibitors of sandfly Toscana virus (TOSV), a neurovirulent tri-segmented single-strand negative-sense RNA genome, as well as other bunyaviruses. These results indicate that amiloride might affect these viruses through additional or broader modes of action. Interestingly, amiloride also inhibited the replication of the vesicular stomatitis virus, a single-stranded negative-sense RNA virus. However, amiloride did not affect the activity of T7 polymerase, a bacteriophage DNA-dependent RNA polymerase, suggesting a possible specificity towards RNA-dependent RNA polymerases. Attempts to create amiloride-resistant TOSV using serial passaging are ongoing and are expected to reveal further mechanistic details. Taken together, our approach may reveal the mode of action of a broad-spectrum antiviral.

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# Characterization of neutralization epitopes on the envelope protein of the Hepatitis C virus

**Manar Alkhateb**[1], Haneen Faris-Gadban[1], Noam Ben Shalom[2], Natalia T. Freund[2], Meital Gal-Tanamy

[1]*Molecular Virology Lab, Azrieli Faculty of Medicine in Galilee, Bar Ilan University, Israel*

[2]*Department of Microbiology and Immunology, Sackler School of Medicine, Tel Aviv University, Israel*

Background & Aims: Hepatitis C virus (HCV) is a major public health problem, with over 70 million people infected worldwide. HCV infection can progress to cirrhosis and hepatocellular carcinoma. No vaccine is available, and immunity against the virus is not well understood. Approximately 25% of infected patients clear the virus spontaneously. Therefore, we aim to generate and investigate antibodies specific for immunodominant epitopes on the HCV-E2 envelope protein associated with viral clearance.

Methods: Previously, we identified a broadly neutralizing epitope for HCV that is associated with HCV clearance, termed as SC-Unique epitope 1. Here, we aim to characterize SC-Unique-epitope-1 by generating specific monoclonal antibodies. Antigen-specific B cells from SC-Unique-epitope-1-immunized mice were isolated by FACS, and transcripts encoding the antibodies` variable regions were amplified by RT-PCR and cloned into expression vectors for antibody production. The constructed antibodies characterized using ELISA and Neutralizing assays. We also performed competition assays to define their antigenic regions.

Results and Conclusion: We generated 10 monoclonal antibodies. Few of these share identical alleles for V and J locus and a similar complementarity-determining-region-3, which indicates a common clone. Some of these antibodies showed specific binding to E2-protein and SC-Unique-Epitope1-peptide, and high neutralization breadth for the seven genotypes of HCV, and for the HCVpp panel. Competitive ELISA revealed no binding competition between the isolated antibodies, and known antibodies to other epitopes, while competition with antibodies that share residues with SC-Unique-epitope-1 was detected. These antibodies will aid in further evaluating their epitopes, guiding the design of an effective HCV vaccine.

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# Structural studies of Thrips Cyclophilin A, an Interactor of Tomatto Spotted Wilt Virus (TSWV) Glycoprotein N

**Adarsh Patel**[1], Marlonni Arajo[2], Anna Whitfield[2], Moshe Dessau[1]
[1]*Faculty of Medicine, Bar Ilan University, Israel*
[2]*Entomology and Plant Pathology, North Carolina State University, United Stated of America*

Members of the Tospoviridae family poses a serious threat to global food security as it endangers the health of many field crops. The representative member of this family, the Tomato Spotted Wilt Virus (TSWV), primarily infects plants through the western flower thrips (Frankliniella occidentalis), resulting in decreased crop yield that leads to an economical burden. The TSWV envelope glycoprotein N (GN) facilitates entrance into the insect host cell and is a crucial for the viral life cycle. Recent studies have shown that the thrips' Cyclophilin A interacts with TSWV GN. To better understand the mechanisms behind TSWV viral entry and infection, structural studies of the proteins involved in the host-virus interaction offer a powerful tool for visualizing and understanding these interaction at the atomic level. In this study, we report the crystal structure of Cyclophilin A of thrips at 1.5 Å resolution which is the first structure of thrips origin in the database. Interestingly, the crystal packing allows a direct view into the active site which harbors, in our case, a substrate peptide. Our high-resolution data will set the framework to develop new approaches to safeguard crops against tospovirus infections, ultimately assisting in tackling the problems associated with food security.

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## Structural-Evolution Investigation of Phenuiviridae Envelop Protein

**Suryakant Tiwari**[1], Hossin Bouz[1], Joel Alter[1], Moshe Dessau[1]
*Faculty of medicine, Bar-Ilan University, Israel*

Arboviruses are evolving pathogens causing various illnesses in animals and plants, which can lead to health crises and significant economic burdens. Unlike many insect-borne animal pathogens, there are many arboviruses that are restricted to their insect host. Viral envelope glycoproteins have a key role in recognizing the host-cell attachment and internalizing the virus particle into the host cell, followed by membrane fusion releasing the viral genome. We investigate the emerging insect-restricted Badu phasivirus (BADUV) of family Phenuiviride order: Bunyavirales, close related to other infectious viruses of the same family. The structural basis of evolutionary processes of virus host selection and restriction remains poorly understood. In this context, the two enveloped glycoproteins, GN and GC, emerge as pivotal in virus cell entry. Understanding the mechanistic roles of GN and GC is essential for unraveling evolutionary dynamics shaping virus-host interactions. Using structural and function approaches, we aim to depict a detailed mechanistic narrative of BADUV's entry process, highlighting the role of GN and GC. We characterized and measure the activity of these glycoproteins` ability to cell membrane fusion process in different cell lines. In parallel, we were able to crystallize ectodomains of GN and GC. In the foreseeable future, we will determine GN and GC structure unraveling the new aspects of the mechanism of virus host-range selection. Deciphering the structural basis of the mechanistic differences between human-infecting viruses and insect-restricted viruses will unravel novel evolutionary paths of virus host-range selection.

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# Interaction of the Kaposi’s sarcoma-associated Herpesvirus vBcl-2 with the Cellular Protein KLHL-9

**Sapir Ohev**[1], Anastasia Gelgor Dontsov[1], Coral Haddad[1], Inna Kalt[1], Ronit Sarid[1]
*Life Sciences, bar ilan university, israel*

Kaposi`s sarcoma-associated herpesvirus (KSHV) is an oncogenic virus causally associated with several human cancers. The vBcl-2 protein encoded by KSHV is a structural and functional homologue of the Bcl-2 family, capable of negatively regulating apoptosis and autophagy. Expression of vBcl-2 is essential for the production of infectious progeny during lytic reactivation; yet, this essential role does not depend on its cell death regulatory functions.

In this study, we report the identification of an interaction between KSHV vBcl-2 and KLHL-9, and potentially with Cullin 3 (CUL3). CUL3 participates in ubiquitin-mediated cellular processes, acting as a scaffold that binds a RING protein to catalyze the transfer of activated ubiquitin, along with various substrate adaptors and/or receptor proteins such as KLHL-9. The interaction was initially identified by mass spectrometry and confirmed by co-immunoprecipitation in uninfected cells and during lytic reactivation. Immunofluorescence assays demonstrated vBcl-2 and KLHL-9 colocalization during infection. Using a panel of vBcl-2 mutants, we mapped the interaction to amino acid 16-20 and 26-30 of vBcl-2. In line, vBcl-2 mutants that failed to interact with KLHL-9 did not colocalize with KLHL-9. Knockout of KLHL-9 resulted in decreased expression of lytic viral proteins and reduced production of infectious progeny viruses, while KLHL-9 complementation reversed this phenotype. Notably, this finding is similar to the phenotype observed with vBcl-2-null viruses, suggesting that the interaction of vBcl-2 with KLHL-9 may have essential functions during infection.

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## Fate of the KSHV Tegument Proteins ORF45 and ORF52 During de novo Infection

**Hanan Abu-Hassan**[1], Dana Dünn-Kittenplon[1], Inna Kalt[1], Ronit Sarid[1]
*Life-science, Bar-Ilan University, Israel*

Kaposi`s sarcoma-associated herpesvirus (KSHV) is an oncogenic virus associated with Kaposi's sarcoma. The KSHV infectious particles consist of double-stranded DNA enclosed within an icosahedral capsid, which is coated with a tegument layer and wrapped within a phospholipid envelope.

This study focused on two tegument proteins ORF45 and ORF52 during de novo infection of SLK cells, where KSHV establishes latency. ORF45, an immediate-early lytic protein, inhibits host type I interferon induction, while ORF52, a late lytic protein, suppresses the enzymatic activity of cGAS. Confocal microscopy revealed that capsids docked at nuclear pores lacked both ORF45 and ORF52, indicating their dissociation from the capsids before nuclear docking. ORF45 accumulated in the nucleus over time, whereas ORF52 initially surrounded the nucleus before diminishing. Cycloheximide treatment abolished ORF45 nuclear accumulation, confirming its de novo expression post-infection. Western blot analysis revealed an early increase in ORF45 followed by a decline, along with evidence suggesting post-translational modifications. No expression of the late lytic protein ORF65 or infectious virion production was observed. Cells treated with endosome acidification inhibitors demonstrated no dissociation of either ORF45 or ORF52 from the capsids, which failed to dock at the nuclear pores. This hindered the newly expressed ORF45 and the establishment of latent infection.

Taken together, our findings reveal the transient expression of ORF45 post-infection prior to the establishment of KSHV latency, and highlight that the dissociation of ORF45 and ORF52 from capsids depends on endosome acidification.

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08 Plant-microbe interactions

## Exploring Wheat Rhizosphere Microbiome to Enhance Sustainable Production

**Sharon Samuel**[1], David Zeevi[1], Avraham Levy[1]
*Plant and Environmental Sciences, Weizmann Institute of Science, Israel*

Beneficial soil bacteria are essential for agricultural sustainability, as they enhance nutrient uptake and utilization by plants. The rhizosphere, the narrow soil zone around plant roots, serves as a central hub for these advantageous interactions, thereby regulating plant health and productivity. These symbiotic relationships have evolved over millions of years, yet they are assumed to be influenced by domestication and modernization. This is particularly crucial for staple crops like wheat, which sustains about 35% of the world's population. This research aims to explore how domestication-driven changes in wheat, including genetic variation reduction and excess fertilizer use, have impacted the composition of the rhizosphere microbiome, specifically the bacterial community, and potentially reduced beneficial relationships. By comparing ancient and modern wheat cultivars, we aim to determine whether these changes have altered the bacterial diversity and function within the rhizosphere. In this study, 12 wheat lines were grown in different soil types and fertilization levels, with rhizosphere samples collected for metagenomics analysis to focus on bacterial populations. Preliminary observations indicate variations among wheat lines, suggesting differing dependencies on soil bacteria or fertilizers for optimal growth. Understanding the dynamics between bacterial communities, wheat genetics, and environmental conditions can provide insights into enhancing sustainable wheat production through microbial management

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08 Plant-microbe interactions

## Systematic exploration of plant-microbe antagonism using the unicellular algae *Chlamydomonas reinhardtii.*

**Tzila Vikniansky**[1], Elad Meilin, Naomie Alon, Dr. Michal Breker, Dr. Omri Finkel
*Department of Plant and Environmental Sciences, The Hebrew University of Jerusalem, Israel*

Microorganisms dramatically influence plant growth, development and resilience. Research on negative microbial effects on plants is largely focused on acute plant diseases, but many microbes in the soil can cause more subtle, chronic effects on plants. In order to systematically delve into the complexity of the plant-microbiota interaction network, we established unique genome-wide approaches utilizing the unicellular green algae *Chlamydomonas reinhardtii*. We developed both agar-based and soil-based assays and screened ~150 bacterial strains isolated from *Arabidopsis thaliana* roots for effects on *Chlamydomonas* growth. Interestingly, we found a strong correlation between antagonism towards both *Chlamydomonas* and *Arabidopsis*. We identified eight bacterial strains that exhibited antagonism towards *Chlamydomonas*, seven of which were pathogenic to *Arabidopsis*as well. Next, we screened UV-mutagenized *Chlamydomonas* strains for colonies resistant to bacterial growth inhibition, and detected several candidates. In parallel, in order to identify genes important for healthy plant-microbiome interactions, we grew a large null mutant *Chlamydomonas* collection on sterilized and unsterilized soil. After 2 weeks of growth, *Chlamydomonas* mutants were collected and sequence for relative abundance. *Chlamydomonas* mutants that were underrepresented in non-sterile soil were considered to be disrupted in a gene important for plant-microbiome interactions. Such genes which have *Arabidopsis* homologs were chosen for further investigation in order to see whether they influence *Arabidopsis*-microbiome interactions as well. Genetic characterization to follow in both algae and bacteria should be a promising path to elucidate additional pathogenic mechanisms. Target genes identified can be potential subjects for genetic engineering to improve crop yield and fitness.

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## Synergistic Interactions in Duckweed Microbiome Facilitate Cobalamin Production

**Dutta Rhishika**[1], Osnat Gillor[1], Inna Khozin-Goldberg[1]
*Zuckerberg Institute of Water Research, Ben Gurion University of the Negev, Israel*

Duckweed, the smallest aquatic flowering plant, boasts a rich nutritional profile with nine essential amino acids, polyphenols, iron, dietary fibers, and omega-3 and omega-6 fatty acids. Wolffia globosa Mankai, a high-yielding duckweed species, was recently discovered to contain a water-soluble, bioavailable vitamin B12, also known as cobalamin. We hypothesized that the cobalamin in duckweed is produced by the plant endophytes to maintain the plant microbiome. To test our hypothesis, we first characterized the diversity and function of the endophytes in W. globosa Mankai, focusing on the biosynthesis of cobalamin. We sequenced the genomes of the endophytes and identified genes related to the cobalamin pathway. Our results suggested that the microbiome of W. globosa Mankai comprises a unique community distinct from the external medium and the epiphytic community. This microbiome consists of approximately 30 species, mainly affiliated with the phylum Proteobacteria. Surprisingly, the genes encoding cobalamin synthesis were distributed among different bacteria within the microbiome, each containing portions of the pathway. We hypothesized that the synergy between these bacteria results in the production of cobalamin. To test this, we selected the five most abundant bacterial strains detected in the duckweed: Caulobacter vibrioides, Hyphomicrobium zavarzinii, Hydrotalea flava, Sphingopyxis terrae, and Methylobacterium oryzae. We found that only one of these strains could grow in the absence of cobalamin, indicating an inability to synthesize this essential vitamin when grown alone. We tested the synergistic growth among the strains by growing them in pairs in all possible iterations. As a control, we added cobalamin to the medium or used the known cobalamin-synthesizing strain Mesorhizobium loti. Our results showed that certain combinations led to mutual growth of the strains and successful synthesis of cobalamin. Our study demonstrates the crucial role of synergistic interactions within the duckweed microbiome for the production of essential nutrients like cobalamin. This highlights the potential for utilizing plant-microbe partnerships in enhancing nutrient availability suggesting new avenues for agricultural and biotechnological applications.

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09 The microbiome

## Establishing an experimental system to investigate sponge-microbe recognition mechanisms

**Chiara Conti**[1], Sofia Sizikov[1], Tzipora Peretz[1], Sagie Schif[2], Rinat Bar-Shalom[1], Laura Steindler[1]

[1]*Department of Marine Biology, Leon H. Charney School of Marine Sciences, University of Haifa, Israel*

[2]*Department of Biology, Faculty of Natural Sciences, University of Haifa, Israel*

Marine sponges, by filtering seawater, feed on particulate matter, including bacteria. Yet, they harbour a phylogenetically diverse and stable symbiotic microbial community. How can these animals discriminate between food bacteria and bacterial symbionts? Sponges are complex models to study, also due to the lack of sponge cell cultures and the uncultured nature of their symbionts. Accordingly, most present studies are based on culture-independent techniques (-omics studies) which resulted in diverse theories on the recognition mechanisms between sponge hosts and their microbiome. Metagenomic studies have found that sponge-associated bacteria, compared to free-living relatives, display genetic adaptations to the sponge-environment. For example, they lack genes for the biosynthesis of L-rhamnose, which is part of the lipopolysaccharide structure found on the membrane of free-living bacteria. It has been hypothesized that lacking L-rhamnose helps the symbiont escape from host phagocytosis. The focus of my research is to establish an experimental system to test these hypotheses. Our approach is to use ‘symbiont mimics’- free-living bacteria, phylogenetically closely related to sponge symbionts, that are culturable and can be genetically modified. We designed a knockout mutant of the free-living cyanobacterium Syenchoccoccus WH8102, that lacks the gene that codes for L-Rhamnose. We then compared phagocytosis of Syenchoccoccus WH8102 wild type and mutant and show that the mutant lacking L-rhamnose are less prone to be phagocytosed by the animal.

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## Reverse-engineering the structure of social networks via microbial strain-sharing

**Amiyaal Ilany**[1], Prameek Kannan[1], Omry Koren[2]
[1]*Faculty of Life Sciences, Bar-Ilan University, Israel*
[2]*Faculty of Medicine, Bar-Ilan University, Israel*

Understanding the intricacies of animal social interactions and population connectivity is crucial for the study of many aspects of social behavior, yet direct observation and genetic analyses often present logistical challenges. We introduce an approach leveraging the strain-sharing of microbes as indirect indicators of social behavior and connectivity among animal populations. By analyzing the microbiomes of individual rock hyraxes from several groups in a population using shotgun metagenomics, we identify shared microbial strains that suggest historical or ongoing social interactions. This microbial fingerprinting allows us to infer patterns of social association and movement across landscapes that are otherwise difficult to document. This approach offers a unique lens through which to view the social and ecological dynamics of animal populations, providing insights into the unseen connections that bind individuals and populations. Our approach has implications for understanding disease transmission dynamics, conservation strategies, and the evolution of social behavior.

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## Microbial dark matter sequences verification in amplicon sequencing and environmental metagenomics data

**Hana Barak**[1], Naomi Fuchs[2], Michal Liddor-Naim[2], Irit Nir[2], Alex Sivan[2], Ariel Kushmaro[2]

[1]*Department of Civil and Environmental Engineering, Ben Gurion University, Israel*
[2]*Avram and Stella Goldstein-Goren Department of Biotechnology Engineering, Ben Gurion University, Israel*

Although microorganisms constitute the most diverse and abundant life form on Earth, in many environments, the vast majority of them remain uncultured. As it is based on information gleaned mainly from cultivated microorganisms, our current body of knowledge regarding microbial life is partial and does not reflect actual microbial diversity. That diversity is hidden in the uncultured microbial majority, termed by microbiologists as “microbial dark matter” (MDM), a term borrowed from astrophysics. Metagenomic sequencing analysis techniques (both 16S rRNA gene and shotgun sequencing) compare gene sequences to reference databases, each of which represents only a small fraction of the existing microorganisms. Unaligned sequences lead to groups of “unknown microorganisms” that are usually ignored and rarefied from diversity analysis. To address this knowledge gap, we analyzed the 16S rRNA gene sequences of microbial communities from four different environments—a living organism, a desert environment, a natural aquatic environment, and a membrane bioreactor for wastewater treatment. From those datasets, we chose representative sequences of potentially unknown bacteria for additional examination as “microbial dark matter sequences” (MDMS). Sequence existence was validated by specific amplification and re-sequencing. These sequences were screened against databases and aligned to the Genome Taxonomy Database to build a comprehensive phylogenetic tree for additional sequence classification, revealing potentially new candidate phyla and other lineages. These putative MDMS were also screened against metagenome-assembled genomes from the explored environments for additional validation and for taxonomic and metabolic characterizations. This study shows the immense importance of MDMS in environmental metataxonomic analyses of 16S rRNA gene sequences and provides a simple and readily available methodology for the examination of MDM hidden behind amplicon sequencing results.

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## Agricultural ecosystem restoration increases soil microbial diversity and reduces nitrogen loses

**Sweta Kumari**[1], Max Kolton[1], Orah Felicia Rein-Moshe[2]
[1]*The Jacob Blaustein Institute for Desert Research, Ben-Gurion University of the Negev, Israel*
[2]*Soil Erosion Research Station, State of Israel Ministry of Agriculture and Rural Development, Israel*

Microbial communities regulate ecosystem services and sustain ecological balances. However, human population growth, higher food demands, and environmental changes impair these balances. Intensive agricultural production has degraded soil health. Therefore, soil restoration is necessary to enhance ecosystem services and recover biodiversity. Despite the central role of microbes in ecosystem functioning, assessments of their contributions to restoration are limited. Thus, to quantify microbial contributions to soil restoration and ecosystem services, we compared the effects of soil management practices on soil properties, microbial activity and biodiversity, and ecosystem functions. A 1 km stretch of the Nahalal river bank, Israel, was divided into ten plots and investigated comparing a conventional Agriculture Treatment (wheat crop) and a Restoration Treatment (mix of 8 seeded native plants). A significant effect of soil management practices was observed on microbial abundance, diversity, and activities after 3 years of restoration efforts. Restored soils hosted a higher number and diversity of culturable bacteria, while agricultural soils exhibited higher potential nitrification rates and nitrogen losses. Restored soils also showed potential for utilizing complex carbon efficiently and storing it, suggesting long-term carbon sequestration. These findings show the potential of soil microbes to maintain the multifunctionality of Ecosystem Restoration. These findings support development of new guidelines to enable policymakers to incorporate into future soil ecological restoration programs.

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# Bacterial DNA inversions and functional plasticity during inflammation and encounter with bacteriophages

**Shaqed Carasso**[1], Rawan Zaatry[1], Haitham Hajjo[1], Dana Kadosh-Kariti[1], Nadav Ben-Assa[1], Rawi Naddaf[1], Noa Mandelbaum[1], Sigal Pressman[2], Yehuda Chowers[2], Tal Gefen[3], Kate Jeffrey[4], Juan Jofre[5], Michael Coyne[6], Laurie Comstock[6], Itai Sharon[7], Naama Geva-Zatorsky[3]

[1]*Medicine, Technion - Israel Institute of Technology, Israel*
[2]*Clinical Research Institute, Rambam Health Care Campus, Israel*
[3]*Medicine, Technion - Israel Institute of Technology, Israel*
[4]*Program in Immunology, Harvard Medical School, United States of America*
[5]*School of Biology, University of Barcelona, Spain*
[6]*Duchossois Family Institute and Department of Microbiology, The University of Chicago, United States of America*
[7]*Molecular and Computational Biosciences and Biotechnology, Migal Galilee Technology Center, Israel*

DNA inversions play a key role in governing bacterial molecular phase-variations, often involving promoter regions dictating transcription by functioning as `ON`/`OFF` switches of outer-surface components which can interact with the host immune-system. The gut microbiota is considered as a major environmental factor in inflammatory bowel diseases (IBD). A mechanistic analysis of potential bacterial functional alterations will allow us to better understand the role of the gut microbiota in IBD.

We have analyzed publicly available metagenomic databases to identify and quantify the prevalence of DNA inversions in the Bacteroidales order. Additionally, we examined the patterns of DNA inversions in humanized mice under chemically-induced gut inflammation. Specifically, we focused on associations between genomic invertible regions with potential effects on gut inflammation.

Our findings revealed that gut Bacteroidales exhibit distinct DNA inversion patterns in response to inflammation, both in mouse models and in IBD patients. We identified DNA inversions controlling the expression of various outer-surface components, including the anti-inflammatory polysaccharide-A (PSA) of Bacteroides fragilis. We also detected increased abundances of B. fragilis associated bacteriophages in patients with the PSA ‘OFF’ orientation. Isolation of a B. fragilis associated bacteriophage revealed that it induced the PSA ‘OFF’ switch, accompanied by an alteration of the bacterial induced immune modulation.

We reveal large-scale dynamic and reversible bacterial phase variations driven both by bacteriophages and the host inflammatory state signifying gut bacterial functional plasticity. Our results have implications for understanding the interplay between gut inflammation, bacteriophages, and bacterial phase variation, particularly in the context of IBD.

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## Metaproteomic Analysis of Immunotherapy Modulation by the Microbiome

**Tal Nissenbaum**[1], Yochai Wolf[2], Lior Lobel[1]
[1]*The Goodman Faculty of Life Science, Bar-Ilan University, Israel*
[2]*The Ella Lemelbaum Institute for Immuno-oncology, Sheba Medical Center, Israel*

The gut microbiome has a crucial role in modulating various physiological processes, including the initiation and progression of cancerous tumors. Previous studies showed the potential of harnessing the microbiome to fight cancer. However, these studies provided limited insights, as they were based on metagenomic sequencing alone. We employed a metaproteomic approach to identify differentially expressed proteins and metabolic pathways in stool samples from melanoma patients responding to immune checkpoint blockade (ICB) therapy and non-responding, to gain deeper insights into the molecular mechanisms.

Proteins and DNA were extracted from stool samples, and tryptic peptides derived from protein fractions were labeled with tandem mass tags (TMT) for multiplexed LC-MS3 analysis. Quantitative analysis was conducted using Fragpipe, and statistical analyses were performed with MSstatsTMT using R. A comprehensive cohort-specific protein database for metaproteomic analysis was generated by shotgun DNA sequencing.

Our metaproteomic analysis unveils a wealth of new information, shedding light on how the microbiome influences the response to ICB. We`ve discovered hundreds of differentially abundant bacterial proteins and dozens of host proteins in responder versus non-responder stool samples. Moreover, we`ve unearthed unique expression patterns in bacterial metabolic pathways between the microbiomes of responders and non-responders: the glycolysis and methanogenesis pathways. The glycolysis pathway is not only biologically intriguing but also a testament to the strength of our approach, as metagenomic analyses failed to identify such an enrichment due to the ubiquitous nature of glycolysis genes.

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09 The microbiome

## The microbiome affects obesity-related metabolites in the process of aging

**Dana Binyamin**[1], Sondra Turjeman[1], Nofar Asulin[1], Ron Schweitzer[1,2], Omry Koren[1]
[1]*Azrieli Faculty of Medicine, Bar-Ilan University, Israel*
[2]*Galilee Research Institute, MIGAL, Israel*

Introduction:

Aging involves changes in the gut microbiome that impact health and longevity, however which microbial metabolites play a role remain understudied. Here we examine the contribution of the microbiome to the metabolic profile in aged mice.

Methods:

Fecal samples were collected from 8-week-old (young) and 18-month-old (aged) Swiss-Webster mice raised conventionally (Conv) or germ free (GF). Fecal samples were also collected from Conv mice after antibiotic treatment. Bacterial DNA from fecal samples were sequenced and microbiome analysis was done using QIIME2. Liquid chromatography mass spectrometry was used for untargeted metabolomics on Conv, before and after antibiotic treatment, and GF samples.

Results:

Significant differences were observed in bacterial composition and predicted functional pathways between young and aged mice. Age-related metabolome variance was greater in Conv compared to GF mice, highlighting the microbial contribution to metabolic changes. Moreover, microbiome-associated metabolites, predominantly lipids, were higher in aged mice compared to young mice, with the linoleic acid metabolism pathway being enriched in the aged group. A decrease in these metabolites was also observed in aged mice after antibiotic treatment.

Conclusions:

These findings underscore the intricate relationship between aging, the gut microbiome, and metabolic changes. The observed age-related shifts in bacterial composition and metabolites, coupled with the enrichment of microbiome-associated lipids in aged mice, emphasize the potential role of the gut microbiome in modulating metabolic health during aging.

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10 Applied microbiology and biotechnology

## A novel method for microbial pathogenic cultures inactivation

**Dror Halachmi**[1], Mahmoud Abu-ghannam[1], Marina Feingold[1], Amal Shibli[1], Elad Bar-Cohva[1]
*Biotechnology Production, Merck Sigma-Aldrich Israel, Israel*

Sodium hypochlorite (bleach/NaClO) or Sodium hydroxide (NaOH) are common chemicals used for inactivation of live microbial cultures. The scientific literature harbors a lot of experimental data regarding inactivation of microorganism cultures using bleach or NaOH. Such inactivating solutions can be very efficient in a small-scale laboratory. On the other hand, when there is a demand to inactivate large volumes of microbial culture (I.e., at the biotechnology industry) there are limitation enforced by the local regulation prohibiting discard of large volumes of Sodium ion solutions to the municipal sewage. Exceeding the acceptable concentrations may result in harsh sanctions being taken against the polluting plant.

In contrast, Potassium hydroxide (KOH) can be used for inactivation of microbial cultures. And there are no threshold limits for potassium ions disposed into the municipal sewage. Hence neutralization with KOH in much larger quantities can be used.

The scientific literature is lacking information regarding the effectiveness of the neutralization of cultures of microorganisms with KOH. Therefore, our work was targeted to test the effectiveness of KOH inactivation of several microbial cultures (some are pathogens and even defined as “selected Agents”). Such as Bacterial strains (I.e., Proteus vulgaris, Micrococcus lysodeikticus, Bacillus subtilis, Staphylococcus aureus and Vibrio cholera). Streptomyces strain (S. roseus) or Fungi (Yeasts and Molds) – Pichia pastoris, Engyodontium album.

In our experiment all cultures were subjected for incubation with the inactivating agents (I.e., NaClO 0.5% or 1.0%; NaOH 0.1M and KOH 0.5M). Incubation period ranged between 2 or 5 hours or for Overnight (ON). Incubation was performed at room temperature.

Our results show that 5 hours incubation with KOH 0.5% are sufficient to achieve full inactivation of 90% of the tested cultures. Only the spore forming Bacillus subtilis survives the treatment even after overnight treatment.

In Conclusion it seems that using KOH to inactivate large volumes of microbial cultures is a good alternative for avoiding using Sodium hypochlorite or Sodium hydroxide.

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# Pipeline for rapidly evaluating activity and inferring mechanisms of action of prospective antifungal compounds

**Judith Kraut-Cohen**[1], Omer Frenkel[2], Shay Covo[3], Evgeniya Marcos-Hadad[3], Shmuel Carmeli[4], Eduard Belausov[5], Dror Minz[6], Eddie Cytryn[6]

[1]*Institute of Soil, Water and Environmental Sciences, Agricultural Research Organization, Volcani Center, Israel*

[2]*Department of Plant Pathology and Weed Research, Institute of Plant Protection, Agricultural Research Organization, Volcani Center, Israel*

[3]*Department of Plant Pathology and Microbiology, Robert H. Smith Faculty of Agriculture, Food and Environment, The Hebrew University, Israel*

[4]*Raymond and Beverly Sackler School of Chemistry, Faculty of Exact Sciences, Tel Aviv University, Israel*

[5]*Confocal Microscopy Unit, Agricultural Research Organization, Volcani Center, Israel*

[6]*Institute of Soil, Water and Environmental Sciences, Agricultural Research Organization, Volcani Center, Israel*

Fungal phytopathogens pose a serious risk to agricultural production and food security and effective, environmentally sustainable compounds are urgently needed. However, in-planta screening assays that identify of antifungal compounds are extremely laborious and time-consuming, hindering the capacity for high throughput evaluation of potential candidates.

We developed an in-vitro screening pipeline that integrates five rapid quantitative and qualitative methods to evaluate the efficacy and mode of action of potential antifungal compounds. Proof of concept was achieved by screening five known antifungal compounds (benomyl, catechol, cycloheximide, 2,4-diacetylphloroglucinol, and phenylacetic acid) against the model soil borne fungal pathogen Fusarium oxysporum f. sp. radicis cucumerinum (FORC). The capacity of these compounds to inhibit sporulation and hyphae growth, and impede fungal metabolic activity was achieved using GFP-labeled FORC and PrestoBlue™-labeled-FORC multiwell plate assays, respectively. This high-throughput approach enabled us to test several compound`s concentrations and incubation times, simultaneously. Subsequently, we employed Fun-1 and SYTO9/propidium iodide staining followed by confocal microscopy, to distinguish between fungal growth suppression and cellular death. Finally, we applied a reactive oxygen species (ROS)-detection assay to quantify ROS production in response to compound application. The proposed pipeline provides a rapid means for screening natural or synthetic compound libraries for antifungal activity, enabling the selection of the most promising candidates, which can be tested for in-planta antifungal activity.

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## Utilizing the beneficial bacterium B. subtilis to reduce chemical and biological corrosion.

**Avichay Nahami**[1,2], Yosi Shacham[1], Yuval Dorfan[2], Ilana Kolodkin-Gal[1]
[1]*Scojen institute for synthetic biology, Reichman, Israel*
[2]*Electrical engineering, HIT, Israel*

Corrosion of metallic materials causes significant socio-economic damage and contributes to about one-third of the global annual scrap metal generation. The impact of corrosion extends beyond economic losses, posing environmental risks like health hazards, pollution, and releasing toxic substances. Our results suggest that B. subtilis can inhibit both chemical corrosion and microbially induced corrosion robustly and that this suppression could be enhanced with the support of a community member from the seawater. We now aim to analyze seawater microbiomes for effective corrosion prevention strategies in marine environments and to identify symbiotic interactions that contribute to the oxidation of carbon steel. The goal is to create sustainable methods for protecting metals against corrosion.

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# Crustaceans as a source of new bistable rhodopsins for optogenetics

**Alina Pushkarev**[1], David Buhrke[1], Johannes Vierock[1], Peter Hegemann[1], Camille Brouillon[1,2], Sonja Kleinlogel[2], Moran Shalev Benami[3], Marjorie Lienard[4], Megan Porter[5]

[1]*Experimental Biophysics, Humboldt University of Berlin, Germany*
[2]*Institute of Physiology, University of Bern, Switzerland*
[3]*Department of Chemical and Structural Biology, Weizmann Institute of Science, Israel*
[4]*Department of Biology, University of Lund, Sweden*
[5]*Department of Biology, University of Hawai'i at Mānoa, United States*

Bistable rhodopsins are important for use in the field of optogenetics for controlling neuronal activity by expressing light-activated proteins in neurons. Bistable rhodopsins are useful since two wavelengths can be used to elicit different reactions from one protein. Only a few bistable rhodopsins were characterized so far. Stomatopod crustaceans (a famous member is the peacock mantis shrimp) can detect wavelengths ranging from UV (310 nm wavelength) to infrared (beyond 700 nm) by using different rhodopsins and complex filtration mechanisms.

A phylogenetic tree comparing a list of ~600 crustacean opsins was created in collaboration with a group of Prof. Porter in Hawaii, and 35 genes were chosen for expression in human cells. Out of chosen genes, 11 have absorption spectra indicative of a well-folded protein. From initial biochemical characterization, we observe a variety of different mechanisms for the action of these opsins. Intriguingly, 8 of these proteins show bistable properties. We found a crustacean rhodopsin belonging to fish louse that can be expressed well in yeast and has a slow photocycle and is bistable. The wavelengths that activate and deactivate the protein are very far apart, which makes it a potential optogenetic tool in human neurons.

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# High CO2 levels increase reactive oxygen species (ROS) generation in cyanobacteria

**Myint Khin**[1]
*FAAB, Ben-Gurion University of the Negev, Israel*

Reactive oxygen species (ROS) are a naturally occurring consequence of respiration and photosynthetic electron transport in cyanobacteria. Inhealthy cells, ROS are tightly controlled to prevent ROS-caused damage of cell components. However, excessive light, UV radiation, pollutants and other environmental stressors can alter the cellular homeostasis of photosynthetic cells and lead to ROS-mediated oxidative stress. We have shown that excess availability of the primary photosynthetic substrate CO2 inhibit growth of some cyanobacterial strains presumably via the activity of BicA bicarbonate transporter. However, the inhibition mechanism remains unknown. Here, we tested the hypothesis that excess availability of CO2 causes ROS accumulation and oxidative stress in cyanobacteria. To test this hypothesis, we measured ROS production in four S. 6803 strains: (1) a wild type (WT) strain, (2) BicA+-G mutant that has an unregulated extra copy of bicA, and (3, 4) the bicA knockout mutants, ΔBicA and ΔBicAr, which were subjected to increasing concentrations of CO2. We found that some WT S. 6803 cells accumulated ROS at 5% and 10% CO2. BicA+-G cells were stressed much stronger as they accumulated ROS not only during continuous growth with high CO2 but even after short exposure to 7.5% CO2. On the contrary, DBicA and DBicAr knockout mutants exhibited minimal oxidative stress even at the highest CO2 concentrations. Our results confirm that excess availability of CO2 induces oxidative stress in S. 6803 cyanobacteria and that the stress can be linked to the activity of BicA transporter.

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## Secretion of Functional Interferon by the Type 3 Secretion System of Enteropathogenic Escherichia coli

**Ira Rostovsky**[1]
*Faculty of Health Sciences, Ben Gurion University of the Negev, Department of Microbiology and Immunology*

Type I interferons (IFN-I) – a group of cytokines with immunomodulatory, antiproliferative and antiviral properties – are widely used as therapeutics for various cancers and viral diseases. Since IFNs are proteins, they are highly susceptible to degradation by proteases and by hydrolysis in the strong acid environment of the stomach, and they are therefore administered parenterally. In this study, we examined whether the intestinal bacterium, enteropathogenic Escherichia coli (EPEC), can be exploited for oral delivery of IFN-Is. EPEC survives the harsh conditions of the stomach and, upon reaching the small intestine, it expresses a type III secretion system (T3SS) that is used to translocate effector proteins across the bacterial envelope into the eukaryotic hosst cells.

Results: In this study, we developed an attenuated EPEC strain that cannot colonize the host but can secrete functional human IFNα2 through the T3SS. We found that this bacteria-secreted IFN exhibited antiproliferative and antiviral activities similar to commercially available IFN. Conclusion: These findings present a potential novel approach for the oral delivery of IFN via secreting bacteria.

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## The effect of storage temperature on salmonella survival and VBNC state on carrots

**Chani Bronshtein**[1,2], Tehila Behar[2], Lea Chuwer[2], Carmit Ziv[2], Danielle Duanis-Assaf[2]
[1]*Food and Environment, The Robert H. Smith Faculty of Agriculture, Israel*
[2]*Postharvest and Food Science, Volcani Center, Israel*

Salmonella, a common foodborne bacterial pathogen causing acute gastroenteritis, is linked to approximately 10% of outbreaks associated with fresh produce. Studies investigating Salmonella survival on different vegetables indicated decreased viability on carrots during storage. Recently, evidence suggested that bacterial cells, including Salmonella, can enter a viable but non-culturable (VBNC) state under unfavorable conditions, posing detection and quantification challenges. This study aims to investigate Salmonella`s survival and VBNC state on fresh carrots peel during storage. Salmonella Typhimurium SL1344 was incubated at 2°C (simulating cold storage) and 22°C (simulating shelf life) in buffered peptone water (in-vitro) and on fresh carrots. Salmonella levels were quantified using colony-forming unit (CFU) and viable-qPCR assays to assess survival and identify VBNC cells. At both temperatures, there was an increase in Salmonella counts at the first three days of storage following a reduction in CFU of half-log at 22°C and 2-logs at 2°C. Interestingly, Salmonella remained culturable on carrots even after one month at 2°C. Viable-qPCR detected higher levels of viable Salmonella than CFU counts under both conditions. Significant differences were observed at 2°C, suggesting induction of VBNC state.

The difference between Salmonella growth in-vitro and on fresh carrots at 22°C for 8 days indicates that other factors, beyond temperature, may trigger the VBNC state. Overall, these results highlight the complexity of Salmonella`s survival strategies on fresh produce and emphasize the importance of employing multiple detection methods. Understanding Salmonella`s survival mechanisms on fresh produce is crucial for ensuring food safety and protecting public health.

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## Live microbiome profiling and PMA-qPCR monitoring of bacterial indicators for food safety and spoilage detection

**May Cohen Hakmon**[1], Keren Buhnik-Rosenblau[1], Hila Hanani[1], Hila Korach-Rechtman[1], Yechezkel Kashi[1]
*Biotechnology and Food Engineering, Technion, Israel*

Early detection of spoilage microorganisms and food pathogens is major in preventing food recalls and outbreaks. Despite the constant effort invested in R&D for food safety testing, none of the current methods detect food pathogens within a few hours. Herein, we suggest a novel strategy for early detection of food production defects by harnessing the product microbiome. Based on the establishment of microbiome profiling datasets of proper and defective batches, indicator bacteria signaling production errors can be identified and targeted for rapid quantification as a routine practice. Employing pastrami production as a model, we characterized its microbiome profiles along the production stages. We used propidium monoazide treatment followed by 16S rRNA gene sequencing to characterize the live microbiota. Pastrami demonstrated product-specific and consistent microbiome profiles with distinct microbial signatures identified at all production stages. Based on the established microbiome dataset, we identified shifts in the microbiome profile of a defective batch produced under potassium lactate deficiency. The most substantial shifts were observed in the relative abundances of Vibrio and Lactobacillus which were therefore defined as candidate lactate deficiency indicators. PMA-qPCR reactions efficiently detected these shifts, which can be used as an effective tool to rapidly pinpoint the defective production event. The suggested strategy allows the identification of such events in-house, with results obtained on the same day, promoting safe and efficient food-production systems.

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## The root microbiome and mitigation of nitrous oxide (N2O) emissions from cereal fields

Ada Viterbo[1], **Omri Vardi-Perlstein**, Rona Ziskin, Dror Minz
*Soil, Water and Environmental Sciences, Agricultural Research Organization, Volcani Center, Israel*

Agricultural soils are the most dominant global sources of nitrous oxide (N2O) emissions, originating particularly from cereal fields, due to their high areal coverage and nitrogen-rich fertilization regime. N2O is a potent greenhouse gas, ca. 265 times more active than carbon dioxide (CO2) per unit mass and cause damage to the ozone layer. The primary N2O producers in soil are the denitrifying, nitrifying and methanotrophic bacteria, while only part of the denitrifying prokaryotes are able to also reduce N2O to nitrogen gas (N2). Strategies for partial mitigation of N2O emission from agricultural soils include the use of nitrification inhibitors, improved fertilization regime management, replacement of urea with ammonium sulfate, and biochar application. Microbiological mitigation methods involving soil inoculation with N2O-reducing denitrifiers have rarely been described. In a previous work we reported the isolation of two bacteria from wheat roots carrying the nitrous oxide reductase encoding gene nosZ, capable of decreasing N2O emissions in wheat mesocosms, in laboratory-controlled conditions, by approximately 40%. Here we will present the optimization of the application of a particular bacteria and its performance in greenhouse experiments in different soils. We optimized the amount of bacteria to be used, and the methods of application. We show that these bacteria, although isolated from wheat roots, are effective also when applied to corn. New candidates capable of reducing N2O emissions were recently also isolated from wheat and maize roots, which may even have superior attributes as future biological products.

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# Combined Arachidonic acid, triclosan and fluoride treatment enhanced the anti-bacterial activity against Streptococcus mutans

**Avraham Melkam**[1,2], Ronit Vogt Sionov[1], Miriam Shalish[2], Doron Steinberg[1]
[1]*Faculty of Dental Medicine, Institute of Biomedical and Oral Research (IBOR), The Hebrew University of Jerusalem, Israel*
[2]*Department of Orthodontics, Faculty of Dental Medicine, Hadassah Medical Center, The Hebrew University of Jerusalem, Israel*

Dental caries is a global health problem that requires better prevention measures. One of the goals is to reduce the prevalence of the cariogenic Gram-positive bacterium Streptococcus mutans. We have recently shown that arachidonic acid (AA; ω-6) has both anti-bacterial and anti-biofilm activities against this bacterium. An important question is how these activities are affected by other anti-bacterial compounds commonly used in mouthwashes. Here, we studied the combined treatment of AA with triclosan and fluoride. Checkerboard microtiter assays were performed to determine the effects on bacterial growth and viability. Biofilms were quantified using the MTT metabolic assay, crystal violet staining, and live/dead staining with SYTO 9/propidium iodide (PI) visualized by spinning disk confocal microscopy. The bacterial morphology and the topography of the biofilms were visualized by high-resolution scanning electron microscopy (HR-SEM). The effect of selected drug combinations on cell viability and membrane potential was investigated by flow cytometry using SYTO 9/PI staining and the potentiometric dye DiOC2(3), respectively. We found that the triple treatment of AA, triclosan and fluoride was more effective in preventing bacterial growth and biofilm formation than either compound alone or the double treatment. AA caused significant membrane hyperpolarization, which was not significantly enhanced by either triclosan or fluoride. We observed an increase in the percentage of PI-positive bacteria, indicating increased bacterial cell death by the triple treatment. This was supported by HR-SEM imaging. In conclusion, our data suggest that AA can be used together with triclosan and fluoride to improve the efficacy of oral health care.

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11 Bacterial physiology

## MinD-RNase E interplay controls localization of mRNAs to the *E. coli* cell poles

**Omer Goldberger**[1], Shanmugapriya Kannaiah[2], Nawsad Alam[3], Anat Nussbaum-Shochat[1], Ora Schueler-Furman[1], Tamar Geiger[4], Orna Amster-Choder[1]
[1]*Faculty of Medicine, The Hebrew University, Israel*
[2]*School of Medicine, Washington University, Missouri, USA*
[3]*Department of Biochemistry, University of Oxford, UK*
[4]*Department of Molecular Cell Biology, Weizmann Institute of Science, Israel*

Research in the past few decades has shed light on the intricacy of bacterial cell organization, mainly focusing on the subcellular distribution of proteins and its implication on their activity. However, unlike RNAs in eukaryotic cells, bacterial RNAs were not assumed to have specific distribution patterns. A recent study from our lab has shown that the *E. coli* transcriptome is unevenly distributed within the cell, with the polar transcriptome showing unique composition compared to the membrane and cytosolic ones.

Several factors were suggested to mediate mRNA localization to the membrane, but the mechanism of localizing mRNAs to the poles is not known. We combined a biased candidate system approach with an unbiased high-throughput proteomic approach to identify factors that mediate polar mRNAs localization. We identified MinD, a pole-to-pole shuttling protein, and RNase E, previously shown to interact with MinD, as essential for proper localization of polar mRNAs, with MinD binding indirectly to the mRNAs. Using in silico modeling followed by experimental validation, the membrane-binding site in RNase E was found to also mediate binding to MinD. Intriguingly, not only does MinD affect RNase E interaction with the membrane, but it also affects its mode of action and dynamics. Polar accumulation of RNase E in Δ*minCDE* cells resulted in destabilization and depletion of mRNAs from the poles. We could further show that mislocalization of polar mRNAs may prevent polar localization of their protein products.

Taken together, the interplay between MinD and RNase E determines the composition of the polar transcriptome, thus affecting the polar proteome. Notably, our study assigns novel roles to MinD and RNase E, never assigned to them before.

Summarily, our research provides valuable insights into bacterial cell organization and suggests a novel mechanism for mRNA localization to the poles, which determines protein localization.

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11 Bacterial physiology

## The Ecophysiology of bacterial rosettes

**Tamar Szoke**[1], Einat Segev[1]
*Plant and Environmental Sciences, Weizmann Institute of Science, Israel*

Bacterial multicellularity challenges the common perception of single celled bacteria When bacteria come together to form multicellular arrangements, they gain the ability to display complex behaviors, like coordinated responses to changes in their environment and increased resilience to external stresses.
These multicellular structures can take various forms, including aggregation, chaining, clustering, or filamentation. One particular type of aggregation involves the formation of rosettes, where bacteria attach to each other at their poles in a star like pattern. In the case of Phaeobacter inhibens bacteria, which commonly associate with algal partners, rosettes are formed through the attachment of individual cells rather than through clonal cell division. Despite the unique organization of bacteria into rosettes, the abundance and possible advantages of this multicellular form are largely unknown. Here, we characterize the abundance of rosettes in the bacterial population and algal bacterial cultures, and we reveal differences between bacterial cells in their single form versus in rosettes. We found that rosettes abundance is increased under nutrient rich conditions both in a mono-culture, while bacteria are in their logarithmic phase, and in co-culture with the algae Emiliania huxleyi after algae die and release their content.

Our findings demonstrate differential gene expression for selected pathways in single versus rosette attached cells. Physiological differences are observed as well bacterial cultures that were initiated using only rosettes exhibit slower growth rates in comparison to cultures initiated with single cells only.
Gaining insight into the conditions that encourage rosette formation and the impact of rosettes on the bacterial population will provide valuable information about the ecophysiology of this unique multicellular bacterial structure.

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11 Bacterial physiology

## The potential contribution of SAR11 to global warming via methyl-phosphonate biosynthesis

**Alhan Abu Hamoud**[1], Rinat Bar- Shalom[1], Tzipora Perez[1], Laura Steindler[1]
*Department of Marine Biology, Leon H. Charney School of Marine Sciences,, University of Haifa, Israel*

Many marine regions are characterized by extremely low concentrations of bioavailable phosphate (P), thereby limiting the primary and secondary microbial productivity that forms the base of the oceanic foodweb. Nevertheless, tiny genome-streamlined bacteria thrive in these conditions by utilizing alternate forms of phosphorus such as phosphonates (PHNs). Interestingly, when one such PHN type, methyl-phosphonate (MPn), is degraded by bacteria to retrieve P as a nutrient, methane is also released, and the latter is a potent greenhouse gas. Greenhouse gases are causing an increase in temperature that causes ocean stratification, which in turn results in phosphate limitation and further MPn degradation. Very little is known about PHN production, even though the presence of relatively high standing stocks of PHN in marine dissolved organic matter (DOM) suggests that these compounds are constantly produced in ocean surface water. The main source of MPn in DOM is still to be discovered. Here, we propose to test whether the most abundant heterotroph of the global surface ocean, the SAR11 clade, represents the primary source of PHNs that accumulate in marine DOM, and thus may play a role in global warming via both MPn biosynthesis and degradation. To reach this objective we are combining microbiology (culturing of the fastidious SAR11 bacteria: strain HTCC7217 that harbors genes for MPn biosynthesis) and advanced organic chemical analysis (chemical purification and identification of PHN-macromolecules produced). Further, we will test the environmental conditions that trigger MPn biosynthesis.

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## Does the precise mid-cell division ensure equal sharing between daughter cells?

**Sharanya Namboodiri**[1], Alexander Aranovich[1], Mario Feingold[1], Itzhak Fishov[2]
[1]*Department of Physics, Ben Gurion University of Negev, Israel*
[2]*Department of Life Sciences, Ben Gurion University of Negev, Israel*

Phenotypic variability in isogenic bacterial populations helps them to cope with external stress. This variability can stem from the low frequency of gene expression (gene expression noise) and/or unequal partitioning of low-copy-number freely diffusing proteins during cell division. However, some high-copy-number components are permanently/transiently associated with immobile large assemblies, called hyperstructures, and thereby are not equally distributed. We hypothesize that this hyperstructure organization of bacterial cells can contribute to bacterial phenotypic variability. We focus on the nucleoid hyperstructure containing many DNA-associated proteins. Our previous findings suggested that replication initiation timing may be the main source of variability for nucleoid segregation events. The variability in the number of nucleoid-associated DnaA and its effective local concentration may serve as an essential source of variability of the replication initiation event and the ensuing chromosome segregation. To uncover the role of DnaA distribution in phenotypic variability in E. coli, we follow the DnaA distribution at different physiological states of the cell within and outside the nucleoid to understand the degree of its association with DNA. The mobility of DnaA is low, corresponding to a diffusion-binding mode, but faster than that of HU. The analysis of intracellular content asymmetry indicates that the distribution in amounts of DnaA may not necessarily follow the distribution of HU, which reflects the unequal DNA content of nucleoids in future daughter compartments. Thus, the DnaA/DNA ratio is unequal in segregating nucleoids. On the other hand, concentrations of DnaA, DNA, and HU are distributed homogeneously within a cell, but the intercell variation in DNA concentration is much larger than that of DnaA.

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## Investigating The Construction of The Bacterial Envelope

**Gideon Mamou**, Federico Corona[1], Ruth Cohen Khait[2], Nicholas Housden[3], Waldemar Vollmer, Colin Kleanthous
[1]*Centre for Bacterial Cell Biology, Newcastle University, UK*
[2]*The Department of Molecular and Computational Biosciences and Biotechnology, MIGAL, Israel*
[3]*Department of Biochemistry, University of Oxford, UK*

The envelope of Gram-negative bacteria is a tripartite compartment which plays several key physiological roles and determines the efficacy of many antibiotics. The hallmark of the Gram-negative envelope is the Outer Membrane (OM), an impermeable barrier that excludes antibiotics and protects the organism against mechanical deformation. Although biochemical studies have exposed the lipid and protein content of the OM and structural works unveiled the mechanism of action of outer membrane proteins, we still know little about how these constituents are organized and assembled into the rapidly growing envelope. In the current work we studied the assembly of new β-barrel Outer Membrane Proteins (OMPs) in live cells and examined the distribution and activity of the essential β-barrel Assembly Machinery (BAM) which inserts them. We discovered that BAM forms clusters all across the surface of the cell and the size and density of these clusters depends on bacterial physiology. Importantly, we discovered that not all the BAM proteins are active at any given time and the cell cycle has a major impact on the assembly of new OMPs by the BAM complex. Consequently, we found that BAM activity is spatiotemporally coordinated with the assembly of new peptidoglycan cell wall and managed to reveal the mechanism coordinating the construction of these two key envelope components. Taken together, these findings open new avenues for studying the coordination among envelope components and might reveal potential weaknesses in the bacterial protecting shell as future targets for antibiotics.

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## Unifying principles of bactericidal versus bacteriostatic antibiotics

**Elizabeth Vaisbourd**[1], Anat Bren[1], David S. Glass[1], Yifan Yang[1], Avi Mayo[1], Uri Alon[1]
*Molecular Cell biology, Weizmann Institute of Science, Israel*

A common way to classify antibiotics is based on their effect on bacteria: bactericidal antibiotics (cidals) kill bacteria, whereas bacteriostatic antibiotics (statics) inhibit growth but do not kill. In medical practice, only cidals are generally effective in immunocompromised patients and brain infections. In over a century of research, much was learned about antibiotics` mechanisms of action, however, is still unclear why some antibiotics lead to death, and others inhibit growth. For example, the bactericidal streptomycin and the bacteriostatic spectinomycin which bind the same target, the ribosomal small subunit. To address this, we aim to find principles explaining the difference between cidal and static antibiotics. We studied 7 antibiotics from each class, spanning various mechanisms of action. High-resolution robotic growth measurements show clear distinction in growth rates and yield between the two classes, across mechanisms of action. Transcriptomics analysis by RNAseq shed light on potential mechanisms that reconcile the relationship between growth rates, biomass building and death. These findings advance our understanding of static and cidal antibiotics and how they work, with the aim of developing new medicines.

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13 Parasites

## Search, preparation and pesticidal activity of new functionally substituted monosaccharides for plant protection

**Valery Belakhov**[1], Irina Boikova[2]
[1]*Schulich Faculty of Chemistry, Technion – Israel Institute of Technology, Israel*
[2]*Microbiological Plant Protection Laboratory, All-Russian Institute of Plant Protection, Russia*

The widespread use of synthetic organic compounds as pesticides has led to a significant increase in global food production and industrial raw materials. Pesticides used in agriculture to protect plants belong to various classes of chemical compounds, but carbohydrates, in particular monosaccharides, are completely absent from the range of chemical plant protection products. In this regard, we have synthesized low-toxic functionally-substituted monosaccharides based on xylose and ribose containing aryl-substituted, thiophosphate, phosphate, phosphonate, fluorine-, sulfur- and organosilicon groups. Biological studies have shown that functionally substituted monosaccharides have high fungicidal and insecticidal activity against phytopathogenic fungi and harmful arthropods, and also exhibit a phytoregulatory effect. Phosphate derivatives of ribose showed the greatest fungicidal activity among organophosphorus derivatives of the obtained monosaccharides against phytopathogenic fungi of the genera Fusarium, Alternaria, etc. The high effectiveness of phosphate derivatives of ribose, as well as fluorine, sulfur and organosilicon derivatives of ribose against harmful arthropods, in particular against the vetch aphid (Medoura viciae Buckt.), has been revealed. A stimulating effect of phosphate derivatives of ribose on the growth and development of vegetable crops such as cucumber, zucchini and pepper were discovered.

Based on the research conducted, it can be concluded that the obtained functionally substituted monosaccharides, which have pronounced fungicidal and insecticidal activity, as well as phytoregulatory effects, combined with low toxicity, can be considered as promising multifunctional compounds for the creation of effective environmentally friendly preparations on their basis for the protection of agricultural plants.

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## Biofilm formation, interspecies interactions and niche adaptation play fundamental role in shaping dust microbiome structured communities

**Talal Salti**[1,2], Naama Lang Yona[1], Ilana Kolodkin-Gal[2]
[1]*Civil and Environmental Engineering, Technion, Israel*
[2]*Scojen Institute for Synthetic Biology, Reichman University, Israel*

Dust events serve as significant sources of microorganisms (also referred to as bioaerosols), contributing to their global dispersion. The means of survival of dust storm microbiome members and their possible contribution to global processes, ecology and health are still poorly understood.

We characterized biofilm formation and interspecies interactions between dust-borne bacteria, isolated from transitional season dust storms. We specifically studied the role of biofilm formation in Bacillus species, previously identified as a significant component of the bioactive community of the dust microbiome.

While sporulation was frequently considered in this context, biofilm formation can serve as an efficient protective buffer against environmental stressors. We found that most dust Bacillota formed robust biofilm under their own optimal laboratory conditions, which were only partially correlated with their planktonic growth. Furthermore, a biofilm of well-characterized strain of B. subtilis, expressed flagellin and biofilm adhesins robustly on dust. In addition, the sterile dust collected during dust storm strongly induced the transcription of the biofilm associated operon, tapA-sipW-tasA, encoding an accessory protein, a signal peptidase and an amyloid-like adhesin, all essential for biofilm formation. We could also demonstrate sterile the development of sustainable, viable, and robust biofilm in key dust Bacilli. The formation of structured community was also supported by a potential synergy between Bacillus community members and primary producers, as well as significant antagonistic interactions between the Bacillota themselves.

Overall, our results highlight the significance of biofilm formation in adapting to the distinct habitat of dust storms, with niche adaptation potentially playing a role in microenvironments within the particle. Biofilm formation and symbiosis may explain mechanisms by which bacteria not only survive, but also adapt and evolve in this understudied habitat.

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## Abundant Sulfitobacter marine bacteria protect Emiliania huxleyi algae from pathogenic bacteria

**Roni Beiralas**[1], Noy Ozer, Einat Segev
*Plant and environmental sciences, Weizmann institute of science, Israel*

Emiliania huxleyi is a unicellular micro-alga that forms massive oceanic blooms and plays key roles in global biogeochemical cycles. Mounting studies demonstrate various stimulatory and inhibitory influences that bacteria have on the E. huxleyi physiology. To investigate these algal-bacterial interactions, laboratory co-cultures have been established by us and by others. Owing to these co-cultures, various mechanisms of algal-bacterial interactions have been revealed, many involving bacterial pathogenicity towards algae. However, co-cultures represent a significantly simplified system, lacking the complexity of bacterial communities. In order to investigate bacterial pathogenicity within an ecologically relevant context, it becomes imperative to enhance the microbial complexity of co-culture setups.

Phaeobacter inhibens bacteria are known pathogens that cause the death of E. huxleyi algae in laboratory co-culture systems. The bacteria depend on algal exudates for growth, but when algae senesce, bacteria switch to a pathogenic state and induce algal death. Here we investigate whether P. inhibens bacteria can induce algal death in the presence of a complex bacterial community. We show that an E. huxleyi-associated bacterial community protects the alga from the pathogen, although the pathogen occurs within the community. To study how the bacterial community regulates pathogenicity, we reduced the complex bacterial community to a five-member synthetic community (syncom). The syncom is comprised of a single algal host and five isolated bacterial species, which represent major bacterial groups that are naturally associated with E. huxleyi. We discovered that a single bacterial species in the reduced community, Sulfitobacter pontiacus, protects the alga from the pathogen. We further found that algal protection from P. inhibens pathogenicity is a shared trait among several Sulfitobacter species. Algal protection by bacteria might be a common phenomenon with ecological significance, which is overlooked in reduced co-culture systems.

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## The role of secondary metabolites in marine bacterial interactions in the context of an algal host

**Olesia Shlakhter**[1], Einat Segev[1]
*Department of Plants and Environmental Sciences, Weizmann Institute of Science, Israel*

Bacterial secondary metabolites play a key role in microbial interactions. Phaeobacter inhibens is a marine bacterium from the Roseobacter group, commonly associated with algae. P. inhibens produces two secondary metabolites: the antibiotic tropodithietic acid (TDA), and the siderophore roseobactin. On the contrary, Dinoroseobacter shibae, another Roseobacter, is not known to produce similar metabolites. Both bacterial species co-occur with the same algal host, Emiliania huxleyi. How bacterial secondary metabolites impact bacterial interactions in the context of an algal host, is not well understood.

Here, we aim to investigate the influence of bacterial secondary metabolites on bacterial fitness in the algal phycosphere. We therefore generated P. inhibens mutants that do not produce TDA (ΔtdaB) or roseobactin (Δrbt). We evaluated their growth compared to wild-type bacteria (WT) in mono-cultures (P. inihbens only), in co-culture with another bacterial species (P. inhibens and D. shibae), and in tri-cultures where both bacteria rely on the algal host as a nutrient source (P. inhibens, D. shibae, and E. huxleyi). Our approach allows us to assess how TDA and roseobactin influence bacterial interactions within a simplified yet ecologically relevant setup while bacteria interact with each other in the context of an algal host.

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## Visualizing division of labor and filament heterogeneity in filamentous multicellular cyanobacterium Nostoc sp. (Anabaena sp.) PCC7120

**Marharyta Skovorodka**[1], Daniel Dar[1]
*Plant and Environment, Weizmann Institute of Science, Israel*

Phenotypic heterogeneity within clonal bacterial populations arises from variations in gene expression due to intrinsic noise and regulatory mechanisms. This diversity enables a division of labor and risk, benefiting the population due to the lack of genetic conflict. One of striking examples of such a division of labor is seen in multicellular cyanobacteria such as Nostoc. In these organisms, individuals grow in chains that are physically encased by a shared outer membrane and utilize channels for cell-cell transfer. In the absence of soluble nitrogen, a subpopulation of cells will differentiate into a nitrogen-fixing factories called the heterocyst, which supply fixed nitrogen to neighboring cells. The unique architecture suggests that filamentous cyanobacteria are capable of higher levels of cooperation and coordination than planktonic species. However, quantifying this coordination and its dependency on key factors such as the circadian clock and environment necessitates profiling individual cells, in situ, directly within the filament context. In this project, we have adapted a new single cell and spatial transcriptomics approach based on mRNA fluorescence in situ hybridization (mRNA-FISH) to cyanobacteria, overcoming their technical challenges. In a pilot experiment, we captured different stages of heterocyst differentiation in mature filaments at cellular resolution and developed the computational foundation to study individuals in the chain context. We are currently expanding our analysis to capture the majority of physiological processes in this organism. This study aims to deepen our understanding of an environmentally important group of bacteria and shed light on how multicellularity manifests in the microbial world.

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## Systemic Approach to Unravel Biofilm-Amoeba Arms-race

**Prem Anand Murugan**[1], Eva Zanditenas[2], Sergi Ankri[2], Ilana Kolodkin-Gal[1]
[1]*Scojen Institute of Synthetic biology, Reichman University, Israel*
[2]*Department of Molecular Microbiology, Ruth and Bruce Rappaport Faculty of Medicine, Technion, Technion, Israel*

The human gastrointestinal (GI) tract comprises various bacterial populations, forming an intricate ecosystem. Entamoeba histolytica (Eh), a human parasite responsible for 100000 deaths/year, enters the GI tract through contaminated food. On entry into the GI tract, the Eh releases an active parasite called trophozoites. The Eh trophozoites feed on intestinal microbiota for survival and are an essential virulence factor. Most bacteria in the GI tract and the gut prevail largely as biofilms. Bacterial biofilms and Eh trophozoites interact in the GI tract and exist in a delicate balance. Numerous studies on Eh trophozoites and bacterial interactions have been only shown using free-living planktonic bacteria. Our recent study by Zanditenas et al. (1) for the first time showed the interaction between Eh, an amoebic parasite, and the probiotic biofilms of Bacillus subtilis. This study showed the differences in Eh trophozoites` interaction between planktonic and biofilm bacteria by analysing the transcriptome response and the attachment mechanism of the Eh trophozoites on the target. The Eh trophozoites penetrated the B. subtilis biofilm using cysteine proteinases (CPs) by degrading the TasA matrix protein. We now aim to unravel how biofilms can evade predation by amoeba using two independent approaches. First, we aim to map the transcriptome of probiotic bacteria, including B. subtilis, to resolve their resistance mechanisms versus GI-associated parasites and to identify potential targets to modify to allow them to resist amoebiasis better. Second, we found evidence that a small stable molecule secreted by the amoeba serves as a signal to inhibit biofilm formation, allowing the bacteria to avoid altogether the formation of biofilms in the presence of potential predators. Our results can collectively provide additional anti-biofilm treatments and allow novel pathways of engineering probiotic bacteria to overcome GI-associated parasites.

1. Zanditenas, E., Trebicz-Geffen M., Romero, D., Chapman MR, D. Kolodkin-Gal I$ and Ankri S$., Digestive exophagy of Bacterial Biofilms by an Amoeba Predator and its impact on stress tolerance and virulence. Npj Biofilms and Microbiomes 2023.

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## A Burkholderia cenocepacia-like Environmental Isolate Rapidly Kills the Human Fungal Pathogen Candida auris

**Jiawei Li**[1], Tingting Song[1], Marina Rocha[2], Neta Shlezinger[2], Jonathan Friedman[1]
[1]*The Institute of Environmental Sciences, The Hebrew University of Jerusalem, Israel*
[2]*Koret School of Veterinary Medicine, The Hebrew University of Jerusalem, Israel*

Fungal infections pose significant health risks to immunocompromised individuals. Candida auris is an emerging global pathogen that is spreading in healthcare settings due to its high levels of resistance to existing antifungals. Therefore, there is an urgent need for alternative strategies to combat this pathogen. Bacteria are a promising source of novel antifungals. Since they produce a diverse array of secondary metabolites, including ones used to inhibit their fungal competitors. Therefore, I screened a diverse library of ~300 bacterial species and identified two strains, termed Og91 (Pseudomonas aeruginosa) and EC14 (Burkholderia cenocepacia), that showed a strong, robust inhibition of C. auris. Moreover, EC14 did not merely inhibit the growth of C. auris, but actually killed more than 85% C. auris cells via a contact-dependent mechanism within 24 hours. To identify genes driving the fungicidal activity of EC14, I screened a transposon mutant library of 1602 strains and found that Tn5-5, a mutant with a disrupted gene within the biosynthetic gene cluster of terpene, showed a reduced inhibition of C.auris in coculturing. My findings not only provide a promising lead to combating C. auris but also underscore the broader potential of leveraging inter-microbial interactions to address the growing challenge of antimicrobial resistance across a spectrum of infectious diseases.

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## Chemical crosstalk guides the interaction between the common soil bacteria pseudomonas chlororaphis and bacillus subtilis

**Zohar Tik**[1], Shaked Uzi-Gavrilov, Bat El Hagbi-Lazar, Orit Sivan[2], Michael M. Meijler[3]

[1]*Department of Chemistry, Ben-Gurion University of the Negev, Israel*
[2]*Department of Earth and Environmental Sciences, Ben-Gurion University of the Negev, Israel*
[3]*The National Institute for Biotechnology in the Negev, Ben-Gurion University of the Negev, Israel*

Various organisms consider the rhizosphere as home. Those organisms form the rhizosphere microbiome and interact not only with the plant but also with other microorganisms. One of the major bacterial genera inhabiting the rhizosphere is Pseudomonas, of which most species are known to produce phenazines, which carry antibiotic properties. A member of this family, Pseudomonas chlororaphis, a common rhizosphere dwelling species that is considered as plant growth promoting bacteria, is known to produce phenazine-1-carboxamide (PCN).

In this study, we focused on how the production of PCN by Pseudomonas affects its neighbor, Bacillus subtilis. We cultured B. subtilis NCIB 3610 in the presence of P. chlororaphis PCL 1391, the non-phenazine producing mutant, and synthetic PCN. The cultures were subjected to examination by liquid chromatography-tandem mass spectrometry, and the collected data were analyzed with several metabolomics platforms. While conducting these experiments, we observed visible change in the colonies` morphology. Transformation in B. subtilis colonies morphology was observed in all PCN-containing treatments, while growing B. subtilis in the presence of a P. chlororaphis non-phenazine mutant, a less significant change was observed. In addition, we identified a decrease in some compounds production by B. subtilis in the presence of P. chlororaphis, several of them was annotated by GNPS. Our results indicate thus that PCN changes the morphology and signaling of B. subtilis, but not significantly its growth. In the near future we will continue to investigate P. chlororaphis and B. subtilis interactions, with emphasis on social interactions involving antibiotics.

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## Estimating horizontal gene transfer frequency of multidrug-resistant plasmids from wastewater

**Kseniia Erokhina**[1,2], Swapnil Kajale[1], Chagai Davidovich[1,2], Eddie Cytryn[1]
[1]*Institute of Soil, Water and Environmental Sciences, Volcani Institute, Israel*
[2]*Department of Agroecology and Plant Health, The Robert H. Smith Faculty of Agriculture, Food and Environment, The Hebrew University of Jerusalem, Israel*

The rise in antibiotic-resistant infections is a major global health threat. While hospitals are well-known hotspots, natural environments affected by human activity also contribute to antibiotic resistance. Due to freshwater scarcity treated wastewater (TWW) is increasingly used to irrigate crops, potentially introducing antibiotic-resistant bacteria (ARB) and antibiotic-resistance genes (ARGs) to soil and plants. Many clinically-relevant ARGs are found on plasmids that can disseminate to other bacteria through conjugation. We hypothesize that multidrug-resistant plasmids from TWW-derived bacteria can be horizontally transferred to sensitive strains on irrigated produce, or in the gut following ingestion. We, therefore, evaluated the horizontal gene transfer frequency of 9 sewage-derived multidrug-resistant Escherichia coli strains and one Klebsiella pneumoniae strain carrying Extended-Spectrum β-Lactamases (ESBLs) by mating them with a model (sensitive) recipient strain using bi-parental liquid and filter mating platforms. Surprisingly, all 10 strains could transfer resistance to the model recipient. However, conjugation frequencies varied significantly between strains, ranging from 0.001% to 0.5%. Plasmid carriage had a small fitness cost and slightly reduced growth rates of the recipients. Genomes of donors and selected recipients were sequenced in attempt to correlate between donor phylogeny and plasmid composition and conjugation frequencies. These results validate that multidrug-resistant plasmids from sewage can readily transfer multidrug-resistant gene harboring plasmids to sensitive recipients. The variation in conjugation frequencies shows the complexity of horizontal gene transfer and suggests some strains and plasmids are more prone to transferring resistance. Our findings highlight the need for better wastewater monitoring and management to prevent the spread of antibiotic-resistant bacteria and genes.

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15 Bacteriophages

# Phage Therapy and Pulsed Blue Light Synergistic Bactericidal Effect Over *Pseudomonas Aeruginosa* Preformed Biofilm

**Amit Rimon**[1], Jonathan Belin[1], Shunit Coppenhagen-Glazer[1], Lilach Gavish[2,3], Ronen Hazan[1]

[1]*Institute of Biomedical and Oral Research (IBOR), Faculty of Dental Medicine, The Hebrew University of Jerusalem, Israel*

[2]*3Institute for Research in Military Medicine (IRMM), Faculty of Medicine, The Hebrew University of Jerusalem, Israel*

[3]*The Department of Medical Neurobiology, Institute for Medical Research (IMRIC), Faculty of Medicine, The Hebrew University of Jerusalem, Israel*

Introduction: Antibiotic-resistant strains and biofilms of Pseudomonas aeruginosa (PA) are a rising cause of morbidity and mortality. Phage therapy is a promising bactericidal non-antibiotic approach. Pulsed blue light (PBL) has been shown to effectively inactivate certain bacteria.

Objective: To determine if PBL combined with phage therapy culminates to a synergistic bactericidal effect against preformed biofilms.

Methods: PA14 Biofilms were irradiated with 450nm 33kHz pulsed blue light emitting diodes for 1-4 ×30min sessions with 30min 'off' intervals (total dose 0-52 J/cm2). Phage 108 PFU/ml was added 24h or 10min prior to PBL or 10min after. Crystal violet stain was used to evaluate biofilm biomass. Live-dead fluorescent staining was used to measure biofilm viability. n=5 biological repeats. ANOVA and Tukey as post-hoc. Results: By crystal violet stain, we found that both PBL and phage significantly reduced biofilm biomass regardless of the dose (p0.001 across doses, presence of phage, and timing of phage addition) with the most efficient combination when adding the phage immediately before 26J/cm2 PBL. By live/dead stain, the biofilm viability was significantly reduced by each of the treatments (%Live, median[IQR]: Control 80%[10]; PBL 25%[10]; Phage 40%[10]; and PBL&Phage: 15%[21], p0.001 vs control for all). PBL with/without phage was significantly superior to phage alone (p=0.012/p=0.044 respectively).

Conclusions: Phage combined with PBL was found to have a synergistic bactericidal effect in preformed biofilms of P. aeruginosa. Evaluating the safety and effectiveness of Phage/PBL in preclinical models is the next step toward validation of this novel approach for combating antibiotic-resistant bacteria.

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## Structure-guided discovery of viral proteins that inhibit host immunity

**Erez Yirmiya**[1], Rotem Sorek[1]
*Department of Molecular Genetics, Weizmann Institute of Science, Israel*

Viruses encode numerous proteins that inhibit host defenses, but sifting through the millions of viral proteins available in public databases for immune-modulatory proteins was so far impractical. We developed a combination of genomics and large-scale computational structural biology analysis that utilizes AlphaFold to systematically screen virus-encoded proteins for inhibitors that antagonize host immune proteins. Focusing on Thoeris and CBASS, two bacterial defense systems that are the ancestors of eukaryotic TIR- and cGAS-mediated immunity, we discovered numerous families of Thoeris inhibitors and one family of CBASS inhibitors, encompassing tens of thousands of genes widespread in phages. Verified inhibitors exhibit extensive physical interactions with the respective immune protein counterpart, with all inhibitors predicted to block the active site of the immune protein. A second set of verified inhibitors form molecular “sponges” that bind and sequester the immune signaling molecules produced by the defense systems. We further show that phage-encoded inhibitors of bacterial TIR proteins can also inhibit distantly related human and plant immune TIRs, and that the phage-derived inhibitor of bacterial cGAS can inhibit the activity of human cGAS as well. Our results demonstrate that phage genomes are a reservoir for immune modulatory proteins capable of inhibiting bacterial, animal, and plant immunity.

References:

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15 Bacteriophages

## A novel phage-encoded RNA manipulates phage efficiency in bacteriophage Lambda

**Aviezer Silverman**[1], Reut Bruner[1], Raneem Nashef[1], Sahar Melamed[1]
*Department of Microbiology and Molecular Genetics, Hebrew University Of Jegrusalem, Jerusalem*

Gene regulation in bacteria is essential for their adaptation and survival in diverse environments. A key component of this regulation is the post-transcriptional control mediated by small RNAs (sRNAs), which enable rapid and precise responses to environmental stimuli. Bacteriophages, or phages, play a crucial role in the survival of bacterial communities. We hypothesized that phages also exploit sRNA-dependent regulation. To investigate this, we employed RIL-seq, a high-throughput method that identifies RNA-RNA interactions during Lambda phage infection. Our analysis uncovered a novel sRNA, PieR, which interacts with both viral and bacterial transcripts and is expressed exclusively during the lytic cycle. We focused on PieR`s interaction with the host gene dnaN, an essential gene for DNA replication. Translational analysis and western blots demonstrated that PieR upregulates DnaN levels. Further examination revealed that PieR binding alters the secondary structure of dnaN mRNA, enabling enhanced translation. Deletion of pieR resulted in a lower viral count during spontaneous induction of a prophage, suggesting a positive role for PieR in Lambda infection. This study introduces PieR as a novel sRNA that enhances phage efficiency and suggests that it does so by manipulating DNA replication. Understanding the cross-regulation between phages and their hosts on the RNA level adds to the complexity of these relationships, and can have great value in advancing phage therapy.

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## An archaeal CBASS system that eradicates a chronic viral infection

**Deepak Choudhary**[1], Uri Gophna[1], Himani Himani[1], Dana Vassover[1]
*George S. Wise Faculty of Life Sciences, Tel Aviv University, Israel*

Some bacterial type II CBASS Systems defend against viral infections by a TIR-SAVED domain protein that depletes cellular NAD+ levels, eventually leading to cell dormancy or death, which is modulated by a ubiquitin E1-E2 ligase-like protein. Such abortive-infection strategy is beneficial in stopping a fast lytic infection because cells die before spreading the virus to sister cells. Conversely, many archaea are infected by chronic “temperate” viruses that co-exist with their hosts for extended periods. Under such situations, abortive infection could be harmful because the cost of immunity may be higher than that of infection. Here we study an archaeal Type II CBASS System from Haloferax strain Atlit 48N, which we heterologously expressed in the model organism Haloferax volcanii. We show that the system protects against a chronically-infecting virus, HFPV-1, and enables clearing of that virus after several passages, without killing the host. Surprisingly, cloning the E1-E2 ligase of that CBASS system, generally thought to only regulate the system, by itself enabled substantial clearing of virus infection, reaching over 80% of the activity of the complete system. Cell death during viral infection was only caused after extensive incubation with the complete system in lower growth temperatures. This suggests that CBASS systems could be beneficial for archaea that are exposed to chronic infecting viruses, and could explain why such systems are relatively common in archaea.

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## Diversification of molecular pattern recognition in bacterial NLR-like proteins

**Nathalie Bechon**[1], Nitzan Tal[1], Avigail Stokar-Avihail[1], Sarah Melamed[1], Gil Amitai[1], Rotem Sorek[1]
*Department of Molecular genetics, Weizmann Institute of Science, Israel*

Antiviral STANDs (Avs) are bacterial anti-phage proteins that are evolutionarily related to immune pattern recognition receptors of the NLR family. Following recognition of a conserved phage protein, Avs proteins exhibit cellular toxicity and abort phage propagation by killing the infected cell. Type 2 Avs proteins (Avs2) were suggested to recognize the large terminase subunit of the phage as a signature of phage infection. Here, we show that while Avs2 from Klebsiella pneumoniae (KpAvs2) can be activated when heterologously co-expressed with the terminase of phage SECphi18, during infection in vivo KpAvs2 recognizes a different phage protein, named KpAvs2-stimulating protein 1 (Ksap1). We show that KpAvs2 directly binds Ksap1 to become activated, and that phages mutated in Ksap1 can escape KpAvs2 defense despite encoding an intact terminase. Our results exemplify the evolutionary diversification of molecular pattern recognition in bacterial Avs2 proteins, and highlight that pattern recognition during infection can differ from results obtained using heterologous co-expression assays.

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## Identification and investigation of phage and host factors that regulate T4-like cyanophage genome expression

**Maor Sarah Berkovich**[1], Ran Tahan[1], Claudia Steglich[2], Debbie Lindell[1]
[1]*Faculty of Biology, Technion – Israel Institute of Technology, Israel*
[2]*Institute of Biology 3, Genetics & Experimental Bioinformatics, University of Freiburg, Germany*

Cyanophages are abundant viruses in the marine environment and infect a wide range of cyanobacteria from the genera Prochlorococcus and Synechococcus. The transcription of the T4-like cyanomyovirus Syn9 occurs in three defined transcriptional groups: early, middle and late genes, with three defined promoter motifs for each group. However, what factors regulate T4-like cyanophage transcription, and how, is still unknown. We aim to identify and investigate host and phage factors that regulate the expression program of Syn9 during infection of Synechococcus WH8109. Currently, we are focusing on two phage proteins: a putative sigma factor hypothesized to shift transcription from middle to late genes, and a putative Sm-like protein based on structural predictions hypothesized to have a post-transcriptional regulatory role during infection. To assess the role of these genes in the progression of the expression program, we developed a knockdown approach for investigating essential phage genes by inserting a translational riboswitch upstream of the gene of interest. Using this method, we generated knockdown cyanophage mutants for these two genes and expressed them heterologously from plasmids in the host. Analysis of phage infection dynamics revealed that both mutants have a longer infection cycle compared to the wild-type phage. Heterologous expression of the Sm-like protein negatively affected host growth, whereas the sigma factor homolog had a milder effect. These findings suggest that both genes play an important role in facilitating the ability of the phage to infect its host, with the putative Sm-like protein also potentially involved in post transcriptional regulation of host genes.

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# Combating Burkholderia cepacia complex infections by integrating different strategies in the development of phage treatments

**Ortal Yerushalmy**[1,2], Ron Browunshtine[1,2], Chani Rakov[1], Sivan Alkalay-Oren[1,2], Leron Khalifa[1,2], Amit Rimon1[1,2,3], Shunit Coppenhagen-Glazer[1,2], Daniel Gelman[2,3], Saima Aslam[4], John LiPuma[5], Ran Nir-Paz[2,6], Ronen Hazan[1,2]

[1]*Institute of Biomedical and Oral Research (IBOR), Hebrew University of Jerusalem, Israel*

[2]*IPTC, The Israeli Phage Therapy Center of Hadassah Medical Center and the Hebrew University, Israel*

[3]*Department of Military Medicine, Faculty of Medicine, The Hebrew University of Jerusalem, Israel*

[4]*Division of Infectious Diseases and Global Public Health, University of California San Diego, USA*

[5]*Department of Pediatrics, University of Michigan Medical School, USA*

[6]*Department of Clinical Microbiology and Infectious Diseases and the Faculty of Medicine, The Hebrew University of Jerusalem, Israel*

Introduction: Burkholderia cepacia complex (BCC), comprising 24 species, poses a serious threat, especially to those with cystic fibrosis, leading to life-threatening respiratory infections. Due to the challenging resistance of BCC infections, phage therapy is considered a promising solution. However, Burkholderia-targeting phages are rare, with only 35 described in literature by 2020, and just 5 considered lytic. This study aims to create a diverse phage library, employing various isolation and training strategies, to lay the foundation for extensive phage therapy against BCC infections.

Methods: We established a BCC bacterial collection for isolating new phages, followed by comprehensive phage characterization using whole-genome sequencing, electron microscopy, host range determination, lytic activity profiling, and plaque morphology analysis.

Result: Screening hundreds of samples led to the successful identification and isolation of 40 phages, effectively doubling the number of known BCC phages. Of these, 36 demonstrated robust lytic activity against at least one strain, with capable of propagation to titers exceeding 10^7 PFU/mL, making them potential candidates for phage therapy. Using a minimum of 12 phages provided coverage for 70% of BCC clinical isolates.

In addition to isolating new phages, we changed the bacterial host used for phage propagation as an alternative strategy to train the existing phages and investigate their impact on the phage host range. Indeed, there was a significant effect on the host range of a single phage. If we use a single phage grown on a strain, we can achieve up to 30% coverage, and if we use multiple hosts, we can increase the coverage to 45%. Furthermore, in at least one case, the tropism of the phage has completely changed by changing the host on which it was grown.

Conclusions: The results of this project give hope to many cystic fibrosis patients struggling with BCC infections. The creation of the largest BCC phage library in combination with phage training through the strategy of host switching represents a promising tool in the ongoing fight against this pathogen, as well as in addressing future challenges posed by pathogens resistant to both antibiotics and phages.

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16 Beneficial microbes

## A Burkholderia cenocepacia-like environmental isolate strongly inhibits the plant fungal pathogen Zymoseptoria tritici

**Tingting Song**[1], Yael Sorokin[1], Jonathan Friedman[1]
*Institute of Environmental Sciences, The Hebrew University of Jerusalem, Israel*

Fungal phytopathogens cause significant reductions in agricultural yields annually, and overusing chemical fungicides for their control leads to environmental pollution and the emergence of resistant pathogens. Exploring natural isolates with strong antagonistic effects against pathogens can improve our understanding of their ecology and develop new treatments for the future. We isolated and characterized a novel bacterial strain associated with the species Burkholderia cenocepacia, termed APO9, which strongly inhibits Zymoseptoria tritici, a commercially important pathogenic fungus causing Septoria tritici blotch in wheat. Additionally, this strain exhibits inhibitory activity against four other phytopathogens. We found that physical contact plays a crucial role for APO9`s antagonistic capacity. Genome sequencing of APO9 and biosynthetic gene cluster (BGCs) analysis identified nine classes of BGCs and three types of secretion systems (Type II, III and IV), which may be involved in the inhibition of Z. tritici and other pathogens. To identify genes driving APO9's inhibitory activity, we screened a library containing 1602 transposon mutants and identified five genes whose inactivation reduced inhibition efficiency. One such gene encodes for a Diaminopimelate decarboxylase (DAPDC) located in a terpenoid biosynthesis gene cluster. Phylogenetic analysis revealed that while some of these genes are also found across the Burkholderia genus, and as well as in other Betaproteobacteria, the combination of these genes is unique to the Burkholderia cepacia complex. These findings suggest that the inhibitory capacity of APO9 is complex and not limited to a single mechanism, and may play a role in the interaction between various Burkholderia species and various phytopathogens within diverse plant ecosystems.

POSTER SESSION I (Monday, September 9) (Monday, September 9, 2024 12:45)

Poster presentation

16 Beneficial microbes

## Coordinated V-shaped structuring governs mature biofilm development in Lactobacillus plantarum during adaptation to acidic stress

**Athira Venugopal**[1], Ronit Vogt Sionov[1], Yulia Kroupitski[2], Moshe Shemesh[2]
[1]*Institute of Biomedical and Oral Research, The Hebrew University of Jerusalem, Israel*
[2]*Department of Food Sciences, Institute of Postharvest Technology and Food Sciences, Agricultural Research Organization, Israel*

The probiotic bacterium Lactiplantibacillus plantarum evolved efficient adaptation mechanisms to survive in different ecological niches. Yet, tolerance to acidic environment remains a major adaptive trait of this versatile species. Here, we further investigated the previously uncharacterized biological process that enables highly coordinated adaptation of this bacterium to acidic stress. We provide mechanistic evidence for the role of V-shaped multicellular structuring, associated with incomplete daughter cell separation, during successful growth of L. plantarum cells in acidic environment. We also show that this process facilitates mature and coordinated biofilm formation, through activation of luxS dependent quorum sensing (QS) pathway, specified with autoinducer 2 (AI-2) production. Indeed, blocking this pathway results in compromised V-shaped structuring and underdeveloped biofilm formation. Besides, exogenous supplementation of 4,5-dihydroxypentanedione (DPD), the precursor of AI-2, restores a proper V-shaped structuring that culminates with mature biofilm formation. Altogether, we propose that the self-generated V-shaped multicellular structuring, regulated by the luxS-dependent QS pathway, is crucial for coordinated biofilm formation during adaptation to acidic stress.

POSTER SESSION I (Monday, September 9) (Monday, September 9, 2024 12:45)

Poster presentation

16 Beneficial microbes

## Unraveling the sensory mechanisms of bacterial defense systems via co-expression with phage genes

**Hadar samra**, Ilya Osterman, Nitzan Tal, Jeremy Garb, Rotem Sorek

To initiate an immune response, a defense system must first sense phage infection. For many of the recently discovered defense systems it is still unclear what phage component stimulates the system. In this study we co-expressed defense systems with phage genes, enumerating over all genes encoded by the phage against which the system acts. As defense systems that cause abortive infection become toxic when co-expressed with the phage encoded gene they recognize, this approach enables pinpointing phage triggers for defense system activation. Using a subset of defense systems that protect against Bacillus phages, we show that our approach reveals specific structural components of the phage as the PAMP recognized by the defense protein.

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Poster presentation

17 Microbial genetics

# A-to-I mRNA editing can control di sulfide bond formation in bacteria and the toxicity of the HokB toxin

**Liron Didi**[1], Liam Aspit[1], Dganit Melamed[2], Raz Zarivach[3], Orna Dahan[4], Yitzhak Pilpel[4], Dan Bar Yaacov[1]

[1]*Microbiology, Immunology and Genetics, Ben-Gurion University of the Negev, Israel*

[2]*The Smoler Protein Research Center, Technion Israel Institute of Technology, Israel*

[3]*Life Sciences, Ben-Gurion University of the Negev, Israel*

[4]*Molecular Genetics, Weizmann Institute of Science, Israel*

Adenosine-to-inosine (A-to-I) mRNA editing and protein disulfide bonds can each, independently, affect the structure and function of proteins. However, the ability of A-to-I mRNA editing to control disulfide bond formation was never shown. Here, we show that A-to-I mRNA editing constitutes a novel mechanism to control disulfide bond formation, which can affect bacterial viability and growth. We demonstrated that A-to-I mRNA editing recodes a tyrosine to a cysteine in the self-toxin HokB, increasing its toxicity in Escherichia coli. Subsequently, we show that edited HokB can induce bacterial death and early entrance to the stationary phase. We demonstrated that DNA-coded cysteines in HokB, and in vivo disulfide bond formation are essential to edited HokB toxicity. Finally, we show by structural modeling that editing-dependent disulfide bonds might stabilize HokB pores in the bacterial membrane. Our work suggests that novel disulfide bonds can occur across different proteins and organisms by A-to-I mRNA editing.

POSTER SESSION II (Tuesday, September 10) (Tuesday, September 10, 2024 12:45)

Poster presentation

01 Host-pathogen interactions

## Profiling the systemic and gut anti-commensal antibody repertoire of children with Crohn's disease

**Tomer Zur**[1], Lilach M Friedman[1], Lior Levi[1], Oren Ledder[2], Dotan Yogev[2], Raffi Lev-Tzion[2], Esther Orlanski Meyer[2], Amit Assa[2], Shira Yuval Bar Asher[2], Asaf Azlay[2], Tasia Maslov[2], Dan Turner[2], Tomer Hertz[1]

[1]*Department of Microbiology, Immunology and Genetics, and The National Institute for Biotechnology in the Negev, Ben-Gurion University of the Negev, Israel*

[2]*Institute of Pediatric Gastroenterology and Nutrition, Shaare Zedek Medical Center, Israel*

Background: Previous studies have reported that serum and stool antibodies to specific commensal bacteria are associated with inflammatory bowel disease (IBD), however these studies were typically focused on a limited number of bacterial species. Studies on gut antibodies typically quantify IgA bound to specific bacterial species of interest. Associations between unbound IgA and IgG antibodies in the stool with disease severity have not been previously studied.

Methods: Serum and stool samples were collected from 17 children with Crohn's disease, four months post diagnosis and prior to biological treatment. We used antigen microarrays to generate antibody profiles to: (1) a childhood vaccines and viruses; and (2) commensal bacteria and fungi. We profiled IgG and IgA repertoires in both serum and fecal water samples.

Preliminary results: We found extensive heterogeneity in the antibody profiles. IgG and IgA profiles were significantly different from one another, as well as paired samples of stool and serum. IgA levels to CMV (p0.01) and Bacteroides caccae (p0.05), and IgG levels to Veillonella dispar (p0.05) were significantly higher in children with active disease compared to children in remission. We found a correlation between calprotectin levels in the stool and high titer anti-Saccharomycetales IgG (r=0.62, p=0.03) or anti-Bacteroidaceae IgA compared to (r=0.66, p=0.01).

Conclusion: The IgG and IgA antibody repertoires are associated with Chron's disease severity and may identify specific commensals that are associated with disease severity. Further work is required to develop this into a clinical biomarker, as well as to study associations with response to antibody therapies.

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Poster presentation

01 Host-pathogen interactions

## Endogenous ZAP affects flavivirus RNA interactome

Nguyen Phuong Khanh Le, Prince Pal Singh, **Uladzimir Karniychuk**[1]
*Veterinary Biosciences, The Ohio State University, USA*

### Objectives

One of the most recent advances in analyzing viral RNA–cellular protein interactions is the Comprehensive Identification of RNA-binding Proteins by Mass Spectrometry (ChIRP-MS). All ChIRP-MS studies have been conducted only in wild-type cell lines, and comparative studies of viral RNA–cellular protein interactions in wild-type (WT) and knockout (KO) cell lines have not been reported. The zinc finger CCCH-type antiviral protein 1 (ZAP) is a cellular protein with broad antiviral activity. Therefore, we conducted experiments to study ZAP interactomes associated with Zika virus RNA.

### Methods

First, we confirmed the antiviral activity of ZAP against Zika virus, and other flaviviruses Japanese encephalitis virus (JEV) and West Nile virus (WNV). Afterward, we performed ChIRP-MS on ZAP-WT and ZAP-KO Vero cells to identify ZAP-independent and ZAP-dependent interactomes associated with Zika virus RNA.

### Results

The ChIRP-MS analysis showed 209 unique proteins interacting with Zika RNA in only ZAP-positive cells; these interactions were lost in ZAP-KO cells, suggesting the ZAP-dependent Zika RNA interactome. The ZAP-dependent Zika RNA interactome has more proteins than the interactome in ZAP-KO cells—209 versus 116 proteins. For example, we identified 11 RNA helicases enriched only in the ZAP-dependent Zika interactome.

### Conclusions

The ZAP-dependent interactome identified with ChIRP-MS provides potential ZAP co-factors for antiviral activity against Zika virus and possibly other flaviviruses. Identifying the full spectrum of ZAP co-factors and mechanisms of how they act will be critical to understand the ZAP antiviral system and may contribute to the development of antivirals.

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Poster presentation

01 Host-pathogen interactions

## Proteome-wide dynamic mapping of organelle composition during enterovirus infection

**Yael Yair**[1], Ofir Atrakchi[2], Rachel Sharan[1], Tami Geiger[2], Orly Laufman[1]
[1]*Department of Molecular Genetics, Weizmann Institute of Science, Israel*
[2]*Department of Molecular Cell Biology, Weizmann Institute of Science, Israel*

Enteroviruses (EVs) are small positive-strand RNA viruses from the Picornaviridae family that infect millions of people worldwide each year. EV infection can cause severe medical conditions such as meningitis and endocarditis, mostly in neonates and children. Currently, there are no approved drugs for EV infections, highlighting the need for a better understanding of EVs infection mechanism.

Organelles play critical roles in virus infection by providing essential metabolites and serving as physical platforms for diverse viral replication processes. Therefore, taking over host organelles and metabolic pathways is key to establishing a successful infection. Yet, how exactly EVs remodel and recruit organelles their replication is poorly understood and represent a major knowledge gap in the field.

To answer these questions, we designed a proteome-wide dynamic mapping of organelle composition over the course of EV infection. Our model enterovirus is coxsackievirus B3 (CVB3), a leading cause of myocarditis and diabetes in humans. Using a multi-step differential centrifugation followed by mass-spectrometry analysis, we determine how the protein composition of organelles change during CVB3 infection. Our proteomic approach also identifies numerous events where host proteins change their localization in the cell during infection, either as a part of the viral replication plan or as a defense response of the host.

Our proteomic analysis will provide the first-ever comprehensive picture of molecular events occurring in different arenas in the cell over the course of infection. Mapping these events is critical for understanding the cell biology of EV replication and for identifying potential therapeutic targets.

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Poster presentation

01 Host-pathogen interactions

## Generation and characterization of sars-cov-2 neutralizing antibodies associated with clinical outcomes from b-cell repertoires

**Pratik Das**[1], Michal Werbner[1], Modi Safra[2], Pazit Polak[2], Moshe Matan[3], Avi Peretz[4], Moshe Dessau[5], Gur Yaari[6], Meital Gal-Tanamy[1]
[1]*Molecular Virology Lab, Azrieli Faculty of Medicine, BarIlan University, Israel*
[2]*Bio-engineering, Faculty of Engineering, BarIlan University, Israel*
[3]*Clinical Microbiology Laboratory, Baruch Padeh Medical Center, Israel*
[4]*Azrieli Faculty of Medicine, BarIlan University, Israel*
[5]*The Laboratory of Structural Biology of Infectious Diseases, The Azrieli Faculty of Medicine, BarIlan University, Israel*
[6]*Bio-engineering, Faculty of Engineering, BarIlan University, Israel*

Aim and Background: The severe acute respiratory syndrome (SARS-CoV-2) was the leading cause of the recent global catastrophe known as the COVID-19 disease. SARS-CoV-2 enters the host cells via interaction between the angiotensin-converting enzyme 2 (ACE2) receptor and the viral envelop protein ‘spike’. Emerging SARS-CoV-2 variants contain mutations that resist antibody neutralization. The use of neutralizing antibodies is currently the most promising approach to prevent SARS-CoV-2 infection. Here, we aimed to generate novel effective SARS-CoV-2 neutralizing antibodies associated with specific clinical outcomes directly from B-cell receptor (BCR) immune repertoires of infected individuals which is an unbiased method, and investigate their binding and neutralizing abilities, and neutralization breadth.

Methods: For identifying neutralizing antibody clones against SARS-CoV-2, a dataset containing immunoglobulin heavy and light chain sequences from 13 severe COVID-19 patients and 50 mild/moderate patients was collected. By antibody repertoire analysis, we identified 10 neutralizing antibody clones prevalent in COVID-19 patients. The full-length antibodies were generated by cloning the respective heavy and light chain sequences for expression and purification. Neutralization assays were conducted to evaluate the neutralization capacity against SARS-CoV-2 pseudo-particles. In addition, binding assays were performed with spike and receptor binding domain (RBD) to evaluate the binding characteristics.

Results and Conclusion: We identified and generated antibody clones that are prevalent in COVID-19 patients and showed that some antibodies bind to spike only and others binds to both spike and RBD. We demonstrated that these antibodies efficiently neutralize SARS-CoV-2. These results suggest that neutralizing antibodies with mechanism of action other than binding RBD are also prevalent in infected patients. This study is expected to lead towards generation of antibodies with various mechanisms of neutralization that may be efficient as a combination therapy for the cure of COVID-19 patients.

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01 Host-pathogen interactions

## Elucidating the relationship between virus infection and cell-cycle regulation in marine diatoms

**Linoy Bamshid**[1], Amit Tzur[1], Dina Spungin[1], Chana F. Kranzler[1]
*The Mina and Everard Goodman Faculty of Life Sciences, Bar-Ilan University, Israel*

Diatoms are a diverse group of eukaryotic phytoplankton, contributing ~20% of photosynthesis on Earth and forming the base of marine food webs. By translating diverse environmental signals to efficiently regulate cell-cycle progression, diatoms exhibit rapid growth, effectively outcompeting other planktonic microalgae and forming massive blooms across diverse oceanic regimes. Diatoms are also infected by Marnaviridae, a group of enigmatic RNA viruses that are ubiquitously distributed throughout the global ocean. Yet how these viruses shape diatom bloom dynamics is poorly understood. While there is substantial evidence that viruses can alter the cell-cycle in mammalian and plant systems, how virus infection might interface with cell-cycle progression in diatoms is unknown. Here, using the model bloom-forming, centric, diatom Chaetoceros tenuissimus and the RNA virus CtenRNAV, along with a range of cell-cycle analysis techniques, we are exploring the interaction between viral infection and cell-cycle regulation in diatoms. We found that CtenRNAV infection substantially remodels cell-cycle phase distribution, generating a higher proportion of S and G2/M phases. In addition, further analysis revealed faster virus replication in cultures which were artificially arrested at S and G2/M phase. Consistent with prior studies, we also found that cell-cycle progression in C. tenuissimus is regulated by both light and silicon limitation, resulting in a G1/S and S phase arrest, respectively. Together, these findings provide novel insight into the interplay between virus infection and cell-cycle regulation in diatoms, advancing our understanding of how viruses might interface with diatom bloom dynamics across diverse environments in the ocean.

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## Virulence, resistance and coexistence: understanding host-virus infection dynamics in marine diatoms

**Ori Zucker**[1], Dina Spungin[1], Chana Kranzler[1]
*The Mina and Everard Goodman Faculty of Life Sciences, Bar-Ilan University, Israel*

Diatoms are among the most widespread and diverse groups of unicellular eukaryotic microalgae in the ocean, contributing ~20% to global primary production and forming the base of marine food webs. Virus infection in marine microalgae is considered a major conduit of ecological, evolutionary and biogeochemical transformation in the ocean, yet we know little about virus infection dynamics in diatoms. Using the model, bloom-forming, centric diatom, Chaetoceros tenuissimus, we are exploring the life cycle and virus life history traits in CtenRNAV and CtenDNAV, two taxonomically distinct diatom-infecting viruses, and the mechanisms by which diatoms might resist viral infection. While exponential growth in C. tenuissimus continues unabated for several days post infection before coordinated culture collapse, we documented a release of infectious virions within several hours post infection, highlighting a rapid and well-orchestrated viral take-over and raising the possibility of early viral budding in infected diatoms. In addition, we observed clear recovery from virus infection C. tenuissimus that gives rise to resistant and physiologically distinct diatom populations. To better understand how diatoms might resist virus infection, we are working to disentangle the diatom immune response to virus invasion from virus-mediated transcriptional reprogramming of host metabolism. Together, these findings advance our understanding of diatom host-virus dynamics and the underlying factors that drive how this biogeochemically crucial group of marine microeukaryotes might resist, coexist and survive virus predation in the global ocean.

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Poster presentation

01 Host-pathogen interactions

# High Titer Against OC43 Seasonal Coronavirus Enhances Susceptibility to SARS-CoV-2 Infections

**Hanna Oppenheimer**[1], Shosh Zismanov[1], Lilach M. Friedman[1],
Lior Nesher[2], Tomer Hertz[2,3]
[1]*Department of Microbiology, Immunology and Genetics, Faculty of Health Sciences, Ben Gurion University, Israel*
[2]*Infectious Disease Institute, Soroka University Medical Center, and Faculty of Health Sciences, Ben Gurion University, Israel*
[3]*Vaccine and Infectious Disease Division, Fred Hutch Cancer Research Center, Seattle*

Several recent studies reported significant associations between the antibody levels against seasonal human coronavirus (hCoV) OC43 and susceptibility to SARS-CoV-2 infection. While in some studies these cross-reactive antibodies were protective, in others they were detrimental, i.e. increased suceptiblity to SARS-CoV-2. Here we studied their potential role using samples from two independent clinical cohorts.

In the first study, we longitudinally tracked 50 participants comprising university staff and students along 9 months. Both symptomatic and asymptomatic viral infections were identified through weekly symptom questionnaires and oronasal swabs. Cornonavirus viral infections, including SARS-CoV-2 and the four seasonal hCoVs (HKU1, OC43, NL63, 229E), were diagnosed using qRT-PCR. Monthly blood samples were collected to facilitate analyses of peripheral blood mononuclear cells (PBMCs), cytokines, and antibodies. Baseline serum samples were sorted according to their IgG or IgA titers to each of the seasonal hCoVs, and SARS-CoV-2 infection rates were compared in the highest and lowest quartiles. We observed a stark contrast between participants in the highest and lowest quartiles for OC43 IgG: All (100%) individuals in the highest quartile were infected with SARS-CoV-2, whereas the infection rate in individuals in the lowest quartile peaked at 50% (Fisher`s exact test: OR = 0.000, p = 0.01373). In support of these findings, we found that the baseline and pre-infection anti-OC43 IgG antibody levels of individuals who were subsequently infected with SARS-CoV-2 were significnatly higher than those of individauls who were not infected with SARS-CoV-2 (p=0.019).

We then used samples from a multi-center study of 606 healthcare workers folowed over a seven-month period, who were monitored for symptomatic SARS-CoV-2 infections via qRT-PCR. Remarkbly, we observed a similar pattern: Only 52% of individuals with higher anti OC43 IgA titers (highest quartile) were subsequently symptomatically infected with SARS-CoV-2, and 37.5% of individuals in the lowest quartile were infected with SARS-CoV-2 (Fisher`s exact test: OR = 0.554, p = 0.0152).

Our findings suggest a potential correlation between elevated OC43 antibody titers in the serum and increased susceptibility to SARS-CoV-2 infections. These observations underscore the intricate interplay between different coronaviruses and shed light on factors influencing susceptibility to viral infections.

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Poster presentation

01 Host-pathogen interactions

## Characterization of the effect of diet on bacterial virulence

**Dor Braverman**[1], Neta Sal-Man[1]
*Microbiology and Immunology, Ben-Gurion University, Israel*

Plant-based diet is broadly defined as the consumption of mainly plant-based whole foods as a source of macronutrients, micronutrients, and bioactive components. It was found to promote human health by providing protection against various chronic diseases. While the mechanisms regarding these effects are not fully characterized, it is assumed to be associated with changes in the composition of the intestinal microbiome and its metabolic byproducts, which in turn provides antimicrobial and anti-inflammatory responses. Today, most of the existing data regarding the effects of a plant-based diet on enteric pathogens revolves around indirect effects mediated by the mentioned changes in the intestinal microbiome. However, the direct effects of plant- and animal-based diets on the virulence of enteric pathogens remains largely unexplored. Here, I aimed to characterize these effects on various enteric bacterial virulence mechanisms, including type 3 secretion System (T3SS) activity, biofilm formation, toxin production, and host-cell infections. Using peptones derived from different sources (meat, soy, pea and casein), I have observed diverse effects on bacterial virulence. While growth in tryptone (derived from the milk protein casein) did not significantly alter the growth rate of enterohemorrhagic Escherichia coli (EHEC), it had an inhibitory effect on the expression and secretion of T3SS components. This later manifested in significant inhibition on a cell culture infection model. Interestingly, those effects were not observed on the other attaching and effacing pathogen, enteropathogenic Escherichia coli (EPEC). These preliminary results suggest that dietary components might have a direct effect on bacterial virulence, yet not a uniform one.

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## Structural and functional characterization of the EspD protein of the T3SS of enteropathogenic Escherichia coli

**May Morhaim**[1]
*Microbiology, Ben Gurion University, Israel*

Enteropathogenic Escherichia coli (EPEC) is a significant global cause of gastrointestinal illness. This strain utilizes a specialized transport system called type III secretion system (T3SS) to colonize host cells. Its initial contact with the host membrane is mediated by the pore complex of the T3SS, which is formed by two oligomerizing proteins; EspB and EspD. The EspD protein has an unknown structure, predicted to have two coil-coiled domains and two transmembrane domains (TMDs). Using multiple sequence alignment, EspD protein and its homologs in T3SSs of other pathogens were analyzed, and identified with a few highly conserved residues at the N-terminal coiled-coil domain. EPEC is used as a model system to assess whether these conserved residues are critical for EspD function. For that, we cloned single mutants of EspD, expressed them in the DespD mutant, and examined their effect on protein expression, secretion, and ability of the strains to infect host cells. While most strains expressing and secreting mutant EspD versions exhibited normal infection ability, the point mutation E164A abolished the ability of EPEC to infect cells and significantly reduced the ability of the bacteria to form actin pedestals under infection. E164A mutation showed similar expression, secretion, folding, and oligomerization as the wild-type protein. In contrast, EspD and Tir proteins` ability to translocate into the host membrane was significantly reduced. These findings indicate that E164 plays a pivotal role in an intermediate phase and is highly probable to modulate the interactions between pore-forming proteins, EspD and EspB, or pore creation ability.

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## ACE2 is a novel entry factor for HCV

**Samer Ayoub**[1], Moshe Dessau[2], Meital Gal-Tanamy[1]
[1]*Molecular Virology lab, The Azrieli Faculty of Medicine, Bar-Ilan University, Israel*
[2]*The Laboratory of Structural Biology of Infectious Diseases, The Azrieli Faculty of Medicine, Bar-Ilan University, Israel*

Background and Objectives: SARS-CoV-2 entry into host cells is mediated by its interaction with the receptor ACE2. Hepatocytes, target cells for hepatitis C virus (HCV), express low levels of ACE2. We demonstrated that HCV increases ACE2 expression, enabling SARS-CoV-2 and HCV co-infection and co-replication in hepatocytes. We aim to evaluate ACE2`s role in the HCV lifecycle.

Methods & Results: In Huh7.5 cells, ACE2 expression levels affect susceptibility to HCV infection, particularly at the viral entry cell binding step, but not RNA replication, using HCVcc, HCVpp, and replicon systems. HCV envelope protein E2 showed higher binding to ACE2-expressing cells in flow cytometry. In in-vitro assays using Microscale thermophoresis (MST) and ELISA, we measured a direct interaction between these proteins. ACE2 upregulation increased the expression of HCV main receptors CD81 and Claudin, while knocking down ACE2 reduced their expression. Cell-to-cell HCV transmission was upregulated in ACE2 overexpressing cells. Additionally, ACE2 enhanced HCV-mediated cell signaling via EGFR activation. HCV infection upregulates ACE2 through the HIF1-α pathway. Our preliminary data also show that ACE2 increases the infectivity of other flaviviruses, suggesting ACE2 as a novel pan-flavivirus entry factor.

Conclusions: This study reveals that ACE2 is upregulated in response to HCV infection and acts as a novel entry factor for HCV and potentially other flaviviruses. These findings may aid in developing pan-flavivirus drugs to reduce viral infectivity and pathogenicity.

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## Utilizing nutrient type compounds as anti-bacterial compounds: arginine and cysteine inhibit Salmonella survival in egg white

**Nir Ben-Porat**[1], Amital Ohayon[1], Tali Rosenberg[1], Abdulafiz Musa[2], Erik Petersen[2], Erez Mills[1]

[1]*Department of Animal Sciences, Robert H. Smith Faculty of Agriculture, Food, and Environment, The Hebrew University of Jerusalem, Israel*

[2]*Department of Health Sciences, College of Public Health, East Tennessee State University, United States*

Because of growing levels of antibiotic resistance, new methods to combat bacteria are needed. We hypothesized that because bacteria evolved to survive in specific environments, the addition of compounds, including nutrient type compounds, to an environment, might modify that environment, disrupting bacterial growth or causing maladaptive bacterial behavior, i.e., gene expression. As a proof of concept, we focused on the egg white environment and the pathogen Salmonella. Despite egg white's antibacterial nature, Salmonella can survive and grow in egg white, leading to infection in chicks and humans. The 20 L-amino-acids were screened for their ability to affect the growth of Salmonella in egg white. L-arginine and L-cysteine were found to reduce growth in egg white at physiologically relevant concentrations. To determine the mechanism behind L-arginine inhibition, TnSeq was utilized. TnSeq identified many Salmonella genes required for survival in egg white including genes for iron import, biotin synthesis, stress responses, cell integrity, and DNA repair. However, comparing Salmonella in egg white with and without L-arginine identified few differences in the frequency of transposon insertions, suggesting cell envelope perturbations. Finally, both D-arginine and D-cysteine were found to inhibit Salmonella in egg white, implying chemical rather than biological effect. Importantly, these compounds have no inherent anti-bacterial properties and only become anti-bacterial in a specific environmental context. To conclude, these result show that addition of nutrient compounds to an environment can limit bacterial growth. Future research screening for the effects of compounds in relevant environments might uncover new ways to reduce pathogen levels.

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## Deciphering the immune subsets in Salmonella Typhi human infection which underly disease outcome

Noa Bossel Ben-Moshe[1]
*Immunology and Regenerative Biology, Weizmann Institute of Science, Israel*

Management of bacterial infections is becoming increasingly difficult due to rapidly evolving pathogens with increased virulence and drug resistance. Promising alternatives to targeting pathogens, novel anti-infective approaches, harness the host's own response to infection. To use these alternatives, a comprehensive understanding of the complex interaction between different immune cells, and how they translate into infection outcomes is required. Currently, our understanding of human infection is limited to studies performed at disease stages, when symptoms are already evident. We believe that early stages of infection are critical in determining infection outcome. To bridge this gap, models of human disease are required, in order to characterize early immune responses which correlate to infection outcome. For this, we used samples from the human challenge model, in which healthy volunteers are infected with Salmonella Typhi (S.ty). We performed single-cell RNA-seq on PBMCs samples from 6 individuals before and at different time-points post-infection. Three individuals developed disease and three did not. We functionally characterized the immune subsets and found (1) immune subsets that are shared across all individuals and represent global response to S.ty infection; and (2) immune subsets that exhibit inter-individual variability which was connected to infection outcome. Interestingly, some of these states were already evident before challenge, suggesting protective effects to S.ty infection; other states were evident as early as 12 hours post-challenge. These immune subsets can be used to predict the risk of developing disease, and suggest new opportunities to direct the course of infection towards a favorable outcome to the host.

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01 Host-pathogen interactions

## Interactions of the chronic-infecting *Haloferax volcanii* pleomorphic virus 1 (HFPV-1) with its host

**Himani Singla**, Deepak kumar Choudhary, Israela Turgeman grott, Uri Gophna

Studying archaea and their viruses could offer valuable insights into virus evolution and reveal new molecular mechanisms in the virus-host arms race. We studied the interaction of an archaeal virus Haloferax volcanii pleomorphic virus (HFPV-1) with its host Haloferax volcanii, focusing on biofilm formation. Our findings revealed HFPV-1 as the first archaeal virus that promotes biofilm formation, with a similar trend observed in other haloarchaeal species that we infected with HFPV-1. We showed higher biofilm-associated light absorbance in infected cells, early cellular cluster formation and higher surface attachment, confirming that the virus promotes biofilm formation. Additionally, we demonstrated that the virus lowered the motility of the host in motility plate assays. It is therefore highly likely that virus factors interact with or regulate host surface proteins. Furthermore, we observed that more cells (previously non-infected) were infected by HFPV-1 upon co-incubation under static biofilm-forming conditions as compared to shaking culture conditions. Since biofilms are known as zones of increased horizontal gene exchange events, we looked for the function of HFPV-1 in horizontal gene transfer. Surprisingly, we found that this virus slightly reduces the mating efficiency in H. volcanii. Nonetheless, all the screened mated colonies were found to be infected by the virus and thus mating probably aids virus spread. Overall, this indicates that HFPV-1 may act as a barrier to gene transfer, while it may potentially benefit from mating and biofilm formation. Our findings provide new insights into the role of HFPV-1 interaction with its host plays in evolutionary processes.

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01 Host-pathogen interactions

## Characterizing the pathogen niche of Salmonella in the gut at early stages of disease

**Liron Levy-Efrati**[1], Noa Bossel Ben-Moshe[1], Roi Avraham[1]
*Department of Immunology and Regenerative Biology, Weizmann Institute of Science,, Israel*

Salmonella enterica, a gram-negative intracellular pathogen, infects a wide range of hosts, causing diseases from self-limiting gastroenteritis to systemic infections. During the initial infection, Salmonella penetrates the gut tissue, mainly through M cells overlying Peyer's patches, to survive, replicate and promote bacterial spread to systemic organs. However, the identity of host cells that comprise the Salmonella niche, their inflammatory phenotype and metabolic state are not well understood. In my studies, I have characterized the pathogen niches of Salmonella in different gut regions, using two mouse models of oral infection with Salmonella enterica serovar Typhimurium (S.Tm). These models position S.Tm at different gut regions , resulting in distinct disease phenotypes: in the enteritis model, S.Tm primarily colonizes the cecum, leading to local gut inflammation, while in the typhi model, the bacteria predominantly colonize the ileum leading to systemic infection. Using transcriptomics and microscopy, I have characterized the kinetics of infection, the immune responses and changes in immune cell composition, expression, and metabolic state following S.Tm infection. Our results indicate that in the Typhi model, S.Tm predominantly colonizes the ileum without causing inflammation until 72 hours post-infection. I further describe a distinct early signature that emerges in a subset of monocytes within the infected Peyer's patches. We hypothesize that this distinctive signature plays a crucial role in establishing the S.Tm pathogen niche in the gut, and we are currently analyzing its impact on disease progression.

POSTER SESSION II (Tuesday, September 10) (Tuesday, September 10, 2024 12:45)

Poster presentation

01 Host-pathogen interactions

## Algal-Bacterial Host-Pathogen Interactions in the Ocean

**Nitsan Albocher-Kedem**[1], Bareket Dassa[2], Assaf Vardi[1]
[1]*Plant and Environmental Sciences, Weizmann Institute of Science, Israel*
[2]*Life Sciences Core Facilities, Weizmann Institute of Science, Israel*

Phytoplankton are unicellular, photosynthetic microorganisms that dominate the ocean's primary production. Phytoplankton blooms have an important ecological impact on marine food webs. The demise of algal blooms is an essential ecosystem process that fuels the marine food web. The 'microbial loop' consists of marine heterotrophic bacteria responsible for processing most of the primary production material fixed by phytoplankton. Studying interactions of phytoplankton and bacteria in co-culture revealed that some bacteria display a lifestyle switch from mutualism to pathogenicity toward their algal host.

The cosmopolitan coccolithophore Emiliania huxleyi is a unicellular eukaryotic alga responsible for some of the most significant oceanic algal blooms. Sulfitobacter D7 from the algal-associated roseobacter clade isolated from an E. huxleyi bloom in the North Atlantic exhibits a lifestyle switch from mutualism to pathogenicity, which is presented in algicidal effects against E. huxleyi upon co-culturing.

To elucidate the mechanisms of the lifestyle switch and pathogenicity, we constructed a transposon mutants' library of Sulfitobacter D7 and tested the mutants' changes in virulence towards E. huxleyi. Next, we study the changes in the transcriptomes plan leading to the lifestyle switch and pathogenicity by constructing a dual-RNA sequencing library from different time points of E. huxleyi and Sulfitobacter D7 couture representing the coexistence and pathogenicity stats. Together, this leads us to the bacterial genes involved in virulence at sea.

POSTER SESSION II (Tuesday, September 10) (Tuesday, September 10, 2024 12:45)

Poster presentation

02 Microbial ecology and evolution

## Aerobic methane production in Nitrogen-depleted oceanic regions

**Jaime Abdiel Lazaro Garcia**[1], Rinat Bar Shalom, Alhan Abu Hamoud
*School of Marine Science, University of Haifa, Israel*

Phosphorus (P) and nitrogen (N) are common limiting elements for primary and secondary production in aquatic environments. Interestingly, important amounts of the potent greenhouse gas methane are produced under the depletion of these inorganic nutrients. It has been demonstrated that the utilization of dissolved organic nutrient compounds can result in methane emissions. Specifically, under P-limiting conditions, the utilization of methylphosphonates (MPn) as a phosphorus source by marine bacteria leads to methane release. Moreover, it has been suggested that methylamine and other methylated compounds, such as dimethylsulfoniopropionate (DMSP), may result in methane emissions under N-depletion. While methane production via the degradation of MPn has been studied in relative depth, little is known about the specific metabolism, genes, and microbial groups involved in methylamine and DMSP-related aerobic methane production. This research aims to identify whether methylated organic compounds, like methylamine and DMSP, can be utilized as nitrogen and sulfur sources by marine bacteria through the aspartate aminotransferase pathway (AAT) and the DMSP lyase pathway (dddD), respectively, while releasing methane. In this study, we investigate the potential for methane release by marine bacterial isolates that are abundant members of the natural marine microbial community, such as members of the SAR11 clade. The results from this research will enhance our understanding of the sources of methane that contribute to the typical methane supersaturation observed in surface oceanic waters.

POSTER SESSION II (Tuesday, September 10) (Tuesday, September 10, 2024 12:45)

Poster presentation

02 Microbial ecology and evolution

# Impact of Biodegradable and Non-biodegradable Microplastic on Soil Microbial Community Composition and Activity

**Lingyan (Ciel) Zhang**[1], Max Kolton[1]
*French Associates Institute for Agriculture and Biotechnology of Drylands, Ben Gurion University of the Negev, Israel*

Microplastics, plastic pieces smaller than 5 mm, accumulate in soil and alter the structure and function of soil microbial communities. While non-biodegradable microplastics are known to harm soil health, the ecological impacts of both biodegradable and non-biodegradable microplastics in arid agricultural soils remain poorly understood. This study hypothesizes that polymer bioavailability affects soil microbial communities differently. To test this hypothesis, Negev agricultural soil was amended with 0.5% PolyLactic Acid (PLA), PolyEthylene Terephthalate (PET), or cellulose and incubated for 105 days at 25°C. Soil properties, microbial respiration, enzymatic activities, and microbial community structure were assessed five times during incubation. Two phases of microbial response were observed: an initial phase of enhanced enzymatic activity and microbial biomass, followed by a decline in activity, biomass, and diversity. The taxonomic analysis of microplastic-amended soil consistently showed the presence of denitrifying bacteria from the genera Microvirga and Skermanella, as well as autotrophic nitrogen-fixing bacteria from the genus Azohydromonas. Additionally, while PET amendment stimulated the propagation of the strictly anaerobic Lentimicrobium from the phylum Bacteroidota, other treatments induced Dechlorobacter. Moreover, beta-diversity analysis revealed significant microbial community shifts over time. However, incubation time strongly affects microbial community structure, overshadowing the effects of microplastics. These findings suggest that initial microbial activity is stimulated by substrate amendment, but prolonged incubation leads to nutrient depletion and microbial community decline. Future studies should consider continuous substrate supplementation during incubation to mitigate nutrient depletion and provide more precise insights into the impacts of microplastic contamination on soil microbial dynamics.

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Poster presentation

02 Microbial ecology and evolution

## Exploring the influence of plant fiber on gut microbiome diversity

**Sarah Winkler**[1], Omer Lavy[1], Liron Levin[1], Stav Eyal[1], Anke Trautwein-Schult[2], Dörte Becher[2], Sarah Morais[1], Yitzhak Mizrahi[1]
[1]*Life Sciences, Ben Gurion University of the Negev, Israel*
[2]*Microbial Proteomics, University of Greifswald, Germany*

The relationship between gut microbe diversity and dietary habits, particularly high-fiber intake, is widely recognized. However, the precise mechanisms underlying this connection and its consistency across diverse gut ecosystems remain enigmatic. Through meticulous examination of community composition, genetic content, and expression patterns, we aim to elucidate the intricate interplay between diet and microbiome dynamics. Our findings suggest a correlation between heightened diversity and phenomena such as niche differentiation and collaborative behaviors among microbial populations, as evidenced by the distribution of genetic pathways. This association transcends various mammalian species and is evident within individual animals across their lifespan. Moreover, we identify specific pathways driving the selection process within microbial communities, ultimately contributing to enhanced community richness. By framing the genetic interactions of microbes within the context of diet, we envision the potential to engineer synthetic microbiomes tailored to desired phenotypic outcomes based on microbial genetic capabilities.

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Poster presentation

02 Microbial ecology and evolution

## Evolution and Ecology of Environmental Microbiome in Response to Human Interferences

**Guy Tadmor**[1], David Zeevi[1], Assaf Vardi[1]
*Plant and Environmental Science, Weizmann Institute of Science, Israel*

The demand for freshwater is rising as the global population constantly grows, exerting ever-increasing pressure on natural ecosystems through anthropogenic pollution. This pollution poses significant threats to aquatic ecosystems across all trophic levels, including microbial communities. As pollutants accumulate, microbial communities adapt, resulting in an alteration of both microbial species composition and community-level functionality. This study investigates the impact of human activities, specifically anthropogenic pollution, on environmental microbiomes across ecological and evolutionary time scales, focusing on pollution-driven changes and adaptations in constantly contaminated environments. We will take a metagenomic approach applied to time-series datasets to explore changes in microbial dynamics and gene evolution. We aim to identify potential biomarkers and bioremediators for better pollution management.

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Poster presentation

02 Microbial ecology and evolution

## Enhanced microbial succession for oil degradation in Arid region

**Kareem Neiroukh**

In December 2014, the Eilat-Ashkelon oil pipeline breach caused a major crude oil spill in the Ein Evrona nature reserve, emphasizing the need for effective bioremediation strategies. This study investigates microbial succession in oil-polluted arid environments. Using 16S rRNA gene sequencing, we extracted DNA from soil samples under sterile conditions to explore microbial community composition and dynamics. The results revealed significant shifts in microbial diversity and composition, as indicated by Phylogenetic Diversity (PD), Alpha, and Beta diversity metrics, with increased evenness and reduced dominance, suggesting successful microbial adaptation and hydrocarbon degradation. Initially, control samples exhibited the highest evenness, which decreased after exposure to water and nutrients but gradually increased over time. This pattern suggests that the early dominance of fast-growing bacteria reduces evenness, but as slower-growing bacteria proliferate, evenness is restored. The study highlights the crucial role of microbial communities in environmental recovery and demonstrates the potential of leveraging microbial succession to enhance bioremediation efforts in oil-contaminated arid regions. These insights improve our understanding of microbial responses to bioremediation, paving the way for more effective environmental restoration practices.

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Poster presentation

02 Microbial ecology and evolution

# Application of chromosomal barcoding to study the role of horizontal gene transfer in bacterial adaptation

**Elizaveta Miticheva**[1], Shimon Bershtein[1]
*Department of Life Sciences, Ben-Gurion University, Israel*

Horizontal gene transfer (HGT) plays a crucial role in the innovation of prokaryotic genomes. Our current studies focus on unraveling the role of HGT in the dynamics of bacterial metabolic adaptation, particularly in Acinetobacter baylyi ADP1, a gram-negative soil bacterium known for its high natural competence.
In this work, we introduced a high-resolution chromosomal barcoding approach based on natural competence in A. baylyi ADP1, creating a bacterial population with 10^6 distinct lineages. Additionally, we designed a barcoded bacterial population lacking HGT capacity. Using those tools, we created an adaptive laboratory evolution (ALE) experimental design to investigate the role of HGT in bacterial adaptation and the interplay between HGT and point mutations in driving metabolic adaptation.
We performed an ALE experiment to adapt A. baylyi ADP1 populations to resistance against chloramphenicol and trimethoprim antibiotics. It demonstrated that the ability to exchange genes horizontally significantly alters the dynamics of bacterial adaptation.

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Poster presentation

02 Microbial ecology and evolution

## Demystifying Heterotrophic Diazotrophy in Streambeds

**Rand Lander**[1], Eyal Geisler[1], Carla Villamarin[1], Shai Arnon[1], Almog Gafni[1], Hagar Siebner[1], Edo Bar-Zeev[1]
*Zuckerberg Institute of Water Research, Ben-Gurion University of the Negev, Sde Boker, Israel*

Marine N2 fixation by heterotrophic diazotrophs was found to have an important role in primary and secondary production. Yet, In freshwater ecosystems and especially in streambeds, little is known about the role of heterotrophic diazotrophs in the N cycle. Here, we investigate diazotrophy (i.e., N2 fixation, abundance, and diversity) below the streambed, focusing on biofilms attached to the sediments and free-living cells in porewater. Samples were collected from the Dan stream (upstream Jordan river), characterized by pristine, high-flow velocity waters and Gesher Arik, found downstream at the Lake Kinneret estuary. Aerobic and anerobic streambed samples were spiked with 15N and incubated for 24h under ambient conditions. At the end of the incubation, samples were analyzed for N2 fixation rates, diazotroph abundance, and diversity. Results show higher N2 fixation in anaerobic than the aerobic layer (11 and 6.4 nmol N g-1d-1, respectively), and inversely, significantly higher at the aerobic porewater than the anaerobic layer (2.8 and 0.7 nmol N L-1d-1, respectively). Immunolabeled flow cytometry indicated that anaerobic porewater diazotrophs comprised a significant fraction (9%) of total bacteria in the Dan stream, while much higher (26%) in Gesher Arik. The α diversity of diazotrophs within the aerobic porewater in both sites was significantly higher than the anerobic layer likely due to deposition and resuspension events occurring at the sediment-water interface. Our results indicate the significance of subsurface diazotrophy and proffer new insights into how diversity and ecological heterogeneity can affect N cycling across aerobic and anaerobic microenvironments as biofilm or free-living cells.

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Poster presentation

02 Microbial ecology and evolution

# Pear flower and leaf microbiome dynamics during the naturally occurring spread of Erwinia amylovora

**Aia Oz**[1], Orly Mairesse[2,3], Shira Raikin[2], Hila Hanani[1], Hadar Mor[1], Mery Dafny Yelin[2], Itai Sharon[1,4]

[1]*Biotechnology, Migal – Galilee Research Institute, Israel*

[2]*Northern Agriculture Research & Development, Migal – Galilee Research Institute, Israel*

[3]*Sciences and Technology, Tel-Hai Academic College,, Israel*

[4]*Science and Technology, Tel-Hai Academic College, Israel*

Erwinia amylovora is the causal pathogen of fire blight, a contagious disease that affects apple and pear trees and other members of the family Rosaceae. Here, we investigated the population dynamics of the pear flower microbiome in an agricultural setting during the naturally occurring infection of E. amylovora. Five potential factors were considered: collection date, the flower's phenological stage, location on the tree, location within the orchard, and pear cultivar. The phenological stage and the collection date were identified as the most important factors associated with pear flower microbiome composition. The location of the tree in the orchard and the flower's location on the tree had a marginal effect on the microbiome composition. The leaf microbiome reflected that of the abundant phenological stage on each date. The flower microbiome shifted towards E. amylovora, dominating the community as time and phenological stages progressed, but the strain population of E. amylovora remained similar throughout the entire collection period. In contrast, other taxa, including Pseudomonas, Pantoea, Lactobacillus, and Sphingomonas, were represented by dozens of amplicon sequence variants (ASVs), and different succession patterns in their populations were observed. Some of the taxa identified include known antagonists to E. amylovora.

This study is the first to describe the flower microbiome dynamics during the naturally occurring infection of E. amylovora. Strain succession patterns for the different taxa under E. amylovora spread varied across different taxonomic groups, and their characterization may help in choosing candidates for antagonist-based treatments for fire blight.

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Poster presentation

02 Microbial ecology and evolution

# Metabolically-versatile Ca. Thiodiazotropha symbionts of the deep-sea lucinid clam Lucinoma kazani have the genetic potential to fix nitrogen

**Lina Ratinskaia**[1,2], Stas Malavin[1,3], Tal Zvi-Kedem[1,2], Simina Vintila[4], Manuel Kleiner[4], Maxim Rubin-Blum[1,2]

[1]*Department of Biology, National Institute of Oceanography, Israel Oceanographic and Limnological Research, Israel*

[2]*Department of Marine Biology, Leon H. Charney School of Marine Sciences, University of Haifa,, Israel*

[3]*Department of Environmental Hydrology and Microbiology, Zuckerberg Institute for Water Research, The Jacob Blaustein Institutes for Desert Research, Ben-Gurion University of the Negev, Israel*

[4]*Department of Plant and Microbial Biology, North Carolina State University, USA*

Lucinid clams are one of the most diverse and widespread symbiont-bearing animal groups in both shallow and deep-sea chemosynthetic habitats. Lucinids harbor Ca. Thiodiazotropha symbionts that can oxidize inorganic and organic substrates such as hydrogen sulfide and formate to gain energy. The interplay between these key metabolic functions, nutrient uptake and biotic interactions in Ca. Thiodiazotropha is not fully understood. We collected Lucinoma kazani individuals from next to a deep-sea brine pool in the eastern Mediterranean Sea, at a depth of 1150 m and used Oxford Nanopore and Illumina sequencing to obtain high-quality genomes of their Ca. Thiodiazotropha gloverae symbiont. The genomes served as the basis for transcriptomic and proteomic analyses to characterize the in situ gene expression, metabolism and physiology of the symbionts. We found genes needed for N2 fixation in the deep-sea symbiont's genome, which, to date, were only found in shallow-water Ca. Thiodiazotropha. However, we did not detect the expression of these genes and thus the potential role of nitrogen fixation in this symbiosis remains to be determined. We also found the high expression of carbon fixation and sulfur oxidation genes, which indicate chemolithoautotrophy as the key physiology of Ca. Thiodiazotropha. However, we also detected the expression of pathways for using methanol and formate as energy sources. Our findings highlight the key traits these microbes maintain to support the nutrition of their hosts and interact with them.

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Poster presentation

02 Microbial ecology and evolution

## Neutral Dispersal and Host-Mediated Selection Shape Bacterial Metacommunity in Galapagos Marine Iguanas

**Ido Grinshpan**[1], Omer Lavy[1], Alvah Zorea[1], Itai Amit[1], Liron Levin[1], Ori Furman[1], Daniel Somech[1], Otto X. cordero[2], Itzhak Mizrahi[1]
[1]*Life science, Ben Gurion University, Israel*
[2]*Climate, Environment & Life Science, MIT, USA*

Understanding the mechanisms governing microbial metacommunity assembly across ecosystems remains a challenge in microbial ecology. This study explores the microbial metacommunity of the endangered Galapagos marine iguana, an understudied host species. We sequenced over 100 faecal samples using 16SrRNA amplicons and applied ecological models to elucidate community assembly processes. Our findings reveal that neutral dispersal is the primary driver of community assembly. However, the iguana gut microbiome exhibits selective retention of specific bacteria despite continuous exposure to diverse microbial populations, indicating a purifying selection process mediated by host-microbe interactions. Furthermore, we identified phylogenetic clustering suggestive of potentially endemic Clostridia species adapted to these marine iguanas. This research provides insights into the ecological dynamics of this unique metacommunity and underscores the importance of preserving microbial biodiversity in endangered species. Our findings may inform future conservation strategies and the development of tailored probiotic treatments for these iconic reptiles.

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Poster presentation

02 Microbial ecology and evolution

# Diet Driven Selection of Microbial Communities and its functional-ecological outcomes

**Omer Lavy**[1], Sarah Whinkler[1], Sarah Morais[1], Liron Levin[1],
Anke Trautwein-Schult[2], Dörte Becher[2], Itzik Mizrahi[3]
[1]*Department of Life sciencs, Ben Gurion university, Israel*
[2]*Department of Microbial Proteomics, University of Greifswald, Germany*
[3]*Department of Life sciences, Ben Gurion university, Israel*

Diet is a prominent example of an environmental constraint affecting the composition of gut bacterial communities. Specifically, fiber-rich diets have been shown to promote the diversity of microbial species, thus assumed to increase the functional potential in comparison to simple-sugar-based diets (soluble carbohydrates). However, the mechanisms behind these diet-derived selection processes remain unclear. Delineating the diet-microbial community connection, will take us a step forward towards engineering gut microbial communities to desired functions.

To decipher how diet shapes community function, we used a collection of zoo animal fecal samples representing three different digestive strategies (herbivores, omnivores, and carnivores) with different initial pools of microbial communities. These communities were "fed" and selected on fiber-rich and fiber-poor diets. This allowed us to remove the host effect, and isolate the diet effect on gut microbial communities.

This selection process was meticulously monitored using amplicon and metagenomics sequencing. In addition, we monitored community function using a fiber degradation assay on the selected communities.

We find that under the same selection regime, bacterial communities of different animal hosts underwent several selection processes to demonstrate one of two distinguished phenotypic functionalities regarding fiber degradation. These significant differences have enabled us to trace back features responsible for the different selection trajectories and utilize them as predictive indicators for fiber degradation capabilities.

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02 Microbial ecology and evolution

## Diffusivity influences evolutionary interactions of bacteria and bacteriophages in space and time

**Christoph Velling**[1], Roy Kishony[1,2,3]
[1]*Faculty of Biology, Technion - Israel Institute of Technology, Israel*
[2]*Faculty of Computer Science, Technion - Israel Institute of Technology, Israel*
[3]*Faculty of Biomedical Engineering, Technion - Israel Institute of Technology, Israel*

Bacteria and bacteriophages exhibit complex co-evolutionary dynamics in spatially structured environments. A major open question is how and under which conditions these dynamics are present? In this project, we try to tackle this question by studying the invasion of resistant bacteria into a travelling wave of sensitive bacteria and bacteriophages. This process raises an interesting conundrum: phage resistance has an advantage behind the propagating front, where there is a substantial concentration of phages, but can these resistant bacteria take over the front itself? Building an in-silico model allows us to explore the parameter space and to identify those parameters which have the biggest impact on the dynamics. Preliminary results suggest, apart from an obvious impact of the growth rate ratio, that bacterial diffusion strength seems to have a strong impact on the dynamics. Our project will pave the way for experimental projects to confirm observed dynamics in spatially structured environments.

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Poster presentation

02 Microbial ecology and evolution

## A systematic exploration shows that Bacillus subtilis has a readily accessible plastic metabolic network

Shanni Horsntein, Swati Sirotiya, Raul Mireles, Alex Geller, Asaf Levi, **Lianet Noda-Garcia**

Underground metabolism (UM) refers to physiologically irrelevant proteins` weak side functions and protein-free chemical conversions. Although this messiness appears by chance, it is highly relevant to adaptation processes, such as when the environment changes or in response to organismal lesions. The bacterial kingdom is king in metabolic richness. Still, UM has only been systematically explored in Escherichia coli, mainly through exploring adaptation to gene loss using the single gene knockout collection. Recently, a similar collection was built for the gram-positive model bacterium Bacillus subtilis. With it, we have been exploring B. subtilis UM potential. We tested whether B. subtilis could recover from losing 17 enzymes, mostly involved in amino acid biosynthesis. We did this in two genetic backgrounds, one weak and one strong biofilm-producing, expecting biofilms to promote adaptation. The 34 strains were cultivated long in agar minimal media, where the missing enzyme is essential for survival and, in a nutrient gradient environment, to promote adaptation. When growth was observed, we replicated it at least twice. Like this, we evolved 49 different populations of B. subtilis to bypass nine of the 17 functions tested. We sequenced the genome of the 49 populations and two isolated colonies from each, plus their ancestral states, to identify the mutations responsible for the metabolic adaptations. Sequence analysis revealed the putative mechanism for three of the nine bypasses. In the three cases, loss of function mutations revealed alternative metabolic pathways, showing that B. subtilis metabolic network can evolve via reciprocal sign epistasis.

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Poster presentation

02 Microbial ecology and evolution

# Illuminating the Secrets of Eukaryotic Microbial Adaptation: Differential Gene Expression in a Naturally Red-Shifted Pit Lake Under Diverse Light Conditions

**Shai Fainsod**[1], Oded Beja[1]
*Biology, Technion - Israel Institute of Technology, Israel*

Uncovering how microorganisms adapt to different light environments is key to understanding their ecological roles and evolutionary strategies. This study explores the gene expression profiles of the eukaryotic fraction of microbial communities from a naturally red-shifted pit lake when incubated under different light conditions: red, blue, green, darkness, and unmodified natural light in an open lid box. Through high-throughput RNA sequencing we assessed the differential expression compared to natural light, focusing on both general responses and light-specific transcripts and processes. Two rhodopsin proteins were identified as significantly differentially expressed. One was upregulated under both red light and darkness, while the other was upregulated in darkness. This suggests a red light, rhodopsin-mediated process or cellular preparation for rhodopsin-mediated processes to occur once the light changes. We observed a significant upregulation of antenna proteins related to photosystem I (LHCA), under green light, red light, and darkness. However, antenna proteins related to photosystem II (LHCB) were not significantly upregulated in any treatment, and significantly downregulated in darkness. This shows the overall preference of photosystem I in this experimental setup and the usage of different LHCA proteins to ensure optimal absorption in response to changes in light quality. Transcripts involved in the carotenoid biosynthesis pathway showed specific responses to different light conditions. For instance, different transcripts annotated as zeaxanthin epoxidase (ZEP) were significantly upregulated in darkness or downregulated under red light and darkness, adjusting the zeaxanthin and violaxanthin composition to better fit the organism’s needs in the specific light environment.

This study sheds light on the photo-adaptive capabilities of eukaryotes in a red-shifted environment. These findings could further our understanding of microbial ecology in unique light environments and contribute to biotechnological applications exploiting these adaptive traits.

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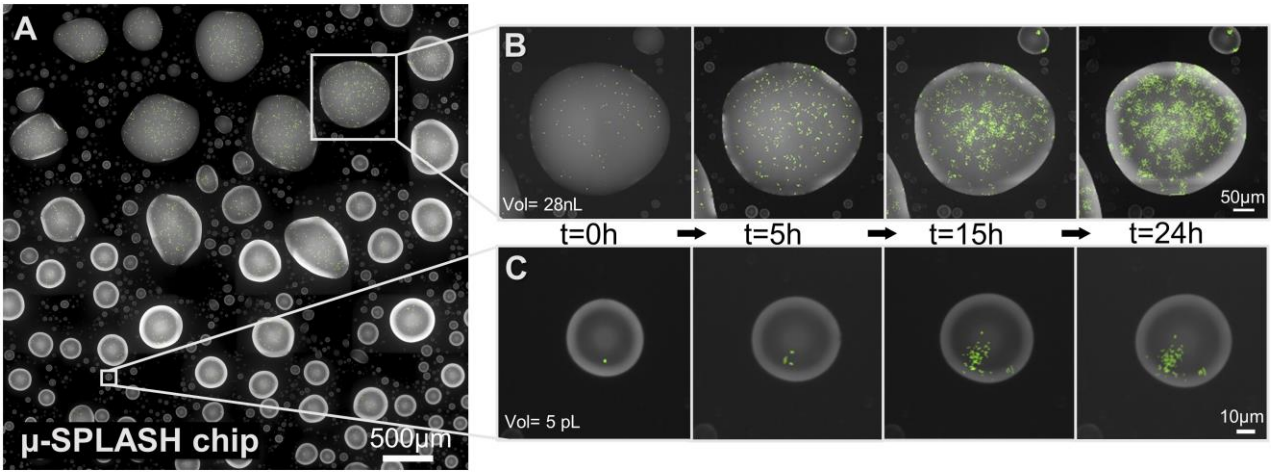
Poster presentation

02 Microbial ecology and evolution

# The impact of micro-habitat fragmentation on microbial populations growth dynamics

**Nadav Kashtan**[1]
*Institute of Environmental sciences, The Herbrew University, Israel*

Microbial communities inhabit almost every habitat on Earth and are essential to the function of diverse ecosystems. Most microbial habitats are not spatially continuous and well-mixed, but rather composed, at the microscale, of many isolated or semi-isolated local patches, resulting in partitioning of microbial populations into discrete local populations. The impact of this spatial fragmentation on population dynamics is not well-understood. Here, we study how fragmentations affect the growth dynamics of clonal microbial populations and how dynamics in individual patches dictate those of the whole metapopulation. To investigate this, we developed the µ-SPLASH, a novel ecology-on-a-chip platform, enabling the culture of microbes in microscopic landscapes comprised of thousands of microdroplets, spanning a wide range of sizes. Using the µ-SPLASH, we cultured the model bacteria E. coli and based on time-lapse microscopy, analyzed the population dynamics within thousands of individual droplets at single-cell resolution. Our results reveal that growth curves vary dramatically with droplet size. While growth rates generally increase with drop size, reproductive success and the time to approach carrying capacity, display non-monotonic patterns. Combining µ-SPLASH experiments with computational modeling, we show that these patterns result from both stochastic and deterministic processes, and demonstrate the roles of initial population density, patchiness, and patch size distribution in dictating the local and metapopulation dynamics. This study reveals basic principles that elucidate the effects of habitat fragmentation and population partitioning on microbial population dynamics. These insights are imperative for a deeper understanding of natural microbial communities and have significant implications for microbiome engineering.



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Poster presentation

02 Microbial ecology and evolution

## Light-harvesting by antenna-containing xanthorhodopsins in cyanobacteria

**María del Carmen Marín Pérez**[1], Andrey Rozenberg[1], Keiichi Inoue[2], Oded Béjà[1]
[1]*Faculty of Biology, Technion-Israel Institute of Technology, Israel*
[2]*The Institute for Solid State Physics, University of Tokyo, Japan*

Microbial rhodopsins are a versatile family of light-sensitive proteins crucial to various phototrophic and sensory processes in microorganisms. Xanthorhodopsins, notable for their dual chromophore system involving retinal and carotenoids, have been predominantly studied in halophilic bacteria where they facilitate light-driven proton pumping and photoprotection. Here we report the finding of a xanthorhodopsin in cyanobacteria with the novel ability to bind a hydroxylated carotenoid. We employed bioinformatic analysis, spectroscopic techniques, and functional assays to characterize this novel xanthorhodopsin. The presence of a hydroxylated carotenoid-binding xanthorhodopsin in cyanobacteria suggests an evolutionary adaptation to diverse and fluctuating light environments, contributing to their ecological success. This discovery provides new insights into the functional versatility of rhodopsins and their evolutionary trajectories.

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02 Microbial ecology and evolution

## Species sorting as the central paradigm modulating microbial assembly in a two-step biofilter with Ulva and periphyton

**Dzung Nguyen**, Matan Masasa, Ofer Ovadia, Lior Guttman

Metacommunity theory, the study of communities spatially connected via dispersal, is among the central pillars in microbial ecology yet remains understudied. In aquatic environments, it is still unclear which of the four paradigms (patch dynamic, species sorting, mass effects, neutral model) best explains microbial community dynamics. While theoretical research endorsed the neutral model, empirical studies primarily support either mass effects or species-sorting paradigms. Here, we study metacommunity theory based on a dynamic biofilter for the ammonia- and nitrate-rich fishpond effluent comprising Ulva fasciata and marine periphyton connected through a sequenced water flux. This setup induces ammonia depletion in the primary Ulva unit and nitrate removal in the polishing unit of periphyton. Samples were collected throughout the five-week experiment, targeting Ulva-associated microbiota, periphyton, and water microbiomes by 16S rRNA gene amplicon sequencing. Our results, including community structure, diversity, and functionality, strongly support the argument that species sorting, operating through environmental heterogeneity, is the central force that drove microbial community dynamics among the three connected patches. Interestingly, determinism was the leading force underlying community dynamics in all three environments. This trend coincides with community diversity, where the less diverse environment tends to impose a more selective force and vice versa. Though supporting species sorting as the majority, our study stood out thanks to the unique small-scale system, where mass effects were expected to overrule. Future research should consider the studied system`s dispersal rate in relation to other ecological parameters for a more comprehensive understanding of metacommunity paradigms and their extent of application.

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02 Microbial ecology and evolution

# Viral Lysis vs. Nutrient Starvation: Effect on Prochlorococcus Macromolecular Structure and the Microbial Community

**Shira Givati**[1], Dikla Aharonovich[1], Natalia Belkin[2], Eyal Rahav[2], Qinglu Zeng[3], Daniel Sher[1]

[1]*Marine biology, Haifa University, Israel*

[2]*National Institute of Oceanography, Israel Oceanographic and Limnological Research, Israel*

[3]*Department of Ocean Science, Hong Kong University of Science and Technology, China*

In marine environments, cyanobacteria supply organic carbon to heterotrophic bacteria not only as they grow and photosynthesize but also as they die. We hypothesized that different types of mortality, for example phage lysis and nutrient starvation, will have contrasting effects on the macromolecular structure of the cyanobacterial cell, and that the subsequent release of organic matter will drive the activity and growth of different heterotrophic bacteria. To test these hypotheses, Prochlorococcus MED4 was infected by a phage (P-HM2) to examine the effect of infection on host macromolecular composition, compared to natural death by nitrogen starvation. We observed that RNA and DNA quotas increased for the infected Prochlorococcus, while protein quotas decreased in the nitrogen starved Prochlorococcus. Then, spent media and lysate from the dying cells was added to surface seawater collected from the Eastern Mediterranean collected in and autumn and spring. In both experiments dark primary production (DPP) significantly increased in the treatments added with spent media from starved Prochlorococcus. Surprisingly, no major differences were observed in the resulting community structure (as assessed by 16S rRNA and rDNA amplicon sequencing) and thus we hypothesize that the functional differences in response to different forms of mortality is due to a shift in community function towards heterotrophic CO2 fixation.

POSTER SESSION II (Tuesday, September 10) (Tuesday, September 10, 2024 12:45)

Poster presentation

03 Fungal biology

## How can you live without Pds5? Finding new genes affecting the maintenance of sister chromatid cohesion.

**Sreejita Dutta**[1], Karan Choudhary, Nir Katz, Martin Kupiec
*Shmunis School of Biomedicine and Cancer Research, The George S. Wise Faculty ofLife Sciences, Tel Aviv University, Tel Aviv University, Israel*

Cohesin, a highly conserved protein complex across all eukaryotes, mediating the sister chromatic cohesion(SCC), the mechanism that holds the two replicated DNA molecules before they are separated at Anaphase. Cohesin also plays pivotal roles in arranging the chromatin without the nucleus, thus facilitating chromatin condensation, DNA repair and transcription regulation. Pds5 is an essential subunit of cohesin. It is a HEAT repeat protein with roles in SCC establishment and maintenance.

A previous screen in the lab found that cells can grow in the absence of Pds5, if they are mutated in the ELG1 and CLN2 genes. Elg1 works as an unloader of the replication clamp PCNA, and Cln2 is a G1/S cyclin. To find additional mutants with similar effects as the elg1 mutants, we have carried out two genetic screens for cells able to grow in the absence of Pds5 and Cln2: 1) Using a high-copy-number genomic library, we found genes that, when overexpressed, allow these cells to thrive. 2) We selected for additional mutations that allow these cells to grow.

We will present the characterization of the genes and mutations found. Our results provide new insights on the mechanisms that link DNA replication and SCC.

POSTER SESSION II (Tuesday, September 10) (Tuesday, September 10, 2024 12:45)

Poster presentation

03 Fungal biology

# Aerial Dissemination of Pycnidiospores in rainless days: A Case Study of Lasiodiplodia theobromae in Avocado

**Satyendra Pratap Singh**[1], Alon Shomron[1], Ran Shulhani[2], Dani Shtienberg[2], Noam Alkan[1]
[1]*Department of Postharvest Science, Agricultural Research Organization, Volcani Center, Israel*
[2]*Department of Plant Pathology and Weed Science, Agricultural Research Organization, Volcani Center, Israel*

Many fungi disperse by asexual pycnidia. For example, Botryosphaeriaceae family disperse through ascospores (sexual) and pycnidiospores (asexual). The rarely reported Lasiodiplodia sexual stage has never been found in Israel. Therefore, the long-distance fungal spread should be associated with pycnidiospores, which known to disperse by water splash. The research aim was to elucidate the mechanism of pycnidial burst and the pycnidiospores spread for long distance in Lasiodilodia theobromae and avocado plant model. Strong correlation was found between L. theobromae disease symptoms and seasons with high relative humidity (RH). Pycnidia bursts and release pycnidiospores in higher RH. After high RH conditions, the pycnidiospores dispersal was linked to reduction of RH along the day. Wind-tunnel experiments showed that as the humidity and wind speed increase the pycnidiospores are dispersed to longer distance. The mechanism of pycnidia burst seems to be based on high molarity of osmolytes as sugars and glycerol (0.17M) in closed pycnidial sap that resulted in 4.18 atmospheric pressure. We propose a mechanism for pycnidiospores release, in high RH, moisture permeates in the closed pycnidia and mix with pycnidial sap, this increases osmotic pressure triggers the pycnidial burst and pycnidiospores release, as the pycnidial sap dries, the wind carry the pycnidiospores.

POSTER SESSION II (Tuesday, September 10) (Tuesday, September 10, 2024 12:45)

Poster presentation

03 Fungal biology

## Antifungal potential of CaEDTA against postharvest pathogen Botrytis cinerea.

**Durga Prasad**[1,2], Carmit Ziv[1]
[1]*Department of Postharvest Sciences, Agricultural Research Organization, Volcani Center, Israel*
[2]*The Robert H. Smith, Faculty of Agriculture, Food and Environment, The Hebrew University of Jerusalem, Israel*

The phytopathogenic fungus Botrytis cinerea causes postharvest gray mold disease in fruits and vegetables. Till now chemical fungicides are the primary means of controlling gray mold disease, but concerns have been raised regarding their safety for the environment and human health, necessitating the development of less toxic and more effective remedies. Calcium chelated with Ethylenediaminetetraacetic acid (CaEDTA) is Generally Recognized as Safe (GRAS). In the current study we found that CaEDTA showed strong antifungal activity against B. cinerea. The minimal inhibitory concentration (MIC) of CaEDTA was 65 mM, which inhibited mycelial growth, spore germination and stimulated cell death when applied to artificial growth medium. CaEDTA inhibition of B. cinerea was accompanied by accumulation of reactive oxygen species (ROS) and specifically hydrogen peroxide, as determined by BesH2O2 staining, indicating induction of oxidative stress. Checkerboard assay was applied with different antifungal compounds in combination with CaEDTA to study potential synergistic effect. These tests indicated a significant increment in the antifungal activity of CaEDTA when combined with Fuconazole, Myriocin, Lantruculin and Caspofungin that resulted in 4-10 times lower MIC of CaEDTA and more than 20 times lower MIC for the fungicides. The Fractional Inhibition Concentration (FIC) index of all the combination of compounds were in range of 0.04-0.26, hence depicting the synergistic effect of combination of compounds on the B. cinerea. These results imply that CaEDTA may be applied as a useful tool in the fighting against B. cinerea, and using it could reduce the use of traditional hazardous chemical fungicides.

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Poster presentation

03 Fungal biology

## Bacterial-fungal crosstalk is defined by a fungal lactone mycotoxin and its degradation by a bacterial lactonase

Livnat Afriat-Jurnou[1,2], Keren Nudel, **Shlomit Dor**, Rami Cohen, Justin L. Eagan, Christina M. Hull, Nancy P. Keller, Dov Pruski
[1]*Department of Molecular and Computational Biosciences and Biotechnology, Migal-Galilee Research Institute, Israel*
[2]*Tel-Hai College, Faculty of Sciences and Technology, Upper Galilee*

Bacteria, fungi, and mammals contain lactonases that can degrade the Gram-negative bacterial quorum sensing (QS) molecules N-acyl homoserine lactones (AHLs). AHLs are critical for bacteria to coordinate gene expression and pathogenicity with population density. However, AHL-degrading lactonases present variable substrate ranges, including degradation of the Pencillium expansum lactone mycotoxin patulin. In Appl Environ Microbiol. 2024. doi: 10.1128/aem.00299-24. we selected Erwinia spp. as our model bacteria to further investigate this interaction. We find both native apple microbiome Erwinia spp. and the fruit tree pathogen E. amylovora to be inhibited by patulin. At patulin concentrations that inhibited E. amylovora growth, expression of E. amylovora lactonase encoded by EaaiiA was increased. EaAiiA demonstrated the ability to degrade patulin in vitro, as well, as in vivo where it reduced apple disease and patulin production by P. expansum. Fungal-bacterial co-cultures revealed that the E. amylovora Δeaaiia strain failed to protect apples from P. expansum infections, which contained significant amounts of patulin. Our results suggest that bacterial lactonase production can modulate the pathogenicity of P. expansum in response to the secretion of toxic patulin. This work supports the potential use of bacterial enzymes and/or the producing bacteria in controlling the post-harvest fruit disease caused by the patulin-producing fungus Pencillium expansum.

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Poster presentation

04 Clinical virology and epidemiology

# cVDPV3 outbreak in Israel (2021-2022) through enhanced environmental surveillance and mutation analysis: a genomic epidemiology study

**Merav Weil**[1], Itay Bar-Or[1], Hagar Morad-Eliyahu[1], Efrat Bucris[1], Leah Weiss[1], Rinat Vasserman[1], Ilana S Fratty[1,2], Ira Aguvaev[1], Zvi Cohen-Said[1], Rua Matar[1], Oran Erster[1], Lester M Shulman[1,3], Ruth Yishai[4], Lior Hecht-Sagie[5], Sharon Alroy-Preis[5], Ella Mendelson[1,3], Yaniv Lustig[1,3], Danit Sofer[1], sNeta S. Zuckerman[1]

[1]*Central Virology Laboratory, Public Health Services, Ministry of Health, Israel*
[2]*The Israel Center for Disease Control, Israel Ministry of Health, Israel*
[3]*School of Public Health, Sackler Faculty of Medicine, Tel-Aviv University, Israel*
[4]*Department of Laboratories, Public Health Services, Ministry of Health, Israel*
[5]*Public Health Services, Ministry of Health, Israel*

## Background

Vaccine-derived Polio virus (VDPV) strains can lead to paralysis akin to wild poliovirus, posing a significant challenge to public health and the eradication of poliovirus. Recently, VDPV outbreaks, have been on the rise worldwide, however outbreaks from type 3 VDPV remain very rare. This study is the first comprehensive analysis of the molecular epidemiology of the widespread VDPV type 3 outbreak in Israel during Sep 2021 to April 2022.

## Methods

VDPV3 detections were monitored and identified via acute flaccid paralysis (AFP) and extensive environmental surveillance (ES) involving 18 sites covering ~70% of Israel`s population. In conjunction, phylogenetic analysis using whole-genome next-generation sequencing of poliovirus isolates was utilized to characterize VDPV3 strain lineages and establish transmission and linkage of the virus.

## Findings

A total of 80 genetically-linked environmental samples and an AFP case of poliovirus and 2 contacts associated with the Sabin type 3 vaccine strain were detected throughout Israel. Most affected locations exhibited a high-density population of ultraorthodox communities. High-resolution genomic characterization of 50 representative sequences including both ES and the AFP case and contacts using phylogenetic analysis facilitated the characterization of three distinct lineages and established connections between different locations in Israel. The in-depth molecular also revealed that one of the positive-AFP contacts is linked to separate chain of VDPV3 infection.

## Interpretation

This study highlights the significance of employing ES for early detection and monitoring of poliovirus circulation, enabling a prompt public health response. WGS offers valuable insights into the outbreak`s origins and linkage across detections.

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Poster presentation

05 Computational biology and genomics of microbes

## Identifying plasmid origin of transfer (oriT) utilizing a machine learning approach

**Ori Ben Shir**[1], Bruria Samuel[1], David Burstein[1]
*The Shmunis School of Biomedicine and Cancer Research, Tel Aviv University, Israel*

Plasmids play a key role in the horizontal mobilization of antibiotic resistance genes between bacteria, including many human pathogens. The conjugation process, which enables the horizontal mobilization of plasmids, begins with the binding to the origin of transfer (oriT) site. However, numerous putative plasmids, including ones with well-annotated conjugative systems, lack any oriT annotations. Recent studies indicate that the leading region, which is adjacent to the oriT, encodes various anti-defense systems. This highlights the crucial role of oriT, not only to study vectors of antibiotic resistance but also to investigate novel anti-defense systems. Despite its importance, no automated tools are currently available to discover and annotate oriT sites. To address this gap, we introduce a machine learning-based approach that utilizes genetic context and DNA physicochemical attributes to identify oriT loci. This innovative approach enables the discovery of novel plasmids and enhances our knowledge of the mobilome. By leveraging these insights, we can improve our ability to manage the spread of antibiotic resistance and expand our understanding of plasmid biology.

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Poster presentation

05 Computational biology and genomics of microbes

## Detecting Positive Epistasis in Chronic Infections of SARS-CoV-2

**Adi Ben Zvi**, Sheri Harari, Danielle Miller, Yael Maoz, Adi Stern

SARS-CoV-2 is characterized by sequential emergence of highly divergent and highly transmissible variants, denoted as variants of concern (VOCs). In parallel, it has been observed that a subset of patients, who suffer from immunosuppression, develop chronic infections. In some case highly divergent variants emerge in these patients with mutations similar to those in VOCs. The similarity between the mutational patterns in VOCs and chronic infections has led to the hypothesis that in some rare cases, chronic infections are the reservoir from which VOCs emerge. However, it remains unclear what are the determinants that allow variants in some chronic infections to become highly transmissible.
Leveraging millions of SARS-CoV-2 sequences, we identified 271 `chronic-like` clades, suspected to represent longitudinal sampling from chronically infected patients. This study aims to test for positive epistasis evidence between mutation pairs in chronic infections. Analyzing mutational pathways, we assessed whether mutation pairs occur more frequently than expected, focusing on mutations with inferred negative fitness and examining the common emergence order.
Preliminary analysis identified at least 29 mutation pairs displaying potential positive epistasis. Notably, the pair S:G446V+S:G476D suggests a combined effect enabling balanced ACE2 binding and resistance to antibody neutralization. Additionally, E:T30I exhibits potential epistasis with various mutations, prompting investigation into its potential ability to infect alternative tissue types.
Ultimately, my plan involves employing a Large Language Model to infer a larger number of chronic-like infections and dependencies between mutations indicative of epistasis. This model-driven approach enables a comprehensive analysis, unveiling hidden patterns and relationships within the extensive SARS-CoV-2 sequence dataset.

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Poster presentation

05 Computational biology and genomics of microbes

# The Role of Environmental Factors in Modulating the Human Breast Milk Microbiome Composition

**Melody Kasher**[1], Shaqed Carasso[1], Tal-Avi Gefen[1], Naama Geva-Zatorsky[1], Kelsley Fehr[2,3], Meghan Azad[2,3], Geoffrey L Winsor[4], Jeffrey R Brook[5]

[1]*Department of Cell Biology and Cancer Science, Rappaport Faculty of Medicine, Technion, Israel*

[2]*Manitoba Interdisciplinary Lactation Centre (MILC), Children's Hospital Research Institute of Manitoba, Canada*

[3]*Department of Pediatrics and Child Health, University of Manitoba, Canada*

[4]*Department of Molecular Biology and Biochemistry, Simon Fraser University, Canada*

[5]*Department of Chemical Engineering and Applied Chemistry, Dalla Lana School of Public Health University of Toronto, Canada*

Background and aims: Breastfeeding is a substantial determinant of the infant gut microbiome, which has important short and long-term health implications. Therefore, it may be crucial to consider potential factors that may impact the milk microbiome and could eventually induce differentiation in the composition of the gut microbiome of the infant. This study aims to investigate associations and the impact of environmental exposures on the breast milk microbiome.

Methods: Data collected from the Canadian Healthy Infant Longitudinal Development (CHILD) cohort study contained 876 participants with milk microbiome data and 1,983 potential environmental exposures. Hierarchical clustering of samples based on their milk microbiome compositions were computed to assess clusters of bacteria that describe the milk microbiome. Analysis of variance was calculated to determine the relationship between milk microbiome clusters and the environmental exposures. Regression analysis was conducted to estimate the correlation between the environmental exposures and the milk microbiome.

Results: Assessment of the milk microbiome composition revealed that 13 emerging bacteria at the genus taxonomic level were abundant. The milk microbiome showed variability where four clusters appeared to describe the milk microbiome. Prominent environmental exposures that appeared to substantially modulate the abundance of the bacteria included: the use of child formula, the use of the breast pump, and the lack of storing baby food.

Conclusions: Environmental exposures suspected to alter the oral microbiome of the infant may contribute to modifications in the breastmilk microbiome`s composition. Perhaps, the infant oral microbiome and the maternal breastmilk are suggestive of a symbiotic relationship.

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Poster presentation

06 Clinical bacteriology and epidemiology

# Comparison of four diagnostic tests for helicobacter pylori infection

**Tsachi Tsadok Perets**[1,2], Rachel Gingold Belfer[3,4], Ram Dickman[3,4], Matan Siterman[3,4], Doron Boltin[3,4]

[1]*Gastroenterology Laboratory, Rabin Medical Center, Israel*
[2]*Digital Medical Technologies, Holon Institute of Technology, Israel*
[3]*Division of Gastroenterology, Rabin Medical Center, Israel*
[4]*School of Medicine, Tel Aviv University, Israel*

Introduction: Due to lower operational costs, HMOs may prioritize stool antigen testing H( pStAg) over 13C-urea breath tests (13CUBT) for the non-invasive diagnosis ofH . pylori infection.

Aims & Methods: We aimed to determine the relative accuracy of the diagnostic tests forH . pylori infection, at our institution. We performed same-day 13C-UBT , rapid urease test (RUT ), histological examination, and HpStAg on consecutive patients presenting for gastroscopy at Rabin Medical Center. 13C-UBT test meal consisted of 4g citric acid. Monoclonal stool Ag test was performed using the LIAISON Meridian chemiluminescent immunoassay (DiaSorin SpA, Italy). Histology was examined with H&E, and additional stains were performed at the pathologist's discretion. For the assessment of 13C-UBT the de facto gold standard was concordant RUT and histology. For the assessment ofH pStAg the de facto gold standard was defined as at the result ofa tleast two of the three remaining tests (RUT , histology and 13C-UBT ).

Results: Overall, 91 patients were included (36 males (39.6%) age 50.1±18.7 years). The indication for gastroscopy was dyspepsia in 57 (62.6%). RUT and histology were discrepant in 3 cases. For1 3C-UBT and HpStAg respectively, H. pylori positivity was 34.5% and 37.1%; sensitivity was 96.6% and 69.2%; specificity was 100% and 95.4%; PPV was 100% and 89.8%; NPV was 98% and 84%; false positive was 0%and 10.1%; and false negative was 1.8% and 16.0%.

Conclusion: The accuracy of 13C-UBT is superior to HpStAg at our institution. Clinicians should be aware of test limitations when interpreting results.

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Poster presentation

06 Clinical bacteriology and epidemiology

# Carbapenemase-producing Kluyvera in Israel - epidemiology of an evolving species.

**Gabrielle Levi**[1], Mor Lurie Weinberger[1], Alona Keren Paz[1], David Schwartz[1], Yehuda Carmeli[1,2]

[1]*National Institute for Antibiotic Resistance & Infection Control, Ministry of Health, Israel*

[2]*Sackler Faculty of Medicine, Tel Aviv University, Israel*

Kluyvera spp., once considered low-virulence, opportunistic bacteria are now recognised for causing significant infections. Recent identification of carbapenemase-producing (CP) Kluyvera spp. supports the theory that they may act as reservoir of resistance genes for Enterobacterales. There are few reports of CP-Kluyvera spp., and little is known about their characteristics.

This study portrays the epidemiology of CP-Kluyvera in Israel, identifies common antibiotic resistance genes (ARGs) and describes the mechanisms of transmission of carbapenemase genes within Kluyvera spp.

A retrospective epidemiological analysis on CP-Kluyvera reported to Israel`s national surveillance system of carbapenem-resistant Enterobacterales from 2006-2023. Additionally, 22 isolates underwent microbiological characterisation to identify carbapenemase genes and determine antibiotic susceptibilities. Whole genome sequencing was performed to identify ARGs, plasmids and phylogenetic relationship between the isolates.

There were 151 incident cases of CP-Kluyvera nationwide in 2006-2023, Kluyvera ascorbata being the most common strain. The predominant carbapenemases were KPC (66.2%) and NDM (25.8%). Molecular characterisation of isolates showed that dissemination of KPC-2-producing K. ascorbata and NDM-1-producing K. cryocrescens was non-clonal and plasmid-mediated. All sequenced isolates harboured at least one plasmid, including IncC (22.7%) and Rep-A type (50%). We detected three outbreaks of CP-Kluyvera, involving 16 isolates from two medical facilities. These isolates clustered into three phylogenetic clades; each comprised from highly related isolates with fewer than 100 SNP differences between them.

Our findings indicate that carbapenemase transmission in Kluyvera species is plasmid- mediated and CP-Kluyvera can cause outbreaks in healthcare settings. Kluyvera spp. may be an under-reported vector for transmission of ARGs within Enterobacterales.

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Poster presentation

06 Clinical bacteriology and epidemiology

## Unraveling the mechanism of antibacterial action of chlorhexidine on enterococcus faecalis

**Nathanyel Sebbane**[1,2,3], Itzhak Abramovitz[4], Nurit Kot-Limon[5], Doron Steinberg[1]
[1]*Faculty of Dental Medicine, Biofilm Research Laboratory Institute of Biomedical and Oral Research (IBOR), Hebrew University-Hadassah, School of dental medicine, Israel*
[2]*Department of Endodontics, Faculty of Dental Medicine, Hebrew University-Hadassah, School of dental medicine, Jerusalem*
[3]*"Bina" Program, Faculty of Dental Medicine, Hebrew University-Hadassah, School of dental medicine, Jerusalem*
[4]*Head, Department of Endodontics, Faculty of Dental Medicine., Hebrew University-Hadassah, School of dental medicine, Israel*
[5]*Department of Endodontics, Faculty of Dental Medicine, Hebrew University-Hadassah, School of dental medicine, Israel*

Endodontic treatment failures are frequently attributed to the persistence of microbial species such as Enterococcus faecalis within the root canal system. Chlorhexidine (CHX) is a widely recognized antiseptic commonly employed in endodontics. This study aimed to elucidate the mechanisms behind the antibacterial effects of Chlorhexidine (0.125-20 μg/mL) on E. faecalis, a Gram-positive bacterium known for its resilience and association with endodontic failures. E. faecalis ATCC 29212 served as the model strain for evaluating CHX`s antibacterial activity using assays such as planktonic growth inhibition, colony-forming units (CFUs), membrane permeability, and quantitative real-time PCR analysis. The results demonsstrated that CHX significantly inhibits the growth of E. faecalis in a concentration-dependent manner, with notable reductions in planktonic growth and CFUs. Additionally, CHX treatment compromised bacterial cell membrane integrity, indicated by increased membrane permeability and depolarization. High-resolution scanning electron microscopy revealed morphological changes and pore formation on the bacterial surface, indicative of membrane disruption. Moreover, confocal microscopy of biofilms showed a reduction in biofilm mass and extracellular polymeric substance (EPS) production, which are critical for biofilm resilience. Gene expression analysis highlighted CHX`s role in down-regulating virulence-related genes while up-regulating stress response genes, suggesting a multifaceted mechanism of action. These findings underscore the potential of CHX as a potent antimicrobial agent in endodontic treatments, capable of significantly reducing E. faecalis populations. By elucidating CHX`s mechanisms of action and its effects on bacterial viability and pathogenicity, this study contributes to the development of more effective endodontic disinfection protocols, potentially improving treatment outcomes and reducing the incidence of treatment failures linked to microbial infections.

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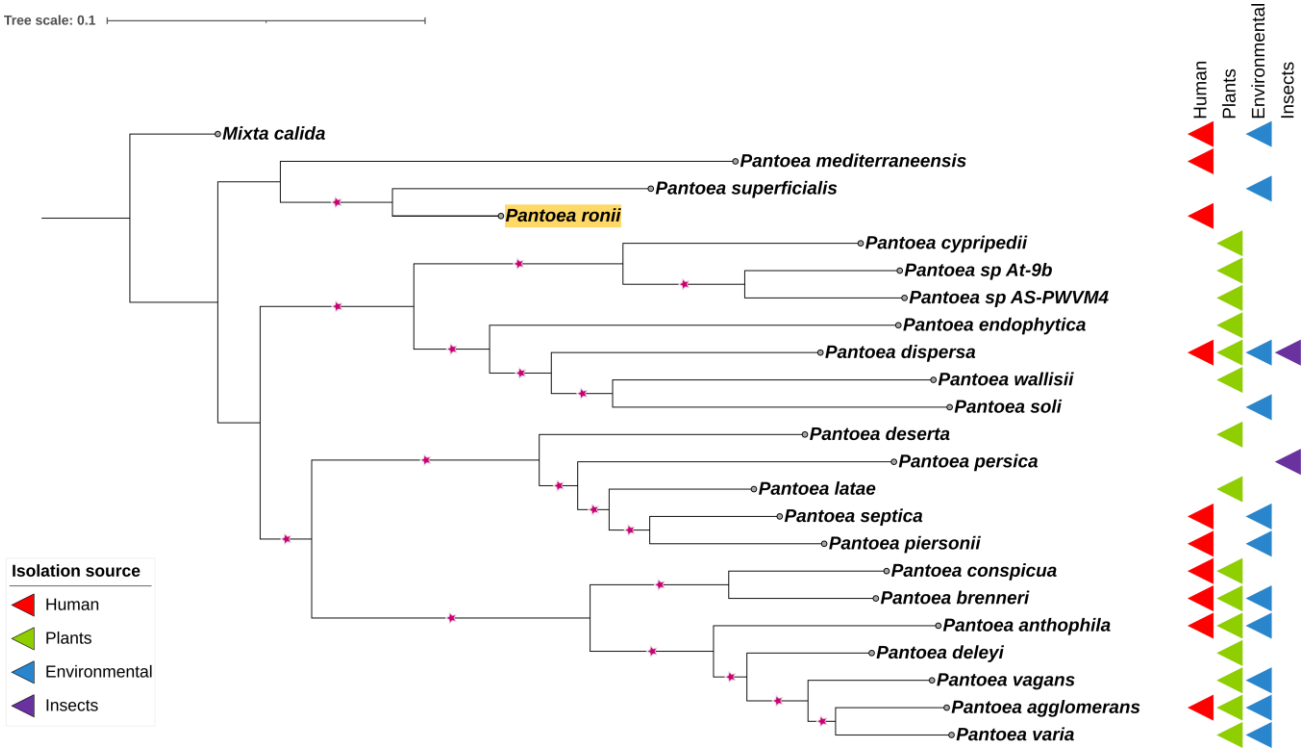
Poster presentation

06 Clinical bacteriology and epidemiology

# Pantoea ronii - a newly identified human opportunistic pathogen identified by whole genome sequencing

**Danielle Keidar Friedman**[1], Daniel Leshin-Carmel[2], Anka Tzur[3], Muriel Amsalem[3], Daria Tolkach[4], Tal Brosh-Nissimov[2], Nadav Sorek[3]
[1]*Emerging Infectious Disease Laboratory, Assuta Ashdod University Hospital, Israel*
[2]*Infectious Diseases Unit, Assuta Ashdod University Hospital, Israel*
[3]*Clinical Microbiology Laboratory, Assuta Ashdod University Hospital, Israel*
[4]*Emerging Infectious Disease Laboratory, Assuta Ashdod University Hospital, Israel*

The emergence of new opportunistic pathogens from the environment is of major importance. Here, we describe a previously unidentified opportunistic human pathogen discovered following a biopsy culture from a 37-year-old woman presenting with chronic osteomyelitis. The infection started twenty years after a leg injury in a field that caused a bone fracture and a large open bleeding wound. The bone biopsies grew Gram-negative rods not identified by MALDI-TOF (matrix-assisted laser desorption/ionization time-of-flight) mass spectrometry. Sequencing of the 16S rRNA gene suggested it belongs to the family Erwiniaceae but could not specify a species. Whole genome sequencing revealed genetic relatedness to several Pantoea species without identifying a known species. While most Pantoea species have been isolated from plants, several known Pantoea species are considered human opportunistic pathogens. We suggest this bacterium represents a new Pantoea species and an opportunistic human pathogen.



POSTER SESSION II (Tuesday, September 10) (Tuesday, September 10, 2024 12:45)

Poster presentation

07 Viruses of eukaryotes

# Diatom and virus succession within a dramatic spring bloom event in the northern red sea

**Jasmin Mellijor**[1], Yael Tennenbaum-Azulai[1], Dina Spungin[1], Yoav Avrahami[2,3], Assaf Vardi[4], Miguel Frada[2,3], Chana Kranzler[1]

[1]*The Mina and Everard Goodman Faculty of Life Sciences, Bar-Ilan University, Israel*

[2]*Department of Ecology, Evolution and Behavior, Silberman Institute of Life Sciences, The Hebrew University of Jerusalem, Israel*

[3],, *The Interuniversity Institute for Marine Sciences in Eilat, Israel*

[4]*Department of Plant and Environmental Sciences, Weizmann Institute of Science, Israel*

Diatoms are a globally distributed and diverse group of phytoplankton that contribute ~20% of primary productivity on the planet and substantial carbon export in the ocean. These aquatic, unicellular eukaryotes are characterized by high growth rates and dominance within seasonal phytoplankton bloom events. While marine viruses are considered key players in phytoplankton bloom demise, little is known about the prevalence and patterns of virus infection within a diatom bloom. To better understand how viruses might shape diatom bloom dynamics we used quantitative metatranscriptomics analysis of the diatom and virus community at high temporal resolution during an outstanding spring bloom event in the northern Gulf of Aqaba/Eilat (GoA/E), Red Sea. Throughout our sampling period, that captured both the peak of the spring bloom and the initiation of its demise, we observed three distinct transitions in active diatom genera, shifting between Skeletonema, Pseudo-nitzchia and Leptocylindrus dominance, indicating rapid and dynamic turnover of diatom taxa within a single diatom bloom event. In addition, we identified multiple cell-associated viruses, with near full-length genomes and close homology to known diatom-infecting Marnaviridae. Intriguingly, temporal fluctuations in the abundance of several dominant viruses corresponded with the observed turnover in diatom taxa, raising the possibility that viruses may drive both the succession of taxa within a diatom bloom and orchestrate its ultimate collapse. Together, these observations suggest a dynamic interplay between diatoms and viruses within a single bloom event and provide additional insight into the role that viruses play in shaping diatom community structure in the ocean.

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07 Viruses of eukaryotes

# The snakehead retrovirus promoter is versatile and generates maternally inherited transcripts in transgenic zebrafish

**Eran Bacharach**[1], Rachel Zamostiano[1], Odelia Pisanty[2], Reem Abu Rass[1], Avi Eldar[3], Marcelo Ehrlich[1], Yoav Gothilf[2]

[1]*The Shmunis School of Biomedicine and Cancer Research, The George S. Wise Faculty of Life Sciences, Tel Aviv University, Israel*

[2]*School of Neurobiology, Biochemistry and Biophysics, The George S. Wise Faculty of Life Sciences, Tel Aviv University, Israel*

[3]*Department of Virology, Kimron Veterinary Institute, Israel*

The Snakehead Retrovirus (SnRV) infects fish and has complex splicing patterns resembling the ones of lentiviruses. Moreover, like lentiviruses, SnRV potentially encodes accessory proteins besides the Gag, Pol, and Env precursors. Based on shared features, the largest accessory ORF (205 codons), named 3` ORF, was suggested to encode a transactivator of transcription (Tat)-like protein. The Tat protein is essential for human immunodeficiency virus type 1 (HIV-1) transcription, enhancing transcriptional initiation and elongation that start at the viral promoter – the long terminal repeat (LTR). Here, we constructed an infectious molecular clone for SnRV and tested the effects of 3` ORF mutations on SnRV transcription. While the replacement of the 3` ORF sequence with foreign sequences with comparable sizes dramatically reduced virus expression and production, an out-of-frame point mutation in the 3` ORF, deleting all but 14 residues of the 3` ORF product, had only a minimal effect on SnRV expression and replication. These results suggest that the 3` ORF sequence may act in *cis*, but the product of this ORF does not function as a Tat protein. Accordingly, the SnRV LTR activity is largely independent of this product. We also show that the SnRV LTR is a versatile promoter, functioning in both fish and mammalian cultured cells. Finally, we demonstrate that in transgenic zebrafish, this promoter is active in sensory organs and gonads and that SnRV LTR-generated transcripts are maternally inherited.

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07 Viruses of eukaryotes

## Up regulation of the long non-coding RNA PURPL by Kaposi's sarcoma associated herpesvirus to suppress p53 activity

**Samia Showgan**, Guy Journo, Diana Meary, Vyacheslav Gurevich, Meir Shamay[1]
*Faculty of medicine, Bar Ilan University, Israel*

KSHV is the causative agent of all forms of Kaposi's sarcoma and is tightly associated with primary effusion lymphoma and multicentric Castleman's disease. Long non-coding RNAs (lncRNAs) are a class of regulatory RNAs with lengths exceeding 200 nucleotides and they can contribute to the pathogenesis of different malignancies. The lncRNA PURPL can repress p53 function, and PURPL over-expression can induce cancer cell proliferation. PURPL is regulated by p53. Transcriptome analysis revealed up-regulation of PURPL in KSHV-infected cells. Using RT-qPCR, we showed that PURPL is upregulated in KSHV infected cells. Anti-sense oligo targeting PURPL reduced PEL cell proliferation. Interestingly, PURPL levels were also up-regulated following KSHV infection in cells that express a mutant p53 and mutation at the p53 consensus binding site abrogated all the induction of PURPL promoter by KSHV. P63 and p73 are homologs of p53 that has been shown to bind the same consensus sequences in some cases. ChIP-seq data re-analysis on PURPL promoter revealed association of p53, p63 and p73 with PURPL promoter. Reporter assays revealed that expression of p63α, p73α and especially p73β leads to upregulation of PURPL in HCT116 p53 KO cell line, and a point mutation at the p53/p63/p73 binding region relieves the PURPL up-regulation. We found higher expression of p73 protein levels in de-novo infected BJAB cells compared to the non-infected BJAB cell-line. In addition, we found that as opposed to p53, p73 is not repressed by PURPL RNA. We propose that KSHV up-regulates p73 to repress p53 via the lncRNA PURPL.

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Poster presentation

07 Viruses of eukaryotes

## KSHV genome harbors both constitutive and lytically induced enhancers

Nilabja Roy Chowdhury, Vyacheslav Gurevich, **Meir Shamay**[1]
*Azrieli Faculty of Medicine, Bar-Ilan University*

Kaposi's sarcoma-associated herpesvirus (KSHV) belongs to the gamma-herpesvirus family and is a well-known human oncogenic virus. In infected cells, the viral genome of 165 kbp is circular DNA wrapped in chromatin. The tight control of gene expression is critical for latency, the transition into the lytic phase, and the development of viral-associated malignancies. Distal cis-regulatory elements (CRE), such as enhancers and silencers, can regulate gene expression in a position and orientation-independent manner. Open chromatin is another characteristic feature of enhancers. To systematically search for enhancers, we cloned all the open chromatin regions in the KSHV genome downstream to the luciferase gene and tested their enhancer activity in infected and uninfected cells. A silencer was detected upstream of the latency promoter (LANAp). Two constitutive enhancers were identified in the K12p-OriLyt-R and ORF29 Intron region, where ORF29 Intron is a tissue-specific enhancer. The following promoters: OriLyt-L, PANp, ALTp, and the Terminal Repeats (TRs) acted as lytically induced enhancers. Expression of the Replication and Transcription Activator (RTA), the master regulator of the lytic cycle, was sufficient to induce the activity of lytic enhancers in uninfected cells. We propose that the TRs that span about 24 kbp region serve as a "viral super-enhancer" that integrates the repressive effect of the latency protein LANA with the activating effect of RTA. Utilizing CRISPR activation and interference techniques, we determined the connections between these enhancers and their regulated genes. The silencer and the enhancers provide an additional layer to the complex gene regulation of herpesviruses.

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07 Viruses of eukaryotes

## Methyl-CpG-binding protein 2 as a Key Player in Kaposi`s sarcoma-associated herpesvirus Latency

**Ankita Awase**, Supriya Bhattacharya, Vyacheslav Gurevich[1], Prof. Meir Shamay
*Azrieli Faculty of Medicine, Daniella Lee Casper Laboratory in Viral Oncology, Bar-Ilan University, Israel*

Kaposi`s sarcoma-associated herpesvirus (KSHV) is responsible for diseases such as Kaposi`s Sarcoma, Primary effusion lymphoma, and Multicentric Castleman disease. The latency-associated nuclear antigen (LANA) protein is crucial for maintaining the viral genome and binds to a number of host chromatin components including MeCP2, RING3, and DEK. Our study investigates the role of methyl-CpG-binding protein 2 (MeCP2) in KSHV genome maintenance and its interaction with LANA using it in order to identify new therapeutic targets. We used ChIP (chromatin immunoprecipitation assay)-qPCR to show that LANA-MeCP2 interacts at LANA binding sites in PEL cells. MeCP2 knockdown in recombinant KSHV-infected cells (iSLK BAC16) that expresses hygromycin resistance gene, lost their ability to grow in the presence of hygromycin. This was supported by experiments in NIH3T3 cells where wild-type MeCP2 maintained the KSHV genome, but T158M mutant failed. We intend to use CUT&RUN assays on iSLKBAC16 cells for identifying regions affected by MeCP2 depletion on KSHV and human genomes thus gaining insights into virus-host interactions

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07 Viruses of eukaryotes

## Systematic functional assay revealed constitutive as well as lytic enhancers within the KSHV genome

**Nilabja Roy Chowdhury**[1], Vyacheslav Gurevich[1], Meir Shamay[1]
*Azrieli Faculty of Medicine, Bar-Ilan University, Israel*

Kaposi's sarcoma-associated herpesvirus (KSHV) belongs to the gamma-herpesvirus family and is a well-known human oncogenic virus. In infected cells, the viral genome of 165 kbp is circular DNA wrapped in chromatin. The tight control of gene expression is critical for latency, the transition into the lytic phase, and the development of viral-associated malignancies. Distal cis-regulatory elements (CRE), such as enhancers and silencers, can regulate gene expression in a position and orientation-independent manner. Open chromatin is another characteristic feature of enhancers. To systematically search for enhancers, we cloned all the open chromatin regions in the KSHV genome downstream to the luciferase gene and tested their enhancer activity in infected and uninfected cells. A silencer was detected upstream of the latency promoter (LANAp). Two constitutive enhancers were identified in the K12p-OriLyt-R and ORF29 Intron region, where ORF29 Intron is a tissue-specific enhancer. The following promoters: OriLyt-L, PANp, ALTp, and the Terminal Repeats (TRs) acted as lytically induced enhancers. Expression of the Replication and Transcription Activator (RTA), the master regulator of the lytic cycle, was sufficient to induce the activity of lytic enhancers in uninfected cells. We propose that the TRs that span about 24 kbp region serve as a "viral super-enhancer" that integrates the repressive effect of the latency protein LANA with the activating effect of RTA. Utilizing CRISPR activation and interference techniques, we determined the connections between these enhancers and their regulated genes. The silencer and the enhancers provide an additional layer to the complex gene regulation of herpesviruses.

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08 Plant-microbe interactions

# The rhizosphere microbiome and exudome of Brachypodium spp. distributed along a steep climatic gradient

**Sheetal -**[1], Peter Reuveni[1], Sofia Arellano[1], Maor Matzrafi[1], Shilo Rosenwaser[2], Daniella Gat[1], Elisa Korenblum[1]

[1]*Plant Sciences Institute, Volcani Center (ARO), Israel*

[2]*Plant Sciences, Hebrew University of Jerusalem, Israel*

Plants that are capable to grow and survive under harsh environmental stresses in different climatic zones, including arid regions, may serve as resources to study microbiome interactions under changing environments. In Israel, Brachypodium spp. are distributed along a steep climatic gradient encompassing Mediterranean climate to the extreme desert within a 400 km range. These wild plants and associated rhizosphere microbiota have co-evolved for millions of years, supporting adaptations to each other and to their local environment. The microbiomes of these wild grasses are unique resources for novel beneficial microorganisms for improvement of plant growth wheat growth, nutrient uptake, and drought-resilience. Our research aims to study the microbiome of the rhizosphere of Brachypodium plants from seven different climatic and rainfall zone locations in Israel using amplicon sequencing of 16S ribosomal RNA gene. Moreover, we extend our understanding of the interactions between plant roots and the root microbiome in the rhizosphere by analysing the root metabolome and exudome of different Brachypodium genotypes. We link the microbiome and exudome results for future application of metabolites as soil prebiotics with bioinoculants as plant growth promoters for sustainable agriculture amid climate change.

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08 Plant-microbe interactions

# Avocado root microbiome for regenerative agriculture

**Daniella Gat**[1,2], Adi Kushmaro Bier[3], Yael Bar-Noy
[1]*Plant Science, Agricultural Research Center (ARO) - Volcani Center, Israel*
[2]*The Robert H. Smith Institute of Plant Sciences and Genetics in Agriculture, The Hebrew University of Jerusalem, Israel*
[3]*Soil, Water and Environmental Science Institute, Agricultural Research Center (ARO) - Volcani Center, Israel*

Interactions between plants and their root microbiota have been proven to have a significant effect on both plant health and the microbial community structure and functions. Accordingly, substantial efforts are dedicated to linking the community composition of various plant roots (microbiomes) to plant health. The objectives of this study are to identify potential microbial populations that are associated with fruit yield in avocado trees. Further, to link those to optimized plant growth conditions, while switching field management to regenerative farming systems. In this study we examined the microbial community composition on the roots of avocado trees in an experimental plot that was treated with different regimes of cover-crop growth. We linked the microbial community composition of avocado tree roots, obtained by barcoded sequencing of 16S ribosomal RNA encoding gene (bacterial taxonomic marker) and the internal transcribed spacer (fungal taxonomic marker). Multi regression analysis was used to test links between the microbial composition and the soil organic carbon, total nitrogen, ammonia and nitrate concentrations, as well as fruit setting, fruit yield and leaf mineral content. The results of this study will assist in switching from an intensive agriculture regime to a regenerative regime, increasing soil health while maintaining similar yield.

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08 Plant-microbe interactions

## Identification of disease suppressive secondary metabolites producing bacteria

**Hildah Amutuhaire**

More than 15% of global crop production is lost to plant disease and most of them are caused by Soilborne fungal plant pathogens. Although chemical fungicides are effective at controlling fungal phytopathogens, public health and environmental concerns are rising from excessive use of these chemicals. Biocontrol is a promising a sustainable alternative.

Majority of bacterial biocontrol agents have the capacity to protect plant roots from attacks by soilborne pathogens through production of antimicrobial secondary metabolites. Addition of compost soil amendment to soil as a way to stimulate the soil microbiome activity and diversity and enrich for disease suppressive microbiome although haven’t been studied specifically for antimicrobial secondary metabolites producing bacteria.

We recently investigated the effect of soil amendment with compost in presence of fungal pathogen FORC on cucumber plant performance, and using amplicon sequencing studied the effect of compost amendment together with pathogen inoculation on rhizosphere microbial community structure and abundance of secondary metabolites encoding genes. Particularly, compost inhibited FORC in pathogen-inoculated cucumber plants in addition to higher plant performance in comparison to the non-amended inoculated plants. Bacterial and fungal diversity were significantly higher in the rhizosphere of compost-amended plants vs. non-amended following FORC inoculation. The diversity of secondary metabolites encoding genes (NRPS and PKS) was also significantly higher in compost amended inoculated plants.

Further research will investigate the ability of these secondary metabolites producing bacteria to inhibit plant fungal pathogens both in-vitro and in-planta with a goal to extract and purify the produced metabolites for commercialization.

This platform provides an opportunity for discovery of novel antimicrobials for use in agriculture that can also be extrapolated to human health.

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09 The microbiome

## The role of the gut microbiota in food allergies

**Nofar Asulin**[1]
*Azrieli Faculty of Medicine, Bar-Ilan university, Israel*

Background: IgE-mediated food allergy (FA) is defined as an adverse immune response to a food protein that occurs within 2 hours of exposure. The gut microbiota is now becoming a research focus in the study of FA and particularly in treatment prognosis. Oral food immunotherapy (OIT) is an active therapeutic strategy for FA which may involve the microbiota.

Methods: We characterized the fecal microbiota (16S rRNA gene sequencing) and the metabolite (untargeted metabolomics) profiles of 17 FA patients undergoing walnut-OIT, before and after treatment, and age-matched controls (n=19). To examine the gut microbiota's mechanistic contributions to FA and to assess whether pre-and post-OIT microbiota differentially impact the allergic response, we preformed FMT from patients before (n=12) and after (n=12) OIT-treatment to germ-free mice.

Results: The microbiota composition between the pre-treatment and the non-allergic control groups were significantly different (beta-diversity), but richness was similar across groups. The metabolic profiles were different between all three groups as well, with metabolites involved in arginine and proline metabolism and in histidine metabolism overexpressed in pre-treatment samples compared to post-treatment and control ones. In the mouse experiment, there were differences in the allergy response phenotype, indicating the role of the microbiota and metabolome in FA and OIT outcome.

Conclusions: Our results demonstrate a link between IgE-mediated walnut FA and the composition of the gut microbiota and metabolome. Because the microbiota is modifiable, this study can set the groundwork for discovery of potential therapeutic interventions that can be used to routinely treat FA.

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09 The microbiome

## The effect of the microbiome on lowering aggressive behavior in Drosophila melanogaster

**Rachel Levin**[1], Sondra Turjeman, Omry Koren
*The Azrieli Faculty of Medicine, Bar Ilan University, Israel*

Aim & Background: The social life of animals depends on communication between individuals, with aggression manifesting as one of the common types of behaviors, preserved among most species. An organism will use aggression for self-defense against conspecifics and predators, in acquisition of territory, food and mates, and in defense of progeny.

Recent findings link variations in gut microbiome composition with animal social behavior such and various cognitive and emotional aspects. This study uses Drosophila melanogaster as an animal model with the aim of determining the effects of the microbiota on aggression.

Methods: Male flies reared under different treatment [control, mix of antibiotics (abx: tetracycline, rifampicin, streptomycin) and germ-free flies] were subjected to behavioral tests to measure aggression. In addition, we used real time PCR to test gene expression, SEM to study the antenna, and untargeted metabolomics.

Results & Conclusions: Flies without a microbiome (abx) are significantly more aggressive than flies with a microbiome. When examining metabolomes, we found that processes associated with aggression, such as dopamine, GABA and serotonin, change depending on the presence or absence of the microbiome. Comparisons of numbers of trichoid sensilla, the sensilla related to aggression, revealed that the absence of bacteria reduces the number of sensilla. Future profiling of receptors in the antenna (by qPCR) that are related to aggression will complete the puzzle of the specific effects of the presence or absence of the microbiome on aggression.

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09 The microbiome

## The neuro-immune effects of the gut microbiota at birth in a germ-free mouse model

**Soha Zgairy**[1]
*Azrieli Faculty of Medicine, Bar Ilan University, Israel*

Introduction: The early life microbiome influences metabolism, immunity, brain development and behavior in the long term. Microbial colonization at birth is significantly affected by birth mode and place. The mechanisms by which this microbiome affects specific host characteristics are just starting to be considered and one important, but understudied mechanism appears to include the immune and nervous system crosstalk. Although the precise pathways of microbiota-immuno-neuronal signaling have not yet been deciphered, it has been reported that changes in specific taxa abundances correlate with effector and regulatory T-cells development through the enteric neurons. In this study, we aimed to study how the birth mode-dependent gut microbiota affects neuronal and immunological development in colonized germ-free (GF) mice.

Methods: Pregnant Swiss Webster GF females were subject to fecal microbiota transplantation (FMT) with fecal samples collected from babies born by cesarean section (CS) in a hospital or vaginally at home (VHOM). Control females were treated with PBS only. Offspring tissues (following sacrifice) and stool were collected at three main time points for assessing neuro-immune development by RNA sequencing and immunofluorescence staining. 16S rRNA gene sequencing was used to detect gut microbiome compositional and developmental differences.

Results: We identified associations between birth mode-dependent microbiota and specific neuronal and immunological gene-sets and pathways.

Conclusion: Birth mode affects the microbial colonization in early life and correlates with long term immunological or neuronal conditions. These findings suggest that early-life microbiota manipulation may be a relevant avenue for alleviating long-term effects of birth mode.

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09 The microbiome

## Resistance and resilience of desert soil microbial communities to prolonged heatwaves

**Leo Shteinberg**[1], Max Kolton[2]
[1]*High School, Kfar Silver Agricultural High School, Israel*
[2]*Department of French Associates Institute for Agriculture & Biotechnology of Drylands, The Jacob Blaustein Institute for Desert Research, Ben-Gurion University of the Negev, Israel*

Billions of microorganisms colonize every corner of the Earth and play critical roles in nutrient cycles and ecosystem stability. However, environmental stressors can negatively affect microbial community abundances, composition, taxonomic and functional diversity, thus compromising the microbes` ability to support the growing human population. Environmental temperature changes are among the most influential factors affecting microbial physiology and survival. Prolonged heatwave events, which characterize ongoing climate changes, may result in the loss of microbial taxonomic and functional diversity, leading to ecosystem collapse. Despite intensive studies on microbial responses to temperature stress, insufficient knowledge about the Negev Desert microbial community`s responses to prolonged heatwaves makes it harder to predict the consequences of ongoing climate changes on human society in arid areas. We hypothesize that prolonged heatwaves will substantially reduce the size of the active microbial community and compromise their ability to maintain ecosystem functionality. In this high school project, Negev agricultural soil was exposed to five severity levels of heatwaves, and microbial resistance and resilience to thermal stress were evaluated after one week of thermal stress and one week after the stress was released. Changes in the number of culturable bacteria, enzymatic activities, basal and induced respiration, and soil organic carbon and nitrogen pools were quantified after each experimental step. The results of these measurements will be used to estimate soil microbial communities` sensitivity to prolonged heatwaves.

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09 The microbiome

## The effect of the gut microbiome on aggression

**Lelyan Moadi**

Background: Numerous studies on animal aggression have been conducted throughout the past century, yet much remains to be deciphered regarding regulation of aggression. The effects of the gut microbiota on metabolism, immunity, general health and behavior have been extensively studied. While there have been indications that the microbiota affects risk taking and mating behavior, its mechanisms and pathways are still a mystery. To date, no study has extensively studied the mechanism by which the microbiota affects aggression in mice.

Methods: To test the effects of microbiota composition on aggression in mice, we modulated the mouse gut microbiome using different antibiotic treatments. We compared the mice's tendencies toward aggression and characterized gut microbiota with 16S rRNA gene sequencing of fecal samples and metabolic profiles of different brain regions using untargeted metabolomics. We also tested aggressive tendencies in offspring of treated mice. The offspring received compromised microbiomes from the mothers without direct exposure outside the uterus.

Results: The antibiotic-treated groups showed significantly lower bacterial diversity and significant differences in bacterial and metabolite composition. These mice were also more aggressive than the control group. The offspring of the dams treated with antibiotics were significantly more aggressive than the control group as well; the number of attacks was higher and the latency to attack was shorter.

Conclusions: Our results indicate that the microbiome affects aggression, and that antibiotics increase aggression via microbiome dysbiosis, but there is likely a critical window for the effect, as well, as seen in in-utero exposed mice.

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## Aerobic microbial methanogenesis and methane oxidation in aplysina aerophoba

**Martha Alejandra Duran Canche**[1], Chiara Conti[1], Jana Milucka[2], Sina Schorn[2], Laura Steindler[1]
[1]*Department of Marine Biology, Leon H. Charney School of Marine Sciences, University of Haifa, Israel*
[2]*De-part-ment of Biogeo-chem-istry, Max Planck Institute for Marine Microbiology, Germany*

Marine sponges interact with a wide range of microorganisms and are considered holobionts. A recent study using microbial metatranscriptomes from the sponge Aplysina aerophoba hypothesized that a hidden methane cycle exists within this sponge due to microbial activity. This sponge is known to thrive in shallow, fully oxygenated waters. The study suggested two potential pathways for aerobic methanogenesis: one involving Alphaproteobacteria using methyl phosphonate (MPN) as a phosphorus source, generating methane, and another involving the use of methylamine (MeA) as a nitrogen source, also producing methane. Additionally, symbionts of the phylum Binatota were suggested to have the genetic capability to oxidize the produced methane. However, this hypothesis has not yet been experimentally tested. In this present study, we performed incubation experiments on three A. aerophoba specimens collected from the Gulf of Trieste (Adriatic Sea). We employed stable isotopes, specifically Carbon-13, as tracers for MPN and MeA substrates. Sponge tissue was exposed to five different treatments in airtight bottles. Seawater was sampled from the incubation bottles at 0, 3, 6, 12, and 24 hours from the start of the incubation. Additional incubations were conducted with $^{13}CH4$ to test for methane oxidation. The samples are currently being analyzed with a methane stable carbon isotope analyzer (Picarro). We expect to observe the production of $^{13}CH4$ when sponge tissue is incubated with $^{13}C$-MPN and $^{13}C$-MeA and the production of $^{13}CO2$ when sponge tissue is incubated with $^{13}CH4$.

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## Microbial terroir across different Israeli vineyard soils

**Omri Bassevich**, Ayala Barkan, Yotam Zeit, Noam Reshef, Elisa Korenblum

Extending beyond traditional terroir concepts—encompassing soil, climate, and topography—microbial terroir includes the diverse microbial communities in the soil, particularly the rhizosphere, on the vine surfaces, and on grape skins. In this study, we aim to analyze the core rhizosphere microbiome of Israeli vineyards, and to establish a link among the microbial populations, soil organic carbon and nitrogen belowground and the plant traits, such as petiole mineral content, water potential, yield and fruit (and wine) quality aboveground. We have extensively analyzed the rhizosphere microbiomes of Cabernet Sauvignon vineyards located in four different geographical regions and characterized by different soil properties. By leveraging the intricate dynamics of microbial terroir, winemakers can enhance the distinctiveness and authenticity of their wines, making microbial terroir a crucial aspect of modern viticulture and oenology.

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10 Applied microbiology and biotechnology

# Etiology of Postharvest Decay of Long-Term Stored Carrots and the Development of Sustainable Control Measures

**Nadav Smila**[1,2]
*Department of Postharvest Science, Agricultural Research Organization, Volcani Institute, Israel*
*Department of Agroecology and Plant Health, the Robert H. Smith Faculty of Agriculture, Food and Environment, Hebrew University of Jerusalem, Israel*

On 2022, the fungicide Rovral® (a.i. Iprodione 50%) was banned for use as postharvest treatment before long-term cold-storage of carrots (0-2°C for 6-10 months) and was replaced by the fungicide Scholar® (a.i. Fludioxonil 230 SC). Subsequently, reports of molds and rots in stored carrots increased. The goal of this research is to identify the pathogens causing carrot decay in cold storage, in order to develop and fine-tune sustainable diseases control methods. To this aim, 24 fungal isolates were collected from infected carrots from various commercial warehouses in Israel. Initial molecular identification was conducted using ITS-1,4 primers, followed by analysis of specific primer sets for each of the pathogens. The main pathogens that were identified included Sclerotinia sclerotiorum (White Mold), Botrytis cinerea (Grey mold), and Alternaria sp. (Black spot). Pathogenicity was evaluated at 5 and 16°C, and was followed by completion of Koch`s postulates. Furthermore, growth inhibition by Rovral® and Scholar® was determined on poisoned agar media, identifying S. sclerotiorum, B. cinerea and Alternaria sp. with tolerance to 10ppm Scholar®. One S. sclerotiorum isolate was resistant to both Rovral® and Scholar® at 1000 ppm. Future research will investigate resistance mechanisms in Botrytis, Sclerotinia and Alternaria strains using molecular and physiological approaches. Uncovering resistance mechanisms of postharvest pathogens of carrot, alongside testing combinations of inorganic GRAS salts with Scholar®, would facilitate sustainable disease management to mitigate postharvest losses, while reducing the selective pressure for the development of resistance to Scholar® (a.i. Fludioxonil 230 SC).

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## Bioinspired surfaces with anti-microbial properties for preventing fungal adhesion

**Hanan Abu-Hamad**[1], Elena Prudnikov[2], Iryna Polishchuk[2], Gali Ron[1], Boaz Pokroy[2], Ester Segal[1]

[1]*Department of Biotechnology and Food Engineering, Technion - Israel Institute of Technology, Israel*

[2]*Department of Materials Science and Engineering, Technion - Israel Institute of Technology, Israel*

Fungal infections pose a significant threat across multiple sectors, with far-reaching consequences for global food security and agricultural economics. These sectors vastly rely on chemical fungicides to combat fungal pathogen infection. Yet, the extensive usage of these fungicides results in detrimental effects on ecosystems and contributes to the emergence of resistant strains, rendering these treatments less effective over time.

In response to these challenges, we have developed a sprayable coating that incorporates saturated fatty acids (FAs) as a novel alternative to current state-of-the-art antifungal coatings. Inspired by nature, we develop a sprayable coating incorporating saturated FAs as an alternative to current state-of-the-art antimicrobial coatings. Inspired by nature`s self-cleaning mechanisms observed in plants like lotus leaves, the FAs assemble to form a superhydrophobic coating that impedes pathogen adhesion, the first step in the infection process.

Using Botrytis cinerea as a fungal model, we show that by tailoring the composition of FAs, we can tune the exerted effect of the coating from passive antibiofouling to active antifungal properties. This is achieved by combining long FAs, which crystallize to create a hierarchical microstructure of wax crystals, with short-chain FAs that possess intrinsic antifungal properties. Our results indicate that the coatings can be easily applied to various surfaces, significantly reducing spore adhesion and germination. These preliminary results show the potential to use these coatings as a solution to mitigate fungal infections on different surfaces in an environmentally friendly manner.

Acknowledgment: This work was supported by the Horizon Europe for Research and Innovation Program under grant agreement No. 101099462.

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## Developing a New Protein Delivery Technique, Using a Bacterial Secretion System

**May Tfilin Samuel**[1]
*Microbiology, Immunology and Genetics, Ben-Gurion University, Israel*

Protein and peptide-based drugs (biologics) have emerged as promising therapeutic agents due to their high selectivity, efficacy, and reduced side effects. The oral delivery of biologics offers several advantages over parenteral administration including improved patient compliance, ease of administration, and reduced production costs. However, the widespread application of oral biologics is limited by the harsh environment of the gastrointestinal tract (GIT), which can lead to chemical and physical instabilities, enzymatic degradation, and low permeability across the intestinal epithelium. Various strategies have been developed to overcome these barriers, such as chemical modification, particulate delivery systems, and bacterial delivery systems. Among these, bacterial delivery systems have shown promise for the oral administration of biologics, with the ability to protect the therapeutic cargo from the GIT environment and facilitate uptake by intestinal cells. This study characterizes a novel bacterial secretion system as a platform for the efficient secretion and delivery of medically relevant proteins. The secreted protein exhibited strong biological activity, as indicated by the production of a predicted response in treated cells and the activation of downstream signaling cascades. This innovative approach highlights the potential of bacterial secretion systems as a versatile platform for the oral administration of biologic drugs.

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## Restricting bacterial bioreporter survival following field dispersal

**Liat Moscovici - Vald**[1]
*1Department of Plant & Environmental Sciences, Hebrew University of Jerusalem, Israel*

Synthetic biology is a broad and dynamic field that combines principles from biology, engineering and other domains for the design and construction of biological systems with novel functions, or to redesign existing biological systems for specific purposes. It offers the potential for revolutionary impacts across industry, healthcare and the environment. However, it necessitates careful consideration of ethical, safety and regulatory concerns. Some of these concerns relate to potential environmental or health risks that may arise from environmental release of genetically engineered microorganisms.

In recent years our focus has been on developing Escherichia coli-based microbial bioreporters for buried landmines detection. These strains harbour several genomic deletions, as well as an engineered plasmid carrying a bioluminescent luxCDABE reporter gene cassette downstream to a 2,4-dinitrotoluene (DNT)-responsive gene promoter, alongside an ampicillin resistance gene.

To address potential concerns regarding environmental release of this sensor bacterium, we have employed three different molecular strategies: (a) removing the ampicillin resistance gene from the plasmid, and replacing it with tryptophan trpED genes and their original promoter. The host strain was rendered auxotrophic to tryptophane by deleting the same genes from the chromosomal trp operon; (b) leucine synthesis by the host was disrupted by deleting the lue gene from the bacterial genome; (c) ensuring complete elimination of the released bacteria, by introducing a “suicide cassette”, consisting of a toxin-encoded gene controlled by a stress-inducible promoter.

We expect that these modifications will render these strains safer and more compliant with both regulatory requirements and public perception.

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# Performance enhancement of bacterial bioreporters for the detection of trace explosives

**Etai Shpigel**[1], Benjamin Shemer[1], Tal Elad[1], Shilat Dadon-Simanowitz[1], Liat moscovici[1], Lidor David[2], Itay Levin[2], Lior Zimmerman[2], Shimshon Belkin[1]
[1]*Department of Plant & Environmental Sciences, The Hebrew University of Jerusalem,, Israel*
[2]*Enzymit, Enzymit Ltd., Israel*

Landmines and explosive remnants of war pose a global problem which claims numerous casualties long after the conflict has ended. Current approaches for the location of landmines, such as metal detection, require physical presence at the minefield and therefore involve high risk to personnel; these methods are also costly, time consuming, and have a high rate of false positive results. The development of alternative technologies for efficient remote detection of buried explosive devices is thus urgently needed.

Most landmines contain 2,4,6-trinitrotoluene (TNT) as the main explosive component, as well as its production by product 2,4-dinitrotoluene (DNT). Over time, the more volatile DNT leaks out of the landmine casing and accumulates in the soil above it, serving as an excellent "signature molecule" for its presence.

We have developed Escherichia coli-based microbial bioreporters which is a basis for a promising technology for future detection of buried landmines. These sensor strains harbour a gene promoter (yqjF) as a sensing element, along with its transcription factor gene (yhaJ). In the presence of DNT metabolites, the yqjF gene promoter is activated, and drives the transcription of a microbial bioluminescence luxCDABEG reporter gene cassette. In order to enhance the bioreporter’s activity, the promoter and the transcription factor were subjected to several rounds of mutagenesis, which resulted in a 100-fold reduction in the DNT detection threshold, and over a 10,000-fold increase in bioluminescent signal intensity. In addition, the integration of luxCDABEG reporter genes from different bioluminescent bacteria has allowed a significant expansion of the temperature operation range.

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## From raw ingredients to product- *Salmonella* survival during chocolate production

Tehila Behar, Zipora Tietel, **Danielle Duanis-Assaf**[1]
*Department of Food Science, Agricultural Research Organization (ARO), The Volcani Center, Israel*

In recent years, *Salmonella* outbreaks in low-water-activity food products have increased steadily. In 2022, two *Salmonella* outbreaks in chocolate manufacturing in Israel and Europe led to a massive recall of many products, resulting in high economic losses. The European outbreak led to over 400 illness cases in 17 countries, with a 41% hospitalization rate. *Salmonella* contamination can occur at every step in the production procedure, and the source of contamination may affect *Salmonella* survival during chocolate manufacturing. The stsudy aims to elucidate the effect of raw ingredients` chemical composition on Salmonella`s survival during chocolate production. We observed a significant decrease in bacterial survival during cocoa powder (CP) storage compared to dry milk powder (DMP). The reduction in cell viability was also affected by the type of CP, where lower survival rates were observed in the dark CP compared to the Dutch CP. This result can be correlated to a higher concentration of antibacterial compounds, such as protocatechuic acid in Dark CP, found by GC-MS metabolomic profiling. During storage, the *salmonella* survival rate was similar in two different DMPs (Israel vs. USA). However, a significant difference in bacterial survival was found during the thermal production processes. While the nutritional composition of both DMPs was similar, metabolic profiling found differences in the profiles of sugars and fatty acids. Overall, our results indicate that *Salmonella*`s survival rate depends on the raw ingredient chemical composition, which might contribute to bacterial defense mechanisms to abiotic stresses at high temperatures.

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# Isolation and characterization of novel methanol tolerant lipases from environmental bacteria for biodiesel production

**Aviv Fainstein**[1], Yoram Gerchman[1,2]
[1]*Department of Biology and Environment, Faculty of Natural Science, University of Haifa, Israel*
[2]*Department of Biology and Environment, Oranim College, Israel*

Israel is importing large amount of diesel for transportation each year, and this situation is not likely to change in the foreseen future. Biodiesel is a green diesel replacement that can be made from waste oil and is currently produced in large amounts in the EU and the USA. Currently, biodiesel is produced mostly by chemical transesterification but there is much interest in enzymatic processes due to their many advantages. Nevertheless, the use of enzymes is hampered by the rarity of enzymes tolerant to methanol, a component in the transesterification reaction.

In this research, the phyllosphere of several plants explored as a source of microorganisms that can produce methanol tolerance lipases.

The results showed that, the species Staphylococcus aureus, exhibited lipolytic activity in the presence of 20% methanol. Tests for characterizing the enzymatic activity revealed that these Staphylococcus aureus isolates exhibit both extracellular and intracellular lipase activity. These results are consistent with genomic search, indicating that this bacterium has at least three genes predicted to code for lipase enzymes: lip1, lip2, and lipA. Structural analysis, demonstrated similarity between Lip1 and Lip2, but very different structure of LipA.

Cloning and expressing each enzyme separately in E. coli revealed that both Lip1 and Lip2 have lipolytic activity. Of the two, Lip2 showed much higher methanol tolerant activity, with tolerance in the presence of 50% methanol. Finally, FAME was produced, using extracellular fluid, agreeing with the proposed use of S. aureus extracellular lipase (and probably Lip2) for biodiesel production.

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Poster presentation

10 Applied microbiology and biotechnology

# Integrated use of electrochemical anaerobic reactors and genomic-based modeling for characterizing methanogenic activity in microbial communities exposed to BTEX contamination

**Jenny Yusim**[1,2,3], Raphy Zarecki[2], Shlomit Medina[2], Sari Mousa[3], Aviv Kaplan[1], Igal Gozlan[1], Mahdi Hassanin[3], Zeev Ronen[4], Katie Baransi-Karkaby[3], Dror Avisar[1], Isam Sabbah[3], Keren Yanuka-Golub[3], Shiri Freilich[2]

[1]*Water Research Center, Tel-Aviv University, Israel*

[2]*Newe-Ya'ar Research Center, Agricultural Research Organization, Ministry of Agriculture, Israel*

[3]*Institute of Applied Research, The Galilee Society, Israel*

[4]*Department of Environmental Hydrology and Microbiology, Ben-Gurion University of the Negev, Israel*

Microbial community functionality is a critical factor towards biodegradation enhancement of anthropogenic contaminated substances in soil ecosystems. In such polluted environments, aerobic respiring bacteria rapidly consume oxygen, resulting in a redox zone gradient across the contaminant plume. This generally leads to the prevalence of methanogens near the polluted source. Methanogens have an important role in enhancing the degradation rate keeping the hydrogen concentration low and the entire process thermodynamically favorable. While it is known that methanogenic hydrocarbon metabolism takes place, explicit metabolic partnerships in such systems are far from being understood. To capture community function, we used a combined reactor system that included an Anaerobic Bioreactor (AB) and two Microbial Electrolysis cell (MEC) chambers, each representing a different spatial zone along the redox gradient. This research aims to characterize key steps in the methanogenic activity taking place in native soil community following BTEX exposure in terms of the core microbial composition and interspecies interactions. This is achieved through a combination of integrated chemical-biological monitoring techniques and the utilization of genome-scale metabolic modeling. In our research we not only generated a simplified functional representation of complex community but also created a tool for formulating predictions that can be further supported by additional layers of experimental information.

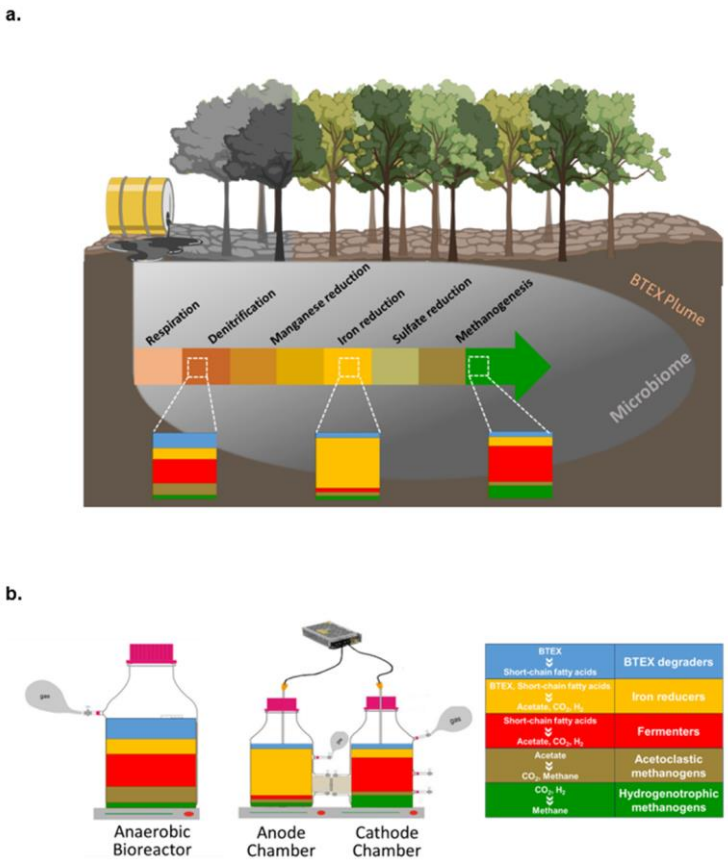


Illustration of key biochemical processes in the reactor system. a. A redox gradient developed in contaminated soils. Each colored bar represents the enrichment of typical microbial functional groups, known to participate in BTEX biodegradation; b. Three-chamber bioreactor system that is designed to capture natural processes within contaminated soils.

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Poster presentation

10 Applied microbiology and biotechnology

# Improvement in aqueous extractable caffeine content using solid-state fermentation was justified to be higher in green tea waste

**Bixia Qiu**[1,2], Avi Shpigelman[1], Yigal Yigal Achmon[1,2]
[1]*Department of Biotechnology and Food Engineering, Technion - Israel Institute of Technology, Israel*
[2]*Department of Biotechnology and Food Engineering, Guangdong Technion Israel Institute of Technology, GTIIT, China*

Green and black tea, coffee waste, and a mixture of both at a solid content (15%) underwent natural fermentation to assess its potential for increasing extractable caffeine in micro-fermenter simulation systems. After a 28-day fermentation, the content of extractable caffeine showed a significant increase in all systems, where green tea waste exhibited the highest, with a rise of 59%. Measuring the respiration of these systems during fermentation indicated that the metabolic activity of microbes in green tea waste was the highest. Development of volatile organic compounds during the process was monitored and three compounds with the highest contribution were tentatively identified as hydrogen sulfide, methanol, and acetaldehyde. These compounds can potentially serve as indicators for the onset of different fermentation stages. The bacterial profiles changed in all systems after fermentation, resulting in dominant strains and microbial diversity changes.

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Poster presentation

10 Applied microbiology and biotechnology

## Alcanivorax dominates the Hydrocarbon degrading community in Ex situ soil treatment.

Swati Gupta, Shlomit Medina, Shiri Freilich, **Zeev Ronen**[4]
*Zuckerberg institute for Water Research, Ben Gurion University of the Negev, Israel*

Bioremediation of soil and sludges polluted with oil products has become a standard practice around the world and in Israel as well. In this treatment, indigenous hydrocarbon degraders are stimulated by adding fertilizer (nitrogen and phosphorus), adjusting water content, and maintaining aeration. Nevertheless, the microbiome of the polluted matrix is viewed as a "black box," and the metabolic potential of the degraders is not optimized. In the current study, we aimed to follow the microbiome dynamic during bioremediation of 12 tones of polluted sludge and soil in a treatment facility. Our goal was to identify the main degraders and their dynamics during the process. We followed the bioremediation and analyzed viable counts, enzymatic activities, qPCR of functional genes, and amplicon sequencing. The data will then be used to optimize the degrader activities via metabolic modeling. The results from this field experiment showed a 75% reduction of the initial 70,000 mg/kg total petroleum hydrocarbons (TPH); however, the branched alkanes, such as pristane, were persistent. We have seen a dramatic change in the microbiome structure of the treatment time. After three months, Alcanivorax species dominated, with a relative abundance of between 46 to 33%. The taxa are highly similar to Alcanivorax dieselolei - known to be a marine organism, and it is able to degrade long-chain alkanes. Its appearance coincides with the degradation of long-chain alkanes in the polluted soil. Our findings suggest that metabolic modeling for specific stimulation of the growth of Alcanivorax in the contaminated soil can accelerate the biodegradation rate.

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Poster presentation

10 Applied microbiology and biotechnology

## Toxicity of Smoke Residues and their Toxicity Elimination Using the Human Microbiome

**Tal Bar**[1], Karina Golberg[1], Robert Marks[1,2], Ariel Kushmaro[1,2]
[1]*Avram and Stella Goldstein-Goren Department of Biotechnology Engineering, Ben-Gurion University of the Negev, Israel*
[2]*The Ilse Katz Center for Nanoscale Science and Technology, Ben-Gurion University of the Negev, Israel*

Billions of people around the world are suffering from smoking toxic effects. Cigarettes are composed of thousands of chemicals which after long or short period of time lead to toxic effects. For toxicity monitoring a panel of bio reporter was used as toxicity monitoring device and it was determined that cigarettes initiates mostly oxidative stress while hookah initiated cytotoxic and genotoxic damages. Healthy heavy smokers were hypnotized and found out to comprise along their respiratory system bacteria strains which digest cigarettes residues and by that reduce smoke toxicity, which can be used as treatment for smokers.

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- Pulmonary department (Soroka):
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- Mrs. Meirav Peretz

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Poster presentation

11 Bacterial physiology

## Metabolomics of anandamide-treated streptococcus mutans reveals mechanism of action

**Goldie Wolfson**[1], Ronit Vogt Sionov[1], Ori Shalev[2], Itzhack Polacheck[3], Maya Korem[3], Doron Steinberg[1]

[1]*Faculty of Dental Medicine, Institute of Biomedical and Oral Research (IBOR),The Hebrew University of Jerusalem, Israel*

[2]*Faculty of Medicine, Metabolomics Center, Core Research Facility, The Hebrew University of Jerusalem, Israel*

[3]*Department of Clinical Microbiology and Infectious Disease, Hadassah-Hebrew University Medical Center, Israel*

Streptococcus mutans is a key pathogen in the oral cavity, involved in dental plaque and caries formation that affect people of all age groups worldwide. Our previous work showed that Anandamide (AEA), a natural endogenous lipid, inhibits S. mutans growth, biofilm formation and production of extracellular polymeric substances, at 12.5 µg/mL. Additionally, AEA increased membrane potential, permeability and cell division gene expression.

Here, we explore AEA`s mechanism of action. We measured membrane fluidity using Laurdan, a fluorescent probe, gene expression of autolysis, linoleic acid and biotin metabolism using Real-time PCR. Autolysis was tested with Triton X-100 treatment, and hydrolytic activity was analyzed with a zymogram assay. Metabolomics investigated metabolic pathways.

We found that AEA increased the membrane rigidity, hindering S. mutans stress adaptation. Genes related to autolysis (lytR, atlA, atlE, rgpG) were significantly upregulated. Treatment with Triton X-100 indicated AEA induced autolysis after 2.5 hours. The zymogram assay revealed a prominent autolysin (AtlA) involved in cell separation, biofilm formation and autolysis in AEA-treated cells. Metabolomics indicated a significant upregulation of linoleic acid metabolism and downregulation of biotin metabolism, supported by gene expression changes (birA, atpA, codY, and fabK).

In conclusion, AEA targets S. mutans membrane, causing a disruption in cell division, increased permeability, and interruption of key metabolic pathways, such as linoleic acid and biotin metabolism) leading to autolysis and ultimately cell death.

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11 Bacterial physiology

# Growth-phase-specific induction of viable but non-culturable state in Listeria innocua by a nature-based antimicrobial formulation

**Esther Mwangi**[1,2], Victor Rodov[1], Moshe Shemesh[1]
[1]*Department of Postharvest and Food Sciences, Agricultural Research Organization, The Volcani Institute, Israel*
[2]*Department of Biochemistry, Food Science and Nutrition, The Robert H. Smith Faculty of Agriculture, Food and Environment, Hebrew University of Jerusalem, Israel*

An important adaptive strategy of bacteria under adverse conditions is entering a viable but non-culturable (VBNC) state. In this form, the bacteria display growth arrest according to culturability tests while retaining metabolic activity manifested in viability assays. The capacity of VBNC pathogenic bacteria to resuscitate and to regain virulence poses an obvious public health risk. We have recently developed a novel synergistic antibacterial triple formulation (TF) comprising 8 mM natural polyphenol gallic acid (GA) and generally recognized as safe (GRAS) materials, 1 mM hydrogen peroxide (HP) and 20 mM DL-lactic acid (LA). The TF reduced Gram-negative (Escherichia coli, Pseudomonas syringae and Pectobacterium brasiliense) and Gram-positive (Listeria innocua and Bacillus subtilis) bacteria by 4-8 log CFU mL-1. Its decontamination capacity towards baby spinach leaves exceeded that of commercial synthetic sanitizers. We further found that the TF effect involved ROS generation and was bactericidal against all species tested except for L. innocua. In L. innocua, the TF effect was dependent on growth phase, being bactericidal towards logarithmic phase cells but demonstrating the VBNC pattern with stationary phase cells. Despite the culturability loss of 7 log CFU mL-1, the TF-treated stationary phase L. innocua maintained high ATP content and membrane profiles similar to non-treated (control) cells: high membrane integrity, stable membrane potential and efficient electron transport mechanism. Hence, our findings demonstrate that in response to TF challenge, the stationary-phase Listeria invokes physiological adjustments to accommodate VBNC development hence enhanced stress adaptation.

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11 Bacterial physiology

# Biofilm formation correlates with resistance to human serum in multidrug resistant clinical isolates of Klebsiella pneumoniae

**Hadas Fulman-Levy**[1], Polina Geva[1], Shiri Navon-Venezia[1,2]
[1]*Molecular Biology, Ariel University, Israel*
[2]*Adelson School of Medicine, Ariel University, Israel*

Multidrug resistant Klebsiella pneumonia (MDR-Kpn) is a major causative pathogen of bloodstream infections with limited therapies and fatal outcomes. The pathogenicity underlying MDR-Kpn survival in the blood is still elusive. We hypothesized that biofilm production promotes MDR-Kpn ability to thrive in serum.

We focused on 12 MDR-Kpn clinical isolates (different genetic lineages) that although exhibited similar growth fitness in defined media, possessed variable growth in human serum. The isolates were divided into three phenotypes based on their response to serum after 3 hours; serum resistant (logCFU change of 1), medium sensitivity (logCFU of 1 to -2) and high sensitivity (logCFU change of -2). Biofilm production by the isolates in the presence of human serum (40% and 80%), assessed by crystal violet and confocal microscopy, revealed variability in biofilm production and volume. We discovered a linear correlation (R2 = 0.9) between biofilm formation and serum resistance. Moreover, increased expression levels of mrk genes, that encode Type-3 pili involved in MDR-Kpn biofilm formation, were found in a serum resistant isolate compared to a serum sensitive isolate, further supporting the involvement of biofilm in serum survival. Overexpression of mrk genes in a serum sensitive strain increased both biofilm production and subsequently serum resistance.

This study describes for the first time in MDR-Kpn a direct linear correlation between mrk operon-mediated biofilm production and serum survival. These findings may be used for prediction of serum survival of clinical strains and may be used for future design of targeted therapies against MDR-Kpn.

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11 Bacterial physiology

# Identification of Universal stress proteins in multidrug-resistant Klebsiella pneumoniae and their role in nutrient limitation

**Tsfia Kohen**[1], Polina Geva[1], Shiri Navon-Venezia[1,2]
[1]*Molecular Biology, Ariel University, Israel*
[2]*The Adelson School of Medicine, Ariel University, Israel*

Universal stress proteins (USPs) are cross-kingdom highly conserved gene superfamily found in bacteria, archaea, fungi, protozoa and plants, involved in stress response. Multidrug-resistant Klebsiella pneumoniae (MDR-Kp) is a causative pathogen of many infectious diseases. Despite the importance of USP function in various pathogens, these genes in MDR-Kp are understudied.

Our hypothesis is that MDR-Kp employs the USP family to effectively cope with various environmental stressors encountered within the body. We aimed to investigate these proteins in MDR-Kp and decipher their involvement in response to nutrient limitation.

Seven usp genes were identified in K. pneumoniae genomes including uspA, uspC, uspE, uspF and three alleles of uspG. Clustal Omega protein comparison revealed high sequence similarity of the identified MDR-Kp USPs with four clinically important pathogens of the Enterobacteriales family. Percent similarities found: UspA-99.3; UspC-77.7-79; UspE-95.9-97.2; UspF-75.7-91; UspG1-74.1; UspG2-92.3-93.7; UspG3-68.5-71.3. Multiple sequence alignment of the seven USP proteins revealed two conserved hydrophobic motifs which may play a role in their function and will be further investigated. Comparative transcriptomic analysis using qRT-PCR experiments demonstrated a significant higher expression level of uspA, uspC, uspE and uspF genes, during bacterial growth in BM2 defined media compared to LB media. In addition, growth kinetic experiments and usps expression levels quantitation under carbon, nitrogen and phosphorous limitations, supported their involvement in MDR-Kp response in nutrient limitation.

Understanding the role of USPs in MDR-Kp contribute to the fundamental knowledge on adaptation systems in this pathogen and could promote the identification of novel potential therapeutic targets in this bacterium.

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11 Bacterial physiology

## Repression by DNA Looping: Functional and Structural Analysis of the Gram Positive Global Transcriptional Regulator ScoC

**Smadar Shulami**, Noam Hadad, Mnar Ghrayeb, Sergei Pomyalov, Yael Pazy, Liraz Chai, Yuval Shoham, Gil Shoham

ScoC of the MarR family is a global regulator of transition phase pathways in Gram positive bacteria and in Bacillus subtilis it is estimated to regulate more than 500 genes. Transcriptional lacZ-fusions analysis demonstrated that ScoC from Geobacillus binds to two operator sites in the promoter region of the oligopeptide permease oppA, and both binding sites are necessary for repression. Gel retardation assays, atomic force microscopy (AFM) and fluorescence resonance energy transfer (FRET) analyses showed that ScoC can induce DNA looping. The rate of change in fluorescence intensity initially increased with increasing concentrations of ScoC and decreased at ScoC concentrations above 10 nM. It is likely that at high ScoC concentrations, two independent ScoC tetramers (rather than a single tetramer) bind to the two operators, thus preventing DNA looping. Indeed, the data could be modeled with a typical Michaelis-Menten kinetics, exhibiting substrate inhibition. The crystal structures of ScoC and ScoC complexed with a 23-bp symmetric palindromic DNA were determined at 3.15 Å and 3.50 Å resolution. The structures revealed a tetrameric X-shape assembly composed of two dimers in which each dimeric unit comprises a winged helix-turn-helix DNA-binding motif. The ScoC wings interact with the DNA bases at the minor groove via a highly conserved Arg100 residue, whereas the recognition helix in the major groove interacts with Ser75 and Asn79. To the best of our knowledge, ScoC is the only reported protein of the MarR family that operates as a tetramer and represses expression via DNA looping.

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Poster presentation

13 Parasites

## Single Vesicle Approaches for Characterization of Nucleic Cargo in Malaria Parasite-Derived Extracellular Vesicles

**Ewa Kozela**[1], Ekaterina Petrovich-Kopitman[2], Tali Dadosh[3], Abel Cruz Camacho[1], Yuval Berger[1], Yaara Shoham[1], Mattia Morandi[4], Neta Regev-Rudzki[1]
[1]*Biomolecular Sciences, Weizmann Institute of Science, Israel*
[2]*Flow Cytometry Unit, Weizmann Institute of Science, Israel*
[3]*Chemical Support Unit, Weizmann Institute of Science, Israel*
[4]*Institute of Organic Chemistry and Biochemistry, Czech Academy of Science, Czech Republic*

Extracellular vesicles (EVs) are membrane-bound nano-organelles released by living cells across various kingdoms, including human parasites. EVs facilitate cell-to-cell communication by transferring bioactive molecules, thereby influencing cell function and fate. Combining cellular, biophysical and omics approaches, we have shown that malaria parasite (Plasmodium falciparum, Pf) while residing inside the human red blood cells (RBCs) utilizes EVs to regulate its growth and interaction with host immune cells. Here, we employed two advanced single vesicle technics, spectral flow cytometry and super resolution microscopy (dSTORM), to enable Pf EV (sub)phenotyping. We used these approaches to detect EVs carrying nucleic cargo and isolated from Pf infected human RBCs. We performed double EV tagging which combines co-staining of membrane lipids and RNA or DNA molecules. Using spectral flow cytometry we observed that Pf EVs are enriched in RNA cargo as compared to EVs isolated from non infected cultures. We also identified Pf EV subpopulations carrying DNA cargo. dSTORM nanoscopy showed that the detected nucleic signal is indeed encapsulated within parasitic EVs.

Our results indicate that single vesicle technologies assist in the detection, quantitation and monitoring of nucleic acid cargo in EVs, advancing our understanding of EV-mediated host-pathogen cross talk.

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Poster presentation

14 Microbial societies and microbial interactions

## Competitive interactions between culturable bacteria are highly non-additive

**Amichai Baichman-Kass**[1], Tingting Song[1], Jonathan Friedman[1]
*Plant Pathology and Microbiology, Hebrew University of Jerusalem, Israel*

Microorganisms are found in diverse communities whose structure and function are determined by interspecific interactions. Just as single species seldom exist in isolation, communities as a whole are also constantly challenged and affected by external species. Though much work has been done on characterizing how individual species affect each other through pairwise interactions, the joint effects of multiple species on a single (focal) species remain underexplored. As such, it is still unclear how single-species effects combine to a community-level effect on a species of interest. To explore this relationship, we assayed thousands of communities of two, three, and four bacterial species, measuring the effect of single, pairs of, and trios of 61 affecting species on six different focal species. We found that when multiple species each have a negative effect on a focal species, their joint effect is typically not given by the sum of the effects of individual affecting species. Rather, they are dominated by the strongest individual-species effect. Therefore, while joint effects of multiple species are often non-additive, they can still be derived from the effects of individual species, making it plausible to map complex interaction networks based on pairwise measurements. This finding is important for understanding the fate of species introduced into an occupied environment and is relevant for applications in medicine and agriculture, such as probiotics and biocontrol agents, as well as for ecological questions surrounding migrating and invasive species.

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14 Microbial societies and microbial interactions

## Multi-species biofilm characterization through ecological and evolutionary timescales using Raman spectroscopy

**Matvey Nikelshparg**[1], Adrian Lehvy[1], Shimon Bershtein[1]
*Department of Life Sciences, Ben-Gurion University of the Negev, Israel*

Formation of microbial communities naturally results in the emergence of complex structures known as biofilms. Biofilm consists of cells and extracellular matrix that provides a structural foundation for the community. In the biofilm, multiple species interact with each other, however, little is known about their interactions within the biofilm matrix. Moreover, it is poorly understood whether and what changes occur in the complex matrix both on the ecological and evolutionary timescales. Here, we devised a non-targeted approach to study multi-species biofilm matrix, using Raman spectroscopy. This method allows us to simultaneously observe structural properties of polysaccharides, proteins, and lipids in the biofilm matrix. We found that Pseudomonas aeruginosa, Pseudomonas fluorescens, and Klebsiella pneumoniae each possess a unique biofilm matrix composition. Furthermore, we were able to characterize matrices of species combinations and accurately predict the species composition, using ratiometric analysis. We aim to combine biofilm matrix profiling with the recently developed Tn7 DNA barcoding technique which allows for high-resolution population dynamics tracking. By integrating these novel methods, we would like to characterize the identity of the biofilm matrix and correlate it with the inter-species interactions through time. This research will contribute to a deeper understanding of the ecological and evolutionary dynamics of complex biofilm communities.

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## Characterizing ecological and evolutionary dynamics in multiple-species biofilm using high-resolution barcodes

**Adrian Lehvy**[1], Matvey Nikelshparg[1], Shimon Bershtein[1]
*Life Sciences, Ben Gurion University, Israel*

Microbial interactions are routinely characterized by quantifying relative species abundance in planktonically-grown cultures. Changes in community properties have been studied, separately, on ecological and evolutionary time-scales. However, since ecological and evolutionary factors form a feedback loop, they must be studied together. Moreover, in addition to quantifying species abundance, measuring changes in lineage frequencies demonstrates the effects of selective pressure. We aim to characterize ecological and evolutionary factors that shape microbial interactions, in a multiple-species biofilm model, which more accurately represents bacterial communities found in nature. Barcodes, designed with identifiers for both species and disparate lineages, enable us to track the intra-species diversity, alongside species abundance. Remarkably, the lineage diversity of the cloned barcodes was Nx10^5, two orders of magnitude higher than previously reported in K. pneumoniae, P. aeruginosa, and P. fluorescens. Using the high-resolution barcoded libraries, all possible multiple-species combinations were grown and passaged until ecological equilibrium. In ~100 generations, there was a significant decrease in intra-species diversity, the rate of which changed based on ecological interactions with growth partners. The loss in intra-species diversity was non-random, indicating evolutionary selection as a result of standing genetic variation (SGV) in the starting populations, as the ecological time-window lacks de novo mutations. The population dynamics of all three species reflected the interplay between ecological and evolutionary forces in shaping the biofilm communities. In future work, we aim to model the eco-evolutionary factors involved in shaping a microbial community, using barcode sequencing together with phenotypic characterization.

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14 Microbial societies and microbial interactions

## The ANMEsphere at the core of the cold seep microbial community

**Stas Malavin**[1,2], Maxim Rubin Blum[2]
[1]*Department of Desert Studies, Ben Gurion University of the Negev, Israel*
[2]*Department of Biology, Israel Oceanographic and Limnological Research, Israel*

Cold seep ecosystems form in sites where cold fluids or gases rich in low molecular weight hydrocarbons, mostly methane, leak through the sea bottom. The influx of hydrocarbons fosters the development of dense chemosynthetic microbial communities characterized by elevated metabolic rates. Consortia of anaerobic methane-oxidizing archaea (ANME) and sulfate-reducing bacteria (SRB) remove methane from seeps anaerobically, yet their interactions with other microbes are not well understood.

Here, we aimed to characterize the seep microbial community, focusing primarily on its less studied heterotrophic component. For that, we collected brine-infused sediment saturated with methane, sulfide, and ammonium at millimolar levels, found near brine pools at the toe of Palmahim Disturbance offshore Israel (1150 m water depth), as well as control non-seep sediments. We assembled 244 and 288 prokaryotic genomes, from seep and control sediments, respectively. Consortia of anaerobic methane oxidizers (ANME) and sulfate reducers (SRB) were prominent, and their species composition varied with the depth. Diverse and abundant heterotrophic lineages fermented various macromolecules. Metabolic reconstruction of Campylobacterota genomes suggests both metabolic specialization and functional redundancy in these communities. Co-abundance network analysis of Palmahim samples and available data on similar seeps indicates the presence of the core microbial community associated with the ANME-SRB consortium that we propose to call “the ANMEsphere”.

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14 Microbial societies and microbial interactions

## Insights into marine plasmid ecological dynamics: a global metagenomic perspective

**Lucy Androsiuk**[1,2], Shay Tal[2], Tal Shay[3]
[1]*Marine Biology and Biotechnology Program, Department of Life Sciences, Ben-Gurion University of the Negev, Eilat Campus, Israel*
[2]*National Center for Mariculture, Israel Oceanographic & Limnological Research, Israel*
[3]*Life Sciences, Ben-Gurion University of the Negev, Israel*

Marine environments host a diverse microbial community, with plasmids influencing microbial adaptability. Despite increased research on marine microbiomes, marine plasmids remain poorly understood and underrepresented in databases. This study aimed to address this gap by characterizing marine plasmids through metagenomic analysis.

We performed de novo assembly of plasmids from publicly available marine metagenomic datasets. Plasmids were characterized, and their distribution patterns were studied. Functional analysis was conducted to assess the legitimacy and environmental role of identified plasmid candidates. Our analysis revealed a diverse array of marine plasmid candidates, many of which represented novel entities. These plasmid candidates exhibited geographical diversity and harbored unique functional gene cassettes. Distinct distribution patterns were observed, correlated with environmental variables such as depth, temperature, geographical location, and oxygen zones. Additionally, regions enriched for plasmid-derived antibiotic resistance genes also exhibited enrichment for genes conferring resistance to metals.

The association of antibiotic and metal resistance suggests a site-dependent role of plasmids in adapting to polluted ecological niches. Our study emphasizes the importance of investigating marine plasmids despite challenges in functional annotation. The novel plasmids identified may encode proteins with unique functions, aiding in predicting antimicrobial resistance dissemination and understanding plasmid-bacterial interactions. Overall, our work encourages further exploration to uncover the ecological importance of marine plasmids, providing insights into microbial ecosystem dynamics.

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14 Microbial societies and microbial interactions

## Tangential Flow Filtration for Concentrating Marine Microbiota: Implications for Physiological Experiments and Microbial Composition

**Tzipora Peretz**

Tangential Flow Filtration (TFF) is a method used to separate particles in liquid solution. Originally designed for the purification and concentration of biomeolecules, this method has also proven suitable for concentrating live aquatic bacteria for physiological experiments. Many bacterial studies require cell concentrations higher than those typically found in marine environments, limiting much environmental research to bioinformatics. Here, we examined the potential of using TFF to concentrate marine bacteria. Previous studies have shown that approximately 60% of bacteria are lost during the TFF process, but the effect of the TFF procedure on the microbial composition has not been investigated. To determine whether the concentrated seawater accurately represents the natural microbial community, we compared microbial composition of three samples of seawater before TFF with three samples after TFF. By analyzing 16S amplicon sequences we found there was no significant difference in the microbial diversity (p value  0.05). Alphaprotobacteria and Cyanobacteria, that are highly abundant in seawater (before TFF) become even more relatively abundant after TFF. This at the expense of less relatively abundant taxonomies, such as Verrucomicrobiae and Bacteroidia.

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## The plastisphere: from ecosystem dynamics to pure cultures of “plastic-loving” microbes

**Matan Oren**[1]
*Molecular Biology Department, Ariel University, Israel*

Every year, millions of tons of plastic waste infiltrate our oceans, persisting for decades. Since the mid-20th century, when plastic first entered marine ecosystems, microorganisms have adapted to thrive on this synthetic material. Within minutes of entering the marine environment, microorganisms begin colonizing plastic surfaces, initiating a succession process. This involves the formation of an early biofilm that matures into a diverse community comprising phototrophs, heterotrophs, predators, decomposers, and symbionts. Our research employs long-read DNA prokaryotic and eukaryotic metabarcoding, integrated with customized bioinformatics analysis, to explore the plastic microbiome in marine environments and the factors shaping its structure. Our results show that the plastisphere is a complex ecosystem with taxonomic structures and dynamics distinct from the surrounding water. Geography, seasonality, and the type of plastic surface significantly impact the plastic microbiome. Using weighted gene co-expression network analysis (WGCNA), we tracked unique succession signatures of bacterial genera on different plastic polymers. In accordance to the WGCNA results, pure cultures of hydrocarbon-degrading bacterial isolates from the genus Alcanivorax showed colonization preference toward polyolefin polymers. Moreover, UV treatment increased Alcanivorax colonization rates specifically on PET but not on polyethylene. Together, these findings deepen our understanding of the interactions and temporal dynamics of marine microbes on plastic surfaces and the mechanisms governing microbial community assembly and succession.

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# *Alcanivorax*, a genus of marine bacteria with varied tastes for hydrocarbons

**Aiswarya Kartha**[1], Keren Davidov, Sheli Itzhari, Matan Oren
*Department of Molecular Biology, Ariel University, Israel*

**Abstract**

Over the past few decades, marine pollution has become a major global concern. Two significant pollutants that pose a constant risk to the marine environment are petroleum and its products, and plastic debris from various land and marine sources. Both types of materials are composed of hydrocarbons and originate from crude oil. We have isolated different strains of hydrocarbon-degrading bacteria of the genus Alcanivorax from marine plastic debris. These strains were tested for their ability to metabolize various types of liquid and solid hydrocarbons, including crude oil. The Alcanivorax strains were categorized into 3-4 groups based on their hydrocarbon metabolism profiles, which correlated with their colonization ability on different plastic polymer types. To understand the molecular basis of these findings, we obtained the full genomes of the strains and conducted a comprehensive screen for hydrocarbon-degrading gene candidates. The leading candidates will be further tested for their function using a knockout approach and through the expression and purification of relevant enzymes.

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Poster presentation

14 Microbial societies and microbial interactions

## Unveiling the Mechanisms Behind Positive and Negative Interactions in Marine Microbial Communities: A Mathematical Exploration of *Prochlorococcus* -Heterotroph Interactions

**Osnat Weissberg**[1], Daniel Sher[1], Mick Follows[2]
[1]*Leon H. Charney School of Marine Sciences, Marine biology, University of Haifa, Israel*
[2]*Department of Earth, Atmospheric and Planetary Sciences, MIT, United States*

Phytoplankton drive the marine food web through carbon fixation, but their success relies on intricate bacterial partnerships. This study explores the mechanisms governing interactions between the cyanobacterium *Prochlorococcus* and co-occurring marine heterotrophic bacteria, using mathematical models. We ask whether mathematical models explicitly representing four putative mechanisms of interaction (overflow metabolism, mixotrophy, exoenzymes and detoxification) are able to recapitulate the results of laboratory co-cultures. Our results reveal two key mechanisms where heterotrophs support *Prochlorococcus*: i) Cross-feeding, whereby *Prochlorococcus* supplies fixed carbon to heterotrophs, while heterotrophs recycle essential nitrogen through exoenzymes or overflow metabolism, creating a mutually beneficial loop; ii) Detoxification, whereby heterotrophs detoxify harmful byproducts (e.g., reactive oxygen species) produced by *Prochlorococcus*, leading to a positive interaction. These mechanisms differ in the overall productivity of the co-culture system. Finally, the models are able to capture also negative interactions, but these are very rare within the range of growth parameters tested, suggesting that negative interactions observed in laboratory cultures are likely due to additional mechanisms not represented in these models, such as antibiotic production.

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Poster presentation

15 Bacteriophages

## Solid-state NMR-based phage structural virology at atomic resolution

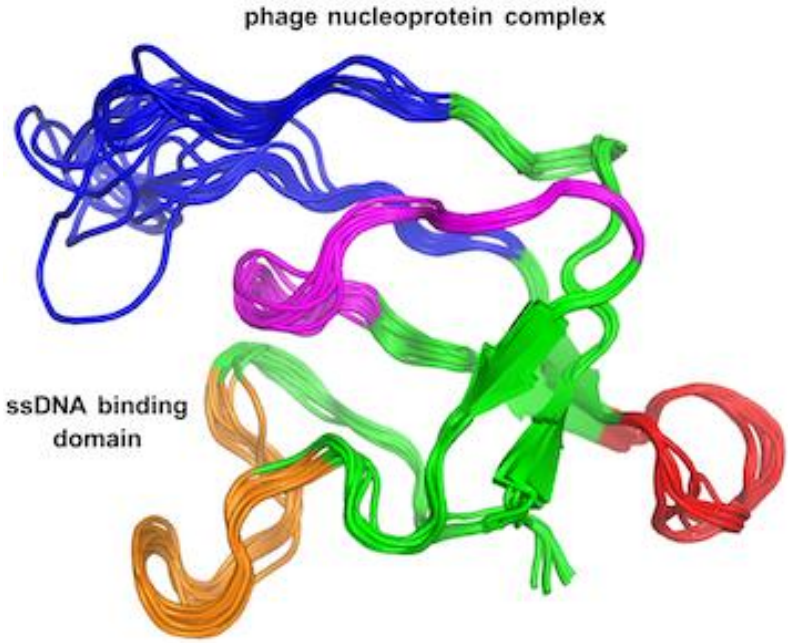
**Prof. Amir Goldbourt**[1], Orr Lusky[1], Yoav Shamir[1], Omry Morag[1]
*School of Chemistry, Tel Aviv University, Israel*

A major tool for structural virology, along with X-ray crystallography and cryo-EM, is the solid-state nuclear magnetic resonance (SSNMR) technique. It provides structural and dynamical information on an atomic-resolution scale for complex systems, with no size limit on resolution, and without the need for crystallization or cooling. Here we show the strength of the technique to study complex intact phage systems.

Filamentous bacteriophages (phages) are micron long ssDNA viruses that infect bacterial cells that possess pili organelles. Their ssDNA (~8000 bases) is wrapped by several thousand copies of the major coat protein, gVIIIp. During replication, the non-structural gene 5 protein (gVp) attaches to a ssDNA creating a nucleocapsid that facilitates secretion.

Using SSNMR we showed that the structure of the M13 phage is dominated by strong hydrophobic stacking interactions and by pointing positively-charge C-terminal lysines towards the DNA lumen. Advanced experiments also report on capsid dynamics showing a highly mobile N-terminus, while protein-DNA interactions induce restrictions on the motion of some of the positively charged lysines.

We also determined the structure of gVp in the gVp-ssDNA complex (see image), obtaining a backbone RMSD of 0.37Å for the well-defined regions, and good convergence criteria. Interestingly, free and ssDNA-bound gVp show significant changes in key locations having an RMSD of 6.5Å. The changes point to a more compact DNA binding pocket, allowing the protein to bind ssDNA. Furthermore, the structural changes facilitate the reported cooperative binding by flattening the protein side facing the dimer-dimer interface, promoting the cellular assembly of this pre-mature virus.



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15 Bacteriophages

## The first gene in a T7-like cyanophage genome is critical for efficient infection and fitness

**Bashir Said**[1], Debbie Lindell
*Faculty of Biology, Technion – Israel Institute of Technology, Israel*

Viruses are the most ubiquitous biological entities in the biosphere. In marine environments, viruses that infect the picocyanobacterial, cyanophages, are highly abundant in the oceans. Cyanophages must take-over host metabolic processes and direct them toward phage progeny production. Despite the importance of the host take-over process, there is no existing knowledge regarding this process in marine cyanophages. The aim of this project is to unravel the role of gene 1 in host take-over in the T7-like cyanopodovirus, S-TIP37, that infects Synechococcus WH8109. Gene 1 (g1) is physically the first gene in the genome and is expressed first, and is thus thought to function in host take-over. This gene has an unknown function despite being conserved among T7-like cyanophages. We generated a phage deletion mutant for g1 and assessed its infection properties. This mutant showed a 45% decrease in virulence compared to the wildtype cyanophage. The g1 mutant also showed a one hour (33%) longer latent period. However, this mutant had a 2-fold larger burst size than the wildtype phage. Overall, this mutant had significantly lower fitness than the wildtype, being outcompeted entirely within 24 hours. Our results indicate that this potential host take-over gene strongly impacts infection dynamics and phage fitness and is vital for efficient infection of its Synechococcus host. This study also suggests that the role for the first gene in the genome of ecologically important T7-like cyanophages is uniquely different to that in the T7 coliphage which functions in protection against host restriction.

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## Diversity and evolution of phage sponge proteins that cancel bacterial immune signaling

**Romi Hadary**

The production of immune signaling molecules is an antiviral strategy conserved between animals, plants and bacteria. At least 20% of bacteria express anti-phage defense systems that involve the production of small signaling molecules. These systems, including CBASS, Pycsar and Thoeris, produce a variety of molecular signals comprising cyclic nucleotides and derivatives of NAD+. The immune signaling molecules are generated in response to phage infection, and then activate downstream effectors that execute the immune function.

It was recently shown that phages can encode sponges, small proteins that bind and sequester the signaling molecules produced by bacteria, thus inhibiting bacterial immunity. In this study we combine phylogenetic analyses with high throughput phage infection assays to systematically study the evolution of the molecular specificity between phage sponges and immune signaling molecules. By experimenting with 85 homologs of phage sponges, we show how members of the same sponge family evolved to recognize different molecules produced by different defense systems. Our results explain how phages can broadly mitigate bacterial immune signaling, and exposes a new layer in the intricate arms race between bacteria and phages.

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15 Bacteriophages

## Exploring cheater virus dynamics in a long-term evolution experiment of MS2 bacteriophage

**Arielle Kahn**[1], Moran Meir[1], Noam Harel[1], Uri Gophna[1], Adi Stern[1]
*Life science, Tel-Aviv University, Israel*

RNA viruses are the most abundant group of subcellular parasites. They are particularly notorious for their remarkable genetic diversity, a result of continuous mutation and natural selection. This capacity for rapid evolution is the basis of their adaptability to novel environments, enabling them to sometimes jump between host species and create global pandemics. However, the rapid evolution of RNA viruses comes at a cost. Most point mutations are deleterious, i.e., exert a fitness cost on the virus bearing the mutation, leading to so-called defective viruses. One category of defective viruses is known as defective interfering particles (DIPs), that can only persist during co-infection with wild-type (WT) virus by subverting its resources. These are also known as “cheater viruses”.

In my research I use the model virus MS2, which infects Escherichia Coli bacteria, to study the dynamics of cheater viruses. Previous experiments in our lab showed there are many different cheaters that emerge during experimental evolution of MS2. Per definition, these cheaters bear a defect but also have some advantage over the WT during co-infection. One of my main goals is to test whether cheater viruses can “fix” their defect and evolve to become potent non-cheating viruses.

We conducted a long-term serial passaging evolutionary experiment and utilized a novel long-read method called LoopseqTM to sequence the entire MS2 genome as one haplotype, keeping track of the different mutations in the population and their frequencies over time. I am particularly interested in one mutant, previously shown to be a cheater, that attained a frequency of around 95% in later passages. We focused our analysis on four mutations that displayed recurring high frequencies in later passages and were present on the background of the cheater virus. I show experimental and computational analyses that investigate the relationships among the mutations and their potential functions. Additionally, I discuss an ongoing experiment designed to test how a cheater achieves such a high frequency.

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## Cyanobacteria lysogens evolution: phenotypic changes and their genetic background

**Esther Cattan- Tsaushu**[1], Israa Yasin[1], Sarit Avrani[1]
*Department of Evolutionary and Environmental Biology,The Institute of Evolution, Haifa University, Israel*

Phages are viruses that infect bacteria, and represent the most abundant and genetically diverse entities in the planet. Phages can have different life cycles: lytic or lysogenic. Lytic phages replicate within their host and then lyse and kill the host cell to release the phage progeny to the surrounding environment. By contrast, lysogenic (temperate) phages have the ability to integrate their genome into the host's genome and stay (mostly) dormant as a prophage that replicates alongside the host, until a biotic or abiotic signal leads to the induction of the prophage to enter a lytic cycle. While lysogeny was studied widely in various heterotrophic bacteria, very little is known on lysogeny in cyanobacteria.

Here we followed the evolution of four clones of the filamentous cyanobacteria Leptolyngbya boryana, lysogenized by the phage LPP-2 for 113 days, which has a very distinctive plaque phenotype composed of concentric rings, known to correlate with light/dark regimen. We assessed the change over time in the ability of the phage to excise and the change in plaque phenotype (ring formation/clear/turbid). Moreover, we assessed the lysogeny ability of the excised phages, as well as the mutations in their genomes.

Our results suggest that the "ring" phenotype is not correlated with the ability to enter lysogenic cycle. Out of 17 sequenced evolved phages, only a single clone carried a single mutation that was connected to the loss of the "ring" phenotype, but not to the lysogeny ability. Further study is needed to identify the genes essential for lysogeny.

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## Aspects of lysogeny in cyanobacteria

**Yara Nwatha**[1], Sarit Avrani[1], Esther Cattan-Tsaushu[1]
*Department of Evolutionary and Environmental Biology, and the Institute of Evolution, University of Haifa, Israel*

Cyanophages are viruses that infect cyanobacteria. All cyanophages can infect their host via the lytic cycle, while some of the cyanophages can “choose” between entering the lytic cycle or the lysogenic cycle, in which the genome of the phage is integrated into the host genome in a locus called integration site. While integrated, the genome of the phage is not active, until an external signal drives its induction and the phage enters a lytic cycle. Prophage induction can be triggered by various environmental factors. Moreover, prophage integration confers the host cell resistance against infection by additional phages identical or similar to the integrated phage. This resistance, called superinfection exclusion, can result of different mechanisms, in which infection is arrested at different stages.

Here we focused on different aspects of the lysogenic cycle in two genera of filamentous nitrogen fixing cyanobacteria and two of their phages. We aimed to identify the integration site of the phages, as well as the factors promoting their induction. Moreover, we studied the mechanism/s that confer superinfection exclusion in those strains.

The integration sites we identified suggest that the integration mechanism in both phages differ, despite the fact that both phages belong to the same family. Similarly to that, the factors that affected the induction of the phages differed between the two phage-host systems as well. Despite those differences, both phages were able to adsorb to the resistant lysogen host, suggesting that in both systems the lytic infection is blocked at an internal stage of infection.

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## Entrance to the lysogenic cycle in cyanobacteria: the factors that affect phage decision whether to enter a lytic or a lysogenic infection cycle

**Hoda Madi**[1], Sarit Avrani[1], Esther Cattan-Tsaushu[1]
*Department of Evolutionary and Environmental Biology, and the Institute of Evolution, University of Haifa, Israel*

Cyanobacteria are photosynthetic bacteria that can form harmful blooms. Their phages (cyanophages) may affect bloom dynamics by selectively killing their hosts during lytic infections. Some cyanophages can “choose” to use the lysogenic cycle, in which the phage genome is integrated into the host genome and the host survives. Moreover, phage integration provides the host with resistance against lytic infections by similar phages. The “decision” between entering the lysogenic or lytic cycles can be influenced by various environmental factors, and thus impacting the cyanophage infectivity, host tolerance, and the propagation of the phage.

Here we aimed to study the factors affecting the decision whether to enter lytic or lysogenic infection cycles in the nitrogen fixing filamentous Leptolyngbya. Moreover, as part of our effort to isolate host strains lysogenized by the phage, we isolated strains resistant to the phage, most of which were non-lysogens. Mutations specific to the resistant strains were identified, to assess whether patterns similar to those previously identified in heterocystous cyanobacteria (nitrogen fixation is performed in specialized cells) can be found in this strain that performs nitrogen fixation in all its cells.

Our results suggest that both light and host density affect the rate of lysogeny in Leptolyngbya infected by LPP-2. Moreover, resistance in non-lysogen strains was conferred many times by mutations in cell surface related genes, suggesting that adsorption of the phage was impaired. While this was similar to the observed in heterocystous (and other) cyanobacteria, the cost of resistance in these strains differed.

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16 Beneficial microbes

# Modifying Nitrous Oxide Emissions by Manipulating Bacterial Communities in Wheat Roots and Soil

**Rona Ziskin**[1], Ada Viterbo[1], Omri Vardi-Perlstein[1], Yitzhak Hadar[2], Roi Ben-David[3], Maya Ofek-Lalzar Maya Ofek-Lalzar[4], Shahar Baram[5], Dror Minz[1]
[1]*Institute of Soil, Water and Environmental Sciences, Volcani Institute, Israel*
[2]*Department of Plant Pathology and Microbiology, The Robert H. Smith Faculty of Agriculture, Food & Environment*

*The Hebrew University of Jerusalem, Israel*
[3]*Department of Vegetables and Field crops, Volcani Institute, Israel*
[4]*Bioinformatic Services Unit, University of Haifa, Israel*
[5]*Institute for Soil, Water and Environmental Sciences, Volcani Institute, Research teams-Newe Yaar, Israel*

Nitrous oxide (N2O) is a long-lasting greenhouse gas, about 265 times more potent than carbon dioxide (CO2) over 100 years and damages the ozone layer. Anthropogenic sources contribute to a significant emission, with agricultural nitrogen fertilizers being particularly responsible. This study aims to investigate strategies for decreasing N2O emissions through application of efficient N2O reducing bacteria. The experimental system was based on pots containing 200 grams of natural soil with the specific bacterial strain amended as 30ml of OD 0.6. Wheat was sown in these pots and grown for 10 days in a controlled chamber. After growth, each pot was sealed in a glass jar for 24 hours, and the accumulation of N2O in the jar was measured using Fourier-transform infrared (FTIR) spectroscopy. In this study, N2O emissions were measured in four different soil types, each inoculated with two strains of N2O reducing bacteria (belong to Alcaligenes (AU14) and Agrobacterium (AU243)). These bacteria have the genes for producing, as well as efficiently reducing N2O. Detailed analyses of soil structure and chemical parameters have been conducted to understand the interactions between soil characteristics and bacterial effectiveness in decreasing N2O emissions. Preliminary results indicated that some soils have a higher natural potential for N2O emissions than others. Additionally, we observed a decrease in N2O emissions resulting from the amendment with the specific bacteria. Specifically, AU14 decreased N2O emissions in all soil types compared to the control (by up to 53% of the control), whereas AU243 decreased emissions in some soils but increased them in others. This finding will contribute to developing an N2O reducing bacterial treatment to promote sustainable agricultural practices.

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## Selecting for short-term drought-tolerance inducing rhizobacteria via host mediated microbiome engineering.

**Israel (Wray) Ben Avraham (Hansen III)**

Food security will be a major concern throughout the rest of the twenty-first century as the world's population increases. Being one of world's major staples, special attention must be given to wheat. Most of the world's wheat is rainfed and therefore susceptible to drought. Irrigating wheat is potential solution but is often not practical. Another solution is inoculating the roots of cultivated wheat with microbes suspected to confer short-term drought resistance. During domestication, cultivated wheat lost most of its core microbiome that is otherwise found in its ancestors. Some of these microbes may help wheat's ancestral species survive harsh conditions in the wild, including drought. The purpose of the study herein is to see if an isolate of bacterium or fungus or consortia thereof derived from two ancestors of cultivated wheat, Triticum dicoccoides and Aegilops peregrina, collected from 12 locations throughout Israel, can confer short-term drought resistance following inoculation to the roots of cultivated wheat. Three rounds of host mediated microbiome engineering experiments (HMME) will help the selection process. The first round involved growing wheat seedlings in a mix of perlite/vermiculite that included the soils from the 12 locations sampled and seeing which locations did the best following drought treatment. The 2nd and 3rd rounds were like the first but inoculated with the macerated selected roots from the previous rounds. The roots from the final location of the final HMME cycle will serve as the "microbial bank" for isolating candidate microbes for the subsequent in planta study.