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Abstracts

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THE TYPE III SECRETION SYSTEM AND INJECTED EFFECTORS OF ENTEROPATHOGENIC *ESCHERICHIA COLI*

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Pathogenic and symbiotic bacteria of plant, insects and animals maintain an extensive communication with their eukaryotic host cells. This trans-kingdom communication is frequently mediated by an organelle termed type III secretion system (TTSS) that is employed by the bacteria as a nano-syringe to deliver a set of effector proteins into the eukaryotic host cells. By these effectors the bacteria modulate host function to its benefit. TTSS is a major virulence component of enteropathogenic and enterohemorrhagic E. coli (EPEC and EHEC), both cause devastating diseases and constitute global health risk. The TTSS of EPEC and EHEC are also among the best studied systems and our group played a significant role in this field. We were involved in elucidation of the transcription regulation of the TTSS genes, studies of the dynamic of protein translocation and investigation of the function of the injected effectors in the host cell. A recent breakthrough was the discovery that EPEC employ an array of anti-inflammatory effectors. These issues will be discussed.

CYTOMEGALOVIRUS INFECTION IN PREGNANCY – PAST, PRESENT AND FUTURE

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CMV infection is the most frequent congenital infection, affecting 0.2% - 2.5% of all live births, and the most common cause of deafness and impairment of intellectual function among infants. The risk of seroconversion during pregnancy is 1-4%, and the rate of congenital infection resulting from primary maternal infection is 30%. About 30% of congenitally infected infants are symptomatic at birth or at risk of developing late neurologic sequelae.The percentages of seropositive pregnant women undergoing reactivation during pregnancy and the percentages of symptomatic newborns among those infected are unknown.We have demonstrated in a prospective study similar rates of infection and congenital disease among pregnant women with primary or non primary CMV infections. In order to prevent unnecessary termination of pregnancies we conducted a prospective study among women who were infected with CMV in the third trimester. We have found that although CMV infection during T3 is highly transmissible, sequelae were not found among infected offspring. There are a few potential ways to combat congenital CMV. Although advances have been made in vaccine development, licensed CMV vaccine remains years away. Neonatal screening can be performed by universal hearing screening or by molecular screening tests. PCR for detecting CMV DNA on neonatal dried blood spots was found to be superior to the classic methods of virus isolation from neonatal urine. However a recent study performed at SMC on umbilical cord blood, did not found RT PCR as a sensitive method. The recent demonstration that RT PCR detection of CMV in saliva swabs is equally sensitive as virus culture techniques has made wide-scale newborn screening realizable. The issue of whether pregnant women should be routinely tested for CMV immunity is not settled. A possible approach is to serologically screen all pregnant women in early pregnancy. Those women who are seronegative should practice meticulous hygiene with young children. Another strategy is to screen pregnant women for primary CMV infection by maternal serology at the beginning of pregnancy and at 20–22 weeks gestation in order to offer prenatal diagnosis to those who underwent seroconversion during pregnancy. Combined viral isolation and PCR from amniotic fluid after the 21st week of pregnancy and after a mean interval of 7 weeks from infection have been established as the reference method for prenatal diagnosis of CMV infection. The results of ongoing controlled trials involving oral valaciclovir and CMV hyperimmune globulin for prenatal intervention are awaited.

THE HIDDEN LIVES OF MARINE PICOPLANKTON: AN "OMICS" PERSPECTIVE

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Microbial communities regulate the cycling of energy and matter in the marine environment, yet how they respond to environmental change, and the variability of their activities in space and time, is not well understood. Metagenomic methods are now providing new perspective on the distribution of microbial taxa, genes, and processes in the marine environment. Yet one of the larger challenges remaining is defining these taxa, gene and process distributions on appropriate spatial and temporal scales. How much does metabolic activity of a specific planktonic microbial population vary of the course of minutes, hours, days and weeks? Over what spatial scales? Put another way, exactly what is "a day in the life" of wild planktonic microbial species? How does the variation in any specific population correlate with the variability of other co-occurring taxa or populations, and corresponding environmental variation? New robotic sampling strategies, coupled with genome-wide gene expression analyses in wild planktonic microbial populations, have potential to answer some of these questions. New results using such approaches show that individual populations, as well as very different bacterial and archaeal species, display remarkably similar, time-variable patterns of synchronous gene expression over extended periods of time. These new results suggest that specific environmental cues may elicit cross-species coordination of gene expression among diverse microbial groups that potentially enable multispecies coupling of metabolic activity. These new results will be presented and discussed.

STEALTH AND PERSISTENCE: DECODING THE PATHOGENICITY OF *CANDIDA GLABRATA*

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Background: *Candida glabrata* is an emerging fungal pathogen that is currently the second most frequent cause of invasive candidiasis. Infection with *C. glabrata* is associated with poor outcomes and treatment failure due to its intrinsic tolerance to antifungal azoles and ability to persist intracellularly. We explored two novel aspects of *C. glabrata* pathogenicity: population heterogeneity and mitochondrial uncoupling. **Methods:** We determined susceptibility, resistance and heteroresistance to fluconazole (FLC), efflux pump gene expression, and mitochondrial metabolic activity of *C. glabrata* clinical isolates. *C. glabrata* karyotypes were analyzed by CHEF gel electrophoresis, and Southern blotting was used to characterize efflux pump gene translocations. Virulence and response to FLC treatment were determined in immunocompetent BALB/c mice. **Results:** 52% (26/50) of *C. glabrata* clinical strains were heteroresistant to FLC, and overexpressed the efflux-pump encoding gene *PDH1*. Heteroresistance to FLC was associated with a specific *PDH1* translocation, t(L;F). There was no variation in the *PDH1* copy number among *C. glabrata* strains. FLC heteroresistant strains persisted in mouse tissues despite 7 days of FLC treatment, whereas non-heteroresistant strains were eradicated. Finally, *C. glabrata* clinical strains showed evidence of inducible uncoupling of mitochondrial oxidative phosphorylation under oxidative stress. Mitochondrial uncoupling was linked to FLC tolerance, possibly through resistance to reactive oxygen species. **Conclusions:** Heteroresistance to FLC is a frequent phenotype in *C. glabrata* clinical strains, and is associated with translocations involving *PDH1* and overexpression of this gene. Mitochondrial uncoupling is a unique characteristic of *C. glabrata* which may facilitate persistence in the host.

SALIVA rt-PCR CAN REPLACE URINE CULTURE FOR NEONATAL DIAGNOSIS/SCREENING OF CONGENITAL CMV INFECTION

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Urine culture is the gold standard method for congenital CMV diagnosis but it is logistically too challenging for universal neonatal screening. Saliva real time PCR (rt-PCR) appears to be a better method because of the ease of sample collection, high CMV titers shed in saliva of infected newborns and high sensitivity of the assay. We have evaluated the use of saliva rt-PCR compared to urine culture in the contest of universal neonatal CMV screening during a one year period (May 2011- May 2012). Saliva was collected from 10,033 infants born at Sheba Medical Center, the swabs were transported to the Central Virology Laboratory within 24-48 hours, viral DNA was extracted and then tested for the presence of CMV IE and gB genes. Urine culture was performed for infants positive by saliva rt-PCR. 56 saliva samples were rt-PCR positive, and 47 of them were confirmed by urine culture. 7 with a low Ct value in rt-PCR were negative in repeated rt-PCR and/or urine culture, and 2 had high rt-PCR Ct value, 1 of them with evidence of positive maternal amniotic fluid. Overall 49 infants (0.5%) were confirmed as positive. 192 negative urine samples from newborns suspected of congenital CMV infection served as negative controls and were all negative by saliva rt-PCR. Our study shows that saliva rt-PCR is highly sensitive and useful for diagnosis of congenital CMV infection, particularly in the contest of universal screening. Urine culture confirmation is required due to the low but critical false positive rate.

AGEs SECRETED BY BACTERIA ARE INVOLVED IN THE INFLAMMATORY RESPONSE

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Advanced Glycated End Products (AGEs) are formed by non-enzymatic protein glycation and are implicated in several physiological aspects including cell aging and diseases. Recent data indicate that bacteria – although short lived – produce, metabolize and accumulate AGEs. Here we show that *Escherichia coli* cells secrete AGEs by the energy-dependent efflux pump systems. Moreover, we show that in the presence of these AGEs there is an upshift of pro-inflammatory cytokins by mammalian cells. Thus, we propose that secretion of AGEs by bacteria is a novel avenue of bacterial-induced inflammation which is potentially important in the pathophysiology of bacterial infections. Moreover, the sensing of AGEs by the host cells may constitute a warning system for the presence of bacteria.

DEVELOPMENT AND VALIDATION OF A MULTIPLEX PCR ASSAY FOR IDENTIFICATION OF THE EPIDEMIC ST-258 KPC-PRODUCING *KLEBSIELLA PNEUMONIAE* CLONE

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Objectives: The molecular mechanisms responsible for the epidemiologic success of the ST-258 KPC-producing *K. pneumonia* (KPC-KP) clone remained unclear. We have previously identified several unique genes of this clone. Our aims were to test for the presence of these genes in a multinational collection of various clones of KPC-KP isolates and to validate a multiplex PCR assay for the identification of the ST-258 clone. **Methods:** This was a multinational, retrospective study of KPC-KP isolates, both of the ST-258 (group A) and non-ST-258 (group B) clones. KPC-negative ST-258 isolates were also included. Three ST-258 unique chromosomal genes were selected for testing by a multiplex PCR assay: 1) *pilV* homologue, 2) putative transposase, IS66-family (IS66) and a 3) putative phage-related protein (PRP). **Results:** The following 146 isolates were included: 1) Colombia-A-2, B-1; 2) Greece- A-12, B-8; 3) Israel-A-50, B-17 and 9 KPC-negative ST-258; 4) Italy-A-19 and the USA- A-10, B-18. The *pilV* gene was present in 99/102 of ST-258 isolates, including the KPC-negative isolates. It was absent in all 44 non-ST-258 strains, hence having a specificity and sensitivity of 100 and 97%, respectively. The sensitivity values of IS66 and PRP for detecting KPC-KP ST-258 were 87 and 90%, respectively, and the specificity values were 72 and 90%, respectively. **Conclusion:** This multiplex PCR assay provides a reliable tool for detection of the ST-258 clone among KPC KP strains. The *pilV*-homologue is unique to ST-258 among KPC-KP strains, suggesting a possible role for its epidemiological successfulness.

CHARACTERIZATION OF MACROLIDE RESISTANCE IN *CAMPYLOBACTER JEJUNI* AND *CAMPYLOBACTER COLI* IN ISRAEL

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Introduction: Human *Campylobacter* enteritis is typically caused by *Campylobacter jejuni* (CJ) and *Campylobacter coli* (CC). Antibiotic resistance of *Campylobacter* species has increased worldwide, mainly to tetracyclines and fluoroquinolones, making macrolides the drug of choice. Little is known about the molecular mechanisms of CJ/CC macrolide resistance in local isolates. **Objectives:** To characterize the major molecular mechanisms of macrolide resistance in Israeli human isolates of CC/CJ. **Methods:** 130 clinical isolates of CJ and/or CC were selected from a national collection belonging to the Ministry of Health's Laboratory (collected 1999-2011). Antibiotic susceptibility (Disk diffusion test) was tested according to CLSI standards, and macrolide resistant isolates were subjected to TaqMan Real time PCR to reveal mutations responsible. This assay, using specific probes, can particularly identify three genotypes, wild type and two mutations of the 23S rDNA gene, A2074C, A2075G, both causing macrolide resistance in Campylobacter. **Results:** 99 of 130 (76%) isolates were resistant to ciprofloxacin, 100 (77%) to tetracyclines, and 43 (33%) to macrolides. No difference in resistance to azithromycin and erythromycin was observed. Among all 85 macrolide susceptible isolates, 58 (68%) were resistant to tetracyclines and 58 (68%) to ciprofloxacin. However, among 43 isolates resistant to macrolides, 41 were resistant to ciprofloxacin and 42 to tetracyclines (95% and 97%, respectively). The majority of resistant isolates were CC (76%). The predominant mutation found in those resistant to macrolides was A2075 (90%). **Discussion and Conclusions:** In conjunction with previous international reports, the dominant mutation leading to macrolide resistance was A2075. Macrolide resistance remains minimal despite the extensive use of new macrolides.

WHAT WILL IT TAKE FOR MICROALGAE TO OVERTURN MALTHUS' PREDICTIONS ON RESOURCE INSECURITY?

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Microalgae yielding 10-20 times more than conventional crops, cultivated using seawater with fertilizer and industrial carbon dioxide in closed photobioreactors at desert facilities near oceans have the potential to be the next quantum yield leap that confounds Malthus's predictions of food and fuel insufficiency. Such algae must be domesticated for robust growth at high densities, prevention of contamination by other organisms, and to produce needed feed and fuel products. Deleting unnecessary genes will reduce risks from inadvertent environmental release. The quickest and probably only way to do all this is by genetic engineering. High production costs require engineering solutions that reduce the amounts of water used, the energy costs of mixing the algae and for gas exchange as well for cooling and harvesting. A high density, thin layer, closed, horizontal bioreactor is proposed that should meet these criteria. Substantial progress has been made in the past few years suggesting that such cultivation can economically supply the necessary amounts of feed and fuel in the near future.

THE EFFECT OF PRECIPITATION ON ACTIVE AND DORMANT MICROBIAL COMMUNITIES IN SOIL

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The relationship between total and metabolically active soil microbial communities can change drastically with environment. In drylands, water availability is a key factor in cells' activity. We surveyed the diversity of total and active Archaea and Bacteria in soils ranging from arid desert to Mediterranean forests. Thirty composited soil samples were retrieved from five sites along a precipitation gradient, collected from patches located between and under the dominant perennial plant. Molecular fingerprinting was used to site-sort the communities according to their 16S rRNA genes (total community) or transcripts (active community). The differences in total microbial communities between samples were much higher than those between active communities: differences in DNA fingerprints between sites were 1.2 and 2.5 times higher than RNA differences (in Archaea and Bacteria, respectively). Patch-type discrepancies between DNA fingerprints were on average 2.7 to 19.7 times greater than RNA differences. Moreover, RNA-based community patterns were highly correlated with soil moisture but did not necessarily follow spatial distribution pattern unlike the DNA-based communities. These differences could be attributed to the Actinobacteria phylum domination in all samples (>40% of the community) and their capacity to form spores, which may provide a competitive advantage in arid environments. Although no known archaeal species can form spores, species of this domain were shown to become dormant and reduce their rRNA production in extreme environments. Our results suggest in dry climates microorganisms' diversity patterns may not be elucidated by analysis of the active communities are not as robust as those drawn from total communities.

ADAPTATION OF *BACILLUS SUBTILIS* TO DAIRY-ASSOCIATED ENVIRONMENTS

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Bacillus species present a major concern in the milk industry as they have the capability of forming spores which withstand pasteurization processes. Contamination of milk by these bacteria leads not only to a financial loss due to milk spoilage, but also is a health hazard, since some strains are highly pathogenic to human. Therefore, it is of highest importance to understand how *Bacillus* species survive during milk production and processing in the dairy industry. Using a reductionist approach in which we focused on the model organism *B. subtilis*, we discovered an adaptation mechanism that enables this bacterium to survive in dairy-associated environments. We further report that this adaptation strategy is highly conserved amongst different *Bacillus* species.

A BACTERIAL REPORTER PANEL FOR THE DETECTION AND CLASSIFICATION OF ANTIBIOTIC SUBSTANCES

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The ever-growing use of pharmaceutical compounds, including antibacterial substances, poses a substantial pollution load on the environment. Such compounds can compromise water quality, contaminate soils, livestock and crops, enhance resistance of microorganisms to antibiotic substances, and hamper human health. We report the construction of a novel panel of genetically engineered *Escherichia coli* reporter strains for the detection and classification of antibiotic substances. Each of these strains harbours a plasmid that carries a fusion of a selected gene promoter to bioluminescence (*luxCDABE*) reporter genes and an alternative tryptophan auxotrophy-based non-antibiotic selection system. The bioreporter panel was tested for sensitivity and responsiveness to diverse antibiotic substances by monitoring bioluminescence as a function of time and of antibiotic concentrations. All of the tested antibiotics were detected by the panel, which displayed different response patterns for each substance. These unique responses were analysed by several algorithms that enabled clustering the compounds according to their functional properties, and allowed the classification of unknown antibiotic substances with a high degree of accuracy and confidence.

LEGIONELLA MONITORING IN A DRINKING WATER SYSTEM IN ISRAEL: UNDERSTANDING THE PATHOGEN'S ECOLOGY

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Legionella cause waterborne infections resulting in severe pneumonia. Our aim is to advance the current knowledge on the aquatic ecology of the bacteria in order to better understand its behavior as a pathogen. In this first part of the research, the water system at Oranim College (Tivon) was monitored for *Legionella* presence. Tap water and biofilms were analyzed. Results from two seasons (summer and autumn 2012) have been obtained. *Legionella* was detected in 64.2% of the water and biofilms samples. In water samples, legionella was found in concentrations between $1.2x10^2$ to $4.74x10^3$ cfu/l. The predominant species was *L. pneumophila* serogroup 1. However, in two sampling points *L. pneumophila* serogroup 3 and *L. sainthelensis* were identified. Heterotrophic total counts, temperature, pH, conductivity and free chlorine were also monitored. The heterotrophic total counts demonstrated a moderated bacterial population in the water system. No correlation was detected with pH and conductivity. Nevertheless *Legionella pneumophila* was detected in tap water samples just when total chlorine concentration was less than 0.1 ppm. *Legionella* virulence genes were detected in all the water samples, including the ones with undetectable *Legionella* counts. We conclude that the water system is completely infected. A risk assessment should be done in order to minimize future health problems. Three countries; Germany, Palestine and Israel are involved in this research. The different conditions for the growth and survival of *Legionella* populations in these two regions allow us to have the ideal scenario to do further research in the understanding of the ecology of the bacteria in waters.

INFECTION STRATEGIES IN PLANT AND HUMAN PATHOGENS: THE ROLE OF ANTI-APOPTOTIC MACHINERY

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Botrytis cinerea is a model system to study pathogenicity of necrotrophic fungi. As inferred by the term "necrotrophic", such fungi kill the cells and then obtain nutrients from the dead tissue. Hence, the most critical step in necrotrophic development is fast killing of the host cells. *B. cinerea* promotes apoptotic cell death in infected plants, which facilitates lesion spreading. How the fungus survives the first encounter with living plant tissue remained unclear. We found that hyphae of *B. cinerea* undergo massive PCD during early stages of infection, but fully recover upon transition to second phase of infection. Further studies using the fungal and plant mutants showed that virulence was fully correlated with ability of the fungus to cope with plant-induced PCD. A similar phenomenon was obtained with another necrotrophic plant pathogen, but not with two hemi-biotrophic pathogens, which have an early biotrophic phase. These results imply that the anti-PCD machinery is essential for disease in necrotrophic plant pathogens. In order to extend these finding to other systems, we generated transgenic strains of the human pathogen *Aspergillus fumigatus.* This fungus shares common stages of infection with *B. cinerea* and hence might also need the anti-PCD machinery for infection. Indeed, when conidia of *A. fumigatus* are inoculated on a sensitive tissue hyphae undergo massive cell death, and similar to *B. cinerea* they fully recover later. Collectively, our results support a general role for anti-PCD machinery in pathogenicity of a large group of fungi, which include both plant and human pathogen.

THE MOLECULAR INTERACTIONS BETWEEN *CLAVIBACTER MICHIGANENSIS* SUBSP. *MICHIGANENSIS* AND TOMATO

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Clavibacter michiganensis subsp. *michiganensis* (*Cmm*) is a gram-positive actinomycete, causing bacterial wilt and canker disease in tomato. The genome of *Cmm* strain NCPPB382 consists of a circular chromosome (3.3 Mb) with high G+C content (72.6%) and two circular plasmids, pCM1 and pCM2 harboring virulence genes,*celA* and *pat-1,* respectively. The loss of either plasmid reduced virulence, while curing the bacterium of both plasmids results in a non-virulent endophytic strain. A chromosomal pathogenicity island (*chp/tomA* PAI) of 129 kb with a low G+C content, harboring several serine proteases, was shown to be necessary for pathogenicity and colonization. Colonization patterns of a GFP labeled *Cmm* strains revealed that the pathogen extensively colonizes the lumen of xylem vessels, preferentially attaches to the spiral secondary wall. Acropetal movement of *Cmm* in tomato resulted in an extensive systemic colonization of the whole plant, while strains (lacking the plasmids or the PAI) remained confined to the area surrounding the inoculation site. Transcriptional analysis revealed that *celA* and *pat-1* were significantly induced in *Cmm* at initial 12-72 h, whereas the serine proteases *chpC* and *ppaA*, residing on the PAI, were highly expressed at 96 h after inoculation. Chromosomal genes involved in cell wall degradation (i.e., *pelA1*, *celB*, *xysA* and *xysB*), was also induced at early stages of infection. Different pattern of pathogenicity and gene expression was observed during blisters formation on tomato leaves.These findings suggest that virulence factors located on the *chp/tomA* PAI or the plasmids are required for effective movement, colonization and symptoms development in tomato.

"CHEMICAL ARMS RACE AT SEA": HOW MARINE VIRUSES MANIPULATE THEIR HOST METABOLISM DURING ALGAL BLOOMS IN THE OCEAN

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Marine viruses are major evolutionary and biogeochemical drivers in marine microbial foodwebs. *Emiliania huxleyi* is a globally important coccolithophore forming massive algal blooms in the North Atlantic Ocean that are routinely infected and terminated by coccolithoviruses (EhVs). Genome analysis of EhVs revealed an unexpected cluster of putative sphingolipid biosynthetic genes, a pathway never before described in a viral genome. We demonstrate how lytic viral infection appears to manipulate host sphingolipid metabolism and regulate replication cycle via activation of programmed cell death (PCD) biochemical machinery. Although the origin of PCD in unicellular organisms is still unclear, its functional conservation among phylogenetically diverse lineages suggests key evolutionary and ecological drivers in aquatic environments. To dissect and elucidate these pathways, we established the use of a single host, *E. huxleyi*, infected by either lytic virus or non-lytic virus, all of which have available genomic information. This experimental setup is an ideal system for comparative "omics" that will allow deciphering of PCD machinery and viral genes essential for of lytic infection. We performed a large scale experiment in which we assessed various physiological and biochemical stress markers, and preformed deep RNA sequencing, whole lipidome analysis and detailed electron microscopy examinations. We highlight the importance of redox signaling and lipid metabolism in determining host susceptibility to viral infection and its role in viral replication. We underscore the pivotal role of a co-evolutionary "arms race" of host-virus life cycle strategies in mediating their interactions in the marine environment.

THE PHYLLOSPHERE: A PHOTOTROPHIC NICHE

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To date, microbial rhodopsins have been found exclusively in diverse aquatic habitats. We tested the hypothesis that microbial rhodopsins as well as BChl-based reaction centers also exist and play an important role in terrestrial niches, in particular on plant leaf surfaces (the phyllosphere). A mode of phototrophy that is compatible with the plant's photosynthesis would offer a significant ecological advantage to microbes inhabiting this environment. Based on 454-pyrosequencing-generated data, we report on the existence of genes encoding microbial rhodopsins in all five phyllosphere metagenomes that were tested: tamarisk (*Tamarix nilotica*), soybean (*Glycine max*), Arabidopsis (*Arabidopsis thaliana*), clover (*Trifolium repens*) and rice (*Oryza sativa*). Most sequences were formed distinct clades of both sensory and proton pumping rhodopsins. Interestingly, all reads contained a Leucine residue at position 105 of the translated sequence, suggesting green light absorption, thus avoiding competition with the plant's chlorophyll. Further analysis of the phyllosphere metagenomes revealed the presence of a diverse community of anoxygenic phototrophic bacteria, including the previously reported *methylobacteria*, as well as other known and unknown phototrophs. The presence of anoxygenic phototrophic bacteria was also confirmed *in situ* by infrared epifluorescence microscopy, confirming both the presence and high abundance of these microorganisms in the phyllosphere. Our findings, for the first time describing microbial rhodopsins from non-aquatic habitats along with BChl-based reaction centres, point towards the potential coexistence of microbial rhodopsin-based and anoxygenic phototrophy as well as the plant chlorophyll-based photosynthesis, with the different pigments absorbing non-overlapping fractions of the light spectrum.

COMPARATIVE ANALYSES OF TYPE III-SECRETED EFFECTORS AMONG STRAINS OF *ACIDOVORAX CITRULLI*

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Bacterial fruit blotch (BFB) is a devastating disease of cucurbits, caused by the Gram-negative bacterium *Acidovorax citrulli*. Despite the economic importance of BFB, relatively little is known about basic aspects of the disease. According to host range, biochemical and genetic features, two distinct groups exist within this species: group I includes strains that were mainly isolated from non-watermelon hosts, while group II includes typical watermelon strains. As many Gram-negative plant pathogenic bacteria, *A. citrulli* requires a functional type III secretion system (T3SS) for pathogenicity. The T3SS injects effector proteins directly into the cytosol of the plant cells, which collectively allows the pathogen colonizing the host tissue and manipulating the host cellular activities to its own benefit. The aim of this study was to assess the diversity of eleven putative type III-secreted (T3S) effectors of *A. citrulli*. We cloned and sequenced the effector genes from several *A. citrulli* strains isolated from different host plants and geographic locations. Comparative analyses revealed that T3S effector genes cluster according to the group I/II classification. However, our analysis, together with additional experimental evidence, supports the existence of at least a third group of *A. citrulli*, represented in our study by strains ZUM 4000 and ZUM 4001. In addition we show preliminary data supporting the contribution of one of the effectors, Aave_1548, to virulence of this pathogen.

THE 'OBLIGATE DIPLOID' *CANDIDA ALBICANS* FORMS MATING-COMPETENT HAPLOIDS

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Candida albicans, the most prevalent human fungal pathogen, was considered an obligate diploid that carried recessive lethal mutations throughout the genome. Here, we demonstrate that *C. albicans* has a viable haploid state that can be derived from diploid cells under *in vitro* and *in vivo* conditions and appears to arise via a concerted chromosome loss mechanism. Haploids undergo morphogenetic changes like those of diploids including the yeast-hyphal transition, chlamydospore formation, and a white-opaque switch that facilitates mating. Haploid opaque cells of opposite mating type mate efficiently to regenerate the diploid form, restoring heterozygosity and fitness. Homozygous diploids arise spontaneously by auto-diploidization and both haploids and auto-diploids display a similar reduction in fitness, *in vitro* and *in vivo,* relative to heterozygous diploids, suggesting that homozygous cell types are transient in mixed populations. Finally, we constructed stable haploid strains with multiple auxotrophies that will facilitate molecular and genetic analyses of this important pathogen.

INTERACTION BETWEEN PHENOLIC AND OXIDANT SIGNALING IN *COCHLIOBOLUS HETEROSTROPHUS*

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The transcription factor ChAP1 is an ortholog of yeast YAP1 in the maize pathogen *Cochliobolus heterostrophus*. ChAP1 migrates to the nucleus upon exposure to oxidative stress, inducing antioxidant genes [1]. ChAP1 also localizes to nuclei on contact with the host leaf and during invasive growth. One of the signals responsible is provided by phenolic compounds [1-3]. Using a genetically-encoded ratiometric reporter of intracellular redox state, we showed that maize leaf extract and phenolics, despite their antioxidant properties, promote nuclear accumulation of ChAP1. To study this dual role of ChAP1 we identified genes expressed in response to phenolics. The intradiol dioxygenase *CCHD1* is rapidly upregulated, independent of ChAP1 [2]. Coumaric acid caused rapid upregulation of most beta-ketoadipate pathway genes. A *cchd1* mutant provided genetic evidence that protocatechuic acid is an intermediate in catabolism of many aromatic acids [3]. To identify additional phenolic-induced genes, microarrays were designed from the predicted coding sequences of *C. heterostrophus* [4]. Expression of about 90 genes, many from the beta-ketoadipate, quinic acid and shikimic acid pathways, and transporter genes, was altered by coumaric acid. The ability to respond to phenolics and detoxify or metabolize them may confer an advantage to plant pathogens. [1] Lev et al. (2005) Eukaryot. Cell 4:443-454; [2] Shanmugam et al. (2010) Cell. Microbiol. 12:1421-1434; [3] Shalaby et al. (2012) MPMI 25: 931-940; [4] Ohm et al. (2012) PLoS Pathog 8: e1003037. Supported in part by the Israel Science Foundation. We thank Michal Levin and Itai Yanai for help with microarray hybridization.

FORMATION AND DISSOCIATION OF PROTEASOME STORAGE GRANULES ARE REGULATED BY CYTOSOLIC pH

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The 26S proteasome is the major protein degradation machinery of the cell and is therefore regulated at many levels. One mode of regulation is to accumulate the proteasomes in proteasome storage granules (PSGs) upon glucose depletion. Despite the importance of this mode of regulation, the pathways that mediate partitioning of the proteasome into this subcellular structure are unknown. We devised a systematic robotic screening approach in yeast to identify trans-acting proteins that regulate the accumulation of proteasomes in PSGs. Our data was enriched for subunits of the vacuolar adenosine triphosphatase (V-ATPase) complex, a proton pump required for the acidification of the vacuole. We show that the impaired ability of V-ATPase mutants to properly govern their intracellular pH (pHi), affects the kinetics of PSGs formation. Further characterization identified that the drop of the pHi alone triggers the formation of other protein aggregates that form upon carbon depletion. While it is well established that carbon source availability regulates PSGs formation, we provide evidence, for the first time, that cytosolic pH is the cellular signal for the glucose sensing that mediates PSGs formation. Moreover, we demonstrate that this is a much more general mechanism for signaling carbon source exhaustion.

LOCALIZED TRANSLATION NEAR THE MITOCHONDRIA: CHARACTERIZING A NOVEL RIBOSOMES' RECEPTOR

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Most of mitochondrial proteins are synthesized by cytosolic ribosomes and imported into the mitochondria. Translationally active ribosomes and many mRNAs were shown to be associated with the mitochondria outer membrane, consistent with localized translation near this organelle. Yet, the factors that coordinate this association are largely unknown. A complex that was previously shown to be important for association is the Nascent-chain Associated Complex (NAC). This conserved heterodimeric complex is associated with the ribosome and interacts with nascent chains. NAC deletion lowers ribosomes' association with mitochondria. Importantly, NAC was shown in vitro to stimulate cotranslational import to mitochondria. This role was shown to be dependent on a yet unknown protein receptor on the mitochondria outer membrane. Yet, the mitochondrial receptor of this complex is unknown. Our preliminary data indicate that a mitochondria outer membrane protein of an unknown function (OM14) is a putative NAC receptor. We found that OM14 physically interacts with NAC, its deletion reduces NAC association with mitochondria and affects mRNA association. Moreover, preliminary in vitro assays suggest that OM14 deletion also affect protein import to mitochondria. Taken together, these results reveal that targeting of translating ribosomes to the mitochondria is mediated through interaction between the ribosome-associated complex NAC and the mitochondria receptor OM14. These results imply that proper targeting of mRNA to the mitochondria starts as soon as the nascent chain emerges out of the ribosome.

A MICROSCOPY SCREEN FOR INCLUSION-BODY RESIDENT PROTEINS REVEALS AN UNEXPECTED LINK BETWEEN PROTEIN QUALITY CONTROL AND LIPID DROPLETS

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Protein quality control (PQC) machinery is responsible for clearing the cytosol of misfolded proteins by utilizing chaperones and proteasomes. However, under stress, misfolded proteins accumulate beyond the capacity of the PQC components and aggregates start to form. Due to the toxic effects of these aggregates in the cytosol, they are actively trafficked into inclusion bodies. The mechanisms that govern processing and clearance of proteins inside inclusion bodies are yet poorly characterized. To uncover novel proteins that might function inside inclusion bodies we undertook a co-localization screen in which we visualized every yeast protein tagged with GFP under conditions where inclusion bodies were formed and stained in Cherry. This screen led to the identification of 13 proteins, among which was an uncharacterized protein: Iml2. In Δiml2 strains we could observe a striking effect on PQC efficiency under stress. To uncover the function of Iml2 we mapped its physical interactions under stress and found that it binds to lipid droplet proteins. Since lipid droplets are the cell's lipid storage compartment we wondered whether they could play a role in PQC. Indeed a mutant strain lacking lipid droplets showed a dramatic reduction in PQC efficiency. This work demonstrates a novel role for Iml2 and lipid droplets in the PQC machinery.

A NOVEL MECHANISM FOR GENOME REMODELING: DECONDENSATION OF THE MITOCHONDRIAL NUCLEOID OF TRYPANOSOMES

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Kinetoplast DNA (kDNA), the mitochondrial genome of trypanosomatids, consists of several thousand DNA minicircles and few dozen maxicircles, linked topologically into a DNA catenane. Replication of kDNA minicircles initiates at a conserved sequence, which is recognized by the replication initiator protein UMSBP. In addition to functioning as kDNA replication initiator, UMSBP functions in post-replication processes, such as segregation of the flagellar basal bodies and the kDNA network. It has long been known that kDNA is condensed in the mitochondrial matrix into a disc-shape nucleoid and that a set of mitochondrial histone H1-like proteins function in this process. However, the mechanism that mediates the remodeling of kDNA, promoting its accessibility to the replication machinery, has not been known. The pre-replication requirement for kDNA remodeling and the mechanisms that may function in this process were challenged here, revealing that specific protein-protein interactions between the replication initiator UMSBP and mitochondrial histone-like proteins result in the decondensation of kDNA. Consequently, the kDNA network rendered accessible to decatenation by topoisomerase, releasing free kDNA minicircle monomers, the necessary templates for kDNA replication. We suggest that remodeling is a pre-requisite for kDNA replication and that UMSBP-mediated decondensation of kDNA activates the pre-replication release of minicircles from the network, a key step in kDNA replication, which precedes and enables its replication initiation. These observations demonstrate a case where DNA remodeling is mediated via specific protein-protein interactions between the histone-type DNA-condensing proteins and the replication initiator protein, rather than through the conventional mechanism of their posttranslational covalent modification.

STRESS GRANULE FORMATION IN *ENTAMOEBA HISTOLYTICA*: CROSS TALK BETWEEN EHMLBP, EHRLE3 REVERSE TRANSCRIPTASE AND POLYUBIQUITINATED PROTEINS

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EhMLBP is an *Entamoeba histolytica* protein that binds to methylated repetitive DNA and is a positive regulator of a reverse transcriptase of a long interspersed nucleotide element. This protein protects trophozoites against heat shock by reducing protein aggregation. The presence of EhMLBP and polyubiquitinated proteins in heat shock-induced protein aggregates raised the question whether these proteins interact. Using a docking method, an interaction between the heat shock domain of EhMLBP and a polyubiquitin chain was predicted and experimentally confirmed. Ubiquitinated proteins were located in the perinuclear region of non-stressed trophozoites. In contrast, ubiquitinated proteins were located in the perinuclear region and together with EhMLBP in cytoplasmic vesicles in heat-shocked trophozoites. We proposed that these vesicles are stress granules based on the fact that their formation was triggered by heat shock or sodium arsenate and prevented by cycloheximide, *a* drug that inhibits the formation of *stress granule*s. We also observed that over-expression of the reverse transcriptase of the long interspersed element 3 induced the up-regulation of EhMLBP expression and the formation of cytoplasmic vesicles that closely resemble stress granules. Our data support the notion that polyubiquitinated proteins-containing stress granules are formed in heat-shocked *E. histolytica* and suggest that the formation of these granules is dependent upon EhMLBP and a long interspersed element.

NOVEL SIGNALLING PATHWAY THAT SENSES LACK OF EXTERNAL ARGININE ACTIVATES SYNTHESIS OF ITS TRANSPORTER BY INCREASING mRNA STABILITY IN *LEISHMANIA*

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Arginine is an essential amino acid for *Leishmania* but not its host. It is the sole precursor for polyamine biosynthesis in this organism, a process that initiates inside a unique organelle called glycosome. Previously, our laboratory cloned a high affinity arginine transporter, LdAAP3 that translocate only arginine in *Leishmania donovani* promastigotes (the extracellular form) and amastigotes (the intracellular form). Amino acid availability and mutation that disconnect arginine from the polyamine synthesis pathway affect LdAAP3 activity. Here we further characterized *Leishmania* response to arginine availability. Indirect immunofluorescence and cellular fractionation of *Leishmania* cells indicated that LdAAP3 localizes to the plasma, flagella and glycosome membranes. Starving both promastigotes and amastigotes to arginine induced rapid and concomitant increase LdAAP3 abundance and arginine transport. Addition of arginine to starved cells caused rapid proteasome-dependent degradation of LdAAP3 to its normal level. Our analysis indicate that *Leishmania* cells sense external arginine availability that turns on a signaling pathway that induce LdAAP3 mRNA and protein abundance increase within minutes. In addition, starvation to arginine induced more than 2-fold increase in the abundance of a dozen other proteins including an ion transporter, metabolic enzymes and several proteins with yet unknown function. This is the first report on a starvation signaling pathway in *Leishmania* parasites.

INSULATOR-LIKE PAIRING ELEMENTS REGULATE SILENCING AND MUTUALLY EXCLUSIVE EXPRESSION OF *VAR* GENES IN *PLASMODIUM FALCIPARUM*

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Plasmodium falciparum causes the deadliest form of human malaria. Its virulence is attributed to its ability to modify the iRBC and to evade human immune attack through antigenic variation. Antigenic variation is achieved through tight regulation of antigenic switches between variable surface antigens named PfEMP1. PfEMP1s are encoded by the *var* multi-copy gene family and individual parasite expresses only a single *var* gene at a time, maintaining the remaining *var* genes in a transcriptionally silent state. Strict pairing between the *var* promoters and the promoter within each intron is required for silencing and counting of a *var* gene by the mechanism that control mutually exclusive expression. Using a series of genetic experiments on artificial *var* genes we have identified insulator-like DNA elements that are required for pairing between the *var* promoter and the intron and thus are essential for both silencing and mutually exlusive expression. We also demonstrate that these elements, found in the regulatory regions of each *var* genes specifically bind an unknown protein. We propose a model by which silencing and mutually exclusive expression of *var* genes is regulated by insulator-like DNA pairing elements.

DIRECT EVIDENCE FOR THE ADAPTIVE ROLE OF COPY NUMBER VARIATION ON ANTIFOLATE SUSCEPTIBILITY IN MALARIA PARASITES

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The rapid emergence of resistance to anti-microbials poses an increasingly difficult problem for the treatment of all infectious diseases. In the case of malaria, widespread use of the antifolate compounds pyrimethamine and sulfadoxine increased resistance rates and led to the subsequent discontinuation of antifolates as first line treatments for malaria.GTP-cyclohydrolase (*gch1*), the first enzyme in the folate pathway, exhibits extensive copy number (CN) variation in parasite isolates from areas with a history of longstanding antifolate use. Increased CN of *gch1* is associated with a greater number of point mutations in enzymes targeted by the antifolates pyrimethamine and sulfadoxine. While these observations suggest that increases in *gch1* CN are an adaptation to drug pressure, changes in CN have not been experimentally demonstrated to directly alter drug susceptibility. To determine if changes in *gch1* expression alone modify pyrimethamine sensitivity, we manipulated *gch1* CN in several parasite lines to test the effect on drug sensitivity. We report that increases in *gch1* CN alter pyrimethamine resistance in most parasites lines. However, we find evidence of a detrimental effect of very high levels of *gch1* overexpression in parasite lines with high endogenous levels of *gch1* expression, revealing the importance of maintaining balance in the folate pathway and implicating changes in *gch1* expression in preserving proper metabolic flux. This work expands our understanding of parasite adaptation to drug pressure and provides a possible mechanism for how specific mutations become fixed within parasite populations.

THE MECHANISM OF SPLICED LEADER SILENCING IN TRYPANOSOMES

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Trypanosomes lack conventional transcription regulation, and their genes are transcribed in polycistronic units that are processed by *trans*-splicing and polyadenylation. In *trans*-splicing, which is essential for processing of each mRNA, an exon the spliced leader (SL) is added to all mRNAs from a small RNA, the SL RNA. We demonstrated that under severe stress such as blocking translocation of proteins to the ER, or treatments with drugs that induce unfolded protein response in eukaryotes, the parasites elicit a mechanism we termed spliced leader silencing (SLS) pathway. In SLS, the transcription of the SL RNA gene is extinguished, and tSNAP42, a specific SL RNA transcription factor, fails to bind to its cognate promoter Lustig et al., *EMBO reports*, 2009).SLS leads to complete shut-off of *trans*-splicing. and induces apoptosis (Goldshmidt et al*., PLoS pathpgens*, 2010). Here we show that during SLS a serine/threonine kinase PK-3 is activated, the kinase translocates to the nucleus where it phosphorylates the TATA binding protein leading to dissociation of the entire transcription complex, leading to the shut-off of SL RNA transcription. RNAi of PK-3 abolished the induction of SLS. iTRAQ analysis led to identification of factors essential for executing SLS including a protease that is essential for activating the programmed cell death induced by SLS.

THE TRANSIENT ROLE OF CHROMOSOMAL DUPLICATIONS IN STRESS ADAPTATION

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We conducted a set of lab-evolution experiments in yeast under diverse stress conditions and found that aneuploidy, an abnormal number of chromosomes, was often used as an adaptive tool to cope with the applied stresses. However, after longer evolution periods, still under the stress, the aneuploidy was eliminated and replaced by more efficient solutions at the individual gene level. Furthermore, when the same stress was applied in a gradual manner during evolution, alternative solutions have emerged but not aneuploidy. Our findings indicate that chromosomal duplications are a first evolutionary means to retain survivability under strong and abrupt selective pressures, yet it merely serves as a crude “temporary-fix” while more refined and sustainable solutions take over. Thus, in the perspective of genome evolution trajectory, we suggest that aneuploidy is a crucial, yet short-lived intermediate that facilitates adaptation.

PROMOTER SWITCHING FACILITATES MASSIVE REGULATORY REWIRING IN BACTERIA

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Bacterial plasticity, which enables remarkably rapid adaptation to the environment, is traditionally thought to be driven by rampant changes in protein repertoire. Core genes, which are ubiquitous and show extreme sequence conservation, are believed not to contribute to interspecies phenotypic variability. Here we challenge this widely held dogma, by showing that the regulatory sequences adjacent to core genes are frequently exchanged. Through the analysis of 270,000 regulatory regions across 247 bacterial genomes, we find that regulatory 'switching' is common and occurs across the bacterial domain, even in the highly clonal *Mycobacterium tuberculosis*. Such switching events can replace two or more non-homologous regulatory regions that contain distinct sets of transcription factor binding sites, thus leading to quantum leaps between regulatory architectures. We further show that horizontal regulatory transfer (HRT) is one mechanism responsible for this ubiquitous regulatory switching and that it impacts more than half of all core genes in *Escherichia coli*. We find that HRT enables regulatory regions to move independently of the genes they regulate, transferring across strains and even genera. Our results reveal a highly dynamic bacterial regulatory network, opening a new widow for exploring the role regulatory divergence plays in the emergence of clinically and ecological traits.

GOING A FEW PHENOTYPIC STEPS BACKWARDS BY TAKING A FEW GENOTYPIC STEPS FORWARD: CONTINUOUS EVOLUTION OF *PROCHLOROCOCCUS* STRAINS RESISTANT TO PHAGE

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Prochlorococcus cyanobacteria are extremely abundant in the oceans as are the viruses that infect them. How hosts and viruses coexist in nature remains unclear, although resistance to viral infection accompanied by a fitness cost, often manifested by a reduction in growth rate, may enable this coexistence. However, compensatory mutations can substantially reduce the extent of the growth rate cost. This raises the question as to the role of the growth rate cost in the environment. To address this, we studied the evolution of resistant strains with a significant growth rate cost that were allowed to evolve for over 350 generations. The growth rate, resistance range and sequence of the evolved strains were compared to those of the original resistant strains. Most of the strains showed significantly improved growth rates over time. Moreover, the range of resistance changed, generally toward a reduced range. These phenotypic changes, in most of the strains, were not due to the reversion to the wild type codon, but rather due to additional mutations. These results suggest that on short evolutionary scales metabolic costs play an important role in population maintenance, whereas on long evolutionary scales this impact declines. Furthermore, they suggest that resistant strains keep evolving, and over time, they become more similar to the phenotype of their ancestral strain through new mutations rather than by reverting to the original genotype. Through this evolutionary path, phages continue to influence the appearance of new genotypic and phenotypic compositions and enhance the diversity of *Prochlorococcus* in the oceans.

CONTRIBUTION OF LATERAL GENE TRANSFER TO THE ADAPTATION OF *METHANOSPHAERA STADTMANAE* TO THE MAMMALIAN GUT

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Methanosphaera stadtmanae is a commensal methanogenic archaeon found in the human gut, a niche predominantly colonized by bacteria. It is therefore expected that lateral gene transfer (LGT) from bacteria to archaea will play a role in the evolutionary history of this organism, contributing to its adaptation to its human host. Notably, a recent analysis of *M. stadtmanae*'s closest phylogenetic neighbor, *Methanobrevibacter smithii*, which inhabits the same niche, revealed evidence of extensive LGT between this organism and bacteria. We therefore performed a phylogenomic survey of putative LGT events in *M. stadtmanae*, using a phylogenetic pipeline, and creating phylogenetic trees using both distance-based and likelihood-based methods. Our analysis indicates that a substantial fraction of the proteins of *M. stadtmanae* (about 15%) are inferred to have been involved in inter-domain LGT. Laterally acquired genes have had a large contribution to surface functions, by providing novel glycosyltransferase functions. In addition, several ABC transporters seem to be of bacterial origin, including the molybdate transporter. Thus, bacterial genes contributed to the adaptation of *M. stadtmanae* to a host-dependent lifestyle by allowing a larger variation in surface structures and increasing transport efficiency in the gut niche which is diverse and competitive.

WHY DOES THE ENTERIC PATHOGEN *SALMONELLA ENTERICA* SURVIVE IN PLANTS, AND HOW DOES IT DO IT?

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Fresh fruits and vegetables, and in particular leafy greens and sprouts, have been increasingly recognized as significant sources of foodborne infections. Outbreaks linked to fresh produce have been caused by many pathogens, whereas *Salmonella enterica* is among the most common human pathogens associated with fresh produce. Contamination of fresh produce occurs along the food production chain "from farm to fork", and *Salmonella* is able to survive in/on plants such as parsley, basil and tobacco pre- or post-harvest for more than a month without causing symptoms. Symptoms are not observed even when high levels (>10^7 CFU/gr) exist on/in the plants for weeks. This is achieved by an active suppression of the plant response, a suppression that depends on the activity of several virulence factors such as the SPI-1 and SPI-2 T3SSs of *Salmonella*. Likewise, bacterial expression of stress response systems eliminates potential damage to the bacteria. Moreover, the pathogen has developed resistance to different antibacterial compounds in the plants essential oils. The ability of *Salmonella* to attach, survive and inhibit the plant immune response indicates that plant colonization is an active process, and that plants may serve as vectors or true hosts for this pathogen.

TRI-TROPHIC UNDERGROUND SYMBIOSIS BETWEEM A WEEVIL, BACTERIA AND A DESERT PLANT

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Habitants of arid ecosystems face challenging conditions, in which water and nitrogen are the most important limiting factors. Inventive adaptations by organisms occupying such habitats are essential for their survival. Here, we describe a unique tri-trophic symbiotic interaction between a plant (*Salsola inermis*), a beetle (*Conorhynchus pistor*), and bacteria (*Klebsiella pneumoniae*). *S. inermis* is an exceptional annual shrub that flourishes during the driest period of the year in the saline soils of the Negev desert. The unusual dry-season vitality of this plant is exploited by the weevil, which survives by living within a unique mud structure that it affixes to the plant's roots, thus benefiting from increased carbon, water and refuge from predators. Active nitrogen-fixing bacteria, harbored within the weevil's gut, mediate this interaction, by supplying nitrogen to the system, which promotes seed development in the plant, and consequently increase plant reproduction and distribution. Out of over 60,000 known weevils, this is the first documentation of a weevil living most of its life underground without harming plants. The unique tri-trophic symbiotic interaction described here signifies the important ecological role of desert plant roots and provides an example for a sustainable consortium of living organisms coping with the challenging desert environment.

INSECT-ASSOCIATED BACTERIA AS POSSIBLE CONTROL AGENTS AGAINST PHYTOPLASMA IN GRAPES

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Bacteria from the genus *Phytoplasma* pose a major threat to many agricultural crops including grapevine. The conventional application of chemical sprays for controlling phytoplasma and other phloem restricted pathogens is inefficient, and the role of endophytes in the induction of plant resistance against such pathogens has been suggested. We hypothesized that host plants and insect vectors harbor microbes that affect phytoplasma infection. The current study is focused on the bacterial community composition of the phytoplasma's insect vector, the planthopper *Hyalesthes obsoletus* (Hemiptera: Cixiidae), as a source for such potentially beneficial bacteria. Sequences of 16S rRNA gene obtained from the planthopper showed dominance of *Sulcia* (77.95% of sequences in the sample), *Wolbachia* (4.51%) and a bacterium which belongs to Enterobacteriaceae (16.89%), with the remaining 0.65% of the sequences belonging to Actinobacteria, Alpha, Beta and Gamma-proteobacteria. Subsequently, different bacteria were isolated from the insect on CV agar. One of these isolates belonged to the bacterial family Xanthomonadaceae, and its 16S rRNA sequence resembled one of the rare bacterial taxa found by the mass sequencing analysis. This isolate was introduced to healthy and phytoplasma-infected grapevine-plantlets by submerging the plants roots in the isolate culture for 24h pre-planting. The presence of the isolate in plant tissues was confirmed by specific PCR three weeks post inoculation. The effect of phytoplasma on plant morphology was markedly reduced in the presence of the isolate. Further study is needed to examine the potential use of this isolate as a bio-control agent against phytoplasma.

VIRULENCE GENE PROFILING AND PATHOGENICITY CHARACTERIZATION OF NON-TYPHOIDAL *SALMONELLA* ACCOUNTED FOR INVASIVE DISEASE IN HUMANS

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Human infection with non-typhoidal *Salmonella* serovars (NTS) infrequently causes invasive systemic disease and bacteremia. To understand better the nature of invasive NTS (iNTS), we studied the gene content and the pathogenicity of bacteremic strains from twelve different serovars. Comparative genomic hybridization using a *Salmonella enterica* microarray revealed a core of 3233 genes present in all of the iNTS strains, which include the *Salmonella* pathogenicity islands 1-5, 9, 13, 14; five fimbrial operons (*bcf, csg, stb, sth*, *sti*); three colonization factors *(misL, bapA, sinH*); and the invasion gene*, pagN*. In the iNTS variable genome, we identified 16 novel genomic islets; various NTS virulence factors; and six typhoid-associated virulence genes (*tcfA*, *cdtB*, *hlyE*, *taiA*, STY1413, STY1360), displaying a wider distribution among NTS than was previously known. Characterization of the bacteremic strains in C3H/HeN mice showed clear differences in disease manifestation. Previously unreported characterization of serovars Schwarzengrund, 9,12:l,v:-, Bredeney and Virchow in the mouse model showed low ability to elicit systemic disease, but a profound and elongated shedding of serovars Schwarzengrund, 9,12:l,v:- (as well as Enteritidis and Heidelberg) due to chronic infection of the mouse. Phenotypic comparison in macrophages and epithelial cell lines demonstrated a remarkable intra-serovar variation, but also showed that *S. Typhimurium* bacteremic strains tend to present lower intracellular growth than gastroenteritis isolates. Collectively, our data demonstrated a common core of virulence genes, which might be required for invasive salmonellosis, but also an impressive degree of genetic and phenotypic heterogeneity, highlighting the complexity of the invasive manifestation caused by NTS.

CHARACTERIZATION OF THE SECRETED EGG MASS DEGRADING FACTOR BY *AEROMONAS AQUARIORUM*

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Chironomid females lay egg masses containing hundreds of eggs embedded in a gelatinous matrix. The egg masses are a natural habitat for *V. cholerae* and *Aeromonas*. *V. cholerae* degrades the egg masses with the secreted Haemagglutinin protease. *Aeromonas* isolates also degrade chironomid egg masses. Our aim was to identify the egg masses degrading factor that is secreted by *A. aquariorum* strain Y3K1C15. Following the hypothesis that the degrading factor is a protease, secreted proteases were screened by the zymogram assay. We found that the egg mass degrading factor of *A. aquariorum* was not a protease. The egg masses degrading factor was purified by a mono-Q column. Several chitinases were identified from the column active fraction (fraction 9). Egg masses were strained by Congo-red dye and were degraded by a commercial chitinse, demonstrating that chitin is one of the egg masses components. While *A. aquariorum* secretes the chitinases constitutively, most of the *Aeromonas* isolates were not able to degrade the egg masses. Egg masses degradation was induced in those isolates by the addition of chitin to the bacterial growth media. Induction of the enzyme secretion in nature presumably occurs when the bacteria are attached to the egg mass habitat where chitin is abundant. We suggest that the egg mass degrading mechanism is more complex than previously thought. *V. cholerae* degrades the egg masses with the secreted Haemagglutinin protease*,* however*, V. cholerae* also secrete chitinases. More research is needed to understand the role of chitinases in *V. cholerae* egg masses degradation mechanism.

THE POST GENOMIC ERA IN THE DIAGNOSIS OF INFECTIOUS DISEASES; MASS SPECTROMETERY AND THE MALDI TOF TECHNOLOGY

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Until some 10 years ago we were convinced the future of bacterial identification lies with molecular biology: DNA extraction followed by sequencing and comparison to different data bases assigning a sequence to specific bacteria. This strategy still plays a major role in the identification and quantification of all those infectious agents incapable of growing on solid culture media: viruses, intracellular obligatory parasites such as Rickettsia, Q fever, Chlamydia and Mycoplasma to name just a few. Whatever the identification of microorganisms in the bacteriology laboratory is concerned, in 2006, a technology called MALDI TOF – Matrix Assisted Light Dissociation Ionization Time of Flight using a Mass Spectrometer (MS), existing since the mid nineteen eighties, has emerged, with a platform for the identification of bacteria within seconds. Basically the instrument uses laser radiation to ionize bacteria which are suspended in a cation enriched organic solvent. The so generated ions are accelerated in an electric field and enter an electric tube towards a detector. Small molecular weight ions migrate fast, while bigger ones migrate slower to create a highly specific ionic pattern for each and every bacterium, yeast and mold with outstanding accuracy. The database of one of the MS models includes the ionic profiles of 2500 bacterial yeast and molds species. The MALDI TOF technology and its application in the routine workflow of a clinical microbiology laboratory will be demonstrated and discussed.

A NETWORK OF CFEM CELL SURFACE PROTEINS DIRECTS HEME-IRON ACQUISITION IN *CANDIDA ALBICANS*

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Candida albicans, a normally harmless commensal organism, can cause life-threatening systemic infection among immunocompromised patients. The human host invests substantial efforts to withdraw iron from potential pathogens. To overcome this extreme iron limitation in the host, *C. albicans*, similar to many pathogenic micro-organisms, has evolved several mechanisms for iron scavenging. Hemoglobin is the largest iron pool in the human host. *C. albicans* evolved a mechanism for heme-iron scavenging. Rbt5 is an extracellular GPI-anchored protein of the CFEM family (defined by 8 identically-spaced cysteines) that was identified as a hemin- and hemoglobin receptor. Genetic analysis suggested a mechanism by which hemoglobin or hemin is bound by extracellular receptors, then internalized into the endosome, where it is stripped of its iron by the acidic pH, and the iron is finally transferred to the cytoplasm. However the initial part of the pathway, including the mechanism of transfer of the heme from the receptor to the endosome, remained less well understood. Here we genetically and biochemically analyzed Pga7, another CFEM protein, and found that: it binds directly to heme *in vitro,* is essential for heme-iron utilization *in vivo*, and contributes to pathogenicity in a mouse model of systemic infection. Although Pga7 and Rbt5 are both predicted to be GPI-anchored extracellular proteins, our data suggest a differential localization of these proteins on the cell envelope, consistent with a sequential transfer pathway of the heme from the medium through the cell wall to the endocytic pathway.

IDENTIFICATION OF NOVEL ANTIFUNGAL COMPOUNDS TARGETING THE FUNGAL CELL WALL AND CHARACTERIZATION OF THEIR MODE OF ACTION

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Over the last years, the number of disseminated fungal life-threatening infections among immune suppressed patients has increased significantly. However, the number of antifungal drugs remains low and unsatisfactory. Most of the antifungal drugs have severe adverse effects, negative interactions with other drugs and limited efficacy. Out of the four main groups of antifungal drugs, only the new echinocandins target the cell wall, a fungal-specific target. So that, there is an urgent need to develop additional and novel antifungal drugs, that specifically target the fungal cell wall. In this work we screened 40,000 small-molecular-weight drug-like compounds and identified compounds that inhibit growth of *Aspergillus* molds. These compounds were tested using a conditional protein kinase C (PKC)-expressing strain of *Aspergillus nidulans*. Unlike typical 'blind screens' performed by pharmaceutical companies, our screen takes advantage of a the *alcA-PKC* mutant, developed in our lab, which can be used to rapidly differentiate between antifungal cell-wall damaging compounds and those with 'non-specific' antifungal activity. Also, unlike screens which identify compounds against a single purified drug target, our approach simultaneously defines as targets all the gene products involved in cell-wall biosynthesis and maintenance in an intact live fungus. This greatly increases the chance of successfully identifying a selection of compounds with diverse and unique modes of action. The hit compounds yielded by the screen were tested for basic features such as cytotoxicity and range of activity among fungi. Following basic characterization, we are now focused on characterizing the mode of action of these compounds using genetic approaches.

CANDIDA ALBICANS ISOLATES FROM CANDIDEMIA AND SUPERFICIAL CANDIDIASIS: COMPARISON OF VIRULENCE ATTRIBUTES AND GENOTYPING

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Candida albicans causes mucocutaneous and systemic infections; the latter generally in immunocompromised patients. The aim of this study was to investigate whether *C. albicans* strains isolated from patients with systemic candidiasis differ from those isolated from patients with mucosal infection regarding virulence attributes and/or genotype. We tested 44 strains: 20 blood isolates from candidemia patients (S strains) and 24 from mucosal infections (M strains). Two standard strains served as controls. We assessed the following virulence characteristics using in vitro assays: **1**. Adhesion to BEC: showing that a larger percentage (30%) of S strains had higher adherence values than the controls vs. 16% of M strains; **2**. Biofilm formation: 80% of the S and 58% of the M isolates had higher biofilm formation; **3**. Evaluation of phospholipase activity showed that 55% of S and 12.5 % of M strains had increased activity; **4.** Evaluation of SAP activity revealed low activity in both groups (S-10%, M- 4%). We also have preliminary data from in vivo experiments. Genotyping of the strains was performed using MLST. The MLST analysis showed that the most prevalent clonal complex found was 124 for both groups. It was also noted that the clonal complex 918 was only found among the S strains while clonal complex 69 was more prevalent among the M isolates. In summary: although a higher number of isolates should be analyzed to draw clear conclusions, it can be noted that more strains from systemic infection have a tendency to a more pronounced expression of characteristics associated with virulence.

RELEASE OF *PLEUROTUS OSTREATUS* VERSATILE-PEROXIDASE FROM Mn^{2+} REPRESSION ENHANCES ANTHROPOGENIC AND NATURAL SUBSTRATE DEGRADATION

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The versatile-peroxidase (VP) encoded by *mnp4* is one of the nine members of the manganese-peroxidase (MnP) gene family that constitutes part of the ligninolytic system of the white-rot basidiomycete *Pleurotus ostreatus* (oyster mushroom). VP enzymes exhibit dual activity on a wide range of substrates. As Mn^{2+} supplement to *P. ostreatus* cultures results in enhanced degradation of recalcitrant compounds and lignin, we examined the effect of Mn^{2+} on the expression profile of the MnP gene family. In strain PC9, *mnp4* was found to be the predominantly expressed *mnp* in Mn^{2+}-deficient media, whereas strongly repressed in Mn^{2+}-supplemented media, having negligible activity. We tested whether release of *mnp4* from Mn^{2+} repression alters the activity of the ligninolytic system. A transformant over-expressing *mnp4* (OE*mnp4*) under the *β-tubulin* promoter was produced. Now, despite the presence of Mn^{2+} in the medium, OE*mnp4* produced *mnp4* transcript as well as VP activity as early as 4 days after inoculation. The level of expression was constant throughout 10 days of incubation and the activity was comparable to that of PC9 in Mn^{2+}-deficient media. *In-vivo* decolorization of the azo dyes Orange II, Reactive Black 5 and Amaranth by OE*mnp4* preceded PC9. OE*mnp4*and PC9 were grown for 2 weeks under solid-state fermentation conditions on cotton stalks as a lignocellulosic substrate. [^{14}C]-lignin mineralization, *n-vitro* dry matter digestibility and neutral detergent fiber digestibility were found to be substantially higher in OE*mnp4*-fermented substrate, relative to PC9. We conclude that releasing Mn^{2+} suppression of VP4 by over-expression of *mnp4* improved *P. ostreatus* ligninolytic functionality.

THE HITCHHIKER'S GUIDE TO THE PHYLLOSPHERE - VOLATILE MEDIATED BACTERIAL INTERACTIONS

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For many years bacteria were considered to live solely as individual organisms, which strive to outcompete their neighboring siblings. However, this notion is no longer valid and bacterial colonies are now regarded as social communities, which integrate intercellular signals in order to coordinate gene expression for the benefit of the colony. One fascinating phenomenon that clearly projects such cooperative nature is swarming motility exhibited by several bacterial species. Although highly influential in many host-pathogen interactions and mechanistically simple, much remains unknown about this behavior. To date it is believed that the stimuli inducing the required cell differentiation for commencing swarming motility is the contact of cells with a surface. In the described study we show that environmental cues from neighboring bacterial colonies also provide a stimulus for differentiation and swarming. Moreover, we show that non-swarming bacteria from the genus *Xanthomonas* secrete such cues, attract swarming bacteria and "hitchhike" on their surfactants in order to migrate on solid surfaces. Notably, these cues induce differentiation of swimmer-to-swarmer cells even in liquid media. Chemical analyses of swarming-inducing cues produced by *Xanthomonas* spp. suggested them to be volatile methylketones. The described interaction shows that, in addition to intra-species signaling molecules, inter-species signaling molecules also have an important role in bacterial swarming. Additionally, such interactions serve as an example of interspecies cooperation and combination of skills, which most probably prevail in the heterogeneous bacterial consortiums common to natural environments.

MICROBE SPECIFIC BIOLOGICAL FILTRATION AT THE SUBMICRON RANGE

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The ocean is numerically dominated by < 2 micron and submicron size cells collectively known as picoplankton that play a key role in biogeochemical cycling and marine food webs. Marine suspension-feeding organisms must process large quantities of water in order to obtain microscopic food particles from highly diluted suspension. These biological filters handle thousands of particles simultaneously, including not only potential food particles, but also toxic, inert, and poorly digestible particles. For pumping suspension feeders, retention mechanism has been assumed to be strictly mechanical, based only on size. Flow cytometry analysis of samples collected *in situ* from the water inhaled and exhaled by undisturbed suspension feeder (ascidians, bivalves, and sponges) demonstrate large variations in filtration efficiencies of different picoplanktonic groups irrespective of the cells size. Using Pyrosequencing we examine the *in situ* microbial diet of the ascidian *Microcosmus exasperatus* at the phylotypes level. *M. exasperatus* efficiently filtered nearly 100% of the photosynthetic cyanobacteria *Prochlorococcus* and *Synechococcus* as well as some other bacterial phylotypes (e.g., Sphingobacteriales and Flavobacteriaceae). In contrast, similar sized bacteria species, most notably *Pelagibacter ubique*, the most abundant marine bacterium in the ocean, and other SAR11 phylotypes, were not removed. The study of species specific and size independent filtration should clarify predator-prey relationships at the basis of the marine food-web and may shade new light on the phenomenal success of SAR 11 clade in the ocean.

GRAZERS MAY SERVE AS IMPORTANT TRANSMISSION VECTORS FOR MARINE VIRUSES THAT REGULATE PHYTOPLANKTON BLOOMS

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Marine viruses infecting phytoplankton are a major biological and evolutionary force shaping communities structure, species evolution and driving nutrient cycling in the oceans. Little is known about how marine viruses propagate and induce the demise of algal blooms stretching over hundreds of kilometers in the ocean. Here we show that marine copepods, which are major grazers of *Emiliania huxleyi*, a cosmopolitan bloom forming coccolithophore, serve as key vectors carrying and spreading *E. huxleyi* viruses (EhV's) in the water column. Using PCR, we detected DNA of both the algal host and its specific virus in single copepods isolated during a bloom in the North Atlantic. These viruses were probably concentrated within infected *E. huxleyi* cells inside the copepods' intestine. Furthermore, we were able to isolate a new *E. huxleyi* virus from a copepod lysate and to demonstrate that these viral particles carried by copepods are indeed infectious to specific *E. huxleyi* lab strains. Typically copepods migrate vertically in the water column, covering vast areas and depths on a daily basis during which they consume phytoplankton cells. During these process copepods release fecal pellets potentially containing infected cells or infectious viral particles ingested at previous sites. We propose that marine copepods are important transmission agents accelerating the rate of viral dispersion within large microbial food webs, impacting viral-host interaction and, ultimately, the life span and fate of phytoplankton blooms.

EXPLORING THE BOVINE RUMEN BACTERIAL COMMUNITY FROM BIRTH TO ADULTHOOD

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The mammalian gut microbiota is essential in shaping many of its hosts' functional attributes. One such microbiota resides in the bovine digestive tract in a compartment termed the rumen. The rumen microbiota is necessary for the proper physiological development of the rumen and for the animal's ability to digest and convert plant mass into food products, making it highly significant to humans. The establishment of this microbial population and the changes occurring with the host's age are important for understanding this key microbial community. Despite its importance, little information about colonization of the microbial populations in newborn animals, and the gradual changes occurring thereafter, exists. Here, we characterized the overall bovine ruminal bacterial populations at five ages, from 1-day-old calves to 2-year-old cows. We describe the changes occurring in the rumen ecosystem after birth, reflected by a decline in aerobic and facultative anaerobic taxa and an increase in anaerobic ones. Some rumen bacteria essential for mature rumen function could be detected as early as 1 day after birth, long before the rumen is active or even before ingestion of plant material occurs. We observed an age dependent shift towards a mature-like bacterial configuration. Additionally, the diversity and within-group similarity increased with age, suggesting a more diverse but homogeneous and specific mature community, compared to the more heterogeneous and less diverse primary community. These findings have also been reported for human gut microbiota, suggesting that similar forces drive the establishment of gut microbiotas in these two distinct mammalian digestive systems.

A NEWLY ISOLATED GREEN ALGA FROM DESERT CRUSTS - AN UNUSUAL COMBINATION OF A UNIQUE SURVIVAL CAPABILITY AND RECORD HIGH GROWTH AND PHOTOSYNTHETIC RATES

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The challenges faced by organisms inhabiting extreme niches can help us to identify unique properties in those that can handle the stress. Desert Biological Sand Crusts (BSC) represent one of the harshest environments. Organisms inhabiting this ecosystem must cope with extreme temperatures from freezing during some winter nights up to 60^{o}C in the summer, excess light intensities and large changes in osmotic potential, from close to pure rainy water to salt crystals in the crust's upper layer. There are numerous examples whereby a superb ability to acclimate to a certain environmental condition is achieved at the cost of reduced performance under optimal conditions. We are clarifying the mechanisms whereby a small eukaryote green alga, *Chlorella* sp., isolated from BSC in the NW Negev desert, is able to cope with these harsh conditions in one hand and perform one of the fastest rates of photosynthesis and growth ever recorded, on the other, when maintained under optimal conditions. This unusual combination is particularly intriguing since this *Chlorella* sp. exhibits high resistance to photodamage even under extremely high light intensities such as 3000 µmole photons m^{-2} s^{-1}; no loss of oxygen evolution even after 2 h. The photosynthetic performance and certain structural aspects such as density of the thylakoids are strongly affected by the growth conditions; Sequencing of this *Chlorella* sp. is in progress. We shall present our view on the means by which this unique organism is able to exhibit such unique properties.

GETTING TO THE ROOT OF FLAVOBACTERIA: ECOLOGICAL, PHYSIOLOGICAL AND FUNCTIONAL CHARACTERISTICS OF A DOMINANT RHIZOSPHERE GENUS

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Members of the Gram-negative genus *Flavobacterium* inhabit a wide array of terrestrial and aquatic ecological niches. They appear to play a fundamental role in nature due to their enhanced capacity to degrade complex polymers such as chitin and cellulose. Flavobacteria possess a unique form of gliding motility tightly associated with a novel protein secretion system that is partially linked to their enhanced metabolic capacity. Our comprehensive analyses has determined that flavobacteria are extremely abundant (relative abundance sometimes exceeded 30% of the total defined genera) in the rhizosphere of greenhouse tomatoes and peppers. Prominence of flavobacteria in the rhizosphere, and as endophytes within roots, of agriculturally-relevant crops is supported by a wide range of previously cited studies, suggesting that this genus plays an important role in plant-microbe interactions. Furthermore, research conducted in our lab and other studies around the world indicate that certain members stimulate plant growth promotion and plant protection. Over the past three years, we have applied a holistic approach combining microbial ecology, physiology and genomic analyses, in order to enhance comprehension of flavobacterial-root interactions. This lecture will summarize our current understanding of flavobacterial community dynamics in the rhizosphere, the genomic evolution of soil flavobacterial strains as associated to terrestrial niche adaptation and the potential role of unique cellular mechanisms from these strains in rhizosphere competence and plant protection.

ANALYSIS OF THE MAGNETOTACTIC BACTERIA CATION DIFFUSION FACILITATOR, MamM

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Magnetosome is a subcellular organelle that consists of a linear-chain assembly of lipid vesicles. Each magnetosome is able to biomineralize and enclose a ~50-nm crystal of magnetite or greigite. Magnetosome allows magnetotactic bacteria, a diverse group of aquatic microorganisms, to orientate themselves along geomagnetic fields in search of suitable environments. MamM and MamB are abundant magnetosomal membrane proteins that share similarity to cation diffusion facilitator (CDF) proteins. Both proteins are multifunctional and presumed to have a role in iron transport and biomineralization stages during magnetosome formation. Recent *in vivo* studies have demonstrated MamM ability to form dimers as well as revealed its role in proper magnetite and not haematite crystal formation. Here we present the structural, biochemical and biophysical analysis of the cytosolic domain of MamM from *Magnetospirillum gryphiswaldense* (MSR-1). We discuss the protein unique structural features accompanied with in depth *in-vivo* characterization and suggest a new model for CDF ion transport.

LOW SPECIES BARRIERS IN HALOPHILIC ARCHAEA AND THE FORMATION OF RECOMBINANT HYBRIDS

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Speciation of sexually reproducing organisms requires reproductive barriers. Prokaryotes reproduce asexually, but often exchange DNA by lateral gene transfer mechanisms and recombination, yet distinct lineages are still observed. Thus, barriers to gene flow such as geographic isolation, genetic incompatibility or physiological obstacles (an inability to transfer the DNA from cell to cell) represent potential underlying mechanisms behind preferred exchange groups observed in prokaryotes. For Bacteria, the common view is that reduced gene flow is mostly the result of sequence divergence between species. Direct experimental evidence showed that sequence divergence impedes homologous recombination between bacterial species. However, in Archaea this question has not been addressed. Here we study interspecies gene exchange in halophilic archaea (*Halobacteriales*) that possess a parasexual mechanism of genetic exchange that is functional between species. In this process, which is arguably analogous to sexual reproduction, cells fuse forming a diploid state containing of the full genetic repertoire of both parental cells (plasmids and entire chromosomes) that facilitates genetic exchange and recombination. Later, cells separate (de-fuse) occasionally resulting in hybrids of the parental strains. We show high recombination frequencies between *Haloferax volcanii* and *Haloferax mediterranei*, two species that have an average nucleotide sequence identity of 86.6%. Whole genome sequencing of a *Haloferax* interspecies hybrids revealed the exchange of fragments ranging from 310kb to 530kb chromosomal sequence fragment. These results show that recombination barriers may be more permissive in halophilic archaea than they are in bacteria.

ARCHAEAL VIRUSES, PLASMIDS AND VIRUS-LIKE MEMBRANE VESICLES: INSIGHTS INTO THE ORIGIN AND NATURE OF VIRUSES AND RELATED ELEMENTS

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Archaeal viruses produce virions with unusual morphologies and their genomes encode proteins with few homologues in other viruses or cells. Recently, more surprises emerged from studying of archaeal viruses such as the discovery of a new egress mechanism for virion production (the formation of pyramidal structures that open for virion exit) or else the largest viral single-stranded DNA genome. The study of archaeal viruses and plasmids has also confirmed an intimate evolutionary connection between these two entities. Recently, we have found that plasmids and viral genomes can be found associated to membrane vesicles produced by archaea of the order Thermococcales and that membrane vesicles can be used for gene transfer between cells. The production of membrane vesicles is a universal process and an evolutionary connection has already been proposed between eukaryotic exosomes and enveloped viruses. I will discuss all these results in the framework of current debated about the origin and nature of viruses and related elements. I will argue that virus could be considered as living organisms if we focus on the infected cell (the virocell) instead to focus on the virion. I will also present a new hypothesis for the origin of viruses: the early escape hypothesis.

METAGENOME AND METATRANSCRIPTOME ANALYSES OF ROOT BACTERIAL COMMUNITIES

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This study describes plant species-specificity in root bacterial communities and transcriptional activity of dominant root microbial populations. Libraries of 16S rRNA gene and transcripts as well as metagenomes and metatranscriptomes were analyzed from soil and root samples of different plants. Results revealed distinct plant species-specific patterns of root colonization. Wheat and maize root communities were characterized by high dominance, particularly of *Pseudomonas stutzeri* (48% and 68% respectively). Cucumber and tomato roots were characterized by more even distribution among members, with relatively dominant *Cellvibrio* (cucumber) and Burkholderiales (tomato). High-stringency mapping of metatranscriptome sequences to published genomes of close relatives of the two dominant root populations, *Cellvibrio* (*C. japonicus*) and *Pseudomonas* (*P. stutzeri*) was performed. For both populations, a distinct functional profile was revealed at different niches examined: cucumber roots, wheat roots and respective soils. This was highly indicated by the pattern of RNA polymerase sigma factors. For example, expression level of the sigma 32 was higher for both taxa when colonizing cucumber compared to wheat roots. In *Pseudomonas*, rpoE (sigma 24) was the most highly expressed sigma factor in wheat root, and was expressed at a normalized GEI more than six times higher than in cucumber roots. Expression levels of several functional groups of genes, previously identified as critical in plant-bacteria interactions, were selectively expressed by these bacteria on the roots and will be discussed. In conclusion, plant species-specificity was manifested by both distinct patterns species association as well as by differential transcriptional responses to conditions dictated by the plant genotype.

YEAST ADAPTATION TO STRESS: A ROLE FOR ANEUPLOIDY AS A FAST EVOLUTIONARY INTERMEDIATE

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During the process of evolution and malignant transformation, the structure of a genome may undergo substantial changes including alterations in chromosome number (aneuploidy). Previous work has documented that aneuploidy can confer fitness advantages under certain environmental conditions by increasing the dosage of key genes and allowing a concentrated change in the expression level of many genes at once. However, aneuploidy also comes with a cost as it introduces gene expression imbalances within the cell. This dichotomy raises the question whether aneuploidy could serve as a stable and sustainable adaptation for prolonged evolutionary periods? To address this question and understand the balance between gain and cost that govern chromosome duplication dynamics, we took advantage of the well established in-lab evolution approach. In this work, we evolved *S. cerevisiae* cells for more than 2000 generations under diverse conditions and followed the long-term evolutionary dynamics of changes in chromosome number and gene expression. We found that specific stresses select for rapid duplication of particular chromosomes. Yet, these duplications are reversible events even under the stressful conditions in which they confer an advantage. We show that following prolonged evolution under stress, duplications are eliminated and replaced by more “refined” solutions. These findings suggest that chromosomal duplication, selected under stress, is a transient trait which serves as a "quick and dirty" evolutionary intermediate to help cells cope with stress until a more cost-efficient solution can be established which conveys a growth advantage and take over the population.

STRESS-INDUCED EVOLUTION OF ANTIBIOTIC RESISTANCE

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The development of antibiotic resistance by bacteria poses a major threat to human health worldwide, and is also one of the best examples of ongoing adaptation available for study. Several studies have demonstrated that, even in the absence of antibiotic treatment, resistant mutants increase in frequency in response to different stresses (e.g. starvation, low pH and high salt concentrations). Since bacteria are often exposed to various stresses, such stress-induced accumulation of antibiotic-resistant mutants likely greatly affects the general dynamics of antibiotic resistance emergence and spread. Currently our understanding of the scope of this phenomenon, of its causes, and of its significance for the spread of resistance within natural bacterial populations is very limited. In my talk I will describe how we are addressing these gaps in our understanding of the acquisition of antibiotic resistance through the combination of microbiology experiments, whole-genome sequencing, and analyses of metagenomic datasets.

MAPPING THE DYNAMIC REDOX PROTEOME OF MARINE DIATOMS IN RESPONSE TO OXIDATIVE STRESS

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Diatoms form an important group of algae in the oceans, being responsible for about 20% of global primary production. Accumulation of Reactive Oxygen Species (ROS) has been implicated in various responses to changes in environmental stresses in marine phytoplankton. Very little is known about thiol-based regulation and its role as metabolic switches during perception of environmental stress conditions in the marine environment. Here, we combined high throughput approaches to quantify and map the *in vivo* redox "landscape" of diatom cells by assessing GSH redox potential in various subcellular organelles using redox-senstive GFP (roGFP) sensor and quantification of the whole redox proteome in the model diatom *Phaeodactylum tricornutum*. We quantified the degree of oxidation of 3845 cysteine containing peptides with average basal oxidation of 19%, therefore they are kept under highly, but not completely reduced state under rest conditions. Analysis of the redox proteome under oxidative stress revealed that most proteins were kept under their reduced state and only a subset of proteins are redox sensitive and may participate in redox regulation under stress conditions. Functional analysis of these redox sensitive proteins revealed the involvement of these proteins in many cell function categories suggesting that redox signaling functions as a key posttranslational regulation in diverse metabolic and signal transduction pathways. We mapped the redox-sensitive proteins into metabolic pathways to assess the affect of alteration in redox state on the metabolic flux through these pathways. This ROS-responsive metabolic network provides insights into diatom's unique acclimation to diverse environmental stress conditions in the marine ecosystem.

INSIGHTS INTO THE BOVINE RUMEN PLASMIDOME

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Plasmids are self-replicating genetic elements capable of mobilization between different hosts. Plasmids often serve as mediators of lateral gene transfer, a process considered to be a strong and sculpting evolutionary force in microbial environments. Our aim was to characterize the overall plasmid population in the environment of the bovine rumen, which houses a complex and dense microbiota that holds enormous significance for humans. We developed a procedure for the isolation of total rumen plasmid DNA, termed rumen plasmidome, and subjected it to deep sequencing using the Illumina paired-end protocol and analysis using public and custom-made bioinformatics tools. A large number of plasmidome contigs aligned with plasmids of rumen bacteria isolated from different locations and at various time points, suggesting that not only the bacterial taxa, but also their plasmids, are defined by the ecological niche. The bacterial phylum distribution of the plasmidome was different from that of the rumen bacterial taxa. Nevertheless, both shared a dominance of the phyla Firmicutes, Bacteroidetes, and Proteobacteria. Evidently, the rumen plasmidome is of a highly mosaic nature that can cross phyla. Interestingly, when we compared the functional profile of the rumen plasmidome to two plasmid databases and two recently published rumen metagenomes, it became apparent that the rumen plasmidome codes for functions, which are enriched in the rumen ecological niche and could confer advantages to their hosts, suggesting that the functional profiles of mobile genetic elements are associated with their environment, as has been previously implied for viruses.

MOLECULAR SELF-ASSEMBLY OF SHORT PEPTIDES: IMPLICATIONS FOR MICROBIOLOGY AND BIOTECHNOLOGY

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The formation of ordered amyloid fibrils is a process of immense biological importance. While it is related to major degenerative human disorders on the one hand, it is important for physiological processes in microorganisms on the other hand. Most notably, amyloid self-assembly is associated with biofilm formation in both Gram positive and Gram negative bacteria. Thus, study of the process is important for the study of bacterial physiology as well as a way to control biofilm formation on medical devices. In spite of it central physiological and pathological role, the mechanism of amyloid formation is not fully understood. We have suggested, based on experimental and bioinformatic analysis, that aromatic interactions may provide energetic contribution as well as order and directionality in the molecular-recognition and self-association processes that lead to the formation of these assemblies. This is in line with the well-known central role of aromatic-stacking interactions in self-assembly processes. Our work led to the development of amyloid formation inhibitors that could be useful for the treatment of amyloid human diseases and the process of biofilm formation. Our works on the mechanism of aromatic peptide self-assembly, lead to the discovery that the diphenylalanine recognition motif self-assembles into peptide nanotubes with a remarkable persistence length. Other aromatic homodipeptides could self-assemble in nano-spheres, nano-plates, nano-fibrils and hydrogels with nano-scale order. We demonstrated that the peptide nanostructures have unique chemical, physical and mechanical properties including ultra-rigidity as aramides, semi-conductive, piezoelectric and non-linear optic properties.

CHARACTERIZING VIRAL-HOST INTERACTIONS IN THE MEMBRANE CONTEXT USING MICROFLUIDICS

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Mapping viral-host interactions is crucial for understanding the viral life cycle on a molecular level. In general, there are several high throughput technologies available for detecting protein interactions. The gold standards being Yeast two hybrid screens (Y2H) and affinity purification coupled with Mass spectrometry (AP-MS). There are several bottle necks for these high throughput technologies. Low sensitivity is one. Membrane proteins is another. When it comes to viral-host interactions the success of these technologies is very limited. A major reason could be the poor compatibility with membrane proteins. For example, about half the proteins of viruses such as HIV, HCV and influenza are membrane associated. Over the last few years we have been developing microfluidic tools to study protein-protein interactions. Our main goals were to increase sensitivity, get quantitative data and be compatible with membrane proteins. In this presentation, I will explain the principles of our microfluidic technology, and demonstrate how we used it thus far for characterizing interactions of viral proteins in the context of both viral and host membrane proteins. Finally, I will demonstrate the quantitative nature of this platform on a specific interaction detected in our screens between RSV matrix protein and Caveolin.

RAPID DETECTION OF BACTERIA BY 'DIRECT-CELL-CAPTURE' ONTO 2D LAMELLAR MACRO-POROUS SILICON PHOTONIC CRYSTAL GRATINGS

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The present work focuses on the design and synthesis of a new optical biosensor based on a two-dimensional (2D) lamellar photonic grating for rapid bacteria detection. The biosensor consists of a 2D periodic structure of macro-PSi with a characteristic pore diameter of 1-3 μm, to allow facile entrapment of the bacteria cells inside the pores. In turn, the captured bacteria induce a change in the effective optical thickness (EOT) of the grating that is monitored and quantified via reflective interferometric Fourier transform spectroscopy (RIFTS). We show that monitoring changes in the optical interference spectrum of the macro-PSi sensors enables a simple and sensitive detection scheme of bacteria. Our preliminary optical studies demonstrate the applicability of these lamellar gratings for the detection of *E. coli* K-12 bacteria. We show that upon proper design, this novel biosensing scheme allows simultaneous measurements of EOT and reflectance intensity changes. Our preliminary results demonstrate a detection limit of 10^5 cell/ml for *E. coli*. It is important to note that no affinity capture molecule is used in these preliminary experiments to bind the E. coli to the PSi. Current experiments explore the use of specific antibodies with high affinity for *E. coli* to capture and concentrate bacteria inside of the pores and achieve higher detection sensitivity. This proof of concept work provides a generic sensing platform that is applicable for rapid detection and identification of a variety of microorganisms.

HIGH RESOLUTION CRYSTAL STRUCTURE OF THE ACETYLXYLAN ESTERASE AXE2 FROM *GEOBACILLUS STEAROTHERMOPHILUS*

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The thermophilic soil bacterium *Geobacillus stearothermophilus* T-6 produces an intracellular acetylxylan esterase, Axe2, which removes acetyl groups from xylo-oligosaccharides. The enzyme is a GDSL hydrolase, using the catalytic triad Ser-His-Asp for hydrolysis, and presents a new undefined family of carbohydrate esterases in the CAZy database. Axe2 was biochemically characterized and its regioselectivity was determined on xylopyranoside per acetate (Alalouf *et al*. *JBC* 286:41993, *2011).* The crystal structures of sel-Met Axe2 and its catalytic mutant, S15A, were determined at 1.7 and 1.9 Å, respectively, and contain a dimer in the symmetric unit cell. Conserved residues associated with the oxyanion hole in the GDSL hydrolase family are positioned at distances appropriate for hydrogen bonds stabilizing the tetrahedral intermediate in the reaction mechanism. Indeed, a single phosphoric acid molecule was found in the active site, simulating the position of acetyl group in the oxyanion hole during catalysis. Native Axe2 appears to have a Mw of 200,000 based on gel filtration chromatography suggesting that the enzyme is an octamer. This octameric structure was predicted by the PDBePISA server, and transmission electron microscopy (TEM) images of the native protein revealed doughnut-like shape structures of 8-10 nm in diameter in agreement with the crystal structure. Based on the data, the octamer structure is held by several conserved residues that form salt bridges, hydrogen bonds and stacking interactions. These residues are now being replaced to verify their exact role in the formation of the octamer and their contribution to the thermal stability of the protein.

DIRECTED EVOLUTION OF LIPASE T6 FROM *GEOBACILLUS STEAROTHERMOPHILUS* FOR ENHANCED METHANOL TOLERANCE

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Enzymes ability to catalyze reactions in non-natural environment of organic solvents has opened new opportunities for enzyme-based industrial processes. However, the main drawback of such processes is that most enzymes have a limited stability in polar organic solvents. An example for such a process is the enzymatic production of biodiesel by transesterification of oil and alcohol which involves lipases as catalysts in a non-aqueous environment. Methanol is utilized frequently as the alcohol in the reaction and the limited stability of the lipases in high methanol concentrations prevents full industrial implementation of the process. In this research we used protein engineering methods to evolve a lipase for enhanced stability in high concentrations of methanol. We have chosen to work with an unexplored lipase from a thermophilic bacterium, *Geobacillus stearothermophilus* T6. Protein engineering by random mutagenesis (error prone PCR) and structure guided consensus was performed on the lipase T6 gene expressed in *Escherichia coli* BL21. A high throughput colorimetric screening assay in 96-well plates was developed to evaluate lipase activity after an incubation period in high methanol concentrations. We have found two variants which had significantly improved activity after 1 hour incubation in 70% methanol compare to the wild type enzyme. Variant Q185L (random mutagenesis) and variant A269T (structural guided consensus) showed improved activity of 37- and 29-fold, respectively. The double mutant Q185L/A269T exhibited a synergistic effect of 69-fold improved activity. This novel variant will be purified and lyophilized for its evaluation in biodiesel production.

PROTEINS AND DNA ELEMENTS ESSENTIAL FOR THE CRISPR ADAPTATION PROCESS IN *ESCHERICHIA COLI*

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The clustered regularly interspaced short palindromic repeats and their associated proteins (CRISPR/Cas) constitute a recently identified prokaryotic defense mechanism against invading nucleic acids. Activity of the CRISPR/Cas system is comprised of three steps: (i) insertion of alien DNA sequences into the CRISPR array to prevent future attacks, in a process called "adaptation", (ii) expression of the relevant proteins, as well as expression and processing of the array, followed by (iii) RNA-mediated interference with the alien nucleic acid. Here we describe a robust assay in *Escherichia coli* to explore the hitherto least-studied process, adaptation. We identify essential genes and DNA elements in the leader sequence and in the array which are essential for the adaptation step. We also provide mechanistic insights on the insertion of the repeat-spacer unit by showing that the first repeat serves as the template for the newly inserted repeat. Taken together, our results elucidate fundamental steps in the adaptation process of the CRISPR/Cas system.

RISE AND FALL OF MICROBIAL MULTICELLULARITY: LESSONS FROM GRAM-POSITIVE BACTERIA

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Bacillus subtilis forms highly organized multicellular communities known as biofilms wherein the individual cells are held together by a self-produced extracelluar matrix. The environmental signals that promote matrix synthesis and complex colony architecture remain largely unknown. We discovered that one such signal is impaired respiration. Specifically, high oxygen levels suppressed synthesis of the extracellular matrix. In contrast, low oxygen levels, in the absence of an alternative electron acceptor, led to increased matrix production. The response to impaired respiration was blocked in a mutant lacking cytochromes caa_3 and *bc* and markedly reduced in a mutant lacking the membrane-embedded kinase KinB. We propose that KinB is activated via a redox switch involving interaction of segment II with one or more cytochromes under conditions of reduced electron transport. We note that complex communities formed by *Bacillus subtilis,* as well as other gram positive bacteria, such as the medically relevant pathogen *Staphylococcus aureus* have a limited lifespan, disassembling as nutrients become exhausted and waste products accumulate. We would discuss a specific cue of disassembly, norspermidine, allowing the deformation of complex structures in "aging" bacterial communities.

STRUCTURAL AND FUNCTIONAL DYNAMICS OF NhaA A PROTOTYPE Na^+/H^+ ANTIPORTER IN HOMEOSTASIS OF pH, Na^+ AND VOLUME IN CELLS

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Living cells are critically dependent on processes that regulate intracellular pH, Na^+ content and volume. pH regulated Na^+/H^+ antiporters play a primary role in these homeostatic mechanisms (1). They are found in the cytoplasmic and organelle membranes and have long been human drug targets. NhaA, the principal Na^+/H^+ antiporter in *Escherichia. coli*, has orthologues throughout the biological kingdoms (1). The crystal structure of NhaA (2) provided insights into the mechanism of action and pH regulation of an antiporter. However, because an antiporter is a nano-dynamic machine that exchange Na^+/H^+ across the membrane we attempt to solve the structures of all active conformations in addition to elucidate the functional dynamics of the antiporter. We use: 1. Mutagenesis to identify the important residues (2). 2. Structural based scanning accessibility of Cys replacements to various SH reagents to trace water filled domains in the protein that change conformation (3) 3. Site specific Trp fluorescence to report upon the active site and the pH sensitive domain in the protein (4). 4. Structure based computation to model the active conformation of the antiporter (5).

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THE ROLE OF NANORNASE IN MEDIATING BIOFILM FORMATION VIA C-DI-GMP IN THE BACTERIAL PATHOGEN *PSEUDOMONAS AERUGINOSA*

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The Gram-negative bacterium *Pseudomonas aeruginosa* is recognized for its ability to colonize diverse habitats. *P. aeruginosa* is an opportunistic bacterial pathogen capable of establishing both acute and persistent chronic infections, while the later usually is the result of biofilm formation. We screened 3552 *P. aeruginosa* PAO1 transposon mutants for altered biofilm formation in a low nutrient medium. One of the mutations, which mapped to the gene oligoribonuclease (*orn*), resulted in hyper-biofilm and in addition caused aggregation, impaired surface motility and increased exopolysaccharide production. Expression of *orn* in *trans* restored all wild-type phenotypes. *P. aeruginosa* Orn was found to possess nanoRNase activity *in vitro* and *in vivo* but was also able to degrade longer RNA substrates. The phenotypes observed in the mutant were previously reported in relation to the second messenger cyclic-di-GMP (c-di-GMP) accumulation. Overexpression of an EAL-domain containing protein (PA2133), which is known to degrade c-di-GMP, in the *orn* mutant restored biofilm formation and aggregation to wild-type levels but not surface motility and exopolysaccharide production. Biochemical analysis identified that the loss of Orn indeed increased cellular c-di-GMP and pGpG (the first c-di-GMP metabolic degradation product) and decreased GMP concentrations. Furthermore, purified Orn was shown to degrade only pGpG. Taken together our results suggest that Orn can impact the c-di-GMP-dependent cascade through pGpG, and the uncoupling between c-di-GMP levels and motility vs. biofilm phenotypes may suggest that additional signals other than c-di-GMP may be involved.

CROSS-REGULATION OF METABOLISM AND VIRULENCE IN THE HUMAN BACTERIAL PATHOGEN *LISTERIA MONOCYTOGENES*

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Intracellular bacterial pathogens are metabolically adapted to grow within mammalian cells. While these adaptations are fundamental to the ability to cause disease, we know little about the relationship between the pathogen's metabolism and virulence. Here we used an integrative Metabolic Analysis Tool that combines transcriptome data with genome scale metabolic models to define the metabolic requirements of *Listeria monocytogenes* during infection. Twelve metabolic pathways were identified as differentially active during *L. monocytogenes* growth in macrophage cells. Intracellular replication requires *de novo* synthesis of histidine, arginine, purine and branch chain amino acids (BCAAs) as well as catabolism of L-rhamnose and glycerol. The importance of each metabolic pathway during infection was confirmed by generation of gene knock out mutants in the respective pathways. Having established these metabolic requirements, we investigated their association with virulence. Surprisingly, limiting BCAA concentrations, primarily isoleucine, were found to trigger robust induction of the master virulence activator PrfA and the PrfA-regulated genes. This response was specific and required the nutrient responsive regulator CodY, which is known to bind isoleucine. Further research has shown that CodY binds directly the *prfA* promoter, thus demonstrating an example of how metabolism and virulence are tightly interlinked. We further propose that CodY serves as a metabolic sensor that informs the bacteria of its intracellular location.

Lobel L, Sigal N, Borovok I, Ruppin E, Herskovits AA (2012) Integrative Genomic Analysis Identifies Isoleucine and CodY as Regulators of *Listeria monocytogenes* Virulence. PLoS Genet 8(9): e1002887. doi:10.1371/journal.pgen.1002887

HCMV (HHV5) ENCODED microRNAs - IN VIRAL REPLICATION AND LATENCY

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Human cytomegalovirus (HCMV or HHV5) is a highly prevalent human pathogen, usually acquired at early age. Following primary infection, the virus establishes a life-long latent infection in peripheral blood monocytes, with episodes of reactivation, mostly in the immune compromised host. More recently, HCMV as well as other members of its *Herpesviridae* family have been found to encode for microRNAs (miRNAs). Using an integrated bioinformatic/biological approach that relies heavily on deep sequencing for the analysis of HCMV Micronome, we have found, several novel miRNAs and moRs not found before, as well as, an extremely high expression of some mature viral encoded miRNAs. Next, we analyzed the expression of HCMV-micRNAs *in vivo* during: 1) acute infection 2) in isolated PBMC from HCMV donors that are carriers of HCMV with latent infection (anti-CMV positive healthy donors) and 3) after induction of reactivation of viral replication in the macrophages of these donors. The study demonstrates that the major miRNAs of HCMV expressed *in vivo* diverge from the micRNAs expressed *in vitro* in human fibroblasts.

RAB1 AND ITS GAP TBC1D20 ARE RECRUITED TO LIPID DROPLETS BY THE HEPATITIS C VIRUS NONSTRUCTURAL PROTEIN 5A

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Replication and assembly of Hepatitis C virus (HCV) depends on the host's secretory and lipid-biosynthetic machinery. Viral replication occurs on endoplasmic reticulum (ER)-derived modified membranes while viral assembly is thought to occur in the vicinity of lipid droplets (LDs). A physical association and coordination between the viral replication and assembly complexes are prerequisites for efficient viral production. The ER and LD-binding non-structural protein 5A (NS5A) is a candidate for interfacing with the host membranes as well as secretory and lipid synthesis machineries. The interaction of GFP-tagged NS5A with host cell membranes and binding partners was characterized in living cells. The binding of NS5A to LDs is apparently irreversible, both in HCV-infected cells and when ectopically expressed. In HCV-infected cells, NS5A fluorescence was observed around the LDs and in perinuclear structures that were incorporated into a highly immobile platform superimposed over the ER membrane. The GTPase activating protein (GAP) TBC1D20 and its cognate GTPase Rab1 are recruited by NS5A to LDs. The NS5A-TBC1D20 interaction was previously shown to be essential for the viral life cycle. In cells, expression of a dominant negative Rab1 mutant (Rab1DN) abolished steady state LDs. In infected cells, Rab1DN induced the elimination of NS5A from viral replication sites. Our results support a model where TBC1D20, via its GAP activity dissociates Rab1 from secretory membranes in order to facilitate its recruitment to LD membranes. Thereby promoting LD biogenesis and sustaining the viral life cycle.

MODELING OF HUMAN CYTOMEGALOVIRS (HCMV) MATERNAL-FETAL TRANSMISSION IN EX VIVO INFECTED HUMAN DECIDUAL ORGAN CULTURES

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Human cytomegalovirus (HCMV) is the leading cause of congenital infection, associated with severe birth defects, placental damage, and intrauterine growth retardation. The mechanism of transmission via the maternal-fetal interface is largely unknown, and there are no animal models for congenital HCMV infection. The initial stages of infection are believed to occur in the maternal decidua - representing the maternal aspect of the chimeric human placenta. To gain insight into these critical early events of transmission, we have recently established an *ex vivo* model of HCMV infection in first-trimester decidual organ cultures. Using both laboratory-derived and clinical HCMV strains, we demonstrated the broad viral target-cell range, with consistent cell-to-cell mode of spread in the decidual tissue. Antiviral drugs as well as neutralizing HCMV antibodies exhibited inhibitory activity on viral spread in the decidua. We have further employed the decidual infection model to study the tissue response to HCMV infection, revealing a profound effect of the virus on decidual immune and angiogenic cytokine/chemokine expression profile. Significantly, a unique virus-induced stimulation of a decidual innate immune response, with formation of proinflammatory environment was demonstrated. The *ex vivo*-infected decidual cultures can serve as a surrogate human model that could potentially address viral and tissue determinants of transmission and damage, and could facilitate evaluation of the effects of new antiviral interventions in the maternal-fetal interface.

THE NUCLEOLUS AND HERPESVIRUS INFECTION

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The nucleolus is a multi-protein nuclear structure whose primary function is the biogenesis of ribosomal subunits involving the transcription of pre-rRNA, rRNA processing and ribosome assembly. The nucleolus is also involved in other cellular aspects including cell cycle control, stress responses, and innate immunity. Replication and assembly of most DNA viruses take place in the nucleus and are accompanied with an extensive disruption of the nuclear architecture. This includes changes in the nucleolar morphology that illustrate the redistribution of host nucleolar proteins to different cellular sites, the occurrence of distinct modifications and the targeting host proteins to the nucleoli. In addition, viral products may traffic to and from the nucleolus. Our study focused on the cellular localization of the nucleolar protein GLTSCR2/PICT-1 following KSHV and HSV-1 infection. We did not record alterations of the cellular distribution of GLTSCR2/PICT-1 in KSHV-infected cells. However, dispersion of GLTSCR2/PICT-1 to extra-nucleolar compact nuclear spots was documented early after HSV-1 infection. Even during late time post infection, when an extensive destruction of the nuclear architecture was noticed, GLTSCR2/PICT-1 was localized in compact dots. Interestingly, redistributed GLTSCR2/PICT-1 colocalized with the basal transcription factor UBF1 which has been previously colocalized with an active RNA polymerase I. Other nucleolar proteins were also dispersed upon infection, yet distinct delocalization patterns were observed suggesting their different regulation during infection. The similar distribution kinetic and pattern of GLSTCR2/PICT-1 and UBF1 imply that these nucleolar proteins share common processes during infection. The functional significance of nucleolar segregation following infection will be discussed.

EFFECTIVENESS OF FMD VACCINATION IN DAIRY AND FEEDLOT CATTLE

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Inactivated foot and mouth disease (FMD) vaccines are used in non-endemic countries for emergency vaccination during outbreaks in order to prevent virus spread. In endemic countries vaccines are used for routine vaccination. Livestock in Israel are routinely vaccinated with a high potency vaccine ($\geq 6PD_{50}$), which may be used also for emergency vaccination. During 2011 a large outbreak of FMD, occurred in Israel. We investigated an affected feedlot and an adjacent dairy herd. Comparison of morbidity and antibodies to NSP enabled the assessment of the effectiveness of various vaccination statuses. Almost 100% of calves vaccinated twice, at least three months prior to the outbreak, showed clinical signs and the infection prevalence reached 96%. Heifer calves vaccinated 3-5 times, 7 months prior to the outbreak showed 100% infection and 18% showed clinical signs. Testing of cattle sera of the same vaccination status as the affected cattle demonstrated low neutralizing antibody (NA) titers against the field virus strain and an r_1 value of 0.37 compared to the vaccine strain. In contrast, cattle vaccinated only once but up to two weeks before the outbreak, were almost all protected from clinical disease and to a lesser extent, protected from FMD virus infection, despite low NA titers. We conclude that emergency vaccination was highly effective for prevention of infection and should be encouraged during outbreaks, whereas routine vaccination with the same vaccine formulation provided only limited protection due to poor longevity of the elicited immunity and low matching with the field strain.

MOLECULAR CHARACTERIZATION OF ACQUIRED RESISTANCE TO MACROLIDES IN *MYCOPLASMA BOVIS* FIELD STRAINS

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Mycoplasma bovis causes pneumonia, mastitis, arthritis and otitis media. The treatment and control of *M. bovis*-related diseases are based mainly on the use of antimicrobials as no effective commercial vaccine is available. In this study, the molecular mechanism of acquired resistance to tylosin and tilmicosin was investigated. Forty seven *M. bovis* strains showing different susceptibility to these antibiotics and two *M. bovis* PG45 mutants selected *in vitro* were used. Sequence analysis of the 23S rRNAs in a *M. bovis* tylosin-selected mutant revealed the presence of an A2058G substitution in domain V of *rrl3* as well as G748A and A2058G substitutions in domains II and V of *rrl4*, respectively. In a *M. bovis* tilmicosin-selected mutant, only the A2058G substitution in domain V of *rrl3* was identified. No nucleotide substitutions were identified in domains II and V of both alleles of the *rrl* in 23/23 *M. bovis* strains with MIC of 0.5-4 µg/ml to tylosin. However, 14/15 *M. bovis* strains with an 8 µg/ml ≤ MIC < 128 µg/ml to tylosin possessed various mutations either in domain II or in V of the of one or two *rrl* genes. Finally, 8/9 strains with MIC ≥ 128 µg/ml to tylosin possessed nt substitutions in more than one domain of the 23S rRNAs. Results of our work showed that point mutations at positions 748 and 752 of domain II and at positions 2058 and 2059 of domain V of the 23S rRNAs correlates with decreased susceptibility of *M. bovis* strains to tylosin and tilmicosin.

BOVINE MASTITIS ASSOCIATED *ESCHERICHIA COLI*: GENOTYPING, VIRULENCE ASSESSMENT AND PATHOGENOMIC ANALYSIS

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Escherichia coli is a major agent of bovine mastitis, a disease of great economic burden in dairy production. Although usually acute, bovine mastitis caused by *E. coli* may manifest in a range of clinical presentations, with particular strains involved in persistent infections. Specific pathogenic mechanisms by which *E. coli* affects the mammary gland are unknown. Objectives of the present work were (1) to genotype and assess the prevalence of known virulence markers in *E. coli*strains derived from bovine mastitis, (2) to identify novel mastitis pathogenic markers. Genotyping was performed by ECOR phylogenetic grouping, multi-locus sequence typing and pulsed-field gel electrophoresis, and assessment of known virulence markers was performed by microarray. Overall, various genotypes are found in bovine mastitis strains. Mastitis strains mostly lack known virulence markers. The most prevalent markers (*lpfA*, *iss* and *astA*) are not present in the majority of these strains. A cluster of genotypically similar strains was identified and representative strains were selected for virulence assessment *in vivo* using murine and bovine models of intramammary infection. Mastitis strains, representing different clinical presentations of disease (acute, mild, persistent) and a non-pathogenic strain were subjected to phenotypic microarray and whole genome sequence analysis. Phenotypic microarray displayed differences in the biochemical properties of the strains. Genome sequencing resulted in identification of strain-specific sequences, including plasmid- and phage DNA. These findings will contribute for identification of mastitis-specific markers, which might be utilized for early detection of *E. coli* mastitis strains and which may be possible novel virulence factors.

IDENTIFICATION OF *BARTONELLA* SPECIES IN COWS AND THEIR LICE FROM ISRAEL

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A wide range of animals have been identified as reservoirs for more than 30 *Bartonella* species. Cows are known to be hosts for *Bartonella bovis, B. schoenbuchensis* and *B. chomelii*. However, information regarding the distribution of these or other *Bartonella* species in cattle and their possible arthropod vectors is lacking. The prevalence of *Bartonella* spp. infection in cattle in Israel has not been investigated to date. In addition, these animals are reported to be constantly infested with lice. Hence, this study investigated the presence of *Bartonella* spp. infection in cows from Israel and the possible implication of their lice as vectors of these bacteria. EDTA blood samples and the cattle tail lice (*Haematopinus quadripertusus*) from 62 cows were collected in 9 farms in Israel. All samples were screened for *Bartonella* spp. infection by high resolution melt (HRM) real-time PCR assay that targeted the 16S-23S intergenic spacer (ITS) and the transfer-messenger RNA (ssrA) gene. Results show that *Bartonella* spp. DNA can be detected in cow blood samples and lice pools. Preliminary sequence analyses have shown homology with *B. bovis* and *B. melophagi*, respectively. This study demonstrates the presence of *B. bovis* in cattle from Israel and it represents the first report of *Bartonella* spp. in the cattle tail louse (*H. quadripertusus*). The zoonotic potential of *Bartonella* species highlights the importance of these findings. Further studies are required to demonstrate the cattle tailed louse as defined *Bartonella*-vector in these animals.

CARDINIUM IN *CULICOIDES* BITING MIDGES: THE MERRIER THE ENVIRONMENT OF THE HOST- THE MORE OF THE SYMBIONT

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"*Candidatus* Cardinium hertigii" (Bacteriodetes) is a maternally inherited reproductive manipulator symbiont known from several arthropods. As the relationship with the host is facultative, prevalence of infection may vary depending on biotic and abiotic conditions of the host and the symbiont. These include environmental conditions, natural enemies and interactions with the host as well as with other microorganisms. *Cardinium* was previously identified in few *Culicoides* species in Japan with a variable range of frequencies, however, the cause for this range is yet unknown. We identified and localized *Cardinium* in sympatric *Culicoides* biting midge species in Israel, examined its abundance, and studied its association with environmental factors. The prevalence of adult infection differed, with 50.7% from *C. imicola*, 31.4% from *C. oxystoma*, and 0% from *C. schultzei* gp., although phylogenetic analyses showed that *Cardinium* in these species is almost identical. In addition, prevalence of infection differed between climate regions, with lowest prevalence in the arid region and highest prevalence in the Mediterranean region. Multivariate linear regression analysis of *Cardinium* prevalence together with climatic and satellite imagery data-derived environmental variables, revealed that infection prevalence is significantly associated with land surface temperature and explained up to 89.7% of infection variability. These findings suggest that that *Cardinium* infection is favored under the same conditions that are optimal for the midge host's immature stages. Results of this study shed further light on the interaction between environment, host and symbiont, and can contribute to understanding *Cardinium*'s potential role in its *Culicoides* host.

SMALL RNAS USED FOR PATHOGEN DETECTION

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Early and accurate detection of human pathogen infection is critical for treatment and therapeutics. Non-coding RNAs (ncRNAs) account for a large portion of the transcribed genomic output. These diverse families of RNA molecules play a crucial role in cellular function. The use of 'deep sequencing' technology (also known as 'next generation sequencing') to infer transcript expression levels in general, and ncRNA specifically, is becoming increasingly common in molecular and clinical laboratories. We have developed two complementary pathogen identification approaches that overcome the requirement of prior knowledge and culturing of pathogens. These methods are centred on the deep sequencing technology: (i) using a short RNA subtraction and assembly (SRSA) algorithm which is based on analysing pathogen degraded small RNAs after infection; and (ii) monitoring host ncRNA repertoire in infected host cells. We demonstrate the relevance these extensive short ncRNAs analysis in pathogen infected mammalian cells. We further prove our approach's efficiency through identification of a combined viral and bacterial infection. Our long term perspective is to characterize the unique signature of host response from biological samples by using a single unbiased test of deep sequencing and computational analysis.

NEXT GENERATION SEQUENCING OF INFECTIOUS DISEASES - IMPLICATIONS FOR PUBLIC HEALTH

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Next Generation Sequencing (NGS) technology is now enabling routine rapid analysis of complete microbial genomes. Whole-genome sequencing of bacterial pathogens has facilitated recent outbreak investigations, providing timely information on evolutionary origin, transmission route, pathogenic potential, and resistance information of the outbreak strains. Assemblage of genomic and epidemiological data enabled rapid development of strain-specific typing methods. NGS is also used for comprehensive analysis of bacterial diversity from complex environmental samples; vaccine development research, unraveling the complex dynamics of viral evolution and pathogen-host response. The bioinformatics challenges of NGS are still many, including: management of large amounts of data; data storage; integration of data with clinical datasets and surveillance systems. Taking in consideration the continue reduction of genome sequencing cost and development of user-friendly analytical tools, it is expected that NGS will be applied routinely in the near future, not only for research and clinical applications but also for public health actions.

THE EVOLVING EPIDEMIOLOGY OF PERTUSSIS IN ISRAEL

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Background: The introduction of pertussis vaccine to the routine immunization program in Israel in 1957 resulted in a sharp decline in the 1960s-1970s, with an average 1–2/100,000 annual incidence rate for 40 years. Since 1998 pertussis incidence increased to 23/100,000 (2004). Due to the resurgence of pertussis, Israel implemented 2 booster vaccine doses in schoolchildren, in 2005 a 5th dose for second-graders (7-8y), and in 2008 an additional dose for eight-graders (13-14y). **Methods:** Population-based epidemiologic study in the Jerusalem district. **Results:** 2520 pertussis cases were reported during 1990-10/2012, 74.4% were <20 years (median age 11y). The incidence rates increased from 2.6/100000 (1990) to 28.8/100000 (2006). Following the immunization schedule modification, pertussis incidence rates declined to 21.7/100000 (2007), 15/100000 (2009) and 13/100000 (2010). The two age-groups showing significant decline were children aged 5-9y (61.5% reduction) and 10-14y (73.9% reduction). However, in 2011, a rise to 30/100000 occurred, specifically in infants. The incidence rates in infants increased from 61 to 190/100000). Infants <1 year were 12.5% of cases and those <6 months -84.3% of cases <1 year. The vaccination status: unvaccinated –19.2%, partially vaccinated – 7.6%, fully vaccinated – 73.2%. The hospitalization rate: overall- 5.4%; infants <6 months 45%, <3 months 70%. Household transmission occurred in 16.1%. Laboratory confirmation was available in 89%. Low birthweight and increasing birth order were identified as risk markers. **Conclusions:** While additional pertussis vaccine doses in children and adolescents were followed by morbidity decline in these groups, the burden of pertussis is still noteworthy among infants.

ENVIRONMENTAL SURVEILLANCE FOR HUMAN HEPATITIS VIRUS (HAV) USED FOR EPIDEMIOLOGICAL INVESTIGATION OF HAV SPREAD IN DAN METROPOLITAN AREA AND OTHER CITIES

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Hepatitis due to Hepatitis A virus (HAV) is a vaccine-preventable and reportable disease in Israel. Like other enteric viruses HAV can cause subclinical or mild infections and thus spread without being noticed in highly immune populations. HAV can be detected in environmental samples and sewage surveillance can serve to determine its geographical distribution when clinical cases appear. The incidence of HAV hepatitis declined rapidly to nearly zero since the introduction of routine childhood vaccination in 1999. Surprisingly, in 2012 more than 40 clinical cases were recorded in Dan Metropolitan Area (DMA) including a cluster in southern Tel-Aviv. For environmental surveillance we used advanced treatment methods for environmental samples developed for HAV RT-PCR assay. The tap water supply pipes to Tel Aviv southern districts were first checked and found virus free, thereby establishing that water contamination was not the source. To determine the geographical pattern of virus circulation we have collected 12 sewage samples from different locations in DMA sewer system. 9 of the samples were positive and a "Hot-Spot" map could be drawn. In addition, HAV was detected in 4 of 12 sewage samples collected in Oct-Nov in Jerusalem, Haifa and Beer-Sheva. Molecular typing and phylogenetic analysis of all sewage positive samples as well as 5 PCR positive serum samples from HAV patients in DMA is underway to study the viral source and transmission pathways. In conclusion, sewage surveillance using molecular assays is a useful method for detection of HAV circulation and determination of its geographical spread.

EMERGENCE AND INCREASE OF EMRSA15 AMONG MRSA BACTEREMIA ISOLATES IN ISRAEL

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Over 750 *S. aureus* bacteremia isolates were received and analyzed at the Israeli *S. aureus* reference center since October 2010. The *mecA* gene conferring methicillin resistance was found in 45% of the isolates. The Panton Valentine Leukocidin (PVL) gene was found in 5.5% of the MSSA isolates but was very rare (0.6%) among MRSA indicating CA-MRSA scarcity in nosocomial infections. The average age for MSSA bacteremia was 57 compared with 64 for MRSA. The most prevalent spa types among MRSA were t002 (30%), t001 (17%), and t032 (12%). MRSA of the t032 type and related types were confirmed as EMRSA15 – a pandemic strain that originated in the UK in the 1990s, has since become very prevalent there (up to 85% of MRSA) and caused a vast increase in MRSA bacteremias. The analysis indicates the emergence of EMRSA15 in 9 health care centers followed by a marked increase in its occurrence (up to 25% of MRSA bacteremias) during the period analyzed. EMRSA15 strains were resistant only to erythromycin and ciprofloxacin in addition to b-lactams (more sensitive compared to other HA-MRSA). The strains were urease negative and contained SCC*mec* IVh as expected. PFGE analysis showed local EMRSA15 consisted of few clones mostly identical to European strains.

P-1

A LARGE-SCALE OUTBREAK CAUSED BY OXA-48-PRODUCING *ENTEROBACTERIACEAE* IN A NEONATAL INTENSIVE CARE UNIT (NICU): EPIDEMIOLOGICAL AND MICROBIOLOGICAL CHARACTERISTICS

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Objectives: Our aims were to describe the course of an OXA-48 producing *Enterobacteriaceae* (OPE) outbreak in a NICU and to characterize the molecular features of the OPE strains. **Methods:** The course of the outbreak was recorded by the Ministry of Health (MoH). Determination of resistance mechanisms was done by PCR and sequencing of the *bla* genes and typing was performed by PFGE and MLST. The bla_{OXA-48}-harboring plasmids were compared to pOXA-48a, the global bla_{OXA-48}-harboring vector. **Results:** The first 2 cases of bacteremia caused by carbapenem-resistant strains were noted in March 2012. The MoH was notified and intervened by July 2012, as the incidence had increased to 9 cases/week. Outside the NICU, the outbreak had spread to the Pediatric ICU and to another hospital. By August, we identified 49 patients who had acquisition of OPE in the NICU, including 16 with invasive infections, out of a total of 156 patients hospitalized during that period. 31 isolates were available for analysis, 29 *K. pneumoniae* and 2 *Enterobacter cloacae*. All isolates produced OXA-48 and CTX-M-14; the *K. pneumoniae* isolates belonged to a single clone, ST39. The $bla_{CTX-M-14}$ gene was located on a pOXA-48a-like plasmid, combined into an ISEcp1-like transposon. **Conclusion**: Dissemination of OPE by combined clonal spread and horizontal plasmid transfer led to a large-scale NICU outbreak. An intervention was able to contain the epidemic. A plasmid similar to pOXA-48a has now acquired $bla_{CTX-M-14}$, leading to resistance to all β-lactam agents.

P-2

A MULTINATIONAL STUDY OF COLONIZATION WITH ESBL-PRODUCING *ENTEROBACTERIACEAE* IN HEALTHCARE PERSONNEL AND FAMILY MEMBERS OF PATIENTS ADMITTED TO REHABILITATION CENTERS

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Objectives: Our aims were to define the prevalence and the risk factors for colonization by ESBL-producing *Enterobacteriaceae* (ESBL-PE) in healthcare workers (HCW) and family members (FM) of colonized patients admitted to rehabilitation centers (RCs) and to characterize the molecular features of these strains. **Methods:** The study was conducted in 5 RCs: in Tel-Aviv and Ra'anana, Israel; Rome, Italy; Berck, France and Barcelona, Spain. Comparisons of molecular characteristics were done between isolates from patients and their respective FM, and between those from HCW and from patients. **Results:** The study included 286 FM of 194 ESBL-PE carriers. Gastrointestinal carriage of ESBL-PE were detected in 26 (9.1%) FM, mostly *E. coli* (n=20; 76%). Species concordance between patients and FM was found in 23/26 ESBL-PE-positive FM. In multivariate analysis, older age of the FM, longer time spent with the patient and being a daughter/female spouse of a patient were associated with carriage in the FM. In all of the cases, isolates from FM and patients were indistinguishable. The HCW study included 73 physicians, 288 nurses, 179 nurse assistances, 205 physical therapists, 37 janitors, and 219 other staff members. ESBL-PE was detected in 33 (3.3%) of the HCW, including 32 *E. coli*. In multivariate analysis, feeding was associated with ESBL-PE carriage. Only 6 out of 20 subclones identified were also represented in a collection of 376 patient-derived ESBL-PE isolates. **Conclusion:** The molecular and epidemiological data suggest a close relation between ESBL-PE colonization in patients and their FM, but not between patients and HCW.

P-3

LABORATORY BASED SURVEILLANCE OF CIRCULATING SEROTYPES AND CLONETYPES OF *LISTERIA MONOCYTOGENES* IN ISRAEL

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Listeria monocytogenes (LM) is a major food borne pathogen widely distributed in nature, and the etiologic agent of listeriosis: a rare but severe disease, with high fatality rates approaching 20-30%. Clinical and food *Listeria* sp. strains, recovered from human cases and food inspection, are routinely submitted to the National Reference Center for Listeria (NRCL), in conjunction with mandatory reporting to District Health Offices. Following species ID, strain subtyping is performed using conventional serotyping. Pulsed-field gel electrophoresis (PFGE) using *AscI* (and *ApaI* as appropriate) according to PulseNet protocols is performed on all isolates. During a 7 year period (2006-2012), a total of 872 isolates were reported to the NRCL. The number of human derived isolates reported has been growing since 2008: from 39 in 2008 to 73 in 2012. Food isolates accounted for 8 different serovars, with the most common being 1/2b and 4b followed by 1/2a, 4ab, 1/2c, 4e, 4c, and 3b. In human isolates the most frequently serotype identified was 4b followed by 1/2b and 1/2a. Molecular analysis revealed that 68.5% mapped into clusters. A total of 26 PFGE clusters were identified: 5 clusters involved strains recovered during the last 7 years and seven clusters involved strains recovered for over 5 years. Notably, 16/26 clusters (all consisting of > 10 strains) involved both clinical and food derived strains. Three LM serotypes, and in particular 4b, pose a greater threat for public health. Further investigation of respective clones will contribute to the elucidation of the source of infection and outbreaks.

P-4

RELATIONSHIPS BETWEEN A(H1N1)pdm INFLUENZA INFECTION AND INFECTIONS WITH OTHER RESPIRATORY VIRUSES

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In 2009, a new influenza pandemic virus emerged, first named swine influenza virus and later renamed A(H1N1)pdm09. The A(H1N1)pdm09 infection had several unique characteristics which included, a rapid transmissibility and a high morbidity in obese individuals, pregnant women and individuals suffering from chronic diseases. The emergence of the A(H1N1)pdm09 virus provided our group with an unique opportunity to study its impact on other respiratory viruses, such as, respiratory syncytial virus (RSV), human metapneumo virus (hMPV), adenovirus and seasonal influenza. In the current paper, we studied patients' samples prior and following the pandemic influenza A(H1N1)pdm09 infection, for the presence of various respiratory viruses. We demonstrate that when the pandemic influenza A(H1N1)pdm09 was first identified, two major infection peaks were noted. We further show that individuals of various ages were infected; that the A(H1N1)pdm09 infection influenced the infection with RSV, adenovirus and hMPV and that it affected the infection by seasonal influenza virus infection. Interestingly, we observed that the A(H1N1)pdm09 virus lost its dominancy when it reappeared in the winter of 2010-11 and that only RSV infection were affected by the A(H1N1)pdm09 virus of that year.

P-5

SALMONELLA OUTBREAKS IN ISRAEL, 1999-2011: FROM STATISTICALLY GENERATED ALARM TO PATHOGEN`S SEQUENCE.

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Salmonella is the leading food-born pathogen associated with outbreaks worldwide. Effective tools to monitor outbreaks are essential to public health. Computer-supported surveillance of anomalous incidence is commonly used for foodborne outbreaks detection internationally. In Israel, currently, such tool is not in use. In this study we have implemented the well-established Farrington algorithm on 62,554 *Salmonella* isolates submitted to the *Salmonella* National Reference Center (SNRC), 1/1/1995 to 31/12/2011. Validation of detected aberrations was performed using pulse-field gel electrophoresis (PFGE) against 23 different outbreaks, which were intuitively identified by the SNRC. In eighteen out of these twenty-one validated clusters, an algorithmic alarm has been generated, implying good sensitivity of 85%. The precision of the adapted algorithm was examined by five random clusters that were flagged by the algorithm and tested by PFGE. Four out of these five previously unreported outbreaks, noticed only by the algorithm, were indeed confirmed experimentally. To evaluate the overall number of potential outbreaks we independently analyzed the weekly incidence of the 36 most prevalent serotypes in Israel, which accumulatively accounted for 95% of all isolates. Our analysis revealed 488 clusters from 1999 to 2011 in Israel, with 37 (95% CI 32 to 42) potential *Salmonella* outbreaks per year in average. Interestingly, three outbreaks of *S. typhimurium* (identified in 2001, 2005 and 2011) demonstrated identical PFGE profile and were recognized as DT104 MDR strains. This indicates the presence of a dominant *S. typhimurium* clone responsible for reoccurring outbreaks in Israel, currently under molecular and phenotypic characterization in our lab.

P-6

EMERGENCE OF MULTIDRUG RESISTANT *SALMONELLA ENTERICA* SEROVAR KENTUCKY IN ISRAEL

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Salmonella enterica serovar Kentucky (*S.* Kentucky) is an emerging food safety threat worldwide. Once a rare cause of foodborne Salmonellosis in Israel, the prevalence of *S.* Kentucky amongst poultry, food and faecal strains submitted to the National *Salmonella* Reference Centre, Jerusalem (NSRC), has increased >5-fold over recent years. These trends coincide with the recent spread of *S.* Kentucky in North Europe, Africa and the Middle East, involving an epidemic *S.* Kentucky ST198 strain, resistant to fluoroquinolones and other antimicrobials. We sought to determine the epidemiological and microbiological features of this microorganism in Israel. We analysed 78 human isolates from the years 1969-2011, for antimicrobial resistance profile using the Disk diffusion assay and VITEK2 system. In 2004, a unique dominant antimicrobial resistance profile emerged, involving resistance to fluoroquinolones which resembles the *S.* Kentucky ST198 strain. Interestingly, the antimicrobial resistance of *S.* Kentucky strains from the years 1969-2003 comprised agents that were not in clinical use at the time of isolate recovery. Molecular analysis of the recent isolates revealed the presence of ciprofloxacin resistant mutations and class I integron resistance genes similar to the ST198 pandemic strain. PFGE of 45 *S.* Kentucky strains presented 3 main clusters. While historical strains fell within 2 clusters, most recent isolates were assigned to a distinguished cluster resembling international strains. These data indicate that the recent increase of *S.* Kentucky in Israel is related to the international spread of *S.* Kentucky ST198 and highlight the need to closely monitor strain dynamics in the poultry and food industry.

P-7
THE GENOMIC AVENUE TO AVIAN COLISEPTICEMIA

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Avian pathogenic *Escherichia coli* (APEC) strains are the cause of an invasive infection, often leading to septicemia with high mortality and morbidity, resulting in significant economic consequences for the poultry industry. To understand the genetic basis of the virulence of avian septicemic *E. coli*, we sequenced the entire genome of the major pathogenic strain of serotype O78 responsible for about 50% of the colisepticemia cases worldwide. The genome was compared with the genome sequence of an APEC strains of serotype O1 and O2, as well as to the genome of nonpathogenic *E. coli* strain MG1655(K-12). Although there is a large variability in virulence-related genes between the APEC strains, several genes are conserved, and these are probably essential for colisepticemia. The majority of these genes are carried on a conserved ColV plasmid. The most prominent among these genes are the *iss* – increased serum survival and several systems for iron uptake. In order to understand the significance of the various genes for septicemia we constructed a systematic series of deletion of each of the relevant gene/operons and determined their survival in serum – the essential property for septicemia. The results indicate that there is a physiological overlap between the iron acquisition systems and single deletion mutants do not have a phenotypE.

P-8
TOWARDS CULTURING THE UNCULTURABLES

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The ability to culture microorganisms is one of the seminal challenges in microbiology. Culturing of organisms has allowed scientists to perform repeatable experiments on them, to categorize and to probe them, and to form strategies for understanding the microbial world. However, since the very first attempts to grow organisms in a lab, it has been known that the majority of microbes that live in the natural environment cannot be readily cultured. Painstaking work has been done in laboratories to determine nutritional requirements for organisms, typically in iterative and intuition-based fashion which requires a large amount of trial and error over multiple rounds of refinement. Most strikingly, genomic and systems biology tools have yet to enter the arena of medium prediction, despite the tremendous potential for these tools to aid in this important and basic goal. Here we leverage systems biology tools, including functional genome-scale metabolic models built from the genomes of thousands of species (provided to us through a collaboration with the SEED group at Argonne national labs), to build medium predictors for the culturing of organisms based on genomic data alone. We focus on developing minimal and selective media, a subset of which we validate via growth experiments. We then refine the predictors using a database we manually curated of known media for hundreds of microorganisms, and we present learned rules and a framework for improving our predictors and ultimately achieving our goal: prediction of novel media which will enable the culturing of currently unculturable organisms.

P-9

PHENOTYPIC CHARACTERIZATION OF DIFFERENT *HALOFERAX VOLCANII-HALOFERAX MEDITERRANEI* HYBRIDS

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Halophilic Archaea are known for their ability to cross the species barrier and preform inter-species gene exchange. The mechanism appears to involve cell fusion, where the first step includes a formation of an unstable intermediate stage of a hetero-diploid cell (containing the genetic information of both parental cells). These hetero-diploid cells tend to segregate to the original cells. However, sometimes this event leads to the formation of recombinant hybrids. Previous studies from our lab have shown that *Haloferax volcanii* and *Haloferax mediterranei* perform interspecies mating and recombination at relatively high frequencies. 10 different hybrids were generated by *H. volcanii - H. mediterranei* mating and their genomes were subsequently sequenced. Most of the genomic information in these hybrids is similar, but there is variability in the plasmids present in the cells and also in the recombination points in the genome. Here, we tested the different hybrids for their growth rate under different conditions and we show differences in the growth rate of the hybrids, including different response to presence of thymidine in the medium. We also tested the hybrids sensitivity to halocin (a form of "bacteriocin" produced by *H. mediterranei* that inhibits the growth of *H. volcanii* cells), and their ability to produce and secrete this protein compared to the parental strains.

P-10

A COMPARATIVE EVALUATION OF NB30, NB54 AND PTC124 IN TRANSLATIONAL READ-THROUGH EFFICACY FOR TREATMENT OF AN USH1C NONSENSE MUTATION

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Translational read-through-inducing drugs (TRIDs) promote read-through of nonsense mutations, placing them in the spotlight of current gene-based therapeutic research. Here, we compare for the first time the relative efficacies of new generation aminoglycosides NB30, NB54, which were synthesized in our lab, and the chemical compound PTC124 on retinal toxicity and read-through efficacy of a nonsense mutation in the USH1C gene, which encodes the scaffold protein harmonin. This mutation causes the human Usher syndrome, the most common form of inherited deaf-blindness. We quantify read-through efficacy of the TRIDs in cell culture and show the restoration of harmonin function. We do not observe significant differences in the read-through efficacy of the TRIDs in retinal cultures; however, we show an excellent biocompatibility in retinal cultures with read-through versus toxicity evidently superior for NB54 and PTC124. In addition, *in vivo* administration of NB54 and PTC124 induced recovery of the full-length harmonin a1 with the same efficacy. The high biocompatibilities combined with the sustained read-through efficacies of these drugs emphasize the potential of NB54 and PTC124 in treating nonsense mutation-based retinal disorders.

P-11

ANTIFUNGAL ACTIVITY OF NEW GROUP OF THE ORGANOPHOSPHORUS HETEROCYCLES 6-(DIALKOXYPHOSPHORYL)-3*H*-THIAZOLO[3,2-*b*][1,2,4] TRIAZOL-7-YLIUM CHLORIDES

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Five-membered heterocycles with two or three heteroatoms, such as imidazoles, thiazoles, triazoles and others, are key structural units in many pharmaceutical preparations, and especially, in the search and synthesis of antifungal drugs [1]. Recently we synthesized a new group of organophosphorus heterocycles 6-(dialkoxyphosphoryl)-3*H*-thiazolo[3,2-][1,2,4]triazol-7-ylium chlorides [2], which may be interesting as potential antifungal agents. Determination of antifungal activity of synthesized heterocycles relative to 21 pathogens of different mycotic infections was performed by serial dilutions in liquid nutrient medium. We found that diethyl [(Z)-1,2-bis[(1-phenyl-1*H*-1,2,3,4-tetrazol-5-yl)sulfanyl]ethynyl]phosphonate was most active against all test cultures, and especially against yeast-like fungi of the genus *Candida*. Thus, biological studies showed that this organophosphorus heterocycle had moderate antifungal activity against six test cultures of yeast-like fungi of the genus *Candida*: *Candida albicans*, *Candida utilis*, *Candida tropicalis*, *Candida krusei*, *Candida parapsilosis* and *Candida guillermondii*, and minimal fungistatic concentration varied from 6.25 to 50 mkg/ml^{-1}. This novel organophosphorus heterocycle diethyl [(Z)-1,2-bis[(1-phenyl-1*H*-1,2,3,4-tetrazol-5-yl)sulfanyl]ethynyl]phosphonate can be considered as a structural analogue of a well-known synthetic antifungal drug, fluconazole [3]. 1. Lass-Florl C. *Drugs*, vol. 71, No. 18, p. 2405-2419, **2011**. 2. Erkhitueva E. B., Dogadina A.V., Khramchikhin A.V., Ionin B.I. *Tetrahedron Lett.,* vol. 53, No. 33, p. 4304-4308, **2012**. 3. Charlier C., Hart e., Lefort A., et al. *J. Antimicrob. Chemother.,* vol. 57, No. 3, p. 384-410, **2012**.

P-12

FUNCTIONALITY OF MANGANESE PEROXIDASE ISOENZYMES FAMILY UNDER Mn^{2+} DEFICIENT CONDITIONS OF *PLEUROTUS OSTREATUS*

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Biodegradation of lignin is a central process in the global carbon cycle performed exclusively by white-rot-fungi, such as *Pleurotus ostreatus.* The ligninolytic system of *P. ostreatus* contains two types of extracellular peroxidases: five Manganese peroxidases (MnPs) (*mnp3*, *6*, *7*, *8* and *9*) and four Versatile peroxidases (VPs) (*mnp1*, *2*, *4* and *5*). VPs share with MnPs the ability to oxidize Mn^{2+} to Mn^{3+} that act as a diffusible oxidizer of low redox potential compounds. VPsare also able to directly oxidize aromatic compounds probably at two different catalytic sites. The functionality of *P. ostreatus* MnPs is different during its two growth phases in liquid GP medium: logarithmic (I) and stationary (II). In Mn^{2+}-deficient culture the peak level of *in vitro*VP activity was evident in phase II. Also, the azo dye Orange II (OII) was decolorized during phase II only. In contrary in Mn^{2+} sufficient medium the decolorization occurred at phase I. Three known metabolites of OII were identified under both conditions at different growth phases. This suggests that different mechanisms control this activity under the different conditions. Transcription level of the *mnp* gene family members during the incubation period indicates that *mnp4* is the predominantly expressed *mnp* isoensyme, mainly at phase II. Inactivation of *mnp4* resulted in drastic reduction in both VP activity and azo dye decolorization. We assume *mnp4* plays a key role in biodegradation of aromatic compound under these conditions and may possess unique characteristics.

P-13

COMBINATIONS OF NYSTATIN-INTRALIPID WITH OTHER ANTIFUNGAL DRUGS AGAINST *ASPERGILLUS TERREUS*

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Infections caused by *Aspergillus terreus* are difficult to treat. Thus, addition of treatment possibilities against *A. terreus* infections is required. Use of combinations of antifungal agents with different mechanisms of activity could be a rational approach to increase the therapeutic possibilities. Our laboratory has developed a new formulation of a polyene antifungal drug, nystatin: Nystatin-Intralipid (NYT-IL) which can be administered intravenously. NYT-IL showed higher in vitro activity against *A. terreus*, than to Nystatin. The present study focuses on investigation of combinations of NYT-IL with representative antifungals used clinically from 4 different classes: Voriconazole (azoles), Caspofungin (echinocandins), Tebinafine (allylamines) and 5-Fluorocytosine (antimetabolites), all in comparison to combinations with conventional NYT. Three to six clinical isolates of *A. terreus* were tested. In vitro activity of the antifungal combinations was assayed using the checkerboard technique, a method which indicates whether the combinations are synergistic, antagonistic or without effect on the activity of the individual drugs in the combination. The results showed that combination of NYT-IL with caspofungin produced a synergistic effect in 3 of the 6 tested strains while the others showed indifference, similar to the combination of NYT with caspofungin. Combination of NYT-IL or NYT with voriconazole did not change antifungal activity of the individual drugs. Combination of NYT-IL with terbinafine resulted in a strong antagonism, while combination of NYT with terbinafine revealed indifference or slight antagonism (in one strain). Combination of NYT-IL or NYT with 5- fluorocytosine showed indifference. In conclusion, regarding therapeutic options – combinations of NYT-IL and echinocandins seem promising.

P-14

RELEASE OF *PLEUROTUS OSTREATUS* VERSATILE-PEROXIDASE FROM Mn^{2+} REPRESSION ENHANCES ANTHROPOGENIC AND NATURAL SUBSTRATE DEGRADATION

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The versatile-peroxidase (VP) encoded by *mnp4* is one of the nine members of the manganese-peroxidase (MnP) gene family that constitutes part of the ligninolytic system of the white-rot basidiomycete *Pleurotus ostreatus* (oyster mushroom). VP enzymes exhibit dual activity on a wide range of substrates. As Mn^{2+} supplement to *P. ostreatus* cultures results in enhanced degradation of recalcitrant compounds and lignin, we examined the effect of Mn^{2+} on the expression profile of the MnP gene family. In strain PC9, *mnp4* was found to be the predominantly expressed *mnp* in Mn^{2+}-deficient media, whereas strongly repressed in Mn^{2+}-supplemented media, having negligible activity. We tested whether release of *mnp4* from Mn^{2+}repression alters the activity of the ligninolytic system. A transformant over-expressing *mnp4* (OE*mnp4*) under the *β-tubulin* promoter was produced. Now, despite the presence of Mn^{2+} in the medium, OE *mnp4* produced *mnp4* transcript as well as VP activity as early as 4 days after inoculation. The level of expression was constant throughout 10 days of incubation and the activity was comparable to that of PC9 in Mn^{2+}-deficient media. *In-vivo* decolorization of the azo dyes Orange II, Reactive Black 5 and Amaranth by OE *mnp4* preceded PC9. OE *mnp4* and PC9 were grown for 2 weeks under solid-state fermentation conditions on cotton stalks as a lignocellulosic substrate. [^{14}C]-lignin mineralization, *n-vitro* dry matter digestibility and neutral detergent fiber digestibility were found to be substantially higher in OE *mnp4*-fermented substrate, relative to PC9. We conclude that releasing Mn^{2+}suppression of VP4 by over-expression of *mnp4* improved *P. ostreatus* ligninolytic functionality.

P-15

ANTIFUNGAL DRUG INTERACTIONS: REPRESSORS AND ENHANCERS OF THE DEVELOPMENT OF RESISTANCE AND TOLERANCE

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Candida spp. are commensal yeasts in the human gastrointestinal tract, but also opportunistic pathogens, primarily associated with immune deficiency or antibiotic use. *C. albicans* is the most common species to cause systemic infections, followed by *C. glabrata*. The fungistatic drug fluconazole is the most common treatment for candidal infections, although the prevalence of strains that are tolerant or resistant to fluconazole is increasing. Furthermore, the identification of drug targets is complicated by the similarity of the eukaryotic metabolic pathways of fungal and mammalian cells. To extend the utility of existing antifungals, we are testing combinations of antifungal and other drugs for synergistic interactions; in addition, these studies have the potential to identify drug combinations that increase antifungal drug resistance and whose use should therefore be avoided. Examples of both types of interactions will be discussed.

P-16

SOCIAL EVOLUTION OF BACTERIAL REGULATORY NETWORKS - A POSITIVE FEEDBACK NETWORK STRUCTURE PROMOTES THE EVOLUTIONARY STABILITY OF ALTRUISTIC COOPERATION

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Many theoretical studies in the last 50 years have tried to explain the evolutionary maintenance of cooperative altruistic behavior in the face of exploitive behaviors. It is clear that a population structure with high relatedness is crucial for cooperation to be maintained; however, little is known about how this requirement is affected by the regulatory structure of the cooperative trait. In this work we examine the role of feedback regulation in maintaining cooperation in a public goods game model. Our results suggest an important advantage of positive feedback regulation over constitutive expression and negative feedback regulation: a positive feedback structure promotes cooperation even in low relatedness populations, thus relieving a major limitation of explaining cooperation. These results are specifically interesting in the context of bacteria, since they utilize multiple secreted molecules to modify their environment (e.g., exoenzymes, surfactants, antibiotics etc.). These secreted public goods are often self-regulated through sensory mechanisms. Elucidating the role of these feedbacks may lead to experimentally amenable predictions.

P-17

CONTRIBUTION OF LATERAL GENE TRANSFER TO THE ADAPTATION OF *METHANOSPHAERA STADTMANAE* TO THE MAMMALIAN GUT

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Methanosphaera stadtmanae is a commensal methanogenic archaeon found in the human gut, a niche predominantly colonized by bacteria. It is therefore expected that lateral gene transfer (LGT) from bacteria to archaea will play a role in the evolutionary history of this organism, contributing to its adaptation to its human host. Notably, a recent analysis of *M. stadtmanae*'s closest phylogenetic neighbor, *Methanobrevibacter smithii*, which inhabits the same niche, revealed evidence of extensive LGT between this organism and bacteria. We therefore performed a phylogenomic survey of putative LGT events in *M. stadtmanae*, using a phylogenetic pipeline, and creating phylogenetic trees using both distance-based and likelihood-based methods. Our analysis indicates that a substantial fraction of the proteins of *M. stadtmanae* (about 15%) are inferred to have been involved in inter-domain LGT. Laterally acquired genes have had a large contribution to surface functions, by providing novel glycosyltransferase functions. In addition, several ABC transporters seem to be of bacterial origin, including the molybdate transporter. Thus, bacterial genes contributed to the adaptation of *M. stadtmanae* to a host-dependent lifestyle by allowing a larger variation in surface structures and increasing transport efficiency in the gut niche which is diverse and competitive.

P-18

A ONE-SIDED ARMS-RACE BETWEEN *PROCHLOROCOCCUS* AND THE CYANOPODOPHAGES INFECTING THEM

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The abundant marine cyanobacteria of the genus *Prochlorococcus* coexist with their cyanophage parasites. Yet hyper-diversity in phage attachment genes suggests that only a small fraction of the population is actually available for infection to a phage. How then do cyanophages survive on this seemingly small host population? A mutational arms-race is often thought to mediate antagonistic coexistence between parasites and hosts. Therefore, we attempted to isolate cyanopodophage host-range mutants that can infect previously isolated phage-resistant *Prochlorococcus* mutants. While host mutations to phage-resistance are frequent, cyanopodophage host-range mutants were rare and often undetectable, even after increasing mutation frequency with chemical mutagens. Nonetheless, some cyanopodophage host-range mutants that infect viral-resistant strains were observed and isolated. Although isolated phage mutants infect strains to which their wild-type phage ancestor cannot attach, the attachment rate of the mutants to these host strains remained low, being lower than the detection limit of our adsorption assays. Furthermore, no change was observed in either adsorption rate of mutant phages to the wild-type host or latent period length. Genetic identification of cyanophage host-range mutations will soon be completed. These data suggest that a genetic barrier prevents cyanopodophages from reinfecting most phage-resistant hosts, leading us to reject the arms-race model. Instead, we propose that coexistence in this system relies on passive host-switching. In this model a phage lacking a host may gain one due to mutations incurred in a non-host cell that make it susceptible to this phage, but grant it resistance to other phages in the community.

P-19

THE AGENT OF LATE WILT OF CORN, *HARPOPHORA MAYDIS*, PATHOGENESIS AND CONTROL

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Late wilt is a severe vascular disease of maize caused by the fungus *Harpophora maydis*. In Israel, the disease appeared in the Upper Galilee during the 90's, and spread southward. The pathogen is a seed-borne as well as soil-borne, and survives for long periods in the soil. It is currently controlled using maize varieties with reduced sensitivity. However, virulent variants of the fungus may threaten these varieties. We have modified a molecular method for diagnostic assay of disease progress in infested plants. The assay identified the pathogen 50 days after seeding, before the appearance of symptoms, in both susceptible and tolerant host plants. Two plant hormones and several fungicides suppressed *H. maydis* in-vitro. A seedlings pathogenicity assay identified *H. maydis* DNA in the host tissues, mainly in the root, 22 days after sowing, in both susceptible and tolerant maize plants. Although the infested plants showed no wilt symptoms, their roots were significantly shorter in length. This seedlings assay used to demonstrate the suppressive effect on *H. maydis* virulence, by the fungicide Flutriafol. Although this fungicide was less efficient in the field, another fungicide, Azoxystrobin (112.5 g per ha) inhibited the development of wilt symptoms and recovered cobs yield by 100%. However, this treatment didn't result in decreasing amounts of pathogen DNA in the host tissues or delaying its spread.

P-20

ANTIMICROBIAL ACTIVITY IN *PISTACIA ATLANTICA* GALLS

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Gall-formers are considered as parasitic organisms manipulating plant traits for their own benefit. Gall-formers of many domains are known, including more than 1,440 gall-forming species of insect from at least five different orders. Insect's galls have been shown to protect their inhabitants from natural enemies such as predators and parasitoids by various chemical and mechanical means, but much less attention has been given to defence against microbial pathogens likely to grow in the humid and nutrient-rich gall environment. The large, cauliflower-shaped, galls formed by *Slavum wertheimae* on buds of *Pistacia atlantica* have been shown to host thousands of aphids, and their sugar rich secretion for up to 8 month, suggesting such protection could be of benefit to the inhabiting aphids. We have if S. wertheimae galls do indeed have antimicrobial properties using plate diffusion assay and essential oils testes on bacteria and filamentous fungi. Our results suggest that indeed those galls do express antibacterial and antifungal activities distinct from those found in non-galled leaves. Antibacterial activity was especially profound against *Bacillus* spp. (known insect pathogen) and against *Pseudomonas aeruginosa* (known plant pathogen). Antifungal activity was demonstrated against multiple filamentous fungi. Our results provide experimental evidence for a new protective antimicrobial role of galls. These results suggest not only *S. wertheimae* galls as a possible source for antimicrobial compounds but also call for an exmination of other gall systems as a possible source for such compounds.

P-21

CONTROL OF PROTEIN MISFOLDING AND DEVELOPMENTAL CUES IN *LEISHMANIA*

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Trypanosomatids are unicellular, flagellated diploid protists that lead digenetic life cycles, migrating between invertebrate vectors to mammalian hosts. It has been established that shifts in temperature and pH induce the developmental program of gene expression. Temperature elevation causes protein misfolding in all living cells, along with other cellular and molecular damages. Chaperones play a key role in recovery from such stresses, and are therefore highly expressed in these organisms. Thus, a delicate homeostasis is required for maintaining the quality control of proteins in the cell. Here we examine to what extent the temperature switches lead to protein damage in *Leishmania*, immediately after exposure to the environmental stresses, and at later stages during differentiation. This was done by monitoring the activity of a reporter gene (luciferase) at different conditions, and its tendency to aggregate, as a result of misfolding. Transgenic parasites expressing luciferase under control of hsp regulatory signals were tested for reporter activity following gradually increasing incubations at mammalian temperatures. Our results indicate that luciferase activity is dramatically reduced immediately after temperature elevation, and along differentiation, while expression levels remained unaffected. Thus, temperature elevation caused misfolding of the luciferase reporter, and its aggregation. We further examined the effects of temperature elevation on a GFP reporter, specifically testing whether inclusion of polyGln stretches at different lengths contributed to its aggregation, as observed in *C. elegans.* We conclude that *Leishmania* parasites experience a typical heat shock response during differentiation, which they must overcome in order to differentiate into amastigotes.

P-22

BIOFILM FORMATION OF *BACILLUS* SPECIES IN MILK IS GOVERNED BY THE *TAPA-SIPW-TASA* OPERON

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Bacteria of the *Bacillus* genus present a major problem in the milk industry. Contamination of milk by *Bacillus* species leads not only to a financial loss due to milk spoilage, but also is a health hazard, since some strains are pathogenic to humans. Moreover, *Bacillus* species have the capability of forming spores and hence withstand pasteurization processes. Among various species colonizing surfaces of dairy industry, *Bacillus subtilis* is one of the most ubiquitous bacterium. This bacterium is capable of forming biofilms, which are elaborate multicellular communities. Another related bacterium, *Bacillus cereus,* is also involved in spoilage of dairy products. Moreover, some of *B. cereus* strains are considered highly pathogenic to human. Biofilm formation depends on the synthesis of an extracellular matrix that holds the constituent cells together. The matrix has two main components, an exopolysaccharide synthesized by the products of the *epsA-O* operon, and amyloid fibers encoded by *tasA* located in the *tapA* (formerly *yqxM*) operon. In the presented research, we studied the ability of *B. subtilis* and *B. cereus* to form biofilms within milk. We show that when grown in milk, the cells of both strains undergo extensive bundling, a structure found to be characteristic for biofilms. Importantly, we demonstrated that within these bundles expression of the *tapA* gene is markedly increased. Our results demonstrate for the first time that formation of biofilm related structures within milk depends on the *tapA-sipW-tasA* operon which is highly conserved amongst *Bacillus* species.

P-23

CASTING A NET: FIBRES PRODUCED BY *MICROCYSTIS* SP. IN FIELD AND LABORATORY POPULATIONS

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The reasons for the apparent dominance of the toxic cyanobacterium *Microcystis* sp., reflected by its massive blooms in many fresh water bodies, are poorly understood. We show that in addition to a large array of secondary metabolites, some of which are toxic to eukaryotes, *Microcystis* sp. secretes large amounts of fibrous exopolysaccharides that form extremely long fibres several millimetres in length. This phenomenon was detected in field and laboratory cultures of various *Microcystis* strains. In addition, we have identified and characterized three of the proteins associated with the fibres and the genes encoding them in *Microcystis* sp. PCC 7806 but were unable to completely delete them from its genome. Phylogenetic analysis of the most abundant one, designated IPF-469, showed its presence only in cyanobacteria. Its closest relatives were detected in *Synechocystis* sp. PCC 6803 and in *Cyanothece* sp. strains; in the latter the genomic organization of the IPF-469 was highly conserved. IPF-469 and the other two proteins identified here, a haloperoxidase and a haemolysin-type calcium-binding protein, may be part of the fibres secretion pathway. The biological role of the fibres in *Microcystis* sp. is discussed.

P-24

INTERACTION BETWEEN *SCENEDESMUS* AND *MICROCYSTIS* MAY BY USED TO CLARIFY THE ROLE OF SECONDARY METABOLITES

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Microcystis sp. are major players in the global intensification of toxic cyanobacterial blooms endangering the water quality of freshwater bodies. A novel green alga identified as *Scenedesmus* sp., designated strain huji (hereafter *S. huji*), was isolated from water samples containing toxic *Microcystis* sp. Withdrawn from Lake Kinneret (Sea of Galilee), Israel, suggesting that it produces secondary metabolites that help it withstand the *Microcystis* toxins. Competition experiments suggested complex interaction between these two organisms and use of spent cell-free media from *S. huji* caused severe cell lysis in various *Microcystis* strains. We have isolated active metabolites from the spent *S. huji* medium. Application of the concentrated allelochemicals interfered with the functionality and perhaps the integrity of the *Microcystis* cell membrane, as indicated by the rapid effect on the photosynthetic variable fluorescence and leakage of phycobilins and ions. Although some activity was observed towards various bacteria, it did not alter growth of eukaryotic organisms such as the green alga *Chlamydomonas reinhardtii*

P-25

BLACK BAND DISEASE BACTERIAL CONSORTIUM IN RED SEA CORALS

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Coral Black Band Disease (BBD) was the first coral disease to be documented; four decades later, BBD is considered a well-described, but as yet, unresolved disease plaguing corals worldwide. To date, a primary pathogen has not been characterized and the disease is believed to be caused by a complex polymicrobial consortium. In this study we demonstrate that the microbial communities associated with necrotic tissue of BBD-affected coral colonies are diverse and dynamic. In the highly-active (summer) stage BBD samples showed low microbial richness and diversity; a higher microbial richness and diversity was evident in the dormant (winter) phenotype in the same corals the following winter. Three main components of the BBD mat investigated here include cyanobacteria, SRB and vibrios. Cyanobacteria-OTU (99% homologous to *Pseudoscillatoria coralii*) in the BBD mat was several orders of magnitude higher than those in the healthy-apparent tissues of the affected corals. *P. coralii* cells showed temperature-independence and dominated the BBD-affected coral samples in both highly-active and dormant black band phenotypes – indicating that infected coral skeleton may serve as a reservoir for BBD cyanobacteria. In addition, *Vibrio*-clones associated with highly-active bands were similar to known pathogens, while dormant clones were similar to general aquatic *Vibrio* sp. Cultured BBD isolates of *Vibrio* sp. were highly homologous to previously documented BBD-associated vibrios from the Caribbean, Bahamas and Red Sea, as well as to other known coral pathogens, and showed typical proteolytic activity, which correlated with water temperature elevation. A BBD-unique clade of SRB was observed.

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A SINGLE MOLECULE AMPLIFICATION METHOD REVEALS THAT T7-LIKE CYANOPHAGES ENCODING THE *PSBA* GENE ARE ABUNDANT IN THE RED AND MEDITERRANEAN SEAS

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Bacteriophages are extremely abundant in the marine environment. They influence many aspects of microbial processes including the population dynamics and evolution of their hosts. Cyanobacteria of the genera *Synechococcus* and *Prochlorococcus* are the numerically dominant primary producers in the oceans and contribute significantly to global primary production. They are infected by various phage families including T7-like cyanophages. Based on DNA polymerase gene phylogeny we found that two discrete T7-like lineages exist. Phages from one lineage encode the *psbA* photosystem II gene whereas phages from the other lineage do not. In order to begin understanding the impact these cyanophages have on cyanobacterial mortality we developed a culture-independent method for the identification and quantification of T7-like cyanophages based on the single molecule polony technique. Using degenerate primers for the DNA polymerase gene we identified and quantified T7-like cyanophages from the Red and Mediterranean Seas and used specific probes to differentiate between the two lineages. We found that cyanophages from the lineage encoding *psbA* are considerably more abundant than phages from the lineage lacking this gene. This suggests that cyanophages that have evolved to carry the *psbA* gene are the more successful lineage and infect more cyanobacteria. This methodology has enabled us to determine, for the first time, differential distributions and abundances of discrete lineages of phages that infect a particular host taxon and greatly improves on current culture-dependent methods. We are further developing this methodology for the identification and quantification of other cyanophage families and of infected cyanobacterial cells.

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MICROBIAL COMMUNITIES RELATED TO ELECTRON ACCEPTORS GRADIENT IN LAKE KINNERET SEDIMENT

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Methane (CH_4) is the most abundant organic trace gas in the atmosphere and the second most important greenhouse gas. Methane concentration in the atmosphere more than doubled in the last 300 years. Calculations of the net methane emission from aquatic environment indicate on only ~6% of the total natural methane emission. Methane production (methanogenesis) occurs mainly by microorganisms in anaerobic environments, and the (AOM) oxidation of methane (methanotrophy) is also microbial. In freshwaters, Methanotrophy was shown to be mainly aerobic, while in marine sediments AOM occurs through sulfate by consortium of bacteria and archaea. In this study a novel process of iron oxide reduction coupled to AOM in the sediments of Lake Kinneret (LK) is shown by geochemical and microbial evidences. This novel process can shed more light on why methane emissions are low in this aquatic environment. The results from *in-situ* geochemical analysis of porewater and incubation experiments indicated a sink for methane below the depths at which nitrate and sulfate are completely exhausted and below the zone of methanogenesis. Therefore suggests that there is iron dependent AOM in the deep sediments. 16S rRNA gene libraries sequencing analysis from three different sediment depths show the structure and diversity of bacteria and archaea along the electron acceptors gradient. Together with qPCR of different functional genes, the phylogenic analysis of the microbial communities discovered a community's shift with depth and with different electron acceptors which can help indicate the microbial ecology that involve in the biogeochemical processes in the deep LK sediment.

P-28

NATURAL QUORUM SENSING INHIBITORS FROM PREVIOUSLY UNCULTURED MARINE BACTERIA

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Marine bacteria are emerging as an exciting resource for the discovery of new classes of bioactive agents that may have a therapeutic effect such as quorum sensing (QS) inhibitors and inductors in the marine environment. The goal of this study is to detect new QS inhibition materials mainly from previously uncultured coral bacteria, to isolate and elucidate the structure of those compounds and to produce or synthesize the relevant molecules. The main method used in this study is a novel culturing technique developed in our lab, employing agar spheres in which previously unculturable microorganisms were entrapped. The polymeric membrane coat enables molecules to diffuse in and out of the sphere without losing the encapsulated bacterial cells, enabling the growth of bacteria that have not yet been cultivated. Samples were collected from the coral mucus layer of the stony coral *Favia* sp., from the reef in the Gulf of Eilat. The samples were diluted, encapsulated and returned to the reef for five weeks of incubation. Then the spheres were tested for their QS inhibition or induction abilities using bioreporters strains. The bacteria from the spheres that were found to have inhibition abilities were diluted and encapsulated again for in-situ incubation, then the QS inhibition potential was tested again to show it is reproducibility. The bacteria from the spheres were molecularly analyzed, in order to determine the bacteria population that caused the QS inhibition, the chemical entities will be tested and characterized using chemical analysis in the future.

P-29

MODELING OF BACTERIOPHAGES AND BACTERIA PREDATOR-PREY DYNAMICS IN BIOLOGICAL WASTEWATER TREATMENT PROCESSES

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The microbial community evolving in biological wastewater treatment plants is crucial for their performance. This microbial community is composed of both bacteria and bacteriophages. Until recently, most research efforts have focused on the bacterial community, while bacteriophages were kept in the shade. We believe that bacteriophages may have a major influence on the bacterial population and consequently on process efficiency. In this study, the predation dynamics between bacteriophages and bacteria were investigated using mathematical models. We developed equations that describe both populations and the changes in substrate concentration. The parameters of the equations were calibrated by comparing the calculated results with experimentally achieved results. Our findings show that mathematical models can be used as a viable tool for investigating the influence of bacteriophages on the bacterial population and substrate degradation in wastewater treatment plants.

P-30

ADAPTATION TO LIFE AT HIGH SALT CONCENTRATIONS: GENOMIC AND METAGENOMIC STUDIES OF THE ABUNDANCE OF ACIDIC PROTEINS IN HALOPHILIC MICROORGANISMS

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Two strategies are known in the prokaryotic world to cope with high salt concentrations. One common strategy is the biosynthesis and/or accumulation of 'compatible' solutes (glycine betaine, ectoine, etc.). No drastic modifications of cellular proteins are then needed. The halophilic Archaea (Halobacteriaceae) and the bacterial genus Salinibacter (Bacteroidetes) accumulate KCl and they have adapted their intracellular proteome to function at high salt concentrations. Their proteins show a large excess of acidic amino acids over basic amino acids. Accordingly, the proteins encoded by the metagenome of the Dead Sea were highly acidic, as expected based on dominance of Halobacteriaceae in the community. Unexpectedly, a significantly acid-shifted proteome was also reported in a microbial mat in Guerrero Negro, Mexico, at a relatively low salinity (9%) at which only low-salt-in strategists are expected to develop. Therefore we surveyed the abundance of acidic proteins encoded by the genomes of moderate halophiles (Halomonas elongata, Chromohalobacter salexigens) and marine bacteria (Alteromonas, Aliivibrio); all showed markedly low average isoelectric points. Halophilic fermentative bacteria (order Halanaerobiales) were earlier shown to accumulate KCl, and therefore an acidic proteome was predicted. A large excess of acidic amino acids was also reported in cell hydrolysates.However, the genomic analysis did not show high contents of acidic amino acids in the encoded proteins. The earlier reported excess of acidic amino acids is due to a high content of glutamine and asparagine, which yield glutamate and aspartate upon acid hydrolysis.

P-31
METABOLIC PATHWAY ENRICHMENT IN VIRAL METAGENOMES

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Viral genomes often contain metabolic genes that originate from microbial genomes (auxiliary genes). It is assumed that these genes were fixated in viral genomes as a result of a selective force, favoring viruses that acquired specific metabolic functions. The higher fitness of viruses that carry auxiliary genes results in enrichment of these genes among the viral population, compared to the entire gene pool. While some auxiliary genes are known to be enriched in viral genomes, there is a great importance in assessment of the overall metabolic functions they promote. In this work we wish to indentify enriched viral auxiliary genes and to map these genes to metabolic pathways, to better understand which metabolic functions are important for viral reproduction. We have used >20,000 metagenomic reads from the GOS (Global Ocean Survey) dataset that were previously characterized as viral reads/scaffolds to search for enriched auxiliary genes, compared to the entire GOS dataset. All reads were annotated using the KEGG Ortholog (KO) database and enriched KO's were mapped to KEGG pathways. We have found 140 KO's in the viral reads, 60 of these were found enriched. The enriched KO's were mapped to 33 KEGG pathways. We show that the pathways with the highest enrichment score are Purine and Pyrimidine metabolism and that many other pathways are linked to these pathways. Finally, we hypothesize that many of the metabolic functions found in viral metagenomes serve the overall function of nucleotide biosynthesis and by that increase viral reproduction.

P-32
TEMPORAL ANTHROPOGENIC EFFECTS ON SOIL MICROBIOME- THE CASE OF TREATED WASTE WATER IRRIGATION

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Agricultural irrigation with treated wastewater (TWW) is a common and increasing practice in arid and semi-arid regions, and is used as an alternative to freshwater (FW). However, irrigation with TWW, which may contain dissolved organic matter, salts and microorganisms, may alter the total microbial community composition and function.This work characterized the effects of irrigation of agricultural soils on community composition by the extraction and analysis of DNA and rRNA from these soils. The microbial composition was studied by next generation sequencing using tag-encoded FLX amplicon pyrosequencing (bTEFAP) approach of the PCR amplified 16S gene, 16S rRNA and amoA gene. The soil community composition was highly similar in different soil samples obtained at the end of winter (rain season) regardless of the summer irrigation type. However, while community composition in FW irrigated soils slightly shifted during the summer irrigation season, the community in TWW irrigated soils shifted to a much greater degree, especially when active community was analyzed (based on cDNA obtained from 16S rRNA). The ammonia oxidizing bacterial community reacted less to seasonality but in a much greater degree to TWW irrigation. Further, few ammonia oxidizing bacteria operational taxonomic units (such as those related to *Nitrosomonas nitrosa*) were almost exclusively affiliated to soil irrigated with TWW. Despite the change in composition, the community structure was moderately affected, as diversity indices changes were insignificant. We concluded that the microbial communities in Mediterranean and semi arid agricultural soil are highly influenced by seasonal irrigation and the use of TWW increases these changes.

P-33

ISOLATION AND CHARACTERIZATION OF A CYANOPHAGE CARRYING PSI GENES

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Cyanobacteria play a key role in marine photosynthesis, which contributes to the global carbon cycle and the world oxygen supply. A decade ago, marine cyanophages were discovered to carry photosystem II (PSII) genes as well as genes from the electron transport chain. It was suggested that these genes increase phage fitness. More recently, cyanobacterial core photosystem I (PSI) genes were identified on environmental DNA thought to come from viruses. These genes, including *psaA, B, C, D, E, K* and a unique *J* and *F* fusion, form a unique cluster on cyanophage genomes, suggestive of selection for a distinct function in virus reproduction. Furthermore, the seven proteins encoded by the viral genes are potentially sufficient for forming an intact monomeric PSI complex. However, prior to this study, no virus with PSI genes had been isolated. Using a screening scheme devised specifically to detect these phages, we isolated a phage from phage-concentrates collected from the Line Islands (central Pacific Ocean) with *Prochlorococcus* MIT9515 as host. The existence of the PSI gene-cluster was confirmed by PCR. In order to study the infection cycle, a one–step growth assay was used. Host range analysis determined that the phage is host specific. In addition, transmission electron microscopy and PCR with primers for the portal protein (g20), established that the phage is a T4-like myovirus. Based on these preliminary PCR results we are confident that a T4-like cyanomyophage carrying PSI genes has been isolated. However, confirmation by whole genome sequencing of the phage is still needed.

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CHARACTERIZATION OF THE RUMEN METHANOGENIC COMMUNITIES AND THEIR CONTRIBUTION FOR METHANE EMISSION

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Ruminant livestock produce approximately 80 million tons of methane annually, accounting for roughly 28% of global methane emissions from human-related activities. Methane (CH_4) is a greenhouse gas 21-25 times more potent than carbon dioxide (CO_2) as a heat trapper in the atmosphere- significantly contributing to the greenhouse effect and global warming. Methane is produced by strict anaerobes methanogenic archaea that utilize hydrogen to reduce CO_2 to methane. The ratio of dietary fiber within the animals feed is thought to effect methane emission. Nevertheless, the effect of dietary fiber on the composition and abundance of methanogenic archaea within the rumen is limited. In this work I characterized the kinetics of change in abundance of the three main orders of methanogens that are known to house the rumen, as well as the overall composition of the archaea community within the rumen as a function of fiber content within the diet. My results indicate that overall number of the archaeal communities did not change with the diet nevertheless; substantial changes were documented at the polygenetic composition of the methanogenic communities.

P-35

ANTIBIOTIC RESISTANCE IN UNDISTURBED SOILS: A POTENTIAL SOURCE OF CLINICAL ANTIBIOTIC RESISTANCE?

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Antibiotic resistance (AR) is one of the most important challenges in the treatment of infectious diseases. AR has been documented against natural, semi-synthetic and synthetic antibiotics. A wide range of recently published experimental data strongly suggests that AR mechanisms originated in bacteria from soils and other natural environments and expanded to clinical bacteria via horizontal gene transfer (HGT). In our study, we assess and explore the impact of selective pressure (antibiotic) on soil AR and the scope of the native soil resistome. Thus, in bench-scale microcosm experiments, characteristic Israeli and Hawaiian soils were exposed to clinical doses of ceftriaxone; a third-generation cephalosporin (class of β-lactam antibiotic) with broad spectrum activity. The results obtained indicate that: i) the natural soil microbiome is highly resistant to next-generation semi-synthetic antibiotic compounds (ceftriaxone), ii) soil irrigation with ceftriaxone does not significantly impact microbial biochemical activity or bacterial community composition, iii) only very high doses of ceftriaxone, in slurries experiments, were able to induce changes in microbial community composition, and iv) soil bacteria contain several extended spectrum β-lactamase (ESBL) genes know to be common in multi-resistant human pathogens. We conclude that soil bacteria are highly resistant to antibiotics. Our current studies are focused on assessing the capacity of the soil microbiome to transfer these AR mechanisms to clinical bacteria, and thus attempt to identify the next generation of clinically-relevant antibiotic resistance mechanisms.

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MUTATIONS IN PORIN-LIKE GENES IN THE MARINE CYANOBACTERIUM *SYNECHOCOCCUS* SP. WH8109 CONFER RESISTANCE TO T7-LIKE PODOVIRUSES

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Cyanobacteria of the genus *Synechococcus* are widespread in the oceans as are the podoviruses that infect them. Their co-occurrence in nature suggests that resistant cells live among susceptible cells, but how resistance to podoviruses affects the genome of *Synechococcus* has yet to be explored. In order to learn which host genes are under virus selection whole genome sequencing of resistant *Synechococcus* WH8109 strains was carried out. Analysis of 35 substrains that were selected for resistance to 2 podoviruses from different lineages, S-TIP37 and Syn5, revealed that mutations conferring resistance converged at 2 paralogous porin-like genes encoding for Porin P and SomB. Sixteen strains had mutations in both porin-like genes as well as in other genes, while 2 strains had only a single mutation: one in SomB and the other in PorinP. Cross resistance tests showed that resistant strains selected with S-TIP37 were resistant to Syn5 and vice versa, but not to 4 myoviruses that were tested. These findings indicate that these porin-like genes may play a key role in the infection of *Synechococcus* WH8109 by both podoviruses despite their belonging to different lineages.

P-37

INTERACTIONS BETWEEN *PROCHLOROCOCCUS* AND HETEROTROPHIC MARINE BACTERIA

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Interactions between marine microorganisms such as symbiosis, competition, and allelopathy determine the structure and function of microbial communities. We must understand these interactions at multiple levels in order to predict how marine microbial communities will evolve in a changing world. Here, we propose an approach to parameterize and test mathematical models of microbial interactions, incorporating experimental data on the physiological processes and gene expression patterns occurring during co-culture between *Prochlorococcus,* the most abundant photosynthetic organism in the oceans, and heterotrophic marine bacteria. We aim to explore the complexity needed to represent microbial co-cultures, ideally obtaining a modeling framework that can be generalized and applied to large-scale ecological and biogeochemical models. Finally, the very act of trying to write down a set of equations to represent microbial growth and death brings into focus gaps in our understanding of microbial dynamics in natural environments, and identifies needs for new experimental data on the kinetics of growth, senescence and death in microbes.

P-38

MUNICIPAL WASTES AS A POOL FOR HORIZONTALLY TRANSERABLE ANTIBIOTIC RESISTANCE GENES

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Sewage treatment plants that employ the activated sludge (AS) process produces large quantities of biosolids, which are frequently applied to fields following stabilization. Along with the obvious benefits of using AS as fertilizer, there are several potential disadvantages and risks associated with its long-term application to soil. One of the main risks is that it contains many anthropogenic recalcitrant compounds such as detergents and antibiotics. Bacteria in the AS that are exposed to these compounds may become extensively more resistant to antibiotics due to selective pressure. Antibiotic resistance genes carried on mobile genetic elements (MGEs) such as plasmids or integrons are of special concern, because they can be horizontally transferred to the soil microbiome, which can serve as a reservoir of human pathogens. The goal of this study was to assess the phenotypic and genotypic scope of antibiotic resistance in raw sewage and biosolids and determine whether antibiotic resistant bacteria and MGE-associated antibiotic resistance genes are enriched in the AS process. We isolated *Enterobacteriace* showing resistance to Ciprofloxacin, a member of the quinolone family that was used as a model antibiotic compound. WE detected several variants of the plasmid associated quinolone resistant gene *qnr* (A, B and S), in isolates from both raw sewage and biosolids. Many of the isolates harbored the class 1 integron-associtated *intl1* integrase gene, which is frequently associated with antibiotic resistance. The scope and abundance of antibiotic resistances and of the qnr and intI genes in AS isolates is very similar to that found in raw sewage isolates.

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IDENTIFICATION OF GENETIC TRAITS ASSOCIATED WITH ECOLOGICAL NICHE ADAPTATION IN THE GENUS *FLAVOBACTERIUM*

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Members of the Gram negative genus *Flavobacterium* colonize a wide range of ecological niches. They are ubiquitous in aquatic and terrestrial environments where their abundance can reach up to 30% of the total bacterial community. *Flavobacterium* are well known for their role in degradation/turnover of organic matter, especially poorly degradable macromolecules such as chitin and cellulose. Phylogenetic analysis of the genus based on 16S rRNA genes and whole genomes, revealed two distinct clades, aquatic and terrestrial. Comparative analysis of clades affiliated genomes showed high similarities between housekeeping genes, but significant variation in functional genes. The genomes of the terrestrial strains were approximately twice the size of their aquatic counterparts, and contained significantly higher relative abundance and diversity of genes involved in the utilization of plant related sugars (pectin, xylose and arabinose), denitrification and defense systems especially antibiotic resistance mechanisms. Several functional genes detected in the soil clade, such as certain chitinolytic-encoding genes, were most closely related to non-*Bacteroidetes* sequences, indicating that these were acquired by lateral gene transfer from the other organisms. In contrast, the aquatic genomes were enriched with CRISPRs and genes associated with bacterial capsulation; suggesting that protection against bacteriophages may be more crucial in aquatic environments. Collectively, this data suggests a strong correlation between genomic elasticity, metabolic flexibility and environmental adaptability, even within individual genera. We hypothesize that lower stability and increased spatial and temporal heterogeneity in soil environments necessitates higher flexibility; explaining the larger genome size and broader metabolic capacity of the terrestrial flavobacterial clade.

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SUCCESSION AND ACTIVITY OF RHIZOSPHERE BACTERIAL COMMUNITIES DURING TOMATO PLANT DEVELOPMENT

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Rhizosphere bacteria can positively impact plant health and physiology. Recent studies suggest that biochar soil amendment can increase crop yields and stimulate plant defense systems; a phenomenon potentially attributed to its effect on the rhizosphere microbial community. Despite the advantages of biochar soil amendment, its application is still restricted due to limited knowledge regarding its mode of action. The aim of this study was to estimate the impact of biochar amendment on rhizosphere bacterial metabolic potential and community composition during tomato plant development. To illustrate root-associated bacterial succession during plant development we combined pyrosequencing analyses of 16SrRNA genes with estimations of the microbial metabolic potential at four distinguished time points during tomato plant growth, with and without biochar amendment. This analysis indicated that *Bacteroidetes* increased with time, whereas *Proteobacteria* decreased, and collectively these two phyla represented more than 85% of the total bacterial community. We observed significant differences between metabolic activities and bacteria community diversity in presence of biochar at each time point during plant growth. *Flavobacterium* was one of the dominant genera in the rhizosphere, exceeding 25% of the total bacteria population at all time points measured; and dramatic changes were observed in the abundance of specific *Flavobacterium* taxa (based on 97% OTU level) when biochar was applied. For example, in the presence of biochar some flavobacterial OTU's completely disappeared from the community, while others appeared. In summary, this study revealed that plant age strongly dictates rhizosphere bacterial community composition, whereas the impact of biochar amendment is more moderate.

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CHARACTERIZATION OF NATURAL DEGRADATION OF CHLORO-ALIPHATIC COMPOUNDS IN POLLUTED GROUNDWATER IN THE HOLON INDUSTRIAL AREA

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The aquifer under Holon's industrial area is plagued by high concentrations of trichloroethylene (TCE), which is poisonous and potentially carcinogenic. We combined chemical, isotopic, and microbial analyses to assess the scope of natural attenuation of TCE in the Holon aquifer. Decrease in TCE concentrations were not detected over a three year sampling period, and stable carbon isotope ($\delta^{13}C$) analysis revealed no significant changes in the isotopic composition of TCE along the plume. Furthermore, high-throughput sequencing targeting microbial community composition failed to identify *Dehalococcoides-ethenogenes*-like phyla, the only known bacterium capable of fully reducing TCE to ethylene. Nonetheless, selected wells contained low levels of *Dechloromonas*, associated with partial reduction of TCE to dichloroethylene. Several ammonia- and methane-oxidizing bacteria were also detected in the wells. Ammonia and methane monooxygenases produced by these two groups may generate aerobic TCE degradation, suggesting that this process may be significant in this primarily-aerobic aquifer. A strong correlation was detected between microbial community composition and the geographic location and physiochemical characteristics of the wells. *Acidobacteria* and *Proteobacteria* were the primary phyla in all of the wells. *Proteobacteria* consisted primarily of comomonad and pseudomonads strains, which have been linked to biodegradation of recalcitrant contaminant in aquifers. Microbial, isotopic, and chemical analyses indicate that natural attenuation of TCE in the Holon aquifer is negligible. Nonetheless, the presence of TCE-reducing bacteria suggests the potential for TCE reduction under the proper redox conditions. Currently research is focusing assessing the anaerobic reduction of TCE under different carbon regimes to assess potential bioremediation strategies.

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DISTRIBUTION OF TOXINOGENIC CYANOBACTERIA ACROSS ISRAEL: GEOGRAPHI, ENVIRONMENTAL FACTORS AND WATER BODY TYPES

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Cyanobacteria are dominant primary producers in a large variety of habitats around the world. Many cyanobacteria can produce toxins and other bioactive compounds. When the environmental conditions are right, cyanobacteria can form massive blooms, which, in combination with toxin production, can strongly affect the aquatic ecosystem and create major threats to animal and human health. Our aim was to understand the biogeographic distribution of potentially toxic populations of cyanobacteria, and elucidate the environmental conditions which encourage the presence of seed populations of toxinogenic species across a variety of freshwater body types in Israel. We focus on Microcystins, hepatotoxins produced primarily by *Microcystis* sp. as our model. We sampled ~60 natural springs, agricultural reservoirs and aquaculture facilities, using Polymerase Chain Reaction (PCR) to detect the genes mcyD and mcyA involved in microcystin biosynthesis. To our surprise, we detected "seed populations" of toxinogenic cyanobacteria in about 70% of the samples, suggesting that the distribution of potentially harmful species is much wider than previously thought. Combining phylogenetic analysis with measurements of a-biotic parameters revealed that there are some clades of mcyA that are preferentially found in specific ecosystems such as fish ponds or agricultural water, and that the distribution of toxic cyanobacteria across Israel is not random. Our results raise the hypothesis that the correlation of specific mcyA phylotypes with specific water body types may represents selection at the level of the mcy genes (suggesting adaptation to the environment) or at the level of the organism carrying it (suggesting horizontal gene transfer).

P-43
FUNGAL INDUCTION OF SANDSTONE FORMATION

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Fungi are an important group of microorganisms, known mostly for thier degradation and deterioration inducing activities. Organic substances are readily degraded by fungi, while mineral substrates and rocks usually serve as a physical support for the fungal colony and the growing mycelium. Fungi have also been shown to interact with mineral substates, mainly by contributing to the weathering processes of rocks and other mineral materials. However, a more unique phenomenon is the involvment of fungi in stone formation. Such an induction of sandstone formation by fungal mycelium was recorded in our laboratory when the mycelium grew in the organic-mineral substrate. Fungal biochemical activity, as well as the physio-chemical conditions contributing to the stone formation, will be presented.

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GLYCINE BETAINE IS THE MAIN ORGANIC OSMOTIC SOLUTE IN A STRATIFIED MICROBIAL COMMUNITY IN A HYPERSALINE EVAPORITIC GYPSUM CRUST

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Most halophilic or halotolerant microorganisms use organic ("compatible") solutes for osmotic balance. The first such solute described in prokaryotes is glycine betaine. De-novo biosynthesis of glycine betaine is rarely found in heterotrophs, but most bacteria can accumulate the compound from their medium. Other osmotic solutes are ectoine and hydroxyectone (the most widely synthesized compatible solutes), other amino acids and derivatives, sugars, and polyols. All this information was derived from pure culture studies, and we know little about the distribution of osmotic solutes in microbial communities in hypersaline environments. We therefore examined the organic osmotic solutes content within the stratified communities in an evaporitic gypsum crust in an evaporation pond (~ 194 g/l salts) of the salterns of the Israel Salt Company, Eilat. We extracted solutes from three pigmented layers of the crust: a yellow-orange layer dominated by unicellular cyanobacteria, a green layer with filamentous cyanobacteria, and a layer colored red-purple by purple sulfur bacteria; dense communities of heterotrophs were present in all layers. The solutes were analyzed by Raman spectroscopy, ^{1}H and ^{13}C nuclear magnetic resonance, and HPLC. All layers contained glycine betaine as the only detectable osmotic solute; ectoine and other known solutes were not found in significant amounts. The concept that glycine betaine may be the universal compatible solute in the prokaryotic world, while incorrect at the level of individual cultures, may thus be correct at the level of complex microbial ecosystems where heterotrophs can take up osmolytes produced by halophilic prokaryotic phototrophs.

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THE ISOLATION AND INITIAL CHARACTERIZATION OF CYANOPHAGES FROM LAKE KINNERET

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Lake Kinneret is the largest natural source of freshwater in Israel. The phytoplankton in the lake is composed of a mix of eukaryotic algae and prokaryotic cyanobacteria. The main cyanobacteria species consist of both filamentous types such as *Cylindrospermopsis raciborskii* and *Aphanizomenon ovalisporum*, and unicellular types such as *Microcystis* spp. and *Synechococcus* spp. The various cyanobacterial groups are abundant at different times of the year and at different depths in the water column. Viruses that infect cyanobacteria (cyanophages) are likely to influence the population dynamics, distribution and diversity of their cyanobacterial hosts as well as the biogeochemical cycling of matter in the lake. As a first step towards investigating the role of cyanophages in the Lake's ecosystem, we are isolating viruses that infect various cultured cyanobacterial types. Water was collected from Station A, located at the deepest point of Lake Kinneret, and was incubated with various cyanobacterial hosts. We obtained plaques with a variety of morphologies, or liquid lysates, when using *Cylindrospermopsis, Aphanizomenon,* freshwater *Synechococcus* spp. as well as marine *Synechococcus* spp. as hosts. We are currently in the process of characterizing these cyanophages by transmission electron microscopy and by polymerase chain reaction (PCR) for signature genes from known cyanophage groups. We successfully amplified the signature gene for T4-like cyanophages directly from the viral fraction of concentrated lake water. This suggests that this phage family is likely to be an important component of the viruses that infect cyanobacteria in Lake Kinneret.

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METAGENOMICS OF PHAGE-HARBORED METABOLIC GENES IN THE RED SEA

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Marine viruses comprise the largest reservoir of genetic diversity in the ocean and are major players in oceanic processes. Recently, marine cyanophages carrying photosynthetic genes were discovered, possibly increasing phage fitness. . Phages may contribute to geobiochemical processes not only indirectly by predation of bacteria and affecting bacterial populations, but may also have a direct effect by constantly tinkering genes related to these processes. In order to gain knowledge on the abundance of phage-harbored metabolic genes and the extant to which they are expressed in the marine environment, genomic (gDNA), viral (vDNA) and transcriptomic (cDNA) samples were collected from the Gulf of Aqaba and sequenced using 454 pyrosequencing. Our results show that several metabolic processes are enriched in the viral fraction of both the vDNA and gDNA samples. Photosynthesis is more enriched in the vDNA fraction and Aminoacyl-tRNA biosynthesis is highly enriched in both cases. Cyanophages are highly abundant in both the gDNA and vDNA fraction and contribute the majority of the photosystem II protein D1 genes, suggesting active infection during sampling. Furthermore pico-Eukarya were highly abundant at the cDNA fraction, suggesting an important contribution to active photosynthesis in the marine environment. In addition, bacterial SAR11, SAR86 and archaeal proteorhodopsins as well as recently described viral-like rhodopsins were detected on the gDNA level. On the cDNA level, only SAR11 and SAR86 proteorhodopsin transcripts were detected. Finally, we show that phage harbored metabolic genes are highly abundant in the Red Sea suggesting a significant role of phages in this marine environment.

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RESISTANCE OF BIOLOGICAL SAND CRUST ORGANISMS TO HARSH ENVIRONMENTAL CONDITIONS

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Biological sand crusts (BSC) play an important role in stabilizing sandy desert areas and influence the biotic composition of deserts; their destruction promotes desertification in arid and semi-arid regions. The crusts are formed by the adhesion of sand to extracellular polysaccharides secreted by filamentous cyanobacteria, the main primary producers in these habitats. These organisms acclimate to extreme temperatures, excess light and frequent hydration/dehydration cycles but the mechanisms involved are largely unknown. Surprisingly, the fluorescence yield of BSC taken from sand dunes in Nizzana, NW Negev desert, started to decline at 0730 when the light intensity was about 1/10 full sunlight. This decline in fluorescence was observed regardless of whether the samples were maintained moist or allowed to dehydrate naturally. On the other hand, the fluorescence decline was not observed when the crusts were kept in low light. Isolated strains of several desiccation-tolerant cyanobacteria were less resistant to excess light as compared to freshwater cyanobacteria. Light and oxygen microprofiles showed that at about 1 mm depth peak oxygen evolution was observed although only less than <5% of the surface light was detected. Interestingly, we detected "light pockets", 1-2 mm below the surface, where the light intensity was as high as 10% of the maximal. These "light pockets" may explain the oxygen evolution peaks observed below the photic zone. These results suggest that photosynthetic activity of the cyanobacteria in the crust occurs at low light levels and that protection mechanisms are activated as soon as direct sunlight hits the crust.

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CHARACTERIZATION OF INDICATOR ORGANISMS AND PATHOGENS IN DOMESTIC GREYWATER

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In light of the widespread shortage of drinking water, there is a growing interest in treating greywater for irrigation purposes, in order to reduce its bacterial load, especially that of pathogens. Waterborne pathogens pose a significant threat to human health; a proper assessment of microbial water quality is important for decision-making regarding water infrastructure and treatment investments, and eventually in providing early warning of disease. In our study we tested the greywater samples from small-scale, decentralized greywater treatment systems (recycling vertical-flow constructed wetland) at Sde-Boker Israel, screening for microbial diversity in general and range of waterborne pathogens in particular using classical and molecular methods, such as selective medium plates and 16S rRNA gene sequencing. In the collective tanks we found a total coliform count of 10^5-10^6 cfu/100 ml, of which are $6x10^2$-$6x10^4$ cfu/100 ml fecal coliforms. After the biological treatment, total coliforms decreased to 10^3-10^4 cfu/100 ml, whereas only few fecal coliform were detected. The last step UV or chlorination treatment decreased total bacterial counts to few dozens. Comparison of 16S rRNA sequences retrieved from the water flows before and after the biological treatment displayed 94-100% homology to the *Klebsiella* sp. pathogens, while some others had99-100% homology to the *Pseudomonas* sp.. The research showed that high levels of potable water indicator organisms (*E. coli*, total and fecal coliforms) can be found consistently in greywater; however, they do not necessarily signify the presence of pathogens and therefore, may not be used for pathogen indication in greywater

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EVALUATION OF BIOCIDES ALONE OR IN THEIR COMBINATIONS TO SUPPRESS SULFATE REDUCING BACTERIA

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Hydrogen Sulfide (H_2S) is a major cause of odor nuisance. H_2S production in industrial wastewater evaporation ponds results from biological activity of sulfate reducing bacteria (SRB). Dissimilatory sulfite reductase is one of the key enzymes of SRB in the reduction of sulfate (SO_4^{2-}) to H_2S and is encoded by the *dsrAB* genes. Effect of the metabolic inhibitors, molybdate (as $Na_2MoO_4 \cdot 2H_2O$) and nitrite (as $NaNO_2$) was assessed on the growth of a pure culture of the *Desulfovibrio vulgaris* and subsequent production of H_2S. Nitrite and molybdate reduce H_2S production by interrupting the biological sulfate reduction process. In all experiments, the initial concentration of *D. vulgaris* was ~10^3 cells per ml in the serum bottles and addition of inhibitors alone or in combinations was carried out after 24 hours of incubation at 30°C. The results show that addition of 0.1 mM molybdate or 0.25 mM nitrite in pH 7.8 yielded about 10% of H_2S inhibition, while the first shows about 75% of cells inhibition, the second shows only about 25%. Combination of those two metabolic inhibitors show a synergistic effect with about 55% of H_2S inhibition and about 85% of cells inhibition. Addition of higher concentrations, with 0.4 mM molybdate or 0.5 mM nitrite yielded about 80% and 95% respectively, of H_2S inhibition.

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HOW SV40 ENTERS THE NUCLEUS?

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In non-dividing cells, the nuclear pore complex provides the major route for viruses and viral genomes to enter the nucleus through the nuclear envelope. However, SV40 infection of non-dividing cells is very inefficient suggesting that the nuclear envelope prevents most viral genomes from entering the nucleus. Surprisingly, we observed that following infection of quiescent CV-1 cells with SV40, the nuclear envelope was dramatically deformed, as seen by immunohistochemistry to localize lamins A/C, B1, B2 and the nuclear pore complexes. Accompanying deformation of the nuclear envelope, we also observed fluctuations in the levels of lamin A/C, dephosphorylation of an unknown epitope on lamin A/C and accumulation of lamin A in the cytoplasm. The nuclear envelope deformations occurred just prior to and during nuclear entry of the viral genome and were transient and the spherical structure of the nuclear envelope was restored subsequent to nuclear entry. Nuclear envelope deformation and lamin A/C dephosphorylation required caspase-6 cleavage of a small fraction of lamin A/C. Taken together the results suggest that virus-induced alterations of the nuclear lamina, are involved in the nuclear entry of the SV40 genome in non-dividing cells. We propose that SV40 utilize this unique, previously unknown mechanism for direct trafficking of its genome from the ER to the nucleus.
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LIGAND MIMICRY – A NOVEL MECHANISM OF MICROBE-RECEPTOR INTERACTIONS

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The repertoire of cellular receptors determines host susceptibility to pathogens, a major issue in the field of emerging diseases. The identification of host receptors, which are attractive drug targets, remains however a major challenge. Here we show that pathogens mimic native ligands, a hitherto unknown mechanism, to engage host receptors. Our structural bioinformatics studies revealed that both bacteria and viruses surface proteins structurally mimic native host ligands and use their respective receptors for cell recognition. Contrasting the structural homology, sequence similarity was low, indicating that ligand mimicry arose by convergent evolution. Importantly, this concept may be applied to identify unknown pathogen receptors. Using this concept we identified Axl as a candidate cellular receptor of SV40. We validated the SV40-Axl interaction by biophysical methods and demonstrated its participation in the infection process. Our results suggest ligand mimicry is widespread and present a quick tool for screening pathogen-host receptor interactions.

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RETROGRADE AXONAL TRANSPORT OF VZV IN HUMAN EMBRYONIC STEM CELL-DERIVED NEURONS: KINETIC AND PHARMACOLOGICAL STUDIES

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Varicella Zoster virus (VZV) is a human-specific neurotropic virus that causes varicella (chicken pox) and herpes zoster (shingles). The highly-specific human tropism of the virus and lack of human neurons for study has greatly limited the study of the interaction of VZV with neurons. It has long been postulated that infection of somatic sensory ganglia by the virus occurs by retrograde transport from vesicles in the skin to the ganglia, but no evidence has been obtained. In the present study we demonstrate that human embryonic stem cell-derived neurons can be used to study axonal transport of VZV in microfluidic chambers system. Two types of recombinant VZV-containing MeWo/RPE cells were seeded with separated axons in the axonal compartment. Using inverted imaging system, we monitored single VZV capsids within the axons in the axonal compartment, inside the channels and the subsequent cell-body's infection in the second compartment. Data obtained from about 40 retrogradely moving capsids was analyzed for VZV transport kinetics. Another application of our model is pharmacological interference in retrograde transport of VZV. Application of different concentrations of EHNA (dynein inhibitor) resulted in dose-depended retrograde transport attenuation. Finally, we were able to show that the retrograde transport of ORF7 deletion mutant VZV was not affected by mutation, but in contrast to the WT strain it failed to infect neighboring cells, leaving small unspread plaques in the cell-bodies compartment. Together, these results demonstrate the great potential of hESC-derived neurons for the study of VZV transport and development of new therapeutics.

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ANTAGONISTIC EFFECTS OF HTLV-1 TAX ONCOPROTEIN ON BRCA1 EXPRESSION AND FUNCTION

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HTLV-1 Tax oncoprotein is considered a key factor in HTLV-1 pathogenicity. *BRCA1* gene dysfunction can lead to breast cancer development. In contrast to the tumor suppressor nature of BRCA1, Tax is a potent oncoprotein, most of its activities are strictly opposing those of BRCA1. Therefore, we hypothesize that HTLV-1 Tax expression in breast epithelial cells can antagonize BRCA1 expression and functionality, thereby sensitizing these cells to malignant transformation by environmental carcinogens. So, the main objective of this study is to provide molecular and cellular indications to validate this hypothesis. Based on earlier findings that the milk of HTLV-1 infected women is rich in HTLV-1 infected lymphocytes that can transfer the virus into breast epithelial cells, the outcomes of our present project may point that HTLV-1 can be a risk factor for the development of breast cancer, with a substantially higher risk to women who practice long-term breastfeeding. Our results showed that Tax strongly inhibited both basal and estrogen (E2) induced activation of BRCA-1 expression in breast cells by sequestering CBP/p300 co-activators. Trying to explore the CBP/p300 associated mechanism of Tax effect on this induced BRCA1 expression; our results suggest that Tax does not prevent the binding of CBP/p300 to ER1α but rather physically associates with the ER1α-CBP/p300 to form a tertiaryreporter. Since CBP/p300 have several binding domains, we believe that Tax associates with ER1α-CBP/p300 complex through binding to CBP/p300 rather than to the ER1α protein. We have also found that Tax inhibits BRCA1-mediated activation of p53-target promoters by inactivating p53.

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INVOLVEMENT OF PROTEIN KINASE C IN THE ACTIVATION OF HTLV-1 AND HIV PROMOTERS

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Human T-cell leukemia virus type-1 (HTLV-1) has been implicated with the aggressive malignancy adult T-cell leukemia (ATL) and other clinical disorders. However, following infection, this virus like the human immunodeficiency virus (HIV) inters into a latent state, rendering the infected individuals seropositive asymptomatic carriers. Although the pathogenic mechanism of this virus is not fully clear, the viral transactivator Tax protein is widely regarded as a key element in this mechanism. However, during the latent period, viral gene expression, including Tax gene, in the carrier's infected cells is extremely low and bellow the pathogenically effective threshold. Therefore, it is conceivable that generating an HTLV-1 related disease would require an activation of the latent virus. Thus, investigation of exogenous or intrinsic factors capable of initiating Tax-independent expression is of particular importance. Phorbol esters are exogeneous agents that have been shown to activate the expression of HTLV-1 and reporter genes driven by its long terminal repeat (LTR) of HTLV-1 and HIV. TPA, which belongs to phorbol esters, is a potent activator of protein kinase C (PKC). In the present study we examined involvement of the different PKC isoenzymes in the activation of reporter genes driven by both HTLV-1 and HIV LTRs. Our results proved that high activity of most PKCs significantly prevented the activation of HTLV-1 LTR by treatment with TPA, while this high activity of PKCs was required for the activation of HIV LTR.

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REGULATION OF BETA-CATENIN PROTEIN LEVELS AND UBIQUITYLATION BY E6 AND E6AP

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The development of cervical cancer induced by human papillomavirus (HPV) infection was associated with deregulation of the canonical Wnt cascade. Recent data from our laboratory demonstrated the ability of the HPV E6 oncoprotein to augment Wnt/beta-catenin/TCF transcription, dependent on the E3-ubiquitin ligase, E6AP. The aim of the present study was to elucidate the mechanism of E6/E6AP activity. Coexpression of beta- catenin with E6/E6AP elevated the levels of total, active and nuclear beta-catenin, dependent on E6 ability to bind E6AP. E6/E6AP did not elevate the levels of a mutant beta-catenin resistant to GSK3 beta phosphorylation. Studies with the proteasome inhibitor MG132 suggest that E6/E6AP may protect beta-catenin from proteasomal degradation. Ubiquitylation experiments using cells transfected with E6, E6AP, beta-catenin and ubiquitin indicated that E6AP alone increased the ubiquitylation of beta-catenin. The phosphorylation resistant beta-catenin was also ubiquitlylated by E6AP. A mutant E6AP impaired in its ubiquitin ligase activity did not ubiquitlylate beta-catenin. Studies with MG132 indicated that E6AP ubiquitylation does not protect beta-catenin from proteasomal degradation. Experiments with ubiquitin mutants showed that lysines 11, 29 and 63 are necessary for the E6AP mediated ubiquitylation of beta-catenin. Finally, reporter assays with E6AP alone indicated its ability to activate beta-catenin/TCF transcription in the absence of Wnt. Results of this study suggest that E6AP may activate beta-catenin/ TCF transcription in the absence of Wnt through ubiquitylation of beta-catenin, whereas in cells with activated Wnt signaling E6AP may cooperate with E6 to augment signaling through elevation of the levels of beta-catenin and perhaps other mechanisms.

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MICROARRAY ANALYSIS OF HOST CELL GENE TRANSCRIPTION IN RESPONSE TO VARICELLA-ZOSTER VIRUS INFECTION IN NEURONS DERIVED FROM HUMAN EMBRYONIC STEM CELLS COMPARED TO FIBROBLAST CELLS

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Background: An important aspect of VZV pathology is its ability to establish long-term latency and reactivate in neurons, resulting in painful zoster. Interactions of the human neurotrophic herpesvirus varicella zoster virus (VZV) with neurons has proven difficult to study because the virus shows strict human specificity. In previous studies we showed that neurons differentiated from human embryonic stem cells (hESC) can provide an accessible source of neurons for study of VZV. **Aim and Method:** In order to determine the transcriptional response of human neurons to VZV, we infected hESC-derived neurons and human fibroblasts with GFP-expressing VZV. After 80% of the cells in the culture were infected/fluorescent, RNA was extracted and analysis of gene expression was performed using Affymetrix human genome microarrays. **Results:** Applying a twofold-change as the cutoff, we observed that transcription of 1654 fibroblast and 340 neuronal genes were upregulated, and 955 fibroblast and 38 neuronal genes were downregulated by VZV infection. 223 of these infection-regulated genes were altered specifically in neurons. Gene ontogeny analysis revealed that several clusters of genes regulated by VZV in neurons and fibroblasts differed. For example, neurons did not show upregulation of immune response, NF-kappaB, response to stress and DNA repair, in contrast to fibroblasts. **Conclusion:** Our observations of differences in host gene expression between neurons and fibroblasts may help unravel the pathways used by VZV to establish latency establishment and prevent of apoptosis in neurons. Understanding these phenomena may aid in development of therapeutics for prevention/treatment of common and painful herpes zoster.

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VIROLOGICAL RESPONSE OF HIV-1 PATIENTS TO RALTEGRAVIR TREATMENT- THE ISRAELI EXPERIENCE

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Background: Raltegravir (RAL), the first integrase (IN) inhibitor to be approved for treatment of HIV infection, was introduced in Israel 5 years ago. We aimed to study clinical and virological parameters and to compare the molecular alterations in the HIV integrase gene in RAL treated and naive patients. **Methods:** 197 samples from 166 patients (47 treatment naïve, 75 RAL naïve and 44 RAL treated) were included. DNA/RNA was extracted from lymphocytes/plasma samples. The full length IN gene was amplified and sequenced. Stanford Database was used to identify IN molecular changes and the virus subtype. **Results:** Lower viral load and higher CD4 counts characterized the RAL treated group. The overall number of molecular alterations in the integrase protein was similar in all groups though several IN polymorphic mutations were more frequent under RAL treatment including V101I, I135V, I208L, F100Y and L101I. IN sequence changes were observed in consecutive samples obtained from RAL treated patients. Three non adherent patients developed RAL resistance mutations shortly after treatment commencement. **Conclusions:** Our study shows that RAL treatment is effective in reducing viral load and increasing CD4 counts and that it did not induce the number of polymorphic mutations. The relevance of the more frequent mutations under RAL is unclear and awaits a larger study. The differences in mutations observed between consecutive samples may suggest viral evolution or HIV proviral DNA sampling variability under low viral load. In conclusion, this study demonstrates RAL, a low genetic barrier drug, to be an effective drug for treatment adherent patients.

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REARRANGEMENTS IN THE NON-CODING REGULATORY REGION OF BKV STRAINS OBTAINED FROM A RENAL TRANSPLANT PATIENT ALTER VIRAL REPLICATION, LATE GENE EXPRESSION AND CYTOPATHOLOGY IN VITRO

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Polyomavirus BK (BKV) establishes a latent infection in the majority of the adult population worldwide. Reactivation of BKV may lead to the induction of polyomavirus associated nephropathy (PAN) which is diagnosed in up to 10% of renal transplant (RT) patients. During reactivation, the non-coding regulatory region (NCRR) is prone to rearrangements, although their role in BKV virulence is undetermined. In this study we have examined the rearrangements of our cloned NCRR library isolated from plasma and urine samples of an RT patient. Sequencing of the NCRRs revealed major rearrangements including insertions and deletions. We reconstructed the strains by inserting the rearranged NCRRs into WW backbone and used the recombinant DNA to transfect cell cultures. The transfections analysis revealed that when compared to WW, strains with insertions in the NCRR (insNCRR) replicated faster in cell cultures, created a cytopathic effect more rapidly and had a higher ability to induce late viral gene expression. In contrast, strains with deletions (delNCRR) replicated more slowly, barely induced a cytopathic effect and had a lower ability to induce late gene expression. WW had the highest ability to induce early gene expression when compared to all other variants. Furthermore, in silico analysis demonstrated incorporation of replication factors binding sites (RFBS) in insNCRR strains and deduction of RFBS in delNCRR strains. In conclusion it is apparent that insertions in the NCRR enhance BKV virulence by increasing the viral replication rate, cytopathic effect and the expression of viral proteins, while deletions in the NCRR demonstrate an opposite pattern.

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AGNO PROTEIN IS INVOLVED IN THE INDUCTION OF DNA DAMAGE, LEADING TO THE FORMATION OF TRUNCATED AGNO STRAINS UNABLE TO REPLICATE OR INFECT HOST CELLS

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Polyomavirus BK (BKV) primary infection occurs at an early age; it is usually asymptomatic and results in sero-conversion of 80% of the adult population that leads to a latent state. Reactivation in kidney transplant (KT) recipients is associated with immune-suppression and up to 10% of them may develop polyomavirus associated nephropathy (PAN). BKV encodes a 66 amino acids 7.5 kDa basic (pI=10.6) protein – Agno, which contributes to viral replication, early viral gene expression, processing of capsid proteins and virion assembly. In this study we sequenced the Agno gene from urine and plasma samples of KT recipients with PAN. We were surprised to find mutated strains with major rearrangements in their Agno gene. We reconstructed the Agno strains by inserting the rearranged Agno DNA into a WW backbone. Viral load tests, viral DNA replication and gene expression of the host cell DNA repair mechanisms were used to characterize the effects conferred by the Agno rearrangements. Agno deletions substantially decreased viral yield, but not the viral DNA replication. Trans-complementation with an intact Agno increased viral replication, but was ineffective for infection with third generation viruses. Agno had an effect on the transcription of DNA repair genes which implies its involvement in inducing rearrangements in the Agno gene itself. In conclusion it is apparent that during BKV reactivation, rearrangements occur in the Agno gene and the Agno protein is involved in inducing these rearrangements. We assume that BKV overcomes the negative effect of the deletions by trans-complementation with WW and other intact strains.

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THE ROLE OF AUTOPHAGY IN THE LIFE CYCLE OF A LARGE DNA VIRUS INFECTING MARINE PHYTOPLANKTON

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Marine photosynthetic microorganisms (phytoplankton) are the basis of marine food webs and are responsible for nearly 50% of the global primary production. Viruses that infect phytoplankton are major evolutionary and biogeochemical drivers in marine microbial foodwebs. *Emiliania huxleyi* is a globally important coccolithophore, forming massive blooms in the North Atlantic Ocean that are routinely infected and terminated by coccolithoviruses. We explored the life cycle of the large double stranded DNA virus that infects *E. huxleyi* and leads to bloom termination. Electron and fluorescence microscopy analyses of cells during lytic phase of infection, revealed induction of hallmarks of autophagy, such as double membrane vesicles and enhanced acidification of distinct vacuoles. Concomitantly, we detected upregulation of expression of a suite of genes with high homology to conserved autophagy related genes (the ATG genes). Among these genes, we found profound induction in the protein levels of Atg8, an essential component in autophagosme formation, exclusively during lytic infection. Interestingly, we detected the host-encoded protein Atg8 within purified virions, indicating the pivotal role of this hallmark of autophagy in viral assembly and egress from the cells. Intriguingly, pre-treatment of the cells with an inhibitor of autophagy prior to viral infection causes massive reduction of extracellular viral particles, without affecting viral replication within the cell. Here we show that the autophagic mechanism, which has never before described in marine phytoplankton, is essential in the propagation of coccolithviruses. Future studies will elucidate the unique ecological role of autophagy in host-virus dynamics during algal blooms in the marine environment.

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A NOVEL SYSTEM FOR *IN VITRO* TESTING OF POLIOVIRUS NEUROVIRULENCE USING HUMAN EMBRYONIC STEM CELL-DERIVED NEURONS

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Background: Poliovirus, a pathogenic, human-specific neurotropic virus, can destroy nerve cells causing paralysis and sometimes death. Live polio vaccine can establish persistence in immune-deficient individuals with vaccine-derived poliovirus (VDPV) progeny sometimes reverting to neurovirulence. Human embryonic stem cell (hESC)-derived neurons have been used to study human-specific neurotropic viruses. Aim: To develop a human neuron culture model alternative or complement to poliovirus neurovirulence testing in primates and transgenic mice. Methods: Neuronal precursors were induced by co-culture of hESC with stromal cells and terminally differentiated within 3-4 days by plating on laminin. Neurons were grown on coverslips or in compartmented microfluidic chambers where axons grew from a cell-body to an axonal compartment through microchannels. Sabin 2 vaccine (S2), high (HNv) or low (LNv) neurovirulent, serotype 2 VDPVs isolated from sewage in Israel was used to infect neurons. Infection was documented by immunofluorescence staining for poliovirus. Results: All three viruses applied directly to neurons, infected them within 24 hours. A dramatic difference was revealed when only axons were exposed to virus in microfluidic chambers. Cell bodies became positive for HNv at 2 days post-infection. In contrast, fewer cell bodies were infected by S2 and only on day 3, at which time cell bodies from axons exposed to LNv remained unstained. Conclusion: Infection of hESC neurons in microfluidic chambers allows distinction between high and low neurovirulent serotype 2 strains of poliovirus. Therefore, our new tissue culture model has great potential to provide an ethical alternative for primate and other animal models for testing neurovirulence.

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SUPERINFECTION EXCLUSION OF α-HERPESVIRUSES IN HUMAN FIBROBLASTS AND NEURONS

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Varicella Zoster virus (VZV) is the causative agent of chickenpox and shingles, while Herpes simplex virus type 1 (HSV1) cause the common cold-sores. Both the viruses belong to the α-herpesviruses subfamily, infect more than 90% of the human population and can reside latently in sensory ganglia for decades. However, it is unknown whether these viruses can infect the same cell or different cells of the same ganglion. Superinfection exclusion (SE) is a phenomenon where a preexisting viral infection prevents a secondary infection with the same or a closely related virus, while infection by unrelated viruses can be unaffected. SE was by HSV1 shown to be due to the expression of glycoprotein D on the infected cell's membrane that interferes with free virus entry into the cells. We here examine the phenomenon of SE for VZV and in neurons for the first time. Human foreskin fibroblasts (HFF) and neurons derived from human embryonic stem cells were infected either simultaneously (co-infection) or concomitantly (super-infection) with fluorescently-tagged viruses, either two HSV1 or HSV1 with VZV. An automated color-based analysis was carried out to detect singly and dually infected cells. We found that although both HSV1 and VZV are able to reproduce within the same human cell, they exclude each other efficiently in fibroblasts, and less efficiently in neurons. The finding that HSV1 and VZV can reside inside the same neurons may have clinical relevance: both viruses establish latency in human trigeminal ganglia and reactivate to cause cold sores (HSV1) or zoster (VZV).

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NEWCASTLE DISEASE VIRUS (NDV) – AN ONCOLYTIC AGENT FOR CANCER TREATMENT

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Cancer is a leading cause of morbidity and mortality in western countries. Despite a huge research effort and funding, there is no cure for most advanced stage cancer. Oncolytic viruses may serve as a novel approach for treating cancer where conventional therapies failed. Oncolytic viruses preferentially infect and kill cancer cells while sparing normal ones. When modified, these viruses can be used as vectors enabling gene expression of anticancer proteins to be delivered to the tumour site. The use of a unique ex-vivo tissue model, developed in our lab, as well as an animal model of colorectal carcinomas, enables us to explore the tropism and mechanism of Newcastle Disease Virus (NDV) as an oncolytic virus. NDV is a negative ssRNA paramyxovirus, naturally infects poultry, is not pathogenic to humans and has limited replication capacity in mammalian cells. The oncolytic effect of NDV was previously demonstrated both *in-vitro* and *in-vivo* as well as in clinical trials. NDV-HUJ is an attenuated NDV strain, with a defective replication capacity (single cycle). Studies in our lab showed that the virus causes apoptosis in lung cancer cells as well as in chemo-resistant primary melanoma cells. In-vivo results indicate that treatment with NDV-HUJ in orthotropic colon cancer model caused a major tumour regression and increased survival. Using the ex-vivo system, a different infection and replication pattern was demonstrated both in mice and human tumor tissues as compared to normal ones. This data may lead to new strategies treatment modalities for advanced colorectal cancer.

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HERPES SIMPLEX VIRUS TYPE 1 MODE OF SPREAD IN THE CLINICALLY- RELEVANT TISSUES, SKIN AND BRAIN, DIFFERS IN ACCORD WITH TISSUE TYPE

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Herpes simplex virus type 1 (HSV-1) initially infects the skin to cause cold sores and is subsequently transported to the nervous system to establish latent infection, or in rare cases encephalitis. Much of the work addressing HSV-1 spread has been done using cells in culture, a system that lacks the three dimensional tissue architecture that play important role in viral dissemination. To investigate HSV-1 mode of spread in the clinically relevant tissues, we have established an *ex vivo* infection models, using tissues of mouse and human skin as well as mouse brain tissue in organ culture. In brain organ culture, HSV-1 spread was directed along tissue neuronal networks and was resistant to neutralizing antibodies. In contrast, HSV-1 spread in the skin was concentric and formed typical plaques of limited size. In contrast to the brain, viral spread in the skin tissue was sensitive to neutralizing antibodies. Heparin, on the other hand, inhibited viral dissemination in the two target tissues. Only a negligible amount of the progeny virus was found in media of infected brain tissues, while substantial quantity of virus was released to the media of infected skin tissues. Moreover, HSV-1, that naturally infects only human, infects the mouse and human skin tissues in a similar fashion, thus validating the mouse model. Taken together, the results indicate that HSV-1 utilizes different strategies to spread in the different target tissues.

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PHOTOINDUCED INACTIVATION OF *MYCOPLASMA GALLISEPTICUM*: NEW PERSPECTIVES FOR VACCINE DEVELOPMENT

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Hydrophobic UV-activatable compounds have been shown to partition into the lipid bilayer of a biological membrane and by UV irradiation to react selectively with proteins of the transmembrane domain while surface proteins located outside the bilayer remain unaffected. In this study, we tested a variety of UV activatable - azido and - iodo based hydrophobic compounds for their ability to inactivate the wall-less bacterium *Mycoplasma gallisepticum* (MG), the causative agent of chronic respiratory disease and infection sinusitis in chickens. Treatment of MG with 1-iodo, 5-azidonaphthalene (INA) followed by UV irradiation, resulted in a marked decrease of cell viability. INA treatment of MG resulted in the inactivation of F_0F_1 –ATPase, whereas cytoplasmatic soluble enzymes were little affected. However, both INA-treated and untreated MG maintained their surface lipoproteins, which play an important role in triggering of antibody response. Transmission electron microscopy revealed that whereas untreated MG were intact, the INA treated MG showed a great number of empty membrane vesicles. It is likely that the inhibition of F_0F_1-ATPase obtained by INA treatment resulted in a limited Na^+ extrusion and a tendency of the cells to swell and lyze. So far, vaccination against MG infection was only partially successful and in many cases the efficacy of these vaccines is low. The possibility to induce a strong immune response by using INA treated MG is therefore intriguing and might have a significant potential for the development of a novel and efficient MG vaccine.

P-66

IDENTIFICATION AND CHARACTERIZATION OF NEW T3SS EFFECTOR PROTEINS IN *SALMONELLA ENTERICA*

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Gram-negative pathogens use type III secretionsystems (T3SSs) to inject effector proteins into host cells. The translocated effector proteins manipulate host cell processes. The genes encoding the structural components of the T3SSs are conserved among bacterial species and can be identified by sequence homology. In contrast, the sequences of secreted effector proteins are less conserved and are therefore difficult to identify. *Salmonella enterica* harbors two T3SSs. The aim of this project was to identify and characterize new effectors translocated by these systems. We developed a machine learning approache for identifying new effector proteins in *Salmonella enterica* serotypes. We received a list of suspicious genes and investigated whether they are indeed translocated to the host cells, by using microbiological and molecular biological methods. Each gene was fused to TEM-1, that served as reporter for translocation. We constructed plasmids expressing candidate effector-TEM1 fusions, which were introduced into the WT *S. typhimurium* strain. HeLa cells and macrophages J774 were infected with the bacteria, and translocation of the effector was analysed by microscopy and FACS. In order to confirm that the newly identified proteins are secreted by one of the two *Salmonella* T3SSs, the experiments were repeated with two mutants defective in one of these systems. Currently we characterize the function of the positive effectors. Discovering new effectors and their function will deepen our understanding of the virulence and host evasion mechanisms adopted by *Salmonella* and may provide novel approaches for treating the infections. Moreover, it will provide tools to distinguish between serotypes with different degree of virulence.

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CHARACTERIZATION OF THE BOVINE BACTERIAL COMMUNITY AND ITS RELATION TO ENERGY HARVEST EFFICIENCY

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Plant degradation in cattle is performed by a highly specialized microbial community found in one of the compartments of the digestive tract of ruminants: the rumen. This community allows for the degradation the most recalcitrant carbohydrates found in plants, and through this process, ruminants are able to harvest energy found in plants for growth, making cattle the main source of milk and meat for humankind. The improvement of feed efficiency, and through it, product yield in cattle remains one of the most sought out goal in agriculture today. Until now, this feat was solely accomplished by selective breeding and improvement of feed composition. While the animals are completely dependent on their microbiota, a connection between the bovine capacity for improved energy harvest and its resident bacterial taxa has yet to be established. Using a pyrosequencing approach we characterized the bacterial community composition found in 15 dairy cows, analyzed the degree of divergence that can be found between different animals, and found significant correlations between metabolic parameters of the animals and the abundance of specific bacterial taxa. This work suggests the bacterial community may have a significant role in the efficiency of energy harvest, and a deeper understanding of this process may allow us to improve yield through community design.

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POTENTIAL VIRULENCE FACTORS OF *MYCOPLASMA HYORHINIS*: PHOSPHOLIPASE ACTIVITY, HEMOLYSIS AND THE REVERSE CAMP PHENOMENON

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Mycoplasma hyorhinis has been implicated in a variety of swine diseases and is a frequent cell culture contaminant. *M. hyorhinis* strain MCLD was first isolated by us from a melanoma cell line and was found to be capable of adhering to and invading into host cells. Phospholipases and hemolysins are likely to be involved in the invasion of host cells by bacteria. Indeed, *M. hyorhinis* membranes posses a non-specific phospholipase A activity capable of hydrolyzing both position 1 and position 2 of C12-NBD-PC. *M. hyorhinis* posses also phospholipase C activity as indicated by a potent glycerophosphodiesterase which is capable of hydrolyzing pNP-PC into pNP and PC. Furthermore, beta-hemolysis activity was obtained by *M. hyorhinis* grown on blood agar. Interestingly, out of eight representative *Mycoplasma* species tested, the co-hemolytic CAMP positive effect was detected in *M. hominis*, *M. fermentans* and *M. gallisepticum* (group I) but not in *M. hyorhinis, M. penetrans*, *M. capricolum* and *M. mycoides* (group II). Nonetheless, the rare phenomenon of the reverse CAMP reaction, known to be associated with a potent phospholipase D, was detected in group II *Mycoplasma* species but not in group I. Interestingly, all group II species do not possess the *pld* gene encoding phospholipase D but do possess the *cls* gene encoding cardiolipin synthetase. This enzyme contains two conserved domains of the Phospholipase D motif. Cloning of *M. hyorhinis cls* gene into *M. pneumoniae* and *M. gallisepticum* and its effect on the reverse CAMP phenomenon will be presented.

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GENOME OF *COXIELLA* SP., THE SYMBIONT OF THE TICK *RHIPICEPHALUS TURANICUS*

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Rhipicephalus turanicus (Acari: Ixodidae), is an obligatory blood feeding tick that can transmit a variety of pathogenic organisms to both man and animal. According to our previous studies, the bacterial community of *Rh. turanicus* was found to be dominated by a single population of intracellular *Coxiella* sp. bacteria. We demonstrated that *Coxiella* sp. specifically resides within females Malpighian tubules and gonads and that it is vertically transmitted from mother to her offspring. This and a 100% prevalence of *Coxiella* sp. in field collected ticks, support an obligatory association. The aim of the current study was to further investigate the nature of association between *Coxiella* sp. and its tick host, using a genomic approach. Genomic DNA of *Coxiella* sp. symbionts was extracted from Malpighian tubules and gonads of females. Differential lysis protocol was used to enrich its total DNA relatively to the tick DNA. The DNA served as template for full genome sequencing using the Illumina HiSeq2000 platform. Based on the obtained data, the genome of *Coxiella* sp. is approximately 1.6 Mbp in size, suggesting a possible ~25% genome reduction in comparison to *Coxiella burnetii* which is its closest known relative and a known pathogen. Further comparison revealed that *Coxiella* sp. lost the genes for motility but retained genes for β-lactamase resistance and most of the genes for B vitamins synthesis, that are presumably lacking in the host blood based diet. Altogether the data points out to an obligatory and possibly nutritional association between *Coxiella* sp. and the tick.

P-70

DISCOVERY OF NOVEL TYPE III EFFECTOR PROTEINS IN *PSEUDOMONAS AERUGINOSA*

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Pseudomonas aeruginosa is a gram negative, opportunistic human pathogen that can infect an assortment of organisms. The virulence of *P. aeruginosa* is often associated with the activation of a type III secretion system (T3SS) that injects harmful effector proteins directly into the host cell's cytoplasm. Until today, despite strenuous efforts, only four T3SS effector proteins (ExoU, ExoS, ExoY and ExoT) have been identified in *P. aeruginosa*. The *P. aeruginosa* T3SS seems to have fewer effector proteins than any other well-characterized T3SS. This is especially intriguing in light of *P. aeruginosa'*s ability to infect a wide range of hosts. In this study, we used a machine learning algorithm to predict novel *P. aeruginosa* T3SS effector proteins. Over a 100 parameters were used for the machine learning cycle, providing a list that ranks all *P. aeruginosa* ORFs by their potential to be an effector protein. The top putative effector candidates were then screened for type III dependent secretion from the bacterial cell into the host cell. Using this methodology, we were able to identify three new *P. aeruginosa* proteins that are translocated via the T3SS. Identification of such new effector proteins can substantially increase our understanding of the virulence mechanisms utilized by this organism especially since the last T3SS effector protein, ExoY, was identified more than a decade ago, in 1998.

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A GENETIC SCREEN FOR THE IDENTIFICATION OF *LISTERIA MONOCYTOGENES* DETERMINANTS THAT MODULATE THE HOST NF-κB PATHWAY

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The NF-κB pathway is a central mammalian signaling pathway known to be activated during bacterial infection and is critical in eliciting pro-inflammatory responses against invading pathogens. While it is known that bacterial pathogens develop strategies to manipulate host signaling pathways, including the NF-kB pathway, a comprehensive genome wide screen to identify microbial factors that directly effect NF-kB activation were not reported. In this study we used the human intracellular pathogen, *Listeria monocytogenes,* as a model pathogen to screen for new bacterial determinants that modulate the NF-kB pathway within immune cells. To achieve this goal we infected RAW macrophage cells harboring NF-kB reporter gene with *Listeria monocytogenes* mutant library (~5600 mutants), and searched for mutants that differentially activate NF-kB. This approach led to the identification of two groups of mutants – low NF-kB stimulators and high NF-kB stimulators, compared to WT *L. monocytogenes* infection. We have validated the NF-kB activation phenotype using RT-PCR on several NF-kB- regulated cytokines and by immunofluorescence assay detecting NF-kB translocation to the nucleus. Combination of bioinformatics analysis and experimental assays is being done to gain insights to the role of the identified genes in the interplay between *L. monocytogenes* and the host immune response.

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THE MULTIDRUG TRANSPORTER P-GLYCOPROTEIN CONTRIBUTES TO ACTIVATION OF TYPE I INTERFERON RESPONSE

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Human multidrug resistance transporters are known for their ability to extrude antibiotics and toxic compounds out of cells. However, increasing data indicate that they possess additional functions involving diverse physiological processes, not related to drug efflux. Here we show that the human multidrug P-glycoprotein transporter, P-gp (also named Mdr1, ABCB1), is transcriptionally induced in monocyte cells upon infection with the intracellular bacterial pathogen *Listeria monocytogenes.* We found that the P-gp induction correlates with the cells innate immune response to *L. monocytogenes* invasion, specifically with the activation of Type I interferon response. Wild type bacteria that triggered Type I interferons also induced enhanced P-gp transcription, while bacterial mutants that triggered less or none of this response resulted in corresponding levels of P-gp. Notably, P-gp inhibition by a specific inhibitor or by mRNA silencing, significantly reduced the activation of cytokines of the Type I interferon response, while did not affect other pro-inflammatory cytokines. Overall, this study demonstrates that P-gp plays a role in activation of an innate immune response, thus highlights the complexity in designing therapeutic strategies that involve inhibition of MDR transporters.

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STUDYING THE ROLE OF *LISTERIA MONOCYTOGENES'* MDR TRANSPORTERS AND LTA IN ACTIVATION OF THE TYPE I INTERFERON RESPONSE IN MACROPHAGES

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L. monocytogenes is an intracellular bacterial pathogen that invades and replicates within wide array of mammalian cells. Upon invasion the bacteria induce a robust Type I interferon response that was previously shown to be dependent on the expression of several bacterial multi drug resistance (MDR) transporters. In attempt to understand how these MDRs exert this phenotype, we have screened for mutants that modulate the induction of Type I interferon response by one of these MDRs, MdrM. In this screen we identified genes of the lipoteichoic acid (LTA) biosynthesis pathway as functionally linked to the MDR transporters phenotype. Mutants deleted of the LTA biosynthesis genes activated an even higher level of Type I interferon response, yet this response was abrogated when deletions of the MDR transporters were combined. We further showed that the MDR mutants produce aberrant LTA profile, as only one variant of LTA structure was detected in these mutants, while wild type bacteria produce two distinct variants. Interestingly, the LTA produced by *wild type bacteria* and the MDR mutants induce similar cytokines levels as their cognate bacterial strains. Studying the dissimilarities in the LTA structures within wild type and the MDRs mutants may give us a clue for the role MDR transporters during infection and in general in bacterial physiology.

P-74

NLED, A BACTERIAL METALLOPROTEASE THAT SPECIFICALLY TARGETS AND INACTIVATES HOST CELL JNK AND P38

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Enteropathogenic *Escherichia coli* (EPEC) is an etiological agent of human diarrhea, and remains a significant cause of infant mortality in developing countries. Like many other pathogenic bacteria, EPEC make use of a type III secretion system (TTSS) to translocate toxic effectors to the eukaryotic host cytoplasm, where the effectors modulate cell functions. Twenty one effectors proteins have been described for EPEC, among them is the NleD. We show that NleD is zinc dependent endopeptidases that specifically cleave and inactivate the Map Kinases JNK and p38. This is a remarkable example of a pathogen using effectors to manipulate the host response signaling network.

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THE CLINICAL SIGNIFICANCE OF ISOLATION OF TWO DIFFERENT ORGANISMS FROM THE URINE OF PATIENTS WITH AN INDWELLING CATHETER

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The diagnosis of catheter associated urinary tract infection is usually based on a combination of clinical symptoms and signs and urine culture. Ideally, one organism is isolated, but not infrequently more than one are cultured. Our purpose was to evaluate the clinical significance of urine cultures from patients with an indwelling urinary catheter (UC) out of which two different pathogens were isolated. During six months all consecutive urine cultures from patients with a UC out of which two different organisms were isolated were enrolled. These cultures were randomly divided into a control group and a study group. Endpoints were (1) change in antibiotic treatment and use of broad spectrums agents and (2) the time for clinical improvement as measured by decrease in fever and leucocytosis, and duration of admission. During the study period, 81 cultures met the inclusion criteria and were randomized in 40 study and 41 control cultures. Antibiotic treatment was changed after 72-96 hours in 19 (48%) study patients and 25 (61%) controls, respectively (NS). There was no difference in type of antibiotic change. Similarly, there was no difference between the groups in use of broad spectrum antibiotics. Duration of hospitalization after obtainment of the index urine culture was similar. In each group 15 (36%) patients died (NS). This study did not reveal statistically significant differences between the two groups regarding outcome of hospitalization or use of broad antibiotics. Our findings imply that laboratory work-up of two pathogens from patients with an indwelling catheter may be discarded.

P-76

RECTAL SWABS ARE SUITABLE FOR QUANTIFYING THE CARRIAGE LOAD OF KPC-PRODUCING CARBAPENEM-RESISTANT *ENTEROBACTERIACEAE* (CRE)

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It is more convenient and practical to collect rectal-swabs than stool specimens for studying carriage of colon pathogens. We examined the ability to use rectal-swabs rather than stool specimens to quantify KPC-producing carbapenem-resistant *Enterobacteriaceae* (CRE). We used a qPCR assay to determine the concentration of bla_{KPC} relative to 16S rDNA genes, and a quantitative culture-based method to quantify CRE relative to total aerobic bacteria. Our results demonstrated that rectal-swabs are suitable for quantifying the concentration of KPC-producing CRE and that qPCR showed higher correlation between rectal-swabs and stool compared to the culture-based method.

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BIOFILM FORMATION AND SUSCEPTIBILITY TO GENTAMICIN AND COLISTIN IN EXTREMELY DRUG RESISTANT KPC-PRODUCING *KLEBSIELLA PNEUMONIAE*

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KPC-producing *Klebsiella pneumoniae* is a challenging pathogen. Its ability to form biofilm is not well defined. We characterized the ability of various genetic lineages to form biofilm, and determined biofilm susceptibility to gentamicin and colistin. 46 KPC-producing *K. pneumoniae* isolates were evaluated for their biofilm formation ability. Isolates belonged to the world-wide endemic sequence type (ST)258 (n=28) and to other various STs (n=18). The effect of sub-inhibitory concentrations of gentamicin and colistin on biofilm formation was examined, and MICs and MBECs (minimal biofilm elimination concentration) were determined. All isolates produced biofilm in the range of 0.02 to 0.3 OD, where ST258 isolates produced less biofilm compared to other STs isolates ($p<0.05$). Sub-inhibitory concentration of gentamicin and colistin showed an inconsistent effect on biofilm formation. In planktonic state, ST258 isolates were less often resistant to gentamicin compared to other STs ($p<0.05$). Proportion of isolates susceptible to gentamicin did not change in biofilm state. However, gentamicin resistant isolates showed a dramatic increase in resistance level (up to 234-fold) in biofilm. Colistin resistance level did not differ significantly between ST258 isolates and other STs ($p>0.05$), and resistance level to colistin in biofilm did not change significantly. No correlation was found between biofilm biomass and MBECs of the two antibiotics tested regardless of the isolates genetic lineage. KPC-producing *K. pneumoniae* isolates apparently do not form massive biofilms and specifically the endemic ST258 lineage showed susceptibility to gentamicin also in its biofilm state. Colistin resistance in biofilm was similar to the resistance level observed in planktonic state.

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EXCESS OF Srs2p SENSITIZES CELLS TO DNA DAMAGING AGENTS

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Srs2 is a helicase that participates in the regulation of homologous recombination and in the choice between different repair pathways and mechanisms. It has been shown to down regulate Rad51- dependent homologous recombination, most probably through its interactions with PCNA. We discovered that adding an additional copy of the *SRS2* gene on a one copy vector sensitizes the cells to the DNA damaging agent MMS. Since *Δsrs2* cells are also more sensitive to MMS, this result stresses the need for a tight regulation of Srs2p levels. The sensitization is elevated significantly in *pol30-RR* mutants (an allele PCNA that cannot undergo neither SUMOylation nor ubiquitination) and also in *Δelg1* (an RFC like, large subunit of a PCNA loading complex), suggesting close relationships between these three factors. The double mutant, *pol30-RR Δelg1*, is hypersensitized to MMS in the presence of an extra copy of *SRS2*. Srs2p undergoes several modifications, including phosphorylation at 7 sites of and SUMOylation at another 3 sites. We are currently analyzing whether these modifications play a role in the sensitization by *SRS2*. Additional experiments will dissect and characterize the effect of the excessive Srs2p in the cell.

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NEUROSPORA CRASSA PROTEIN ARGININE METHYL TRANSFERASES ARE INVOLVED IN GROWTH AND DEVELOPMENT AND INTERACT WITH THE NDR KINASE COT1

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The protein arginine methyltransferaseas (PRMTs) family is conserved from yeast to human, and regulates stability, localization and activity of proteins. We have characterized deletion strains corresponding to genes encoding for PRMT1/3/5 (designated *prm-1*, *prm-3* and *skb-1*, respectively) in *Neurospora crassa*. Deletion of PRMT-encoding genes conferred reduced growth rates and altered Arg-methylated protein profiles (as determined immunologically). Δ*prm-1* exhibited reduced hyphal elongation rates (70% of WT). In Δ*prm-3*, distances between branches were significantly longer than the WT, suggesting this it's required for proper regulation of hyphal branching. Deletion of *skb-1* resulted in hyper conidiation (2-fold of the WT) and increased tolerance to the chitin synthase inhibitor. Taken together, all *N. crassa* PRMTs are involved in fungal growth, hyphal cell integrity and affect asexual (but not sexual) reproduction. The PRMTs in *N. crassa* apparently share cellular pathways which were previously reported to be regulated by the NDR (Nuclear DBF2-related) kinase COT1, whose dysfunction leads to a pleiotropic change in hyphal morphology. Using co-immunpercipitation experiments, we have shown that SKB1 and COT1 can physically interact. We have now identified a new , 70kDa, isoform of COT1, whose abundance was increased in a Δ*skb-1* background. This isoform, as well as the two others, are Arg-methylated, as determined on the basis of immunological detection that suggest that methylation observed involves the activity of more than one PRMT enzyme. Based on the highly conserved structure of the PRMTs and the NDR kinases in eukaryotes, it is likely that these proteins undergo similar interactions in other organisms.

P-80

SENSING ENVIROMENTAL STRESSES AND CELL FATE REGULATION VIA REDOX SIGNALING IN MARINE DIATOMS

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Phytoplankton are unicellular photosynthetic organisms responsible for about half of global primary production. One of the most important phytoplankton groups is the diatoms, which often dominate phytoplankton communities. Their blooms in the oceans are of great ecological and biogeochemical importance, as they serve the basis of the oceanic food webs, and sink for atmospheric CO_2. In the ocean diatoms are exposed to a variety of environmental conditions such as abiotic (nutrient limitations, high-light) and biotic stresses (grazing and viruses). Still, the molecular mechanisms by which diatoms perceive the environment distinguish between different stimuli and integrate them to activate signal transduction pathways, are under explored. We use the pennate diatom *Phaeodactylum tricornutum*, as a model system to explore the response to stress conditions. Since sensing environmental stress is mediated via redox signaling, we explored the intracellular redox potential, which can induce signal transduction pathways leading to either acclimation or Programmed Cell Death (PCD). We generated *P. tricornutum* transformants expressing an organelle-specific redox-sensitive Green Fluorescent Protein (roGFP), novel sensitive reporter for oxidative stress. We examined the dynamics of the redox potential on the subcellular compartments and monitored its correlation to cell fate in response to. We found intriguing correlations between the organelle specific early oxidation pattern and later induction of cell death. Furthermore, we found that the cell death had PCD hallmarks, including metacaspase induction and DNA fragmentation which proceeded loss of membrane integrity. We propose that redox metabolism and its tight regulation are key components in phytoplankton response to environmental stress.

P-81

MAS1, A 91AA PROTEIN CONFERS SENSITIVITY OF *NEUROSPORA CRASSA* TO A NOVEL *ASPERGILLUS TUBINGENSIS* METABOLITE

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Sponge-associated fungi are a promising source of natural products, due to the unique ecological niche in which they reside. We have previously isolated several *Aspergillus* spp. from the Mediterranean marine sponge *Psammocinia* sp. One of them, an *A. tubingensis* strain, was found to secrete metabolite(s) that inhibit the growth of several fungi (*Alternaria alternata*, *Rhizoctonia solani* and *Neurospora crassa*). At least two novel metabolites with antifungal activity were purified and their structures elucidated. The compounds inhibited *N. crassa* growth (MIC= 210μM) and affected hyphal morphology. Using random tagged mutagenesis, we have identified *N. crassa* mutants exhibiting resistance to the compounds. Plasmid rescue analysis indicated that a defect in a yet-uncharacterized gene (NCU03140.4), designated *mas-1*, confers resistance to the compound. This was confirmed by analysis of the appropriate *N. crassa* knock-out strain. Furthermore, complementation of the knockout strain restored sensitivity to the compound. Analysis of *mas-1* expression indicates that the gene is highly expressed during conidial germination when compared to young or mature hyphae. In addition, we analyzed the sensitivity of a Δ*mas-1* strain to several fungicides whose function results in impaired hyphal integrity. When grown in the presence of either tebuconazole (an ergosterol biosynthesis inhibitor) or fludioxonil (an activator of the hyperosotic stress response pathway), no significant difference between the Δ*mas-1* strain and the wild type were observed. Unexpectedly, the Δ*mas-1* strain was only about half as sensitive to sublethal concentrations of the chitin synthase inhibitor polyoxin D, suggesting alterations in the cell wall of Δ*mas-1* may involve changes in chitin deposition.

P-82

MOLECULAR CHARACTERIZATION OF *SALMONELLA ENTERICA* SEROVAR INFANTIS UNIQUE PLASMID AND ITS ROLE IN VIRULENCE

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Non-typhoid *Salmonella* is a leading cause of food-borne illness worldwide. *Salmonella enterica* serovar Infantis (*S*. Infantis) is a prevalent serovar in many countries and the most dominant serovar in Israel in the recent years. Surprisingly, only little is known about *S*. Infantis pathogenicity, host specificity and virulence mechanisms. To address that, we have started to study genetic differences between various isolates of *S*. Infantis and between *S*. Infantis and *S. typhimurium*. Using a whole-genome sequencing approach we discovered the presence of a large (280 kb) IncP plasmid conferring a multidrug resistant (MDR) phenotype to the *S*. Infantis emerging strain. Additionally, this plasmid encodes a heavy metal resistance operon (*mer* operon), iron acquisition operon from the *Yersinia* high-pathogenicity island (HPI) and possess two unknown chaperon-usher fimbrial clusters. Adhesion and invasion assays established that the plasmid-harboring strains adhere and invade to host cells more efficiently than plasmid-less strains. Moreover, we showed that the emerging strain is resistant to mercury chloride as opposed to strains that do not harbor the plasmid. Comparison between *S*. Infantis strains and *S*. Typhimurium SL1344 revealed that *S*. Infantis strains are significantly less invasive than *S. typhimurium* but more adhesive to different cell lines. From quantitative RT-PCR we learned that *Salmonella* pathogenicity island-1 (SPI-1) genes are transcribed in significantly lower levels (10-100 fold) in *S*. Infantis vs. *S. typhimurium*. Collectively, the new data is expected to provide novel information about the pathogenicity, virulence mechanisms and host-pathogen interactions of *S*. Infantis as a ubiquitous and important pathogen.

P-83

THE EFFECT OF *PSTS* AND *PHOB* ON *PSEUDOMONAS AERUGINOSA* SWARMING MOTILITY AND BIOFILM FORMATION

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Pseudomonas aeruginosa is an opportunistic pathogen that can cause a wide range of infections and inflammations in a variety of hosts, such as chronic biofilm associated lung infections in Cystic Fibrosis patients. Phosphate, an essential nutrient, has been recognized as an important signal that affects virulence in *P. aeruginosa*. In the current study we examined the connection between phosphate regulation and virulence factors in *P. aeruginosa.* We focused on two important proteins, *pstS*, that is involved in phosphate uptake and *phoB,* a central regulator that responds to phosphate starvation. We found that a mutant lacking *pstS* is constantly starved for phosphate, has a hyper swarming phenotype and is impaired in its ability to develop biofilms. The *phoB* mutant, while also impaired in its ability to create biofilm, did not express phosphate starvation and showed no swarming. After generating a double mutant lacking both genes, we found that the hyper-swarming phenotype observed in the *pstS* mutant is regulated by *phoB* and that this gene is also responsible for swarming motility in phosphate-depleted conditions observed in the wild type. Our preliminary results suggest that PhoB enhances the expression of the Rhl quorum sensing system in *P. aeruginosa*, which results in hyper production of rhamonlipids, biosurfactants that induce swarming motility and are also involved in biofilm development and maintenance.

P-84

PHOSPHOGLYCOLATE MAY SERVE AS THE SIGNAL FOR ACCLIMATION TO CHANGING ATMOSPHERIC CO_2 LEVEL

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In their natural environment photosynthetic microorganisms must cope with vast changes in the abiotic conditions. The nature of the signals that initiates their acclimation, including to the ambient level of CO_2, is for the most part unknown. In cyanobacteria, it was suggested that the rising phosphoglycolate (2PG) level consequent on the enhanced oxygenase activity of Rubisco under declining CO_2 levels may serve as a signal molecule inducing the CO_2 concentrating mechanism (CCM) but experimental evidence is missing. In the cyanobacterium *Synechocystis* sp. PCC6803, we have identified 4 putative phosphoglycolate phosphatases encoding genes- *sll1349*, *slr0458, slr1762* and *slr0586*, all bearing the HAD-like superfamily domain and Thr or Ser residue in the active site. Overexpression of some of them in *E. coli* and biochemical analyses confirmed PGP activity. By deletion and overexpression of sets of these genes we have modified the rate and extent of *Synechocystis* acclimation to changing CO_2 levels as determined by the time courses of expression (transcript abundance) of CO_2-dependent genes and the apparent photosynthetic affinity to external inorganic carbon. Our results clearly indicate that the level of 2PG may serve as a signaling molecule for the CO_2 concentration experienced by the cells in their environment.

P-85

CROSS-REGULATION OF METABOLISM AND VIRULENCE IN *LISTERIA MONOCYTOGENES*

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Listeria monocytogenes is an intracellular pathogen that causes serious illness in immuno-compromised individuals. *L. monocytogenes* replicates within its hosts' cytosol, where it detects various signals; metabolites and nutrients. Those signals modulate its virulence via the activation of the virulence master regulator, PrfA. Phosphorylated sugars, such as glucose-1-phosphate (G1P), activate PrfA and thus the transcription of the virulence genes. Additionally, it was previously demonstrated in our lab that low availability of branched chain amino acids (BCAAs), especially of isoleucine, triggers the activation of PrfA. This response is dependent on the isoleucine responsive regulator, CodY. To gain further insight to the regulation of *L. monocytogenes* intracellular metabolism and virulence, we performed a genetic screen to identify genes that are responsible for the detection of metabolic signals or that encode regulatory factors affecting PrfA activity. A *L. monocytogenes Mariner* transposon based mutant library was generated, harboring a Luciferase reporter system under the *hly* promoter, a gene that is largely regulated by PrfA. The library was screened under two conditions: with G1P as a carbon source, and under conditions of low concentration of BCAAs. Mutants with altered profile of *hly* transcription were chosen for further analysis. So far, 67 mutants were confirmed to differently regulate *hly* transcription and 23 were sequenced. Overall, we found that both core metabolic signals and secondary metabolic messengers influence *L. monocytogenes* virulence. Our aim is to complete the genetic screen and characterize the mutants, eventually characterizing a regulatory network that connects metabolism, signaling pathways and virulence in *L. monocytogenes*.

P-86

A NOVEL PROTEIN INVOLVED IN RIBOSOMAL MATURATION

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YbeY is an *Escerichia coli* protein important for efficient translation at all temperatures and essential at high temperatures. Therefore deletion mutants of ybeY are temperature sensitive. It is highly conserved among bacteria and is a heat shock protein. Here we use genetic and molecular means for analysis of its function and show that it is involved in ribosome maturation.

P-87

IMIPENEM RESISTANCE AND FITNESS COST IN *PSEUDOMONAS AERUGINOSA* DUE TO OprD LOSS ARE PARTIALLY COMPENSATED FOR AT THE POPULATION LEVEL

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The most common mechanism of imipenem (IMP) resistance (R) in *Pseudomonas aeruginosa* (PA) is the loss of OprD, a porin which enables the entry of IMP and basic amino acids into the cell. We aimed to characterize the fitness cost IMP-R-PA. The MICs and the growth characteristics on various carbons and amino acids as the carbon sources were compared between a spontaneous mutant IMP-R -PA due to OprD loss (R3), and a WT PAO1 strain. While growth in enriched media of the two strains were similar, R3 growth was impaired in restricted medium were arg or suc were the carbon source. When sub-inhibitory (0.02 MIC) IMP was added to the restricted media, R3 did not grow, thus suggesting reconstitution of IMP susceptibility. However, MICs at enriched media remained at the resistance level. Population analysis, revealed shift between R and S subpopulations based on growth conditions. Membrane protein analysis identified at least 14 proteins that were induced / repressed in the presence of IMP and / or arginine. This was confirmed by qRT-PCR analysis. Analyses suggest that OprF, a general major porin, is the main candidate for the hypothetical alternative channel for IMP/arg/suc entry in the absence of OprD. We conclude that OprD loss leading to IMP R results in nutritional disadvantage. At the population level, this fitness cost is partially compensated in restricted conditions, resulting in partial regain of IMP susceptibility.

P-88

REGULATORY INTERACTIONS BETWEEN CspC AND THE HEAT SHOCK RESPONCE

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The heat shock response is one of the major global regulatory networks in all organisms, which involves an increased cellular level of chaperones and proteases to enable correct protein folding and balanced growth. We have recently shown the involvement of one of the major RNA chaperones - CspC - in the regulation of the heat shock response. Here we will discuss the regulatory interactions between CspC and the heat shock response.

P-89

DEGRADATION OF THE CYANOBACTERIAL LIGHT HARVESTING COMPLEX: DAVID AND GOLIATH STORY

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The macromolecular pigment complex in cyanobacteria undergoes controlled degradation during nutrient starvation to prevent excessive light excitation and its consequent oxidative damage. An essential component in this process is the 6 kDa NblA protein. To elucidate the role played by NblA in pigment degradation we have expressed and characterized GFP tagged NblA protein (NblA::GFP) in the cyanobacterium *Synechococcus elongatus*. NblA::GFP was found to be localized to the photosynthetic membranes, which anchor the pigment complex. Support for a physical interaction between NblA and the major light harvesting pigment, was obtained by fluorescence lifetime imaging microscopy. Intriguingly, aside from its association with the photosynthetic membranes, NblA::GFP appeared as a single cellular focus in ~ 10% of the cells. The Clp-protease is also localized to cellular foci. Taken together, we suggest that NblA serves as a degradation tag by associating with the pigment and introducing it to the cellular degradation machinery.

P-90

AUTO-INHIBITION OF BIOFILM FORMATION MEDIATED BY A TYPE II PROTEIN SECRETION SYSTEM

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Information regarding the molecular mechanisms underlying cyanobacterial biofilms is very limited in spite of the environmental prevalence of these mats and their industrial implications. We revealed an inhibitory mechanism of biofilm formation in the unicellular cyanobacterium *Synechococcus elongatus* PCC7942. This common model organism exhibits a planktonic phenotype when grown under laboratory conditions. While screening a library of random transposon-insertion strains, we identified a mutant which unlike the wild type, developed biofilms. This suggested that the planktonic nature of the wild type strain is a result of an inhibitory mechanism, which is impaired in the mutant. Molecular analysis revealed that inactivation of a type II secretion system impaired this biofilm inhibitory mechanism. Furthermore, the conditioned medium from a wild type culture repressed biofilm formation by the secretion-mutant. These results indicate that the auto-inhibitory effect depends on the deposition of a factor to the extracellular milieu, which requires an intact type II secretion system. We also identified two genes that are essential for biofilm formation; inactivation of either one of these genes on the background of the secretion-mutant abolishes biofilm development. Transcripts levels of these genes are elevated in the mutant compared to the wild type, and are decreased in mutant cells cultured in conditioned medium of wild type (which inhibits biofilm formation). Taken together, we suggest that the type II secretion system is required for auto-inhibition of biofilm formation by secreting a substance that governs transcription of the biofilm essential genes.

P-91

STRUCTURE- FUNCTION STUDIES OF FTSZ & FTSZM FROM *MAGNETOSPIRILLUM GRYPHISWALDENSE* MSR-1

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Magnetotactic bacteria (MTB) can navigate through the magnetic field of earth. These bacteria synthesize organelles called "megnetosomes", which are contain magnetic nanoparticle (Fe_3O_4) or greigite (Fe_3S_4) and surrounded by lipid membrane. In MTB most of the genes which are involved in magnetosome formation, are located in genomic magnetosome island (MAI). *FtsZ* gene is located outside the MAI while *ftsZ like* gene located in the mamXY operon. Both genes encode to the tubulin-like *ftsZ* protein that involved in cell division in many bacteria. In MTB the function and structure of *ftsZ* is yet to be determined. To understand their function, we initiated biochemical study. Here we present biochemical analysis and structure models to both proteins.

P-92

INTER-SPECIES INTERACTION MEDIATES CELL LYSIS AND BIOFILM FORMATION THROUGH NUTRIENT AVAILABILITY AND SECONDARY METABOLITES

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Inter-species interactions critically influence the development and shape of the community and are significant for both medical and environmental studies.Nutrients availability, including iron, has been shown to play an important role during bacterial interactions. Under aerobic conditions, iron is present in insoluble mineral complexes or, in case of pathogens, bound to a mammalian host's own iron-binding proteins. Thus, many microorganisms have developed efficient means to obtain iron, such as iron-chelating molecules termed siderophores. *Pseudomonas aeruginosa* can utilize siderophores produced by other micro-organisms to facilitate iron uptake. Here we show that a *P. aeruginosa* strain deficient in siderophore production can use the *Vibrio cholerae* siderophore vibriobactin as an iron source. In addition, we identified a *P. aeruginosa* gene, *fvbA*, encoding a protein highly homologous to the *V. cholerae* vibriobactin receptor (ViuA). A *P. aeruginosa* mutant in the two endogenous siderophores (pyoverdine and pyochelin) and in *fvbA* was unable to utilize vibriobactin as an iron source. Additionally, preliminary analyses revealed the involvement of vibriobactin, Fur protein and an IclR-type regulator, FvbR, in *fvbA* regulation. Furthermore, we demonstrate the effect of iron on the interaction between *V. cholerae* and *P. aeruginosa* in planktonic and biofilm cultures. We show that *V. cholerae* lysis depends upon the ability of *P. aeruginosa* to utilize its endogenous siderophores, thus *P. aeruginosa* siderophores mutant reduced *V. cholerae* lysis compared to the wild type strain when grown in planktonic cultures. Our results also suggest that secondary metabolites secreted by *P. aeruginosa* can impact *V. cholerae* viability and biofilm formation.

P-93

MOLECULAR AND CELLULAR COMPARISON OF *SALMONELLA PARATYPHI* A AND *SALMONELLA TYPHIMURIUM* INVASION IN TO NON-PHAGOCYTIC CELLS

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Salmonella enterica serovars are responsible for two distinct clinical manifestations in humans: self-limiting gastroenteritis, caused by a large number of non-typhoidal serovars such as *S. typhimurium* (STM) and enteric fever, a life threatening systemic infection caused by the human-restricted serovars *S. typhi* and *S. paratyphi* A (SPA). Despite the different disease manifestation, invasion into non-phagocytic cells is a common and essential virulence trait of all *Salmonella* serovars. Herein, we compared and analyzed the ability of 17 SPA strains and 3 STM strains to actively invade epithelial cells. Surprisingly we found that while SPA and STM adhere similarly to host cells, SPA was dramatically less invasive than STM under several examined conditions. Additionally, we show that growth conditions have a profound impact on SPA invasion relative to STM. The ability of *Salmonella* to interact with host cells is largely dependent on the optimal function of the *Salmonella* pathogenicity island-1 (SPI-1) encoded type III-secretion system (T3SS-1). In contrast to STM, the role of SPA T3SS-1-dependent invasion was found to be cell type and growth condition dependent. Real-Time PCR analysis revealed an overall lower expression of the SPI-1 regulon in SPA compared to STM. Secretion levels of the sopB effector were also found to be significantly lower in SPA then STM. Furthermore, we observed significant decreased motility of SPA vs. STM. Collectively, our results indicate profound differences in motility, invasion and in the role of T3SS-1 between SPA and STM which may play a role in the unique disease manifestation of both serovars.

P-94

PROTEIN ENGINEERING OF 2-HYDROXYBIPHENYL 3-MONOOXYGENASE FROM *PSEUDOMONAS AZELAICA* FOR SYNTHESIS OF HYDROXYTYROSOL

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Hydroxytyrosol is one of the most powerful antioxidants with a high free radical scavenging capacity. Hydroxytyrosol can be found in olives, virgin oil and wine. It was shown that hydroxytyrosol has many beneficial attributes such as anti-inflammatory activity, cardioprotective capacity, inhibition of platelet aggregation, anticancer and antidiabetic activity. Thus, an efficient biocatalytic route to hydroxytyrosol will have a great potential for both pharmaceutical applications and functional food. 2-Hydroxybiphenyl 3-monooxygenase (HbpA) from *Pseudomonas azelaica* is a single-component enzyme, NADH dependent, that catalyzes the first step in the degradation pathway of 2-hydroxybiphenyl. Our goal is to use both rational design and directed evolution to alter HbpA regiospecificity towards the production of hydroxytyrosol from *p*-tyrosol. *HbpA* was cloned into *E. coli* for overexpression and the protein was purified using a His-tag. The activity on 2-hydroxybiphenyl was measured using a colorimetric assay based on NADH depletion as well as HPLC analysis. To enable a massive and profitable hydroxytyrosol production in vitro, the NADH cofactor must be recycled. A novel NADH recycling system based on a soluble hydrogenase, termed the C@Enzyme system, will be used to allow the continuous synthesis of hydroxytyrosol. The soluble hydrogenase and diaphorase proteins are immobilized onto graphite beads to facilitate electron transfer. The benefit of this system is the use of H_2 as an electron donor; therefore it has 100% atom efficiency without by-products. HbpA can be immobilized onto the beads or employed in the solution bulk in order to transform a *p*-tyrosol into hydroxytyrosol.

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ENZYMATIC MECHANISMS INVOLVED IN CARBAMAZEPINE TRANSFORMATION BY THE WHITE ROT FUNGUS *PLEUROTUS OSTREATUS*

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Carbamazepine (CBZ), a widely used anticonvulsant drug, exhibits very limited removal efficiency in municipal wastewater-treatment plants and shows high persistence in the environment due to its high resistance to microbial degradation. CBZ is found in wastewater effluents, surface water, groundwater, and drinking water. It can also be introduced into agricultural soils via reclaimed wastewater irrigation, and be taken up by crops. CBZ has also been found to have an ecotoxicological impact on aquatic organisms. However, CBZ modification by different fungi has been recently reported. The aim of this study is to elucidate the metabolic pathway of CBZ by *P.* ostreatus. When *P. ostreatus* PC9 was grown in liquid medium which support both cytochrome P450 (CYP450) and manganese peroxidase (MnP), 99% of the added CBZ (10 mg L^{-1}) was eliminated from the solution and transformed to 10,11-epoxycarbamazepine (EP-CBZ). High removal of CBZ was also obtained when either MnP or CYP450 was active. When both CYP450 and MnP were inactivated, only 10 to 30% of the added CBZ were removed. Thus we suggest that these two activities are involved in the oxidation of CBZ in liquid culture. *P. ostreatus* was able to eliminate CBZ in liquid culture even when CBZ was added at an environmentally relevant concentration (10 μg L^{-1}). EP-CBZ accumulated in the medium until day 10, then its concentration decreased and a new metabolite, 10,11-Dihydro-10,11-dihydroxy carbamazepine, was formed. These results highlight the potential of using *P. ostreatus* for detoxification, since 10,11-Dihydro-10,11-dihydroxycarbamazepine, unlike the CBZ and EP-CBZ is not pharmacologically active.

P-96

COPPER ACCUMALATION AND ITS EFFECT ON THE SELECTIVITY OF TYROSINASE FROM *BACILLUS MEGATERIUM*

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Tyrosinase belongs to the type 3 copper enzyme family, containing a di-nuclear copper center, CuA and CuB. It is mainly responsible for melanin production in a wide range of organisms. In the presence of molecular oxygen tyrosinase catalyzes the formation of monophenols to commercially valuable diphenols, but its use is limited due to further oxidation of diphenols to quinones. Although copper ions are essential for the activity of tyrosinase, the mechanism of copper uptake is still unclear. We have recently determined a crystal structure of tyrosinase from *Bacillus megaterium* (TyrBm) and revealed that this enzyme has tighter binding of CuA in comparison to CuB. Investigating copper accumulation in TyrBm, it was found that copper presence has a more significant effect on the diphenolase activity. Therefore, by decreasing copper concentration, the diphenolase/molophenolase ratio was increased by 2-fold. Using a rational design approach, we have identified five variants having an impact on copper uptake. We have found that the role of a highly conserved Asn205 residue is to stabilize His204 in the binding site and thus to allow a proper coordination of CuB. Further investigation of these variants revealed that Phe197, Met61 and Met184 residues, which are located at the entrance to the binding site, not only play a role in copper uptake but are also important for the diphenolase activity. In this study we are proposing a mechanism of copper accumulation as well as an approach to changing the selectivity of TyrBm towards L-dopa production.

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REVEALING NOVAL GLYCOSIDE HYDROLASES BY CONTEXTUAL METAGENOMICS

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Biomass is considered an attractive and immediate source for liquid biofuel that do not contribute net CO_2 to the atmosphere. The hydrolysis of lignocellulose to soluble sugars that can be fermented to ethanol involves a costly enzymatic step. Lignocellulose hydrolysis requires the synergistic actions of several enzymes termed glycoside hydrolases (GHs). In the framework of this research, metagenomics and biochemical approaches are combined to isolate and characterize novel enzymatic systems geared for lignocellulose hydrolysis and biofuel production. We attend to screen metagenomes databases in order to detect novel GHs with targeting on low sequence similarity genes. Our sequence based screening applies the genomic neighboring (GN) approach following a sensitive search. The GN approach searches the metagenome for putative GH sequences located in gene clusters related to lignocellulose utilization. The sensitive search aims to identify distanced GH homologous sequences with low sequence similarity based on phylogenetic relativity. Both algorithms are based on hidden Markov models (HMM) profile searches. In our preliminary results the GN search approach on three nodes of a human gut microbial metagenome had yielded 21 putative novel GH sequences. The profile based search had identified sequences from several GH families. A comprehensive search algorithm consists of several search tools is proposed.

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C-TERMINAL BIOMASS SENSOR OF ANTI-SIGI6 FACTOR RSGI6 IN *CLOSTRIDIUM THERMOCELLUM* RESEMBLES GH10 XYLANASES AND FORMS A STRUCTURALLY NOVEL FOLD

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Scaffoldin protein of cellulosome complex of cellulolytic *Clostridium thermocellum* bacterium have nine cohesins modules to mount nine cellulose degrading enzyme molecules. In contrast, it can express over 70 different enzymes that can degrade diverse types of cellulolytic materials into simpler soluble polysaccharides that the bacteria can use. It was shown recently that to decide what enzymes to express, *C. thermocellum* regulates σ transcription initiation factors (used to enable specific binding of RNA polymerase to gene promoters) sensing nearby biomass composition by virtue of RsgI multi-domain trans-membrane proteins. C-terminal modules of RsgIs comprise proteinaceous modules that act as specific biosensors of specific components of biomass. Here we describe X-ray structure determination of C-terminal biomass sensor of RsgI6 of *C. thermocellum* in space group $P2_1$. The structure contains two molecules in asymmetric unit and was determined by molecular replacement using structure of bioinformatically detected, sequentially similar GH10 xylanase from *Aspergillus nidulans*. The structure represents novel fold with N-terminal and C-terminal parts of the sensor interlaced in the region of N-terminal.

P-99

CROSS-LINKING OF SOY PROTEINS GLYCININ AND β-CONGLYCIN IN USING TYROSINASE FROM *BACILLUS MEGATERIUM*

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Tyrosinases are members of the type-3-copper protein family and are mainly responsible for the formation of melanin. Tyrosinases are polyphenol oxidases that catalyze (i) the *ortho*-hydroxylation of monophenols to *o*-diphenols and (ii) the oxidation of *o*-diphenols to *o*-quinones. The reactive *o*-quinones can crosslink proteins by covalent bonds, or autoxidize, producing brown pigments (melanins). Their ability to cross-link proteins has been proposed for modification of food structure, and several studies have shown this on milk, meat and cereal proteins. Tyrosinase from *Bacillus megaterium* (TyrBm) was isolated, characterized and its crystal structure was determined in our lab. In the present research, we have evaluated TyrBm for its ability to cross-link soy proteins, glycinin and β-conglycinin. Soy protein isolate (SPI) is being used extensively as a functional ingredient in processed foods. However, although SPI functions reasonably well as an emulsifier in concentrated emulsions, its use as a surface active agent in diluted emulsions and foam type products is limited. Our aim is to improve the foaming, emulsifying and gelation properties of soy protein in order to broaden its applications in the food industry. Glycinin and β-conglycinin were purified from defatted soy flakes and subjected to TyrBm's oxidation activity. High-molecular weight fractions of glycinin were formed in the presence of a low molecular weight phenolic reagent as demonstrated from SDS-PAGE analyses, dynamic light scattering and static light scattering. Improved emulsion stability was observed using space- and time-resolved extinction profiles (LUMisizer) and suggested a lower creaming velocity in enzymatic-treated glycinin based emulsions.

P-100

LACTIC ACID BACTERIA FERMENTED PUMPKIN BASED PROBIOTIC BEVERAGE

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The lactic acid fermentation of vegetable products applied as a preservation method for the production of finished ready to use products is considered as an important technology since time immemorial. Nowadays the main reasons for growing consumption and scientific interest on lactic acid fermented foods are their nutritive value, physiological i.e. functional properties, hygienic stability and production costs. In recent years consumers demand for nondairy-based probiotic products has increased. Besides traditionally fermented vegetables or their juices e.g. cabbages, cucumbers, carrots, tomatoes, beets etc., less common vegetable substrates e.g. pumpkin (*Cucurbita pepo*), particularly their juice can be processed by lactic acid fermentation with certain hetero- and homofermentative probiotic cultures to broaden choice of healthy beverages. Pumpkin is one of well-known edible plants and has substantial dietary and medical properties (hepato-protective, anti-diabetic, antioxidant, hypotensive, anti-carcinogenic, anti-microbial, anti-inflammatory) due to the presence of unique natural substances. However application of scientifically proven probiotic cultures for fermentation of vegetable substrates often is difficult due to specific physiological and technological properties of applied probiotic, or probiotic strains containing mixed starter cultures. Therefore selection of appropriated probiotic strains of lactic acid bacteria, in regard of their technological potential, e.g. acidification power etc., and influence on the sensoric properties of the finished fermented product should be performed. This research was supported by ERAF 2.1.1.1. Contract's Nr 2010/0322/2DP/2.1.1.1.0/10/APIA/VIAA/108/pre

P-101

EFFECTS OF NITROGEN AND CARBON SOURCES ON RDX DEGRADATION UNDER ANAEROBIC CONDITIONS IN STRAIN *RHODOCOCCUS* YH-1

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Hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) is widely used explosives, often polluting soils and groundwater; its fate in the environment mainly controlled by microbial biodegradation. *Rhodococcus* YH1 was shown to degrade RDX aerobically through a cytochrome P450 enzyme encoded by the *xplA* gene. In this study, it was found that it is also capable of utilizing RDX as a sole nitrogen source under anaerobic conditions. The RDX ring was cleaved and methylenedinitramine (MEDINA) was detected as an intermediate. Inorganic nitrogen and organic carbon sources had a profound impact on RDX degradation. Biodegradation experiments under anaerobic conditions demonstrated that increasing concentrations of nitrate, nitrite or ammonium as external nitrogen sources delayed the degradation of RDX. When glucose was used as a carbon and energy source for anaerobic biodegradation of RDX, RDX was completly degraded with concurrent formation of mixed organic acids as fermentative end products. However, cyclohexanone, utilized by this strain during aerobic degradation of RDX, did not support the anaerobic degradation of RDX. These findings lead us to hypothesis that similary to aerobic conditions, under anaerobic conditions, external inorganic nitrogen represses *xplA* expression in strain YH1. Further, we hypothesize that biodegradation of RDX under anaerobic conditions by this strain depends on its ability to generate reducing power for reductase-cytochrome P450 complex involved in the initial step of RDX degradation. Thus, under anaerobic conditions the ability of YH1 and other *xplA* carrying rhodococci to degrade RDX depends not only on the presence of alternative sources of nitrogen but also on the type of carbon and energy sources.

P-102

BIODEGRADATION OF CYCLIC AZO DYE BY A NOVEL BACTERIAL CONSORTIUM ABR-1 AND TOXICITY EVALUATION OF METABOLITES BY DIFFERENT BIOASSAY SYSTEMS

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The textile industry actively uses large amounts of azo dyes for the purpose of dyeing fabrics. The term azo dye is applied to synthetic organic colorants that are characterized by a nitrogen to nitrogen double bond (-N=N-). Azo dyes have poor exhaustion properties and as a result 10-15% of the dyestuff used remains unbound to the fibre and is therefore released into the environment leading to ecotoxicity. Biological systems have gained considerable importance over the existing physico chemical techniques (adsorption, precipitation chemical oxidation) for the treatment of these dye containing wastewaters. A novel bacterial consortium (ABR-1) comprising of different *Bacillus* strains with the potential to rapidly decolorize and degrade the cyclic azo dye Direct Blue-1 (DB-1) was developed. The individual strains of the consortia were identified as *Bacillus cereus*, *Bacillus endophyticus*, *Bacillus infantis*, *Bacillus mojavensis*, *Bacillus cohnii* and *Bacillus halodurans*. The consortium was able to completely decolorize 1000 mg l^{-1} of DB-1 in 4 h under static conditions. The biodegradation of DB-1 was monitored by UV-Vis, FTIR, HPLC and LCMS. Possible biodegradation pathway for DB-1 was proposed based on the degradation pattern. Phytotoxicity study with seeds of *Vigna mungo*, *Sorghum bicolor* and *Vigna radiata* revealed less toxic nature of the degradation metabolites compared to the dye. Biotoxicity test with *Artemia salina* proved the lethality of the DB-1. Genotoxicity with *Allium cepa* confirmed the cytotoxic nature of dye by inducing several chromosomal abnormalities compared to the negligible effects of metabolites.

P-103

MOLECULAR AND ISOTOPIC ANALYSIS FOR EVALUATION OF *IN SITU* DEGRADATION OF BROMINATED COMPOUNDS

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We investigated if compound-specific isotope analysis (CSIA) in combination with molecular biological approaches may be used to assess the *in situ* anaerobic biodegradation of brominated compounds. Laboratory reference experiments showed that a significant enrichment of ^{13}C in the added substrate occurred during microbial dehalogenation of brominated ethenes, however, patterns were different from parallel experiments with chlorinated ethenes. These results suggest the potential use of CSIA for *in situ* assessments of reductive debromination, however, further research is warranted for an understanding of the observed differences. To further explore this potential, a field site in Israel, where brominated as well as chlorinated ethenes and benzenes were found in high concentration, was investigated and the isotope pattern of these compounds was analyzed. Preliminary results revealed an *in situ* degradation of vinyl bromide whereas vinyl chloride seems to be a product from the dechlorination of higher chlorinated ethenes (e.g. tri-/dichloroethene), shown by the change of the isotope signature from enriched to depleted in ^{13}C. In contrast, the isotope values of monochlorobenzene as well as brominated toluene did not change along a predicted groundwater flow, suggesting that these compounds were not degraded. Using taxon specific PCR of organohalide respiring microorganisms, *Dehalococcoides*-like bacteria could be detected in all samples whereas *Geobacter*-like bacteria where detected only selectively. Further analysis of the overall diversity as well as detection of the responsible genes (functional marker genes) will contribute to assess whether dehalogenation is taking place and which organisms are involved in this process.

P-104

BIOMASS SENSOR OF ANTI-SIGMA FACTOR IN *CLOSTRIDIUM THERMOCELLUM* CONTAINS TWO INTERLACED PA14 MODULES AND STRUCTURALLY RESEMBLES PART OF ANTHRAX TOXIN

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Scaffoldin protein of cellulosome complex of cellulolytic bacterium *Clostridium thermocellum* has nine cohesin modules to mount nine cellulose degrading enzyme molecules. In contrast, it can express over 70 different enzymes that can degrade diverse types of cellulolytic materials into simpler soluble polysaccharides that the bacteria can use. It was shown recently that to decide what enzymes to express, *C. thermocellum* regulates s transcription initiation factors (used to enable specific binding of RNA polymerase to gene promoters) sensing nearby biomass composition by virtue of RsgI multi-domain trans-membrane proteins. C-terminal modules of RsgIs comprise proteinaceous modules that act as specific biosensors of specific components of biomass. Here we describe X-ray structure determination of C-terminal biomass sensor of RsgI3 of *C. thermocellum* in space group I222. The structure contains one molecule in asymmetric unit and was determined by molecular replacement using structure of bioinformatically detected, sequentially similar PA14 module of toxin from *Bacillus anthracis*. The molecule forms a symmetric dimer related by two fold crystallographic rotation by swapping apparently imitating architecture between two similar tandem domains PA14A and PA14B that are present in RsgI3.

P-105

STUCTURE-FUNCTION STUDY OF GH27 β-ARABINOPYRANOSIDASE FROM *GEOBACILLUS STEAROTHERMOPHILUS* T-6

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Geobacillus stearothermophilus T-6 is a thermophilic soil bacterium possessing an extensive system for the utilization of arabinan. This bacterium produces a limited number of extracellular and intracellular enzymes that cleave the high molecular weight arabinan backbone and its short decorated oligosaccharides, respectively. One of the intracellular enzymes is Abp, a family 27 glycoside hydrolase. Abp has an activity of a β-L-arabinopyranosidase, and is capable of removing arabinopyranose residues from natural arabinose-containing polysaccharides. The crystal structure of Abp was determined at 2.3 Å resolution. The Abp monomer is comprised of a catalytic $(\beta/\alpha)_8$–barrel domain and a C-terminal domain made of eight antiparallel β-strands. The biological unit of Abp appears to be a tetramer, based on gel filtration experiments and bioinformatic analysis. Most enzymes from GH27 family exhibit α-D-galactosidase activity, while some others demonstrate an activity of β-L-arabinopyranosidase. Abp is highly specific to β-L-arabinopyranose and exhibits only marginal activity on α-D-galactopyranose ($(k_{cat}/K_M)_{ara}/(k_{cat}/K_M)_{gal} = 2.52 \cdot 10^5$). Multiple sequence alignment of Abp with representative GH27 family enzymes revealed a single residue that is probably involved in substrate specificity. Galactosidases in GH27 family have an Asp residue in that position, which forms a hydrogen bond with Gal-O^6, thus stabilizing the binding of galactose. In Abp the corresponding residue is Ile67 which interferes with galactose binding. A single replacement of Ile to Asp, I67D, resulted in three orders of magnitude increase in specificity towards α-D-galactopyranose with only 2.7-fold decrease in specificity towards β-L-arabinopyranose ($(k_{cat}/K_M)_{ara}/(k_{cat}/K_M)_{gal} = 1.86 \cdot 10^2$).

P-106

REGULATION ANALYSIS OF CELLULOSE RELATED GENES BY ALTERNATIVE σ FACTORS IN *CLOSTRIDIUM THERMOCELLUM*

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Clostridium thermocellum is a gram positive, anaerobic and thermophilic soil bacterium that secretes a high molecular weight protein complex, the cellulosome, aimed for crystalline cellulose degradation. *C. thermocellum* attracts much interest because of its ability to both hydrolyze cellulose and produce ethanol in one step called consolidated bio-processing. Recently, seven clusters of σ and anti- σ factors that play a role in the regulation of cellulosomal genes have been identified in *C. thermocellum*. These σ factors are positioned adjacent to a downstream gene encoding to a multi-modular protein containing a trans-membrane helix with an anti - σ domain at the N-terminus and a polysaccharide-related function module at the C-terminus, revealing a novel regulatory mechanism, based on sensing the composition of extracellular carbohydrates and regulating the expression of genes encoding for carbohydrate degrading enzymes. In this work, this mechanism was directly challenged by disrupting one of the σ – anti σ regulatory mechanism, SigI6 – RsgI6, by deleting the *rsgI6* gene and the transcription levels were measured by real – time RT PCR. In total, 4 genes, including *sigI6*, exhibited increased transcript level in the *rsgI6* deletion mutant compared to its parental strain. The results from this analysis enabled us to better characterize the consensus promoter sequence recognized by SigI6. The *rsgI6* – deleted strain showed also a significantly increased specific activity on two synthetic substrates: para-nitrophenyl- β-D-xylopyranoside (pNPX) and para-nitrophenyl- β-D-xylobioside (pNPX2), which demonstrated the increased xylanase activity and connected between regulation and biochemical activity.

P-107

BIODEGRADATION OF STYRENE BY NEWLY ISOLATED BACTERIAL LACCASE

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Styrene is an important chemical pollutant exerting toxic effect on the liver, kidneys and nervous systems. Styrene serves as a raw material of polystyrene industry. The annual global demand of styrene in 2010 reached 17.5M tons and its consumption trend is showing a permanent increase. Until recently, only a couple of enzymes that are capable of transforming styrene (styrene mono and di-oxygenases). In the last few years, a new fungal enzyme (laccase) has emerged as a biodegraders, of styrene. This enzyme originated from fungi, such as *Phanerochaete chrysosporium, Trametes versicolor* and *Daldinia concentrica.* We have isolated a soil bacterium which cannot exploit styrene as a sole carbon and energy source but harbors the laccase enzyme as shown by the laccase activity test. We have purified the enzyme by affinity column using Ni-NTA resin while multiplying its activity compared to crude supernatant. This laccase activity shows a 10 fold higher than that of the fungal laccase. Styrene degradation assay shows that the purified regenerated enzyme retains the ability to convert styrene to its products at much lesser concentration than fungi laccase. This laccase seems to be an effective solution for minimizing the styrene waste.

P-108

THE MACROMOLECULAR CRYSTALLOGRAPHY RESEARCH CENTER (MCRC) AT BEN-GURION UNIVERSITY OF THE NEGEV

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The Macromolecular Crystallography Research Center (MCRC) is located in the Department of Life Science of Ben-Gurion University of the Negev. The main goal of the MCRC is to serve as a service center for laboratories inside and outside the university who are interested in structural information as part of their research goals. This information may decipher the structure/function relationship on the macromolecules under consideration. In order to obtain well diffracted macromolecular crystals, the MCRC provides an automated protein crystallization process. This process starts with the design of the experiments, continues with the use of liquid handler and drops dispenser and finishes with automated imaging of the crystallization plates under a user-defined schedule. This process saves time and material as it relies on robotics which uses tiny amounts of a sample. In order to view and score his crystallization plates images, the user can access our RockMaker website (Formulatrix) from any computer inside and outside BGU University. Our preliminary success in crystallization so far includes a photosynthetic protein, synthetic protein and peptides, a protein which involves in the quorum sensing process in bacteria and more. The MCRC is equipped with two X-ray systems which are used for crystals measurements as well as with computer systems and all computer programs which are needed for the structure determination of macromolecules.

P-109

USE OF FORMALDEHYDE-DEGRADING BACTERIA FOR BIODEGRADATION OF CONTAMINATIED WASTEWATER

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Formaldehyde, a volatile organic compound, is widely used in the chemical industry especially in the production of phenolic and urea resins. This toxic substance may become a reason of growth disorder, blindness, and respiratory diseases of living-organisms. Considering its high cytotoxicity toward human health and the environment, the removal of formaldehyde from soil, water, and air has become a necessity. Several formaldehyde degrading-microorganisms were isolated in our lab from soil and identified as belonging to the genera *Pseudomonas, Sinorhizobium* and *Methylobacterium.* The aim of the present work is to utilize these bacterial strains for bioremediation of formaldehyde contaminated-wastewater. The isolated strains were able to grow on agar plates containing 0.25% formalin as the sole carbon source and were able to fully degrade 500 ppm formaldehyde within a few hours. Several methods were developed to quantify formaldehyde. A colorimetric assay (Hantzsch method) based on reactivity with acetylacetone under alkaline conditions was found suitable for detecting formaldehyde at concentrations of 50-1000 ppm. A commercial kit based on a reaction with chromotropic- acid was suitable for low concentrations of 0.02-8 ppm. An HPLC method was developed for the range of 0.1-1000 ppm. This method is based on the formation of hydrazones formed from the complex between aldehydes and 2,4-dinitrophenylaldehydrazine. These products are easily separated by chromatography using a variety of reverse-phase HPLC columns and monitored by wavelength of 360 nm. Immobilization of the degrading bacteria is now underway in our lab for enabling continuous use of the biocatalysts in a bioreactor.

P-110

COHESIN-INDUCED THERMOSTABILITY ENHANCEMENT OF A *CLOSTRIDIUM THERMOCELLUM* DOCKERIN-CONTAINING CELLULOSOMAL ENDOGLUCANASE

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The thermostability of endoglucanase Cel8A, a major component of the cellulosome complex from *Clostridium thermocellum*, was significantly enhanced using a directed evolution strategy. A two-step screening strategy was employed that involved consecutive activity and thermostability assays. We have combined three of the mutations from the thermostability screen to obtain a Cel8A variant with a significant increase in thermal resistance without substantial alteration of kinetic parameters. One of the three mutations provided the highest contribution to enzyme stability. This single mutation served to increase the T_m by 7.0□°C and the half-life of activity by eight fold at 85□°C. The mutant was then incorporated into a designer scaffoldin composed of different cohesin modules. The synergistic activity with other enzymes was tested for degradation of a more recalcitrant substrate. It was combined to different exoglucanases: a recombinant form of the Cel48S exoglucanase of *C. thermocellum* or a recombinant form of the Cel48A exoglucanase of *Thermobifida fusca*. When it was not connected to a cohesin module, the Cel8A mutant was shown to provide higher activity than the wild-type form after a prolonged period of incubation (3 to 4 days). On the other hand, connection to its respective cohesin module confers higher enzymatic activity to the wild-type form. These results demonstrate that the cohesin-dockerin binding event provides stability to the wild-type enzyme which eliminates the advantages of the thermostable mutant. In future screening approaches, the directed evolution process should be achieved on the cohesin-bound form of the enzyme.

P-111
SYNERGY AMONG FAMILY-48 EXOGLUCANASES AND FAMILY-124 ENDOGLUCANASE IN TRIVALENT DESIGNER CELLULOSOME COMPLEXES

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The cellulosome is a multi-enzymatic complex that efficiently degrades the plant cell wall. Cel124 is a newly discovered *C. thermocellum* endoglucanase, with a unique sequence and fold, and it thus belongs to a new GH family. It bears an N-terminal atypical type-I dockerin, which binds preferentially to the cohesin of the cell-envelope protein OlpC, rather than the cohesins in the cellulosomal scaffold protein, CipA. Cel48S is the most abundant exoglucanase and a key component in the cellulosome of *C. thermocellum*. The combined activity of the targeted Cel48S and Cel124 resulted in a 1.9-fold enhancement of cellulose hydrolysis, compared to the additive value when the two enzymes were used in isolation. The synergism between the family-48 and family-124 cellulases was further investigated in designer cellulosome complexes. The addition of the endoglucanase Cel124 to a divalent designer cellulosome complex containing two family-48 exoglucanases, resulted in a 1.7-fold enhancement of cellulose degrading activity, owing to the contribution of Cel124 and to the synergism induced by the combined proximity and targeting effects.

P-112
STRUCTURE OF A NOVEL STAND-ALONE COHESIN PROTEIN FROM *RUMINOCOCCUS FLAVIFACIENS*

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Ruminococcus flavefaciens is a cellulolytic bacterium found in the rumen of herbivores where it plays an important role in cellulose degradation. It produces one of the most elaborate and variable cellulosome systems known in nature, with an unprecedented number of genes encoding cellulosomal proteins of different types. In addition to cellulosomal proteins with known function, several separated proteins of cellulosomal type but of unknown function are expressed by *R. flavefaciens.* One of exceptional gene organizations codes for the single cohesin domain (*Rf*COHG, ZP_06142108) that is classified as type III cohesin. Here we describe structure of type III cohesin module in space groups C2 and P1. These crystals contained eight and twelve copies of the Se-Met derivative and native protein molecules in the asymmetric unit and diffracted to 2.43 Å and 2.03 Å. The structures were determined consequently using SAD and molecular replacement methods respectively. The structures of *Rf*COHG protein revealed the most complex cohesin among all known functional cohesin structures. In addition to a common beta-sandwich structure it possesses four a-helices, while the other known cohesin structures possess up to two a-helices. Biological significance of this helical decoration is discussed.

P-113

THE ACCELERATION OF BIODEGRADATION OF COMPLEX HALOGENATED ORGANIC COMPOUNDS IN INDUSTRIAL WASTEWATER

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Owing to the large scale industrial production and extensive usage of halogenated organic compounds (HOC) over the past few decades, as well as their inherent toxicity and limited biodegradability, those synthetic chemicals have accumulated in almost all compartments of our environment. Therefore, inappropriate treatment of industrial wastewater and waste containing halo-organic compounds causes a great environmental concern. Biological treatment strategies, which are mostly microbially mediated, are considered the preferable approach for clean-up of contaminated sites. At the Ramat Hovav industrial area, many industries are located producing various HOC, of which dibromoneopentyl-glycol (DBNPG) is highly abundant. The wastewater contains a complex mixture of organic pollutants with varying composition over time. Here we report the application of a continuous biological process with complete biomass retention to the biodegradation of DBNPG under controlled laboratory conditions, using a novel mini membrane bioreactor (mMBR) system that allows simulating treatment of wastewater containing HOC. The mMBR was successfully operated for a period of 120 days. Following an acclimation period of about 30 days, the reactor showed long-term stable biodegradation performances of approximately 50%. Key microbial groups, which might take part in the biodegradation process were tracked and quantified by real time PCR (qPCR) using specific primers that were designed by us. This knowledge will aid in future development of efficient treatment processes for wastewater contaminated with HOC using various engineered systems.

P-114

IDENTIFICATION OF GROWTH-RATE RELATED REGULATORY ELEMENTS OF CELLULOSOMAL GENES IN *CLOSTRIDIUM THERMOCELLUM*

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Clostridium thermocellum is an anaerobic thermophile that utilizes crystalline cellulose efficiently. The hallmark of the cellulose degradation system is an extracellular multi-enzyme complex termed the cellulosome. There are approximately 70 genes, mostly monocistronic, that encode cellulosomal enzymatic subunits (including cellulases, xylanases and other hemicellulases). This catalytic variety enables production of cellulosomes with different compositions, depending on the composition of the extracellular polysaccharides. Works by our group and others revealed that many cellulosomal genes are up-regulated during slow growth rates ($0.05 \times hr^{-1}$) in continuous cultures under carbon limitations. This suggests that growth-rate related regulation is involved in the transcription of cellulosomal genes. One of these genes is the anchoring protein *olpB*. In order to detect regulatory proteins that recognize *olpB*'s promoter sequence, transcription factor isolation was performed. Soluble protein extract from *C. thermocellum* was incubated with biotin-labeled DNA fragments containing *olpB*'s promoter region and loaded on a streptavidin column. The bound proteins were eluted, analyzed by LC-MS/MS, and aligned against the *C. thermocellum*'s NCBI-nr database. The most common proteins detected were GntR family transcriptional regulator (Clo1313_0035), and AbrB (Clo1313_2758), a global pleiotropic regulator well studied in *Bacillus subtilis*. AbrB has several putative consensus sequences and it regulates many genes, suggesting that it might have a role in regulating cellulose utilization in this industrially important bacterium. Aligning $AbrB^{Cth}$ with $AbrB^{Bsub}$, revealed a 90% identity in the N-terminal DNA-binding domain and a complete identity in the DNA binding residues (identified by NMR resolved $AbrB^{Bsub}$-DNA structure); thus, suggesting binding to similar sequences in both bacteria.

P-115

FROM SMELLY WINES TO ALZHEIMER- CYSTEINE CATABOLISM TO RELEASE H_2S

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Volatile sulfur compounds are a key component of wine aroma, contributing both attractive and repulsive odours. A prominent unpleasant sulfur compound is hydrogen sulfide (H_2S), which possesses an odour reminiscent that of 'rotten eggs'. The yeast *Saccharomyces cerevisiae* is able to produce H_2S using inorganic sulfur sources as well as organic sulfur sources such as cysteine and glutathione. As *S. cerevisiae* is a model organism of the eukaryotic cell, the study of cysteine catabolism to release H_2S holds a broader relevance, rising from H_2S increasing eminence as an essential modulator of many physiological processes, particularly considering that cysteine is the main source for H_2S formation in mammalian cells. These research interests motivated a comprehensive genome-wide screen of the yeast deletion collection for cysteine catabolism to release H_2S. To this end a novel, patent-pending method was developed, suitable for high throughput detection of cysteine-generated H_2S. Following its verification the method was used for a genome-wide screen for cysteine catabolism to release H_2S. Results of the screen revealed a surprising set of cellular factors affecting this process. The yeast vacuole, not previously associated with cysteine catabolism, emerged as a major compartment for cysteine degradation and the mechanism of vacuole acidification was identified as highly important. Results of this study provide useful advice and techniques for aroma wine management. Additionally, foundations laid here support the use of *S. cerevisiae* as a model organism to study cysteine catabolism and may provide insights into the underlying cause of cysteine accumulation and H_2S generation in eukaryotes.

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CHORISMATE IS A STUMBLING BLOCK IN THE PATH FOR SUSTAINABLE AROMATICS

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Increasing prices and decreasing natural resources availability have inspired the chemical industry to search for more sustainable alternatives for feedstock chemical production. A significant amount of funds have been invested in metabolic engineering of microbial strains to produce aromatics compounds deriving from the shikimate pathway, a pathway used for aromatic amino acid (AAA) synthesis in microorganisms. The shikimate pathway is a seven steps process that ends with the formation of chorismate, which is the branching point for AAA and other metabolites synthesis. These metabolites include p-amino benzoic acid (PABA) and p-hydroxybenzoic acid (PHBA), used in the pharmaceutical and the liquid crystal polymers industries, respectively. *Saccharomyces cerevisiae* strains engineered for increased PABA and PHBA production, deleted for all AAA synthesis pathways, have shown an unexpected accumulation of phenylalanine and phenylpyruvate. *In vivo* enzymatic assays revealed phenylpyruvate accumulation is not a result of enzymatic activity. On the other hand, chorismate stability examination showed a facile spontaneous transformation of chorismate to phenylpyruvate during the course of fermentation. Genetic mutation of the aromatic amino transferase enzymes involved in phenylalanine synthesis from phenylpyruvate (Aro8, Aro9), inferred that phenylalanine synthesis was facilitated enzymatically from phenylpyruvate. These results point out to a major stumbling block in the process of metabolic engineering of any chorismate derived product, one that would require careful balancing of the rate of chorismate production and consumption through genetic or environmental means.