Abstract Title:
Evidence of Three-Way Life Strategies Associated to Complex Bacterial Communities after Succession Under Varying Disturbances

Primary Author Block:
E. Santillan1, H. Seshan2, F. Constancias1, S. Wuertz1; 1Singapore Ctr. for Environmental Life Sci. Engineering, Singapore, Singapore, 2Brown and Caldwell, San Diego, CA

Abstract Body:
Background: Functional tradeoffs are expected after community perturbations since organisms reallocate resources to facilitate recovery. Microorganisms could then adopt distinct life strategies across a disturbance range, which has been suggested but not yet verified. Here we analyzed the effect of disturbance on the assembly, functionality, and distribution of traits in activated sludge bacterial communities, to identify community life-strategies using an ecological trait-based framework. Methods: Sequencing batch microcosm bioreactors (20 mL working volume) were inoculated with activated sludge from a full-scale plant and operated for 35 days. The daily complex feed included toxic 3-chloroaniline (3-CA) at varying frequencies. Eight levels of disturbance in triplicate independent reactors (n = 24), received 3-CA every day (press-disturbed), every two, three, four, five, six, and seven days (intermediately-disturbed), and never (undisturbed). Samples were analyzed using a variety of methods including metagenomics, biomass quantification, and effluent chemical characterization. Results: After 35 days, bacterial communities displayed significant differences in community structure and composition across disturbance levels, which along with trade-offs in community aggregated function and genotypic traits, indicated that communities adopted different life-strategies depending on the disturbance frequency. We semi-quantitatively aligned our results within Grime’s CSR theoretical framework. Ruderal organisms (R) predominated in communities at intermediately disturbed levels, stress tolerants (S) in press-disturbed reactors, and competitors (C) in undisturbed ones.<p><a href="http://files.abstractsonline.com/CTRL/68/c/7c4/842/efa/4b6/2be/2f0/1a6/6b5/e28/65/g5893_1.png" target='_blank' address=no >img src="http://files.abstractsonline.com/CTRL/68/c/7c4/842/efa/4b6/2be/2f0/1a6/6b5/e28/65/g5893_1.png" alt="" border="0" width="600" height="383" /></a></p> Conclusions: We showed how the CSR framework can enable simplification of real complex datasets of microbial traits, function, and taxa, into ecologically meaningful components towards understanding the mechanisms behind the system response to disturbance.
Abstract Title:
Metaproteomics Reveals A Novel Betaproteobacterium with Roles in Metal and Nitrogen Cycling in the Deep Subsurface

Primary Author Block:
N. Szeinbaum1, C. Henny2, S. A. Crowe3, F. J. Stewart1, T. J. DiChristina1, C. T. Reinhard1, B. L. Nunn4, J. B. Glass1; 1Georgia Inst. of Technology, Atlanta, GA, 2Indonesian Inst. of Sci., Cibinong, Indonesia, 3Univ. of British Columbia, Vancouver, BC, Canada, 4Univ. of Washington, Atlanta, GA

Abstract Body:
Background: Microbial genomes assembled from deep subsurface environments are revealing novel respiratory pathways distinct from those of surface-dwelling species. The advent of metaproteomics has enabled detection of novel genes expressed under diverse conditions from microbes that resist cultivation attempts. We evaluated the metaproteomic profile of a sediment microbial community enriched over ~1 year with Mn3+ as electron acceptor. Methods: Deep sediments from Lake Matano, Indonesia were sampled under anoxic conditions, and inoculated into minimal media containing soluble Mn3+-pyrophosphate as sole electron acceptor, methane as sole electron donor, and ammonium as sole nitrogen source (0.2 mM). Cultures were incubated in the dark at 30°C, and diluted 1:10 for three transfers. Changes in community taxonomic composition were monitored by 16S rRNA gene amplicon sequencing. Metagenomic and metaproteomic sequencing was performed on total DNA and protein recovered from the third transfer on day 335. Shotgun proteomics were completed in technical triplicates on a high accuracy tandem mass spectrometer, and spectral counts were used to determine relative quantification of proteins (Nunn, Slattery et al. 2015). Results: Relative quantification of shotgun metaproteomic data revealed that cultures were dominated by protein sequences affiliated with a new species of Dechloromonas (Betaproteobacteria), for which we propose the name “Candidatus Dechloromonas occultata” [occultata (L. adj.), hidden]. “Ca. D. occultata” expressed an uncharacterized cytochrome-c-rich operon containing NHL-repeat proteins and an extracellular undecaheime protein also present in other Betaproteobacteria from metal-rich ecosystems, including uncultivated Gallionellales and Rhodocyclales from the deep subsurface, and the Fe2+- and Mn2+-oxidizer Leptothrix cholodnii SP-6. Paradoxically, the enzymatic machinery for denitrification was among the most expressed proteins despite the lack of nitrogen oxides in the media. A hemolysin-type Ca2+-binding domain protein was also highly expressed. Conclusions: The metabolic potential recovered from metaproteomes thus implicates “Ca. D. occultata” and by extension possibly other Betaproteobacteria like deep subsurface Gallionellales and Rhodocyclales, in oxidative metal and reductive nitrogen metabolisms. Delineating the extent to which these metabolisms can be coupled within “Ca. D. occultata” and other Betaproteobacteria represents an important opportunity for future research.
Session Title: Managing Stress: Microbial Community Responses to Environmental Challenges
Session Start Date Time: 6/8/2018 11:00:00 AM
Session End Date Time: 6/8/2018 12:00:00 PM
Session Primary Track: Microbial Ecology and Evolution
Abstract Control Number: 9000
Poster Board Number:

Abstract Title:
Is Heavy Metal Bad for Your Biomass? Consequences of Metal Exposure to Bacterial Stoichiometry Upstream and Downstream from A Superfund Site

Primary Author Block:
K. Harrington, B. A. Wolff, M. P. Manzella, E. K. Hall; Colorado State Univ., Fort Collins, CO

Abstract Body:
Metal contamination is the most prevalent stress to freshwater ecosystems in Colorado and in large parts of the North American Mountain West. The Upper Arkansas River near Leadville CO, a high elevation headwater ecosystem, has been a site of heavy metal contamination since the mid-1800s and the site of concerted environmental restoration for the past 20 years. Due to intensive restoration efforts the benthic invertebrate communities upstream and downstream of the metal contamination now have similar levels of taxonomic richness, however the composition of both communities remain markedly different. Traditional analyses of metal-contaminated streams have relied exclusively on measuring direct exposure of benthic invertebrates to dissolved metals in the laboratory. However, multiple lines of evidence indicate that dietary exposure is more important for determining toxicity of metals to primary consumers. To address the potential for differences in microbial biomass to affect the invertebrate community composition we sampled benthic microbial communities at multiple sites upstream and downstream of the point source for metal contamination. We analyzed bacterial community and biomass composition from each location. From each site we isolated ~ 300 bacterial strains on a minimal media containing no metals, with copper (Cu), with zinc (Zn), and with Cu and Zn. We chose a subset of bacterial isolates that were phylogenetically similar, but grew on media both with and without metals. For each isolate x media combination we compared differences in biomass phenotypes (i.e. biomass C:N:P stoichiometry and metal content). Environmental communities upstream and downstream of the metal contamination site differed in community composition, as determined by 16S amplicon sequencing. In addition, downstream microbial biofilms were enriched in Zn and Cu relative to upstream microbial biofilms. Bacterial isolates grown in the presence and absence of Cu and Zn differed in both biomass stoichiometry and metal content. Our experiment suggests that microbial biomass composition (both C:N:P stoichiometry and heavy metal content) changes in response to metal exposure. This change in biomass composition alters the quality of the microbial biomass as a food resource for benthic invertebrates. Changes in microbial biomass phenotype in response to environmental stress have the potential to alter important aspects of ecosystem structure through previously unconsidered pathways.
Abstract Title:
Phosphate Addition Increases Tropical Soil Respiration Primarily by Deconstraining Microbial Population Growth

Primary Author Block:
E. R. Johnston1, M. Kim1, J. K. Hatt1, J. R. Phillips2, Q. Yao2, Y. Song2, C. Pan2, T. C. Hazen3, M. A. Mayes2, K. T. Konstantinidis1; 1Georgia Inst. of Technology, Atlanta, GA, 2Oak Ridge Natl. Lab., Oak Ridge, TN, 3Univ. of Tennessee, Knoxville, Knoxville, TN

Abstract Body:
Tropical ecosystems are an important sink for anthropogenic CO2 emissions; however, sustained uptake is increasingly restricted by phosphorus (P) availability. Soil microbiota facilitate the turnover of organic P and carbon (C) compounds and scavenge P from inorganic forms, but their role in tropical C-P-coupled biogeochemistry remains poorly understood. To advance this topic, soils collected from four sites in the El Yunque National Forest in Puerto Rico were incubated with exogenous PO43-. Phosphorus amendment increased CO2 respiration by 13.6-23.3% relative to control incubations for soils taken from all sites except the most P-rich one soil (no difference compared to its control). RNA-seq analysis revealed the increased relative transcription of genes involved in the biosynthesis of essential cell infrastructure components (purines, pyrimidines, phospholipids), cell division, and nutrient uptake and assimilation. A novel methodology assessing population-level gene expression by integrating companion metagenomic and metatranscriptomic datasets revealed that the trend of enhanced growth/respiration was community-wide. P-limited microbial communities possessed a greater abundance of genes for the biosynthesis of α-glucosyl polysaccharides, reflecting a community-wide adaptation to store excess organic carbon substrates under poor growth conditions (e.g., limiting phosphorus). Phosphorolysis genes governing the degradation of α-glucosyl polymers were more abundant in low-P soils and also had increased relative expression with P-amendment; these functions likely regulate the metabolism of stored organic substrates in soils with scarce P-availability. Collectively, our results provide quantitative estimates of increased CO2 respiration upon an alleviation of P-constraints and elucidated the underlying ecological and molecular mechanisms involved.
Outstanding Abstract Award: Cas1-Targeted Metagenome Assembly Reveals the Response of Crispr/Cas to Continuous Soil Warming Treatment

Primary Author Block:
R. Wu1, B. Chai2, E. R. Johnston3, G. Xue4, J. R. Cole2, J. M. Tiedje2; 1The Univ. of Hong Kong, Hong Kong, Hong Kong, 2Michigan State Univ., East Lansing, MI, 3Georgia Inst. of Technology, Atlanta, GA, 4Univ. of Oklahoma, Oklahoma, OK

Abstract Body:
The intriguing ability of bacterial and archaeal CRISPR/CAS systems to excise exogenic elements and integrate them into a collection of CRISPR spacers has been repurposed for more programmable biology. The universal and most conserved Cas protein, Cas1 would be an ideal marker to elucidate the ecology of CRISPR/CAS for study beyond the limited cultured organisms. Therefore, we used the Xander assembler with 8 Cas1 Hidden Markov models (HMM) for more gene-targeted mining of soil metagenomes. Read simulation on 91 genomes from 11 phyla was used to assess the specificity and sensitivity of the models. All 17 subtypes of Cas1, including the newly discovered Archaeal II, CasX and CasY, IA-F, IU, IIA-C, IIIA-D were captured with >88.96% identity. The phylogenetic analysis indicates that Cas1 with same subtype are clustered together allowing emphasis on subtypes of Cas1 using the corresponding model. The Cas1 subtypes, however, do not correspond to the organism’s taxonomy. We then applied HMM-guided assembly method (Xander) to detect the Cas1 in Alaska tundra soils (AK) and Oklahoma temperate soils (OK) both with soil warming treatments. The results revealed that there was a lower Cas1 diversity and abundance in OK which has a more diverse microbiome. To further dissect the response of CRISPR/CAS to soil warming, we detect Cas1 in OK soil over an eight-year continuous warming treatment. The relative abundance of Cas1 adversely changed with increased temperature. Actinobacteria, one of the dominant phyla, had the most positive response to soil warming but less counts of Cas1 compared to Euryarchaeota and Thermotogae, the two less abundant phyla. The distribution of CRISPR/CAS significantly varies among phyla and microbial communities in different environments. This research can provide an alternative method to study Cas1 in metagenomes and enhance the understanding of CRISPR/CAS’ ecological role and phylogenetic distribution.
Session Number: 85
Session Type: Late-Breaker Poster Presentations
Session Title: FRIDAY - MEE Late-breakers
Session Start Date Time: 6/8/2018 11:00:00 AM
Session End Date Time: 6/8/2018 1:00:00 PM
Session Primary Track: Microbial Ecology and Evolution
Abstract Control Number: 7521
Poster Board Number: FRIDAY - MEE LB1

Abstract Title:
Collective Decision-Making in Bacteria, How do Microbes Respond to Increasing Cell Densities and Why?

Primary Author Block:
J. Rattray, S. Brown; Georgia Inst. of Technology, Atlanta, GA

Abstract Body:
Bacteria exhibit many collective behaviors ranging from biofilm formation and virulence factor production to bioluminescence. The study of bacterial communication, termed quorum sensing (QS), has uncovered mechanisms of producing and monitoring signaling molecules. Despite the extensive background of molecular work done on QS, there is still debate over the basic evolutionary function of QS. While signal concentration is traditionally seen as a proxy for population density, potentially allowing single cells to differentially express group-beneficial phenotypes at appropriate cell densities, absolute signal concentration can be altered by changes in the physical (diffusion, flow) and social (spatial patterning, genotypic mixing) environment. This project focuses on both group and individual response to increasing population densities in order to build upon the strong molecular work currently available and forms the foundation for experiments that include spatial dimensions. For this study I utilized a lab strain of Pseudomonas aeruginosa containing a transcription fusion of the QS regulated elastase promoter with a green fluorescent protein (GFP). This allowed for visualization of a passive non-fluorescent QS OFF state and an active green fluorescent QS ON state. Cells were analyzed in bulk via microplate assays as well as individually via single cell microscopy. Population level experiments showed a graded response to increasing density, contrary to traditional concepts of a threshold response. Single cell analysis shows that cells have a heterogeneous response to increasing density, with an increasing proportion of cells in the ON state as density increases. Further analysis reveals a bimodal distribution of ON and OFF cells, with the ON state also increasing as density increases. These two factors when combined, an increase in proportion of cells expressing coupled with a higher expression state, help explain the observed graded population response.
Session Number: 85
Session Type: Late-Breaker Poster Presentations
Session Title: FRIDAY - MEE Late-breakers
Session Start Date Time: 6/8/2018 11:00:00 AM
Session End Date Time: 6/8/2018 1:00:00 PM
Session Primary Track: Microbial Ecology and Evolution
Abstract Control Number: 8016
Poster Board Number: FRIDAY - MEE LB2

Abstract Title:
Selection of Thermotolerant Acetic Acid Bacteria from Fermented Cocoa for High-Temperature Acetic Acid Fermentation

Primary Author Block:
H. X. Phong1, N. M. Tien1, N. N. Thanh1, B. H. D. Long1, K. Matsushita2, T. Yakushi2, N. T. P. Dung1; 1Can Tho Univ., Can Tho, Viet Nam, 2Yamaguchi Univ., Yamaguchi, Japan

Abstract Body:
Background: Temperature is a key factor strongly affects to the fermentation ability of acetic acid bacteria (AAB). Acetic acid fermentation is normally carried out at 30°C, both growth rate and acid production are sharply decreased when temperature increases only 2-3°C. Nowadays, global warming is affecting the process of biological metabolism, especially the acetic acid fermentation. High-temperature fermentation has several advantages such as cost reduction associated with cooling fermentation vats and risk reduction of bacterial contamination. Therefore, selection of thermotolerant AAB for acetic acid production is a key solution for dealing with climate change. In cocoa fermentation, AAB play a role in oxidization of ethanol into acetic acid and lead temperature of fermentation mass increase up to 50°C so that isolation and selection of newly thermotolerant AAB from fermented cocoa is very possible. Methods: Isolation of thermotolerant AAB was carried out on YPGD agar medium containing 4% (v/v) ethanol and 0.5% (w/v) CaCO3 at 35°C. Growth of AAB colonies on YPGD agar at different temperatures (37°C, 39°C, 41°C and 43°C) were applied to evaluate the thermotolerant ability. The screening of acid-forming ability was test on YPGD agar (containing 0.2% ethanol, 0.3% yeast extract, and 0.01% bromocresol green) at 35°C. High-temperature acid production was conducted with YPGD broth (supplemented 4% (v/v) ethanol) at 35°C, 37°C, and 39°C. AAB identification was carried out using the universal primers of α-proteobacteria. Results: Sixteen strains of AAB were isolated from fermented cocoa beans collected from Mekong Delta, Vietnam. Ten AAB strains could grow at 43°C on YPGD agar (added 4.0% v/v ethanol) and produce high acid concentration on bromocresol green ethanol agar medium (the diameter of yellow zone from 19 mm to 23 mm). Three thermotolerant strains of AAB including BT1H, BT2B1 and TG1D1 were selected due to their good performance during fermentation. The concentrations of acetic acid produced by BT1H, BT2B1 and TG1D1 after five days of fermentation could reach at 3.0%, 3.20% and 3.10% (w/v) at 35°C; 4.45%, 3.75% and 3.85% (w/v) at 37°C; 3.30%, 2.35% and 2.65% (w/v) at 39°C, respectively. Strain BT1H was identified as Acetobacter pasteurianus while BT2B1 and TG1D1 were A. tropicalis. Conclusions: Fermented cocoa was recognized as the great source for isolation of thermotolerant AAB. The newly isolated thermotolerant A. pasteurianus BT1H has potential to apply for acetic acid production at high temperature.
Session Title: FRIDAY - MEE Late-breakers

Abstract Title: Characterization of Lineage and Locus-specific Variations Associated with Pneumococcal Carriage Dynamics Post Pcv-13 Implementation in An African Birth Cohort

Primary Author Block: F. S. Dube; Univ. of Cape Town, Cape Town, South Africa

Abstract Body:

Background and Aims: Our understanding of the bacterial genetic determinants of pneumococcal transmission, acquisition rates and carriage duration is limited. This study examines lineage- and locus-specific genetic variation amongst pneumococci colonizing the nasopharynx and interactions with environmental factors amongst African children enrolled in an intensively sampled birth cohort. Methods: Pneumococcal isolates were obtained from nasopharyngeal (NP) swabs collected 2-weekly from 800 infants enrolled from birth through their first year of life in a birth cohort in Cape Town, South Africa. Confirmatory lytA real-time qPCR was performed on all presumptive pneumococci before whole genome re-sequencing (WGS). Detailed metadata were longitudinally collected on selected environmental, infectious, nutritional, genetic, immunological, psychosocial and maternal health determinants. Results: A total of 19 289 NP swabs were collected from infants. Culture has been completed on 15 906 swabs. Pneumococci were isolated from 53% (8 422 /15 906); yielding a point prevalence of 0.5% at birth and reaching a maximum prevalence of 71% at 38 weeks. Of the planned 12,000 pneumococcal isolates to be sequenced, data for 96 isolates are available, with an additional 1330 genomes currently in the sequencing pipeline. Complete results will be presented at ASM Microbe 2018. 314 pneumonia cases occurred amongst enrolled children (incidence 0.27 episodes per child-year, 95% CI 0.24-0.31; median age 5 months [IQR 3-9]) in 967 children during 1145 child-years of follow-up. PCV13 coverage was high (90%, 90% and 81% for 6, 14 weeks and 9-month vaccines respectively). Conclusion: This study will report, lineage and locus-specific variation associated with pneumococcal carriage dynamics in an intensively sampled, PCV-13-vaccinated population.
Abstract Title:
Networks Reveal Patterns of Gene Sharing across Bacterial, Archaeal, and Eukaryotic Viruses

Primary Author Block:
J. W. Shapiro, C. Putonti; Loyola Univ. Chicago, Chicago, IL

Abstract Body:
Background: While phylogenetic methods are valuable for understanding the evolution of specific virus groups, no single gene is shared among all viruses, and in many cases, horizontal gene transfer complicates phylogenetic inference further. These issues make it difficult to address broader questions in viral ecology and evolution. In recent years, networks have emerged as an alternative for depicting relationships among bacteriophage (phage) genomes and also between phages and their hosts.

Methods: Here, we build on our previous work (Shapiro & Putonti, in press) that used a subset of available RefSeq phage genomes to identify gene clusters shared among phages. In this network, each node represented all homologs of a particular gene, and two nodes were connected if the two genes are ever found in the same genome. In the present work, we extend this network to include all available RefSeq virus genomes (including also eukaryotic and archaeal viruses), as well as all metaviromic contigs identified by IMG/VR from the Human Microbiome Project (HMP). Results: New isolate genomes and metaviromic contigs fill in the gaps between previously-identified gene clusters; metaviromic contigs also form a large set of unique groups, emphasizing how little of viral diversity has been cultured in the lab. Overall, the expanded gene network is dominated by one large connected component, which includes nearly all bacteriophages, most archaeal viruses, and a subset of eukaryotic viruses.

Interestingly, not all archaeal viruses cluster together, and two subclusters contain genes with homologs found in both bacterial and archaeal viruses. In one case, homologs related to zot, a common virulence factor associated with pathogenic bacteria, appear to act as a hub bridging viruses that infect archaea and bacteria. Conclusion: This network approach can be updated readily as new sequences are made available. Moreover, metadata for each genome or metagenome can be associated with each node in the network, enabling statistical analysis of associations between gene sharing and viral ecology. Networks, therefore, provide a flexible framework for exploring viral ecology and evolution when phylogenetic methods would otherwise fall short.
Abstract Title:
The minimum InDel pattern analysis of Zika virus

Primary Author Block:
Y-H. Kim, H. Lee, M. Nguyen, Y. Choi; Daegu Catholic Univ. Sch. of Med., Daegu, Korea, Republic of

Abstract Body:
Background: Zika virus (ZIKV) can cause microcephaly and congenital abnormalities in the fetus. Recent studies provide insights on the evolution of ZIKV from the current and previous outbreaks, but the types are not determined. Methods: We analyzed the minimum InDel (minInDel) patterns in 135 ZIKV polyproteins and 5 Dengue virus (DENV) reference sequences. Spearman correlation tests for the minimum InDel (minInDel) patterns were used to assess the type of polyprotein. Using the minInDel frequencies calculated from polyproteins with 11 elements, likelihood estimation was conducted to correct the evolutionary distance. Results: The minInDel-corrected tree topology clearly distinguished between ZIKV types (I and II) with a unique minInDel character in the envelop protein E. From 10-year average genetic distance, African and Asian lineages of ZIKV-II were estimated to have occurred ~1,200 years ago, unlikely ZIKV-I. Conclusions: These results demonstrate that the minInDel in the E protein is targetable for rapid detection and determination of the virus types.
Bacterial Diversity from Two Andean Tropical Volcanoes, the Cayambe and Sumaco

Primary Author Block:
A. Molina1, M. Diaz2, D. Quiroz2, P. Castillejo3, E. Yugsi1, O. Carrasco1, E. Rivadeneira4, P. Jarrín-V.2; 1Central Univ. of Ecuador, Quito, Ecuador, 2Univ. Regional Amazónica Ikiam, Tena, Ecuador, 3SEK Intl. Univ., Quito, Ecuador, 4Salesian Polytechnic Univ., Quito, Ecuador

Abstract Body:
Andean volcanoes are unique ecosystems with extreme environmental conditions, whose microbial communities remain poorly understood. Due to a wide elevational gradient and their geological conditions, these volcanoes harbor diverse habitats. Microorganisms living at these volcanoes, such as mesophilic and psychrophilic bacteria, may have developed strategies to overcome cold and high levels of UV radiation. Cold-adapted microorganisms, such as those that may be found in Andean volcanoes, have evolved to tolerate the inhospitable conditions and are sources of useful enzymes that are key components in biotechnological applications. We analyzed the bacterial diversity at the Cayambe and Sumaco volcanoes in Ecuador, both with unique characteristics. The Cayambe is a dormant volcano, and the third highest mountain in Ecuador with 5,790 m of elevation. Interestingly, this is the only volcano in the world whose summit is traversed by the Equator. Therefore, microorganisms inhabiting this volcano may be adapted to powerful UV radiation. Sumaco is an active stratovolcano with 3,732 m of elevation. It is an isolated volcano in the rain forest rising 2,800 m over the Amazon basin. We analyzed the bacteria present on soil, water and ice (treated as water) from Cayambe and Sumaco by using massive sequencing of the V3 and V4 regions of the 16S rRNA on the MiSeq platform (Illumina). Physicochemical parameters such as electrical conductivity, content of organic matter, humidity, cationic exchange, dissolved oxygen, pH, temperature, and presence of P, N, Ca, Mg, SO4 were used in quantitative approaches in numerical ecology to establish their role in explaining the observed bacterial diversity. A range of standard data processing protocols were applied in QIIME and R, which included denoise and chimera checking, data normalization, OTU picking, taxonomic assignment, biodiversity and phylogenetic analysis, clustering and ordination analysis. Bacterial communities significantly differ in the composition of taxa between volcanoes. Sumaco was more diverse than Cayambe. Dominant groups in Cayambe include Actinomycetales, Acidobacteriales, Pseudomonadales, and Clostridiales. On the other hand, Burkholderiales, Pseudomonales, Actinomycetales and Sphingomonadales were more abundant in Sumaco. This is the first study in Ecuador to report bacterial diversity at extreme environments. Bacteria inhabiting other extreme ecosystems in Ecuador must be studied to understand their role in natural environments and their potential applications.
Characterization of Cyanobacteria Diversity in Shallow Water Shipwrecks

C. G. Daokoru-Olukole; Niger Delta Univ., Yenagoa, Nigeria

The diversity of shipwrecks cyanobacteria in shallow water of New Calabar River, in River State - Nigeria was examined. The 16S rRNA gene sequences from brown rusticles (braided structures attached on the wreck surfaces) and bio-concretions (layered coatings of different concretions) were investigated using Illumina High Thorough-put. The visual examination revealed 3 types of rusticles: brown rusticles, dendritic concretion, biofilm and also diverseness in the cyanobacteria distribution patterns. The diazotrophic filamentous genus Trichodesmium sp., dominated the bio-concretion, having in abundance 4 of its species; T. erythraeum (8.75%), T. hildebrandtii (1.08%), T. contortum (1.04%) and T. tenue (1.02%). The availability of iron on the bioconcretions could explain the reason for the presence of the Trichodesmium clades present. Phormidiaceae, Cyanobacteriacea, Nostocaceace and the nonheterocystous filaments members of the Pseudanabaenaceae were dominant on the rusticle samples. We can associate the formation of rusticles by Cyanobacteria as one of their eroding characteristics on shipwrecks. This study gives insight that the presence of mat-matrix forming cyanobacteria can actually aid aerobic corrosion on shallow water shipwrecks.
Session Title: MEE01 - Biofilms in Ecological and Evolutionary Science: Biofilm Ecology

Session Start Date Time: 6/8/2018 11:00:00 AM
Session End Date Time: 6/8/2018 1:00:00 PM

Session Primary Track: Microbial Ecology and Evolution

Abstract Title: Biofilm Evolved Morphotypes of Pseudomonas Aeruginosa Display Diverse Patho-Immunological Effects

Primary Author Block:
S. Azimi1, A. E. Roberts2, S. P. Diggle1; 1Georgia Inst. of Technology, Atlanta, GA, 2Cardiff Metropolitan Univ., Cardiff, United Kingdom

Abstract Body:
Background: Pseudomonas aeruginosa chronically infects cystic fibrosis (CF) lungs by forming multicellular biofilms. Emergence of phenotypically diverse isolates of P. aeruginosa in chronically infected CF lungs has previously been reported. However, interaction dynamics between diverse isolates, and the effects on levels of induced immune responses, tissue damage and progression of the disease are poorly understood. Methods: Here we tested how the P. aeruginosa strain PAO1 evolves in biofilms over 50 days in synthetic sputum medium. Our long term evolution experiment showed that within biofilms, the emergence of distinct P. aeruginosa morphotypes evolve, which contribute to population level phenotypes. Results: To assess the host response and levels of tissue damage, we used an ex vivo pig lung model and observed differential levels of available IL-8 and different levels of tissue damage in the alveolar tissues infected with different morphotypes. Furthermore, IL-8 levels were negatively correlated with total protease activity. Conclusions: Our work highlights that phenotypic diversity within P. aeruginosa populations can influence how this organism damages lung tissue and that diversity can contribute to higher incidents of acute pulmonary exacerbation during chronic CF lung infection. Investigating interactions between phenotypically diverse isolates may help inform new strategies for the treatment and control of biofilms and infection.
Abstract Title:
Drought Conditions Decrease Respiration Rates of Most Abundant Bacterial Taxa in the Southern California Grassland Litter

Primary Author Block:
Z. Gomez-Lunar, A. B. Chase, J. B. H. Martiny, A. C. Martiny; Univ. of California, Irvine, CA

Abstract Body:
Background: Different microbial communities can carry out litter decomposition at different rates; however, little is known about when and how the composition and physiological traits of litter microbial community affect decomposition. Evidence suggests that drought conditions in southern California reduce grassland litter decomposition rate, change bacterial community composition, and also reduce bacteria population size. However, there is a knowledge gap regarding the mechanisms that regulate microbial drought tolerance as well as their impact on carbon metabolism. In this sense, if bacterial cells require large amounts of energy to drought tolerance traits, then a trade-off against growth traits and resource acquisition may result. The aim of this research is to recognize the mechanisms that regulate drought tolerance, as well as its impact on carbon metabolism in bacteria from litter communities.

Methods: Curtobacterium spp. will be used, as study model given that is the most abundant bacterial taxa in the southern California grassland litter community. Besides, Curtobacterium spp. are cellulolytic bacteria that may contribute to litter decomposition. A microcosm system will be established using grassland litter as carbon substrate in a gradient of water potential. Fifteen Curtobacterium spp. strains from five different sites will be used during two weeks of the experiment. To estimate litter decomposition, respiration measurements will be performed every 24 h, and cell abundance will be measured every 48 h by flow cytometry. To identify drought tolerance and carbon metabolism traits, samples will be analyzed via transcriptomics at two different times (48 and 336 h). Results: Preliminary results indicated that the addition of Curtobacterium spp. to microcosms increase respiration rates in all water potential level tested in comparison to negative control (no inoculated). Curtobacterium respiration rates decrease in drought conditions (water potential from -54 to -12 MPa) in comparison to higher water potential from -6 to -2.5 MPa. The highest respiration rates in all water potential levels were observed after 48 h of experiment and gradually decrease over time. Differences in cell abundance between water potential levels were not observed during the time. Conclusions: Curtobacterium spp. is an important member of litter community that contributes to carbon metabolism by breaking down structural carbon sources of litter. Additionally, drought conditions reduce Curtobacterium spp. metabolism and resource acquisition.
Abstract Title:
Effects of Long-Term Elevated Co2On the Taxonomic and Functional Compositions of Soil Microbial Community in A Californian Annual Grassland

Primary Author Block:
S. Yang1, Q. Zheng1, M. Yuan2, Z. Shi2, N. Chiariello3, K. Docherty4, C. Field3, Y. Gu2, J. Gutzkecht5, B. Hungate6, X. L. Roux7, X. Ma1, A. Niboyet8, T. Yuan2, J. Zhou1, Y. Yang1; 1Tsinghua Univ., Beijing, China, 2Univ. of Oklahoma, Norman, OK, 3Carnegie Inst. for Sci., Stanford, CA, 4Western Michigan Univ., Kalamazoo, MI, 5Helmholtz Ctr. for Environmental Res. - UFZ, Beijing, Germany, 6Univ. of Minnesota, Twin Cities, MN, 7Univ. of Lyon, Beijing, France, 8UMR 7618 Université Pierre et Marie Curie / CNRS / AgroParisTech, AgroParisTech, France

Abstract Body:
The continuously increasing concentration of atmospheric CO2 has considerably altered ecosystems. However, there is only a limited number of studies to examine the long-term (i.e. over a decade) effect of elevated CO2 on soil microbial community. Using 16S rRNA gene amplicons and a GeoChip 4.6 microarray, we investigated soil microbial communities from a Californian annual grassland after 14 years of experimentally elevated CO2 treatment (300 ppm higher than the ambient level). Both taxonomic and functional gene compositions of the soil microbial community were affected. Taxa with higher ribosomal RNA operon (rrn) copy number (e.g. Ferruginibacter) decreased but those with lower rrn copy number (e.g. Acidobacteria Group 1) increased. As a consequence, the abundance-weighted average rrn copy number was significantly (P<0.01??) decreased from 2.46 in ambient CO2 plots to 2.36 in elevated CO2 plots. In addition, a large number of microbial genes related to carbon (C) degradation were affected by elevated CO2, while those related to C fixation remained largely unchanged. The nifH nitrogen (N) fixation gene and the amoA nitrification gene significantly decreased in elevated CO2 plots by -12.6% and -6.1%, respectively. Concomitantly, nitrifying enzyme activity decreased by -48.3%. There was also notable N reallocation from soil to plants as revealed by increased annual grass biomass (+29.3%), perennial forbs biomass (+77.3%) and total root biomass (ca. +30%). The overall changes of microbial communities, plant and soil N pools induced by long-term elevated CO2 treatment were consistent with the N limitation theory.
Abstract Title:
Different Relationships between Climatic Factors and West Nile Virus Disease Incidence in Humans and Mosquitoes among California Counties

Primary Author Block:
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Abstract Body:
The role of several climatic factors on West Nile virus (WNV) cases in California was examined in order to better predict and respond to infections. This is particularly important since WNV is transmitted by mosquitoes, whose life-cycle might be affected by such factors. In this study, correlations between human and mosquito infections was compared to precipitation and temperature data on per county or state-wide basis from 2008-2015 (includes only more recent and relevant data). Correlations between human and mosquito infections per county were also examined. Methods: Reported cases of human and mosquito infections were obtained from ArboNet. Climatic data and numbers of human infections were acquired from a SQL database under development by Malone University that will soon open for public use. Climate data for this database was retrieved from NOAA's climate FTP climate division server. Census data were from the Census Bureau's American Community Survey Application Programming Interface. Results: Correlation coefficients between reported infection in humans or mosquitoes and average, minimum, and maximum temperature exceeded 0.75 in only 5 CA counties: Butte, Glenn, and Yolo in northern-central California and Los Angeles and Orange County in the southern, coastal region. Northern counties also had high correlations between numbers of human and mosquito infections (>0.75), with much lower human/mosquito correlations in the southern region. No such correlation was found state-wide. Maximum and average precipitation did not correlate with numbers of human or mosquito infections per county or state. The northern counties are in the same climate division and the southern counties, in a separate division. Differences between precipitation and temperatures in these climate divisions differed significantly (p<0.0001), indicating that other factors must also affect human infection. Conclusions: Precipitation did not correlate with human WNV infection in CA by state or by county, while temperature strongly correlated with human and mosquito infections in 5 counties, but not state-wide. Strong correlations between human and mosquito infections are seen in the northern, but not the southern, counties. Predicting human WNV infection using climatic data, therefore, is not useful in CA, except in 5 counties.
Abstract Title:
Acclimation of Soil Fungal Community to Soil Transplantation Simulating Climate Changes
Primary Author Block:
M. Zhao1, B. Sun2, J. Zhou3, Y. Yang1; 1Tsinghua Univ., Beijing, China, 2Chinese Academy of Sci., Nanjing, China, 3Univ. of Oklahoma, Norman, OK
Abstract Body:
Although the fungal biomass is less abundant in soils than that of bacteria, they are considered to play more substantial roles in mediating belowground carbon cycling in terrestrial ecosystems. To predict future carbon stability, it is thus imperative to understand their responses to environmental changes, which remains challenging owing to our limited knowledge. As current global warming causes range shifts toward higher latitudes, we conducted three reciprocal soil transplantation experiments over large transects in 2005 to simulate climate changes. In 2011, we collected soil samples to examine microbial biomass, community composition and functional gene inventories. Six years after soil transplantation, fungal biomass of transplanted soils showed a general pattern of changes from donor sites to destination, which were more obvious in bare fallow soils than maize cropped soils. Strikingly, fungal community compositions were clustered by sites, demonstrating that fungi of transplanted soils acclimated to the destination environment. Several fungal taxa displayed sharp changes in relative abundance, including Podospora, Chaetomium, Mortierella and Phialemonium. In contrast, bacterial communities remained resistant to environmental changes. Consistent with the important role of fungi in affecting soil carbon cycling, 8.1-10.0% of fungal genes encoding carbon-decomposing enzymes were significantly (P < 0.01) increased and larger than those from bacteria (5.7-8.4%). To explain those observations, we found that fungal occupancy across samples was mainly determined by annual average air temperature and rainfall, whereas bacterial occupancy was more related to soil conditions, which remained stable six years after soil transfer. Together, these results demonstrate that soil fungal community is highly sensitive to climatic drivers, which may have large consequences for ecosystem-scale carbon cycling.
Abstract Title:
Fecal and Feather Microbiomes of Saltmarsh Sparrows (Ammodramus caudacutus)

Primary Author Block:
E. A. Herder, K. Grond, C. S. Elphick, S. M. Hird; Univ. of Connecticut, Storrs Mansfield, CT

Abstract Body:
The fecal microbiome of Saltmarsh sparrows (Ammodramus caudacutus) was characterized for the first time and feather-degrading bacteria was found on tail and venter feathers. Saltmarsh sparrow populations are in decline with the potential of extinction in twenty years. The sparrows live in salt marshes in northern United States during breeding months and migrate to southern United States during the winter. Saltmarsh sparrows experience nest failure due to flooding events, which are expected to rise due to sea level rises. Ten birds were sampled during the breeding months of 2017. Sample sites were chosen in Hammonasset State Park and Barn Island on the Connecticut coast. Both after-hatch-year (AHY) and hatch-year (HY) birds were sampled, as well as male and female birds. High-throughput sequencing was conducted on the samples and the sequences were processed using Mothur and analyzed using R. No significant correlations were found between age, sex, or location. There is a heavy Proteobacteria, Chlamydiae, and Firmicutes presence in the samples (Figure 1). Several studies have been conducted that show opportunistic pathogens of the phylum Proteobacteria isolated from birds including Campylobacter, Escherichia, Helicobacter, Rickettsia, Salmonella, and Vibrio. Firmicutes, containing members such as Bacillus subtilis and Enterococcus faecium, have been found to help with nutrient uptake and metabolic efficiency when added to chicken diets and may be performing similar functions in these wild birds. Hatch-year birds were very dissimilar from each other. Feather-degrading bacteria use keratinase enzymes to degrade β-keratin in feathers. HY birds show a lower abundance of feather degrading bacteria when isolating and testing feather bacteria in feather medium. This study shows phylum level taxonomic information in the samples and the feather bacteria present in the samples.
Abstract Title:
Outstanding Abstract Award: Cas1-Targeted Metagenome Assembly Reveals the Response of Crispr/Cas to Continuous Soil Warming Treatment

Primary Author Block:
R. Wu1, B. Chai2, E. R. Johnston3, G. Xue4, J. R. Cole2, J. M. Tiedje2; 1The Univ. of Hong Kong, Hong Kong, Hong Kong, 2Michigan State Univ., East Lansing, MI, 3Georgia Inst. of Technology, Atlanta, GA, 4Univ. of Oklahoma, Oklahoma, OK

Abstract Body:
The intriguing ability of bacterial and archaeal CRISPR/CAS systems to excise exogenic elements and integrate them into a collection of CRISPR spacers has been repurposed for more programmable biology. The universal and most conserved Cas protein, Cas1 would be an ideal marker to elucidate the ecology of CRISPR/CAS for study beyond the limited cultured organisms. Therefore, we used the Xander assembler with 8 Cas1 Hidden Markov models (HMM) for more gene-targeted mining of soil metagenomes. Read simulation on 91 genomes from 11 phyla was used to assess the specificity and sensitivity of the models. All 17 subtypes of Cas1, including the newly discovered Archaeal II, CasX and CasY, IA-F, IU, IIA-C, IIIA-D were captured with >88.96% identity. The phylogenetic analysis indicates that Cas1 with same subtype are clustered together allowing emphasis on subtypes of Cas1 using the corresponding model. The Cas1 subtypes, however, do not correspond to the organism’s taxonomy. We then applied HMM-guided assembly method (Xander) to detect the Cas1 in Alaska tundra soils (AK) and Oklahoma temperate soils (OK) both with soil warming treatments. The results revealed that there was a lower Cas1 diversity and abundance in OK which has a more diverse microbiome. To further dissect the response of CRISPR/CAS to soil warming, we detect Cas1 in OK soil over an eight-year continuous warming treatment. The relative abundance of Cas1 adversely changed with increased temperature. Actinobacteria, one of the dominant phyla, had the most positive response to soil warming but less counts of Cas1 compared to Euryarchaeota and Thermotogae, the two less abundant phyla. The distribution of CRISPR/CAS significantly varies among phyla and microbial communities in different environments. This research can provide an alternative method to study Cas1 in metagenomes and enhance the understanding of CRISPR/CAS’ ecological role and phylogenetic distribution.
Abstract Title:
Whole-Genome Sequences of Two Genetically Diverse Bdellovibrio Species Isolated from Soil for the Biol. Control of Gram Negative Bacterial Pathogens

Primary Author Block:
O. O. Oyedara1, A. Segura-Cabrera2, S. B. Akinde1, X. Guo3, T. O. Elufisan3, R. A. Cantu-Gonzalez3, M. A. Rodriguez-Perez3; 1Osun State Univ., Osogbo, Osun State, Nigeria, 2Red de Estudios Moleculares Avanzados, A.C., Xalapa Enríquez, Mexico, 3Inst. Politécnico Natl., Centro de Biotecnología Genómica, Reynosa, Tamaulipas, Mexico

Abstract Body:
Background: Bdellovibrio spp. are small predatory bacteria that survive on nutrients derived from hydrolyzing cellular constituents of other Gram-negative bacteria. Thus, they have potential to serve as an alternative therapy in the biocontrol of Gram-negative bacterial pathogens. In order to provide information that can aid the successful application of Bdellovibrio spp, this study focused on the genomic analysis of two genetically diverse Bdellovibrio spp strains SKB1291214 and SSB218315 isolated from soil in Mexico. Methodology: The whole genome sequencing was performed using the Illumina NextSeq platform. The resulting reads were trimmed and assembled using Sickle and SPAdes software respectively. The NCBI Prokaryotic Genome Annotation Pipeline (PGAP) was used for the genome annotation. The MEGA6 software was used to construct a phylogenetic tree and estimate pairwise evolutionary distance. The orthologous gene cluster analysis and average amino acid identity (AAI) among the Bdellovibrio strains and seven (7) other reported genome sequences retrieved from GenBank database was done using Orthovenn and ANI/AAI-Matrix Genome-based distance matrix calculator respectively. Results: The genomes of the Bdellovibrio spp. have virulence factors that include flagellar, Type IV pilus and chemotaxis factors. They also have genes encoding several serine proteases, DNases, RNases, hydrolases, lipases, peptidase, esterases, RTX toxins, hemolysin, collagenase and siderophores. The 16S rRNA gene sequences of Bdellovibrio spp. strains SKB1291214 and SSB218315 showed 96% similarity with a pairwise evolutionary distance of 0.043. The AAI between strain SKB1291214 and other strains was very low (63.70 - 67.68%) while strain SSB218315 shared high AAI value of 95% with reported strains B. bacteriovorus strains HD100, Tiberius and 109J. Bdellovibrio spp. SKB1291214 also possessed 30 unique orthologous gene clusters that are rich in proteins involved in ion binding and hydrolase activities. Conclusion: The Bdellovibrio spp. have genes encoding collagenase, hemolysin, and RTX toxins that are associated with some bacterial pathogens. It is important to characterize these factors for safe application of Bdellovibrio spp. in humans. The differences observed among the Bdellovibrio spp. based on GC content and AAI suggests future taxonomy review of the genus Bdellovibrio. Our findings also showed that Bdellovibrio sp. SKB1291214 has possibly experienced some lateral gene transfer that can influence its ecological adaptations.
Abstract Title:
Genome Wide Survey of Positively Selected Genes in Livestock Associated Staphylococcus aureus Strains

Primary Author Block:
T. R. Relangi, S. Natesan, J. Kannan; Madurai Kamaraj Univ., Madurai, India

Abstract Body:
The availability of bacterial genomes isolated from different hosts led to comparative genomic analyses that help in understanding the molecular evolution of specific pathotypes. Staphylococcus aureus is a major opportunistic pathogen responsible for wide range of infections in humans and animals. Animal origin S. aureus is also suggested to possess a role as a zoonotic agent. Despite its importance, S. aureus’s evolution of pathogenesis still has to be decoded to better understand.

POTION - 1.1.2 used to detect genome wide positively selected genes. BLAST2GO and COG analysis employed for Enrichment and functional characterization respectively. In this study, a genome wide survey of twelve genomes of animal associated S. aureus revealed presence of recombination in only ~4% homologous groups. Enrichment analysis revealed that four Gene Ontology (GO) terms significantly more represented among recombinant groups. Among these GO terms, most terms noted to be organic and inorganic compound transporters. The search for positive selection of genes among the genomes under this study, revealed 70 groups of homologous genes under positive selection. Among these groups, we found some related to cell metabolism, as well as several un-characterized genes. Also, we identified most genes related to ion transport, amino acid transport and energy production. Interestingly no known virulence genes are positively selected. A GO term enrichment test showed a prevalence of terms related to transferase activity, ATPase activity and ion binding proteins. Finally, the genes with evidence of positive selection retrieved from genomes of human origin S. aureus genomes and tested as were done for Livestock associated S. aureus strains. The result revealed that none of the groups of genes presented evidence of positive selection except glpD and asp3 genes, confirming that the analysis effective in inferring positive selection for animal origin S. aureus and not for S. aureus in general, which means that the study of the genes with evidence of positive selection identified in this study can contribute for the better understanding of the evolutionary trends of animal origin S. aureus.
Abstract Title:
Exploring the Ecophysiology of Acidobacteria and Identifying the Potential Strategies for their Success in Soil

Primary Author Block:
S. A. Eichorst1, D. Trojan1, A. Giguere1, S. Roux2, C. Herbold1, T. Rattei1, D. Woebken1; 1Univ. of Vienna, Vienna, Austria, 2DOE Joint Genome Inst., Walnut Creek, CA

Abstract Body:
Members of the phylum Acidobacteria are abundant and ubiquitous across soils, but the reason for their success remains unclear. We performed a large-scale comparative genome analysis spanning acidobacteria from subdivisions 1, 3, 4, 6, 8, and 23 (n=24) with isolates from environments such as soils, microbial mats, hot springs, aquifer, peat bogs, and acid mine drainage. Our goal was to identify features that could help explain their prevalence in soils and understand their ecophysiology. The genome sizes range from 2.7 to 9.9 Mb; although many are considered draft genomes, CheckM analysis indicated that they are ca. >95% complete. Genomes of acidobacterial strains isolated from soils typically harbored a larger genome size and a larger proportion of paralogous genes. Bacteriophage integration events along with transposable and mobile elements influenced the structure and plasticity of these genomes. Low- and high-affinity respiratory oxygen reductases were detected in multiple genomes, suggesting the capacity for growing across different oxygen gradients, which was supported in growth-based experiments where select strains have the capacity to grow under reduced oxygen conditions. Amongst many genomes, the capacity to use a diverse collection of carbohydrates, as well as inorganic and organic N sources (such as extracellular peptidases), were detected - both advantageous traits in environments with fluctuating nutrient availabilities, such as soils. We also identified multiple soil acidobacteria with the potential to scavenge atmospheric concentrations of H2 due to the presence of a high-affinity hydrogenase. This physiology now encompasses additional mesophilic soil strains within the subdivision 1 and 3, in addition to a previously identified thermophilic strain in subdivision 4. Current efforts are underway to explore this physiology in the wet-lab. This large-scale acidobacteria genome analysis reveals traits that provide genomic, physiological and metabolic versatility, presumably allowing flexibility and versatility in the challenging and fluctuating soil environment.
Abstract Title:
The Biol. of IncI2 Plasmids Shown by Whole-Plasmid Multi-Locus Sequence Typing

Primary Author Block:
R. J. Meinersmann; Agricultural Res. Service, Athens, GA

Abstract Body:
IncI2 type plasmids are medium-sized (~55 - 80 kb) conjugative plasmids that have been found carrying important antimicrobial resistance genes but have also been frequently found as cryptic plasmids. The DNA sequences for 147 fully sequenced IncI2 plasmids were studied by a whole-plasmid multi-locus sequence typing (wpMLST) scheme. A total of 165 loci were identified of which 52 were considered core (carried by greater than 95% of the plasmids). Most of the plasmids carrying the antimicrobial gene mcr-1 were in a distinct clade while most of the antimicrobial gene free plasmids were fairly unrelated. However the parent strains of bacteria were disparate for both groups of plasmids, showing that conjugal transfer of IncI2 plasmid is frequent. The mcr-1 gene was likely to have been introduced into IncI2 plasmids multiple times. It was also observed that the genes for conjugation showed significant linkage disequilibrium despite substantial diversity for most of those genes. Genes associated with biofilm formation were also among the core genes. Given the role conjugation can play in biofilm formation, it was concluded that conjugation is an active survival strategy for IncI2 plasmids.
Session Number: 88
Session Type: Poster
Session Number: 88
Session Type: Poster
Session Title: MEE04 - Comparative and Evolutionary Genomics: Environmental Isolates
Session Start Date Time: 6/8/2018 11:00:00 AM
Session End Date Time: 6/8/2018 1:00:00 PM
Session Primary Track: Microbial Ecology and Evolution
Abstract Control Number: 6879
Poster Board Number: FRIDAY - 960

Abstract Title:
Ribosomal Protein Genes of the Plant Endosymbiont Frankia Are the Products of Horizontal Gene Transfer

Primary Author Block:
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Abstract Body:
The actinobacterial genus Frankia and the proteobacterial rhizobia are the two diverse groups of bacteria that can establish nitrogen-fixing symbioses with the host plants of the Nitrogen-fixing Clade. To date, the phylogenetic position of Frankia has been difficult to resolve within the phylum Actinobacteria (Sen et al., 2014) leaving open questions about the evolution of their symbioses. We have performed sequence-based and phylogenetic analyses on the ribosomal protein genes across the Actinobacteria, which suggest that the genes encoding ribosomal proteins in Frankia genomes are inherited from two separate families: the Streptomycetaceae, to whom the Frankia rRNA genes are closely related, and the Pseudonocardiaceae. Syntenies of horizontally transferred ribosomal protein genes were found to be highly conserved with other actinobacteria, however recombination detection using the program RDP4 (Martin et al., 2015) identified recombination sites within several ribosomal protein genes and conserved adjacent housekeeping genes throughout genus Frankia. The two partial sequences of these recombined genes followed their respective phylogenetic patterns, showing similarity to the Streptomycetaceae or the Pseudonocardiaceae. Together, these findings suggest extensive horizontal gene transfer and recombination in the evolutionary history of genus Frankia and have implications for the impact of horizontal gene transfer on the evolution of symbiosis at the genomic level and for the use of ribosomal proteins as generic markers for phylogenetic reconstruction.
Abstract:
Phylogenetic Distribution of Enzymes for Structural Polymer Degradation

Primary Author Block:
L. Liu1, Y. Bai2, F. C. C. Leung1; 1Univ. of Hong Kong, Hong Kong, China, 2Beijing Genomics Inst., Shenzhen, China

Abstract Body:
Cellulose, hemicellulose, pectin and lignin are major constituents of plant cell walls; chitin primarily constitutes the fungal cell walls and the exoskeletons of arthropods. These structural components are the most abundant biopolymers on earth, hence structural polymer-degrading enzymes are playing a vital role in ecological, industrial, medical and agricultural processes. Considerable research efforts have been dedicated to identifying novel organisms and mining potential genes from omic and meta-omic datasets. However, comprehensive genomic analyses of the potential of structural polymer degradation in nature is lacking. Protein sequences belonging to glycoside hydrolase, polysaccharide lyase and auxiliary activity were retrieved from CAZy database (28-Dec-2017). Sequences of specific CAZy families were identified as potential cellulases, xylanases, pectinases, chitinases, ligninases, and lytic polysaccharide monooxygenases (LPMOs) according to Berlemont, 2017. Partial sequences and duplicated accessions were removed. Then annotation against CAZy HMM models with dbCAN pipeline was performed and sequences with a HMM coverage not lower than 60% were remained. The taxonomic lineages were derived from NCBI Taxonomy database and further analyzed using MEGAN6 and R. 50,268 protein sequences from CAZy database were analyzed, including 13,780 cellulases, 4,874 xylanases, 11,840 pectinases, 11,010 chitinases, 5,428 ligninases and 3,336 LPMOs. These putative enzymes were found across various lineages along the Tree of Life, most belonging to four major bacterial phyla Proteobacteria, Firmicutes, Actinobacteria and Bacteroidetes, as well as two fungal phyla Ascomycota and Basidiomycota. Relatively lower levels of cellulases, pectinases and chitinases were detected in the phyla Arthropoda and Streptophyta, representing the kingdom Animalia and Plantae, respectively. Less than 30 bacterial species were found potentially capable of deconstructing all types of polymers, including the well-known enzyme producer Bacillus licheniformis and plant pathogen Pseudomonas syringae. Two thirds are members of Actinobacteria, mostly streptomycetes and some “rare” actinomycetes, for which only a few studies have been reported. Our analyses present a huge gene pool in nature, enabling large-scale evolutionary studies of structural polymer-degrading enzymes. It also facilitates mining of uncommon potential super degraders, providing great selection for further biochemical characterization for relevant industries.
**Abstract Title:**
Complete Genome Sequence of Streptococcus Ruminantium Sp. Nov. Gut-187T (=dsm 104980T=jcm 31869T), the Type Strain of S. Ruminantium

**Primary Author Block:**
M. Tohya1, T. Sekizaki2, T. Miyoshi-Akiyama1; 1Natl. Ctr. for Global Hlth.and Med., Tokyo, Japan, 2The Univ. of Tokyo, Tokyo, Japan

**Abstract Body:**
Background: Streptococcus ruminantium sp. nov. with the type strain GUT-187T (=DSM 104980T=JCM 31869T) was recently described as a novel streptococcal species by Tohya et al (Int J Syst Evol Microbiol. 2017 Sep;67(9):3660-3665.). S. ruminantium was used to be considered as Streptococcus suis serotype 33. Methods: In this study, we determined complete whole genome sequence of S. ruminantium GUT-187T using combination of Oxford Nanopore and the Illumina platform, and compared with genomes of 27 S. suis reference strains. Results: The genome size of GUT-187T is 2,090,539bp and the GC contents is 40.01%. There are 1,961 CDSs in the genome. In the all CDSs, 1,685 of them showed homology with those of S. suis while rest of CDS, which do not show homology with those of S. suis, 81 showed some homology with those of other streptococcal species. Virulence factors such as ECM binding proteins found in the other streptococcal species are conserved with relatively low homology in the GUT-187T genome, and there was no intact phage in the genome. Numbers of phages and CRISPR spacers as well as the presence or absence of Cas proteins are varied among S. ruminantium and S. suis. Phylogenetic analysis showed that GUT-187T is the most distant strain from the other S. suis strains. A genome rearrange map among S. ruminantium and S. suis suggested that several massive rearrangements among the genomes took place. Ratio of each category of Clusters of Orthologous Groups (COG) is relatively conserved among S. ruminantium and S. suis strains. Conclusions: The complete genome sequence of S. ruminantium further support the distinct species classification, and the sequence data would provide basis to develop rapid diagnostic assays.
Comparative Phenotype Analysis and Genome Completion of Streptomyces Venezuelae Strains

Primary Author Block:
W. Kim, N. Lee, Y. Jeong, S. Hwang, Y. Lee, D. Choe, B.-K. Cho; Korea Advanced Inst. of Sci. and Technology(KAIST), Daejeon, Korea, Republic of

Abstract Body:
Background: Streptomyces venezuelae, the emerging model species of Streptomycetes, is highly profitable with the ability to produce valuable antibiotics, such as chloramphenicol and pikromycin. In addition, S. venezuelae is a promising heterologous expression host due to rapid growth rate and relative ease of genetic manipulation. To understand S. venezuelae, researchers focused on studying S. venezuelae ATCC 10712, the type strain of S. venezuelae. However, based on the StrainInfo database, 29 strains were classified into S. venezuelae species. Those strains produce various secondary metabolites that are different between strains. Much of mechanisms in S. venezuelae ATCC 10712 were revealed, but the other strains are relatively unknown. To reveal the differences between strains and genetic potential of S. venezuelae strains, we compared phenotype and genome sequence of 10 S. venezuelae strains. Methods: We compared phenotype of commercially available 10 S. venezuelae strains. Next, genome sequences of only 2 strains among 10 strains have been reported, one of which is not the complete level. Therefore, we completed genome sequences of 9 of 10 S. venezuelae strains using Pacific Biosciences SMRT and Illumina Hiseq sequencing technologies. Finally, we identified secondary metabolite clusters in each strains using antiSMASH. Results: 10 S. venezuelae strains colonies differ in their color, surface form and radial colony growth rate on MYM agar media. They clustered in two groups with different growth profile in liquid MYM media culture. Next, we completed genome sequences of 9 S. venezuelae strains and annotated an average of 7,400 CDSs, 19.5 rRNA, and 67 tRNA. Interestingly, when we aligned 16S rRNA sequences of 10 strains, 9 strains can be regarded as same species (ANlb>97%) whereas completed genome multi-alignment result revealed that only 3 strains can be considered as same species (ANlb>95%). Based on the result, 10 strains can be divided into three groups (ANlb>90%). We identified total 86 secondary metabolite clusters in 10 strains, with one strain having 28 secondary metabolite clusters on average. The strains belonging to each 3 groups have tendency to share same secondary metabolite clusters. Conclusions: Our results showed that only 3 of 10 S. venezuelae strains can be considered as same species even though 10 strains were classified into same species. However, their genetic components related with growth or novel secondary metabolite cluster can be utilized to improve the S. venezuelae strain as a beneficial industrial platform.
Abstract Title:
Functional Genomics of Novel Lactobacillus Associated with Non-Eusocial Bees

Primary Author Block:
H. Q. Vuong, Q. S. McFrederick; Univ. of California, Riverside, Riverside, CA

Abstract Body:
Lactobacillus micheneri, Lactobacillus timberlakei, and Lactobacillus quenuiae are novel bacterial symbionts found in flowers and abundantly in non-eusocial bee pollen provisions and adults. These three bacterial species form a clade referred to as the L. micheneri clade. Though not found in honey or bumble bees, these bacteria are associated with a rich diversity of wild bees and managed bees that pollinate crops, such as Nomia melanderi and Megachile rotundata. We sequenced the genomes of 29 isolates belonging to the L. micheneri clade to determine the possible ecological function of L. micheneri clade in the flower and bee host niches. We assembled our genomes using the A5-miseq pipeline and annotated our genomes using the RAST pipeline. RAST also reconstructs the metabolism model of each genome based on its annotation via ModelSEED. After annotation, we used the program Count for ancestral genomic state reconstruction to determine genes gained and lost from common ancestor states between different clades of Lactobacillus. Finally, after our gene gain and lost analyses, we classified the genes into their respective cluster of orthologous genes (COG) using eggNOG. From our ancestral genome state analyses, we found that the ancestral state of the L. micheneri clade gained two copies of pectate lyase genes. These genes are found in a honey and bumble bee symbiont named Gilliamella apicola, but have not been found in any other Lactobacillus species outside of the species in the L. micheneri clade. The pectate lyase genes have been suggested to be involved in releasing the protein contents within pollen. In addition, from the ancestral state of distant relatives including L. plantarum, L. brevis, L. buchneri, we find a large loss of genes in the COG groups involved in carbohydrate transport, protein metabolism and biosynthesis, and energy conversion. What pathway remains for ATP production in the genome of L. micheneri clade isolates is glycolysis and fermentation. Based on the pectate lyase genes and the presence of the glycolysis and fermentation pathways, we believe the L. micheneri clade bacteria have adapted to simple carbohydrate and protein rich niches. These niches are most likely the nectaries and anthers of flowers and the pollen provisions and guts of non-eusocial corbiculate bee, all of which contain an excess of pollen or nectar, or both. These inferences serve as hypotheses for future experiments to test whether the functions found from the genomic analyses in the L. micheneri clade improve the fitness of their bee hosts.
Abstract Title:
Diversity and Evolution of Myxobacterial Type Iv Pilus Systems

Primary Author Block:
G. Sharma1, L. L. Burrows2, M. Singer1; 1Univ. of California, Davis, Davis, CA, 2McMaster Univ.,
Hamilton, ON, Canada

Abstract Body:
Background: The myxobacteria (order Myxococcales) are members of the class Deltaproteobacteria, well known for their largest bacterial genomes and complex social behaviors including gliding motility, fruiting body formation, biofilm production, and predation. Gliding motility is an evolutionarily distinctive feature between myxobacteria and Deltaproteobacteria. Gliding motility integrates and coordinates two distinct mechanisms, designated as adventurous and social motility. Myxobacteria lack flagella but have Type IVa Pili (T4aP), which help them, performing social motility on the surface. T4aP are surface-exposed protein fibers that play key roles in the bacterial life cycle via surface attachment/adhesion, biofilm formation, motility, and development. Myxococcus xanthus, the best-characterized member of order Myxococcales, relies on the appropriate expression of 17 T4aP pilus machinery genes organized in a single cluster within the genome. Methods: Here, we compared the organization of T4aP genes within myxobacteria to understand their evolutionary origins and diversity. Using various homology search strategies, we identified the genes encoding key T4aP proteins and examined their genomic organization, generated phylogenetic trees of conserved proteins, and explored their synteny in 28 myxobacterial and 66 closely related outgroup genomes. Results: Phylogenomic, gene organization and syntemic studies of myxobacterial T4P described here revealed that T4aP genes in the Cystobacterineae have an organization comparable to that of members of the order Bdellovibrionales and Desulfuromonadales. We propose that the T4aP system in the Cystobacterineae, Bdellovibrionales, and Desulfuromonadales would have evolved from a common ancestor. Likewise, our results reveal the high degree of relatedness of the T4aP genes organization in suborders Sorangiineae and Nannocystineae, and rest of the orders of class Deltaproteobacteria, implying a common ancestor. Conclusions: Altogether, current analysis proposes that the T4aP machinery within three myxobacterial suborders had evolved bilaterally from the order Deltaproteobacteria, thereby producing two distinct and different genomic organizations of T4aP genes within a single order. This study elucidates the origins and evolution of social motility in myxobacteria and provides a foundation to understand how complex behavioral traits, such as multicellular development, might have evolved in this diverse group of organisms.
Nutrient Level Does Not Determines the Ecological Strategies in Marine Bacterial Communities

Primary Author Block:
Y. Gao, M. Wu; Univ. of Virginia, Charlottesville, VA

Abstract Body:
The copy number of ribosomal RNA operon (rrn) in a bacterial genome is an important functional trait. Studies on isolated bacterial strains have found that the rRNA copy number correlated positively with the cell's maximum growth rate and negatively with its growth efficiency [1]. Therefore, it is hypothesized that nutrient level dictates the ecological strategies of bacteria in nature in that nutrient-rich environments select for bacteria with high rrn copy numbers (high growth rate) while nutrient-poor ones select for bacteria with low rrn copy numbers (high growth efficiency) [2]. It follows that bacterial communities in copiotrophic environments should have higher mean rrn copy numbers than those in oligotrophic environments. We tested this hypothesis using bacterial community profiling data from two large-scale microbiome studies, the Earth Microbiome Project (EMP, 16S rRNA amplicon sequencing) [3] and the Tara Oceans expedition (TARA, shotgun metagenomics) [4]. Mean rrn copy numbers were estimated using phylogenetics-based method [5] for samples in EMP 16S rRNA dataset, and as the proportion of 16S rRNA gene reads for samples in TARA metagenomic dataset. Correlation between the estimated mean rrn copy number and the environmental factors was tested using generalized linear regression. We found significant correlation between the mean copy number and environmental factors such as temperature and salinity. Surprisingly, we found no correlation between the mean copy number and concentrations of inorganic nutrients (phosphate, nitrate, nitrite and ammonium) (Figure 1). Our results suggest that in ocean, nutrient level does not play an important role in determining the ecological strategies of bacteria as hypothesized previously. The lack of selection on rrn copy number by nutrients also suggests that the actual growth rate of bacteria in ocean is determined by environmental factors like temperature and salinity, but not the nutrients.
Session Number: 89
Session Type: Poster
Session Title: MEE06 - Ecological and Evolutionary Modeling and Theory
Session Start Date Time: 6/8/2018 11:00:00 AM
Session End Date Time: 6/8/2018 1:00:00 PM
Session Primary Track: Microbial Ecology and Evolution
Abstract Control Number: 5721
Poster Board Number: FRIDAY - 968

Abstract Title:
Nutrient-Based Drivers of Mutualism Breakdown

Primary Author Block:
J. M. Chacón, S. Hammarlund, W. Harcombe; Univ. of Minnesota, St. Paul, MN

Abstract Body:
Microbes often cross-feed on metabolites released by other species. To predict the dynamics of microbial communities, it is essential that we understand how changes in nutrient availability alter the composition of cross-feeding systems. We used a combination of genome-scale metabolic modeling, classic ecological models, and co-culture experiments to test how the addition of different nutrients alters the population dynamics, inter-dependence, and coexistence of pairs of conditionally-obligate bacterial mutualists. Pairs were formed from strains of Escherichia coli, Salmonella typhimurium, and Methylobacterium extorquens. Each pair formed a cross-feeding mutualism with a different combination of carbon, nitrogen and amino acid being exchanged. In general agreement with previous results, we find that addition of nutrients to a mutualism can lead to the extinction of one species. We extend this result and show that such extinction depends upon the presence of a second ecological mechanism, such as competition, which is only exposed upon nutrient addition. In conclusion, nutrient pulses can break mutualistic associations and alter community composition in ways that may be predictable from metabolic models. This work provides a quantitative foundation for the management of microbial metabolic networks.
Abstract Title:
The Influence of Structured Environments on the Strength of Competition between Colonies of Different Species

Primary Author Block:
J. M. Chacón, W. Harcombe; Univ. of Minnesota, St. Paul, MN

Abstract Body:
Many bacteria grow in structured environments in which they must compete for diffusing nutrients. It is therefore important to quantify how spatial proximities between colonies impact their growth and interactions. Using a combination of theoretical modeling and petri dish experiments, we examined the circumstances in which spatial proximities significantly alter variability in colony yields. For a single genotype, the importance of spatial proximity depended on the distance between colonies, the diffusion coefficient of the resource, and the growth rate of the bacteria. When bacteria grew quickly relative to resource diffusion, or if colonies were well-separated in space, then proximity had a large impact, and relative colony yields were well-predicted by the colony's territory. Spatial proximity was less important when growth rates were low, diffusion was high, or there was high founder density. Additionally, excretion of toxic metabolites, such as fermentation by-products, reduced yield variability caused by spatial structure. In environments with multiple genotypes, the effect of spatial structure is influenced by phenotype. Relative growth rate significantly alters the weighting that must be used to predict colony yield from location information. Our work helps understand the quantitative way spatial structure interacts with species traits to impact growth, with implications for how species should be combined in structured fermentations to achieve desired yields.
Abstract Title:
Outstanding Abstract Award: Metabolic Networks Based on the Substrate-Spatiotemporal Heterogeneity Can Organize Coexistence of Microbes

Primary Author Block:
K. Suzuki1, M. Honjo2, T. Nishimura2, K. Masuda2, A. Minoura2, Y. Tashiro1, H. Futamata3; 1Graduate Sch. of Sci. and Technology, Shizuoka Univ., Hamamatsu, Japan, 2Graduate Sch. of Integrated Sci. and Technology, Shizuoka Univ., Hamamatsu, Japan, 3Res. Inst. of Green Sci. and Technology, Shizuoka Univ., Hamamatsu, Japan

Abstract Body:
Microbial communities are ubiquitously found in nature and maintain the huge diversity. It has been considered that microbial diversity contributes to maintain ecological functions. However, how to coexist multispecies and maintain the microbial diversity are unknown although the competitive exclusion principle demonstrated that only strain exhibiting the highest competitiveness occupies the niche. Cupriavidus sp. P-10 and Comamonas testosteroni R2 were co-cultured with phenol as the sole carbon and energy source in a chemostat to reveal the coexistence mechanism under the substrate-competing condition. Simulation depending on Lotoka-Volterra equations predicted that strain R2 became dominant and strain P-10 was washed out. However, these strains coexisted and strain P-10 became dominant in the chemostat culture. The kinetic parameters for phenol indicated that the phenol concentration exhibiting Vmax of strain P-10 was higher than that of strain R2. It was shown that phenol concentration was spatiotemporally heterogeneous in the chemostat, suggesting that strain P-10 obtained a chance to acquire phenol. Actually, a dominant strain changed from strain P-10 to strain R2 corresponding to the decrease of phenol loading rate. When strain P-10 was dominant, the transcription of the gene encoding phenol hydroxylase (LmPH) was observed in only strain P-10. Interestingly, total cells yield in the coexisting culture was higher than distinct pure culture. These results suggested that spatiotemporal heterogeneity allowed strain P-10 to overcome the competition with strain R2 and that strain R2 could survive using metabolites from strain P-10. Coexistence collapsed when strain R2 dominated, where only strain R2 expressed LmPH and strain P-10 washed out. These results suggested that coexistence was organized when metabolic networks were constructed between microorganisms, which would be one of maintenance mechanism of microbial diversity in the nature.
**Session Number:** 89  
**Session Type:** Poster

Session Title: MEE06 - Ecological and Evolutionary Modeling and Theory  
Session Start Date Time: 6/8/2018 11:00:00 AM  
Session End Date Time: 6/8/2018 1:00:00 PM  
Session Primary Track: Microbial Ecology and Evolution  
Abstract Control Number: 5553  
Poster Board Number: FRIDAY - 972

**Abstract Title:**  
Symmetry in Propagule Pressure Reduces Establishment Success of Engineered Bacteria with An Allee Effect  

**Primary Author Block:**  
M. D. Dressler, R. P. Smith; Nova Southeastern Univ., Fort Lauderdale, FL

**Abstract Body:**  
Background: The global threat of invasive species is a major ecological concern for the preservation of healthy, native ecosystems. However, the factors that favor establishment and subsequent invasiveness remain poorly studied. One candidate that has been proposed to predict establishment success is propagule pressure, or the number of individuals introduced at a given time. While promising, several recent studies have shown no link between propagule pressure and establishment success. As such, the role of propagule pressure in establishment success remains unclear, likely owing to the complexity of studying establishment dynamics in natural systems.  

Methods: To better understand how and when propagule pressure becomes important during an invasion, we used an engineered strain of Escherichia coli. This strain of bacteria has been engineered to have an Allee effect, a density-dependent population growth dynamic that is often observed in invasive species. Using this strain of bacteria as a surrogate for invasions in a natural systems, we measured how the timing between successive introduction events and how differences in the size of the introduced population determines establishment success.  

Results: We observed that, for populations at the Allee threshold, a second introduction event of bacteria can lead to successful establishment, even when a single introduction event fails to allow growth. Moreover, we found that symmetry in the density of bacteria introduced in successive introduction events limits establishment success by reducing the timing between introduction events that allows for successful establishment.  

Conclusions: Overall, our results help to identify conditions where propagule pressure has a positive impact on establishment success, and may have implications in preventing and controlling establishment and spread of invasive species.
Abstract Title:
Periodic Spatial Disturbance Alters Cooperation in Bacteria Engineered with An Allee Effect

Primary Author Block:
R. Smith; Nova Southeastern Univ., Fort Lauderdale, FL

Abstract Body:
Cooperation is fundamental to the survival of many species, including bacteria. Several previous studies have shown that the spatial organization of a bacterial community can impact cooperation. In general, spatial organization, such as biofilms, has been shown to promote cooperation, as they serve to localize small molecules that are required for cooperation. Traditionally, cooperation has been studied in either undisturbed, or continually disturbed environments. However, most environments where bacteria can be found are periodically disturbed. This will, in turn, periodically change the spatial structure of the population. Despite the important role that periodic spatial disturbances play in facilitating critical ecological relationships, it currently remains unclear as to how such disturbances affect bacteria that are dependent on cooperation for survival. To address this question, we used a synthetic biology approach to study the impact that periodic spatial disturbance had on bacteria engineered with a strong Allee effect. Using microplate reader assays, fluorescent microscopy and mathematical modeling, we found that intermediate frequencies of spatial disturbance affect the ability of a bacterial population to cooperate. Our model predicts that periodic spatial disturbance leads to a tradeoff between accessing a small molecule required for cooperation (an acylhomoserine lactone), and accessing nutrients, which impacts growth rate. Based on this relationship, we altered the degradation rate of the acylhomoserine lactone and demonstrated that this enhanced cooperation, but simultaneously reduced the ecological resistance of the system. Finally, we extended our work to biofilms consisting of the opportunistic bacteria Pseudomonas aeruginosa, and observed that intermediate frequencies of spatial disturbance decreased its ability to cooperate using the virulence factor pyoverdine. Overall, our results may have implications in maintaining stability of microbial communities and in infectious disease.
Session Title: MEE06 - Ecological and Evolutionary Modeling and Theory
Abstract Title: Evidence of Three-Way Life Strategies Associated to Complex Bacterial Communities after Succession Under Varying Disturbances

Abstract Body:
Background: Functional tradeoffs are expected after community perturbations since organisms reallocate resources to facilitate recovery. Microorganisms could then adopt distinct life strategies across a disturbance range, which has been suggested but not yet verified. Here we analyzed the effect of disturbance on the assembly, functionality, and distribution of traits in activated sludge bacterial communities, to identify community life-strategies using an ecological trait-based framework. Methods: Sequencing batch microcosm bioreactors (20 mL working volume) were inoculated with activated sludge from a full-scale plant and operated for 35 days. The daily complex feed included toxic 3-chloroaniline (3-CA) at varying frequencies. Eight levels of disturbance in triplicate independent reactors (n = 24), received 3-CA every day (press-disturbed), every two, three, four, five, six, and seven days (intermediately-disturbed), and never (undisturbed). Samples were analyzed using a variety of methods including metagenomics, biomass quantification, and effluent chemical characterization. Results: After 35 days, bacterial communities displayed significant differences in community structure and composition across disturbance levels, which along with trade-offs in community aggregated function and genotypic traits, indicated that communities adopted different life-strategies depending on the disturbance frequency. We semi-quantitatively aligned our results within Grime’s CSR theoretical framework. Ruderal organisms (R) predominated in communities at intermediately disturbed levels, stress tolerants (S) in press-disturbed reactors, and competitors (C) in undisturbed ones.<br />

Conclusions: We showed how the CSR framework can enable simplification of real complex datasets of microbial traits, function, and taxa, into ecologically meaningful components towards understanding the mechanisms behind the system response to disturbance.
**Session Number:** 89  
*Session Type: Poster*

**Session Number:** 89  
*Session Type: Poster*

**Session Title:** MEE06 - Ecological and Evolutionary Modeling and Theory  
**Session Start Date Time:** 6/8/2018 11:00:00 AM  
**Session End Date Time:** 6/8/2018 1:00:00 PM  
**Session Primary Track:** Microbial Ecology and Evolution  
**Abstract Control Number:** 5135  
**Poster Board Number:** FRIDAY - 975

**Abstract Title:**  
Neutral and Selective Dynamics in A Synthetic Microbial Community  
**Primary Author Block:**  
N. Cira1, M. Pearce2, S. Quake2; 1Rowland Inst. at Harvard, Cambridge, MA, 2Stanford Univ., Stanford, CA

**Abstract Body:**  
Ecologists debate the relative importance of selective versus neutral processes in understanding biodiversity. This debate is especially pertinent to microbial communities, which play crucial roles in areas such as health, disease, industry, and the environment. Here we created a synthetic microbial community using heritable genetic barcodes and tracked community composition over repeated rounds of subculture with immigration. Consistent with theory, we find a transition exists between neutral and selective regimes, and the crossover point depends on the fraction of immigrants and magnitude of fitness differences. Subjecting a community to a step increase in carrying capacity resulted in a transition from neutral to selective regimes, highlighting that using the correct model is essential for predicting community response to change. Taken together these results emphasize the importance of including selection to obtain realistic models of even simple systems.
Abstract Title:
Outstanding Abstract Award: Bacterial Collective Decision-Making: A Solution to and A Source of Social Dilemmas

Primary Author Block:
Y. Wang, J. Rattray, S. Thomas, J. Gurney, S. Brown; Georgia Inst. of Technology, Atlanta, GA

Abstract Body:
Many species of bacteria are able to collectively sense and respond to their physical environments. A common solution to the challenge of environmental uncertainty is the evolution of cell-cell communication systems known as ‘quorum sensing’ (QS), where individual cells control gene expression via the secretion and sensing of extracellular signal molecules. Despite detailed understanding of the intra-cellular mechanisms of signal production and signal responses, there remains considerable debate over the functional role(s) of QS, how does QS enhance bacterial fitness - or in short, what is it for? Using an agent-based modeling approach, we investigated how different selective challenges drive the evolution of QS complexity and multi-functionality. Specifically, the in silico simulations enable us to track the evolution of multiple QS component traits underlying signal production, signal response and their interaction (auto-induction) under defined conditions of social and physical heterogeneity. We demonstrated that individual’s production rate and signal threshold co-evolve in a fashion that is sensitive to costs and benefits of cooperation. We found that bacteria can coerce others to produce more signals and become less responsive by increasing their signal threshold to avoid paying for the cost of cooperation while gaining the group level benefits. However, when we continuously decreased the genetic relatedness, we found that the coercion strategy eventually collapsed and a cheat strategy evolved. This phenomenon was observed in the evolutionary scenarios regardless of the presence of QS auto-induction mechanism. However, when we investigated the transition period where the coercion strategy was collapsing, we found that bacteria evolved with auto-induction mechanism generally have both a higher overall cooperation payoff than the ones evolved without auto-induction mechanism. This suggests that the auto-induction architecture provides a robust mechanism to sustain cooperation. This work is important as it sheds light on how simple signal-mediated behavioral rules can shape complex collective behaviors in bacteria.
Abstract Title:
Using Metagenomic Coalitions As Features for Statistics and Machine Learning
Primary Author Block:
G. Al-Ghalith1, L. Harrington2, D. Knights1; 1Univ. of Minnesota - Twin Cities, Minneapolis, MN,
2Dartmouth Coll., Hanover, NH
Abstract Body:
Metagenomic data analysis is characterized by highly multidimensional data such as the genomes present in a microbial community. Furthermore, treating each microbe as an independent variable may be untenable given the ecological dynamics (cooperation, competition) that exist between microbes. This is a particularly important consideration when performing pairwise statistical tests or machine learning, where either multiple hypothesis correction is performed or feature selection is used. However, these strategies often do not account specifically for community dynamics and instead only reduce or account for number of features, but not the higher-order associations between them. We propose WRANGLr, an open-source software in R, which uses coalitional game theory and related concepts such as greedy feature recombination, to facilitate feature creation instead of purely feature selection. By considering microbes as coalitions, we find groups of microbes that form cooperative units through optimization of the system’s Shapley value (each microbe’s marginal contribution to predicting an outcome variable such as disease status). We further demonstrate using public datasets that using these constructed features as compared to traditional feature selection strategies results in modest but consistent performance improvements in certain machine learning tasks.
Abstract Title:
A Machine Learning Approach for Predictive and Explanatory Microbial Ecology

Primary Author Block:
D. DiMucci, D. Segre; Boston Univ., Boston, MA

Abstract Body:
Pairwise co-culture experiments of all microbial species in a community are emerging as powerful tools for understanding the interactions that determine function and dynamics in microbial communities. These studies are performed with the goals of predicting the outcomes of new unobserved interactions and developing plausible explanations regarding mechanism but are often limited in scope by the experimental logistics of handling large communities. We have combined a trait-based representation of microbial interactions with machine learning to develop a model for predicting yet to be seen interactions and the guided generation of mechanistic hypotheses. All pairwise combinations between 100 genome-scale metabolic models of human gut associated bacteria [1] were performed in silico and the relative yield of each model in co-culture with each other model was recorded. Models were negatively affected by their partner 5563 times and unaffected by the presence of an interaction partner 4337 times. Interactions between microbial models were represented as a binary vector where each element corresponds to one of 388 unique metabolic reactions; vectors were subsequently used as predictors for machine learning. Evaluation on the full experimental space with five fold cross validation yielded a balanced accuracy of ~90% when predicting the qualitative outcome of a held out interaction. Furthermore, a learning curve analysis revealed that a balanced accuracy of 78.56% could be attained using as few as 5% (248) of possible interactions to predict the remaining 95% (4702) pairwise interactions. Finally, the application of feature selection methods to generate ranked lists of potential mechanisms results in the discovery of mechanisms of competition in interactions with negative interactions at rates as high as 97 times faster than what would be expected by chance for this data set. Our results indicate that the goals of predictive microbial ecology can be greatly facilitated by combining trait-level representations of microbes with machine learning. Feature selection methods allow us to generate ranked lists of potential mechanisms that are specific to an interaction of interest and expedite the discovery of molecular mechanisms of interaction. This approach should prove useful as a guide for the construction of synthetic microbial communities and for lightening the experimental burden associated with mechanistic inquiries.
Abstract Title:
Immune Loss As A Driver of Coexistence During Host-Phage Coevolution

Primary Author Block:
J. L. Weissman1, R. Holmes2, R. Barrangou3, S. Moineau4, W. F. Fagan1, B. R. Levin2, P. L. F. Johnson1; 1Univ. of Maryland Coll. Park, College Park, MD, 2Emory Univ., Atlanta, GA, 3North Carolina State Univeristy, Raleigh, NC, 4Université Laval, Québec, QC, Canada

Abstract Body:
Bacteria and their viral pathogens face constant pressure for augmented immune and infective capabilities, respectively. Under this reciprocally imposed selective regime, we expect to see a runaway evolutionary arms race, ultimately leading to the extinction of one species. Despite this prediction, in many systems host and pathogen coexist with minimal coevolution even when well-mixed. Previous work explained this puzzling phenomenon by invoking fitness tradeoffs, which can diminish an arms race dynamic. Here we propose that the regular loss of immunity by the bacterial host can also produce host-phage coexistence. We pair a general model of immunity with an experimental and theoretical case study of the CRISPR-Cas immune system to contrast the behavior of tradeoff and loss mechanisms in well-mixed systems. We find that, while both mechanisms can produce stable coexistence, only immune loss does so robustly within realistic parameter ranges.
Tracing the Ancestry of Operons in Bacteria

H. Nguyen, A. Jain, O. Eulenstein, I. Friedberg; Iowa State Univ., Ames, IA

Background: Complexity constitutes a fundamental attribute of life. Complex systems are made of parts that together perform functions that a single component, or most subsets containing individual components, cannot. Examples of complex molecular systems in bacteria include protein structures such as the F1F0-ATPase, the ribosome, or the flagellar motor: each one of these structures requires most or all of its components to function properly. At the molecular level, operons are a classic example of a complex system. An operon’s genes are co-transcribed under the control of a single promoter to a polycistronic mRNA molecule, with its gene products forming molecular complexes or metabolic pathways. With the large number of complete bacterial genomes available, we now have the opportunity to examine the evolution of operons and identify possible intermediate states.

Methods: In this work, we develop a simple vertical evolution model of how operons evolve from individual component genes and orthologous gene blocks or orthoblocks. Utilizing this model, we present two algorithms to reconstruct ancestral operon states using a maximum parsimony approach.

Results & Conclusions: We describe several ancestral states that are plausible functional intermediate forms leading to the full operon. A few interesting observations emerge regarding conservation and ancestry of operons. It appears that essentiality (the trait of being essential to life) and the formation of a protein complex are the main drivers for gene block conservation.

Figure caption: Orthologous gene blocks from species A-E are arranged in a species phylogenetic tree. Species C has an experimentally-determined operon (Black arrows), and serves as the reference taxon. The tree’s inner nodes show proposed intermediate states in the operon’s evolution using maximum parsimony.
Session Number: 89
Session Type: Poster
Session Number: 89
Session Type: Poster
Session Title: MEE06 - Ecological and Evolutionary Modeling and Theory
Session Start Date Time: 6/8/2018 11:00:00 AM
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Session Primary Track: Microbial Ecology and Evolution
Abstract Control Number: 5919
Poster Board Number: FRIDAY - 981

Abstract Title:
Latent Environment Allocation of Microbial Community Data
Primary Author Block:
K. Higashi1, S. Suzuki2, S. Kurosawa2, H. Mori1, K. Kurokawa1; 1Natl. Inst. of Genetics, Mishima, Shizuoka, Japan, 2Tokyo Inst. of Technology, Tokyo, Japan

Abstract Body:
Background: As data for microbial community structures found in various environments has increased, studies have examined the relationship between environmental labels given to retrieved microbial samples and their community structures. However, because environments continuously change over time and space, mixed states of some environments and its effects on community formation should be considered, instead of evaluating effects of discrete environmental categories. Methods: We applied a hierarchical Bayesian model to paired datasets containing more than 30,000 samples of microbial community structures and sample description documents. From the training results, we extracted latent environmental topics that associate co-occurring microbes with co-occurring word sets among samples. Topics are the core elements of environmental mixtures and the visualization of topic-based samples clarifies the connections of various environments. Based on the model training results, we developed a web application, LEA (Latent Environment Allocation). Result: Our tool, LEA, enables researchers to do the following: 1) Clarify the relationship between environments and patterns of microbial community structures. 2) Predict the “latent environments” of new samples from, for example, the ocean, a diseased gut, or another unexpected environment, and quickly compare new samples with tens of thousands of existing samples based on their environmental similarity, which makes it easy to detect dysbiosis of the microbiome in the human gut or contaminants in natural environments. 3) Search for samples in the >30,000-sample dataset based on an environmental perspective, without depending on exact word matching of queries and sample descriptions. Conclusion: LEA provides the way to evaluate typicality and heterogeneity of microbial communities in newly obtained samples without confining environmental categories to be compared.
Session Number: 90
Session Type: Poster
Session Title: MEE07 - 'Omics to Study Ecosystem Function
Session Start Date Time: 6/8/2018 11:00:00 AM
Session End Date Time: 6/8/2018 1:00:00 PM
Session Primary Track: Microbial Ecology and Evolution
Abstract Control Number: 6864
Poster Board Number: FRIDAY - 982

Abstract Title:
Understanding the Effects of Environmental Factors on Microbial Composition and Activity in the Delaware Bay

Primary Author Block:
C. Hall, A. J. Harris, E. M. Weber, S. Katragadda1, B. J. Campbell; Clemson Univ., Clemson, SC

Abstract Body:
Background: Microbes are major contributors to biogeochemical cycling within bodies of water. Members of one bacterial phylum, the Bacteroidetes, are abundant in the Delaware Bay. Metagenome and metatranscriptome analyses from Delaware Bay samples allow for discovery and characterization of respective microbial populations and communities in relation to different environmental conditions.

Methods: Samples from the Delaware Bay were taken under different environmental conditions such as season, time, salinity, and size fraction. The samples were sequenced and assembled into metagenome assembled genomes (MAGs) and annotated. RAxML was used to generate a phylogenetic tree comparing 14 shared genes between the MAGs and their closest relatives. NCBI and RAST databases were used for comparative genome analysis.

Results: Fourteen MAGs were chosen that phylogenetically associate with the Bacteroidetes. Eight MAGs assembled from the summer high salinity conditions usually had Flavobacteriales bacterium HTCC2170 or Rhodothermus marinus DSM 4252 as their closest relatives. MAGs assembled from the spring high salinity conditions had Pedobacter saltans DSM 12145 as their closest relative or were very diverse. All MAGs had less than 55% amino acid similarity to their closest relatives. Some MAGs sampled in similar environments scored higher than 90% in shared amino acids when compared to each other. Genes for proteorhodopsin, carotenoids, motility and chemotaxis, carbohydrate, amino acid, and aromatic compound subsystems were commonly found among the fourteen MAGs. For instance, proteorhodopsin genes were present in 12 out of 14 MAGs.

Conclusions: Similar conditions such as salinity, season, nutrient availability, and size fraction may allow for the selection of certain taxa which are highly present in a niche. Low amino acid similarities to known bacteria indicate the MAGs are novel species. High amino acids similarity scores between MAGs suggest that some samples taken in similar environmental conditions may be the same species. Flavobacteriales MAGs were dominant during the summer, most likely because proteorhodopsin containing organisms benefit from additional light stimulated energy production. The mechanisms explaining light stimulated growth by proteorhodopsin are still relatively unknown and our data may shed additional light on this important mechanism.
Abstract Title:
Unraveling the Microbial and Functional Diversity of Clara Cave and Empalme Sinkhole, Puerto Rico Using Cultivable and Uncultivable Methods

Primary Author Block:
L. E. Rodriguez-Ramos, C. Rios-Velazquez; Univ. of Puerto Rico at Mayaguez, Mayaguez, Puerto Rico

Abstract Body:
Camuy River Cave Park (CRCP) is an underground cave system located at a karst in the subtropical moist forest of northern Puerto Rico. Clara Cave and Empalme Sinkhole are part of CRCP that have not been extensively studied by microbiology and metagenomics. The focus of our research was to explore the cultivable bacterial communities and molecularly, the functional diversity of Clara Cave and Empalme Sinkhole soil and water samples. Thirty-six (36) bacteria were isolated from a total of nine samples, whose pH ranged from 7.45 to 9.93, using LBA incubated at 25°C. It was found that most of them were Gram negative bacteria of variable sizes. In addition, we generated two metagenomic datasets of four water samples between Clara Cave and Empalme Sinkhole using uncultivable methods and DNA shotgun sequencing. The environmental DNA (eDNA) was extracted using Metagenomic DNA Isolation Kit for Water (Epicentre) for 200mL of water sample. Next-Generation-Sequencing technology (Illumina MiSeq) was used to process the isolated eDNA, generating two genomics libraries. Subsequently, the libraries were submitted to MG-RAST online server for taxonomic profile and functional in silico description of the samples. Our analyses showed an abundance of Proteobacteria (96.0%) at the phylum level, followed by unclassified for viruses (1.0%), Bacteroidetes (1.0%), Actinobacteria (1.0%), and Firmicutes (0.3%). At the genus level, Acinetobacter (24.0%) predominate, followed by Pseudomonas (16.0%), Bordetella (7.0%), Acidovorax (6.0%), and Achromobacter (5.0%). Meanwhile, subsystem functional analysis demonstrated that 11.0% of genes belong to clustering-based subsystems, 11.0% to amino acids and derivatives, 10.0% to carbohydrates, and 6.0% to protein metabolism. In addition, the in silico analysis suggest the presence of genes associated with resistance to erythromycin, fosfomycin, methillicin, vancomycin, streptothricin, and fluoroquinolones. Further, gene sequences related to multidrug resistance efflux pumps and anaerobic degradation of aromatic compounds were also found. In silico functional analysis using Non-Supervised Orthologous Groups (NOG) annotation showed that most proteins are poorly characterized, indicating the possibility of potential reservoirs of biomedical, industrial, or biotechnological applications. The datasets will provide a source not only for comparison with other caves studied in Puerto Rico, but also develop strategies to identify bioprospects with biomedical and biotechnological applications.
Abstract Title:
Time Dependent Assessment of Microbial Community Profile in Crude Oil Polluted Mangrove Swamp of Niger Delta

Primary Author Block:
V. I. Obidiugwu1, C. K. Wachukwu2, C. C. Nwankwo1, G. C. Okpokwasili1; 1Univ. of Port Harcourt, Port Harcourt, Nigeria, 2River State Univ. of Sci. and Technology, Port Harcourt, Nigeria

Abstract Body:
The basic knowledge of the natural bacterial population ecology depends on metagenomics approach. This study is aimed at using metagenomics to access the community structure of microorganisms in crude oil polluted sites. The top and subsections of soil samples were taken from three georeferenced crude oil contaminated swamps with 3 week, 14 week and 50 week spill history in Degema Local government Area, of Niger Delta Region of Nigeria and investigated using metagenomics and bioinformatics/mathematical modeling approach. Next Generation Sequencing technique was employed to determine the nucleotide sequences of all microorganisms present in the swamp sample using the Genome sequencers 454 or Illumina whereas, the metagenomics data sets generated were analyzed using bioinformatics. Study of the geochemical parameters of the soil showed variation among the different spill sites. At relative abundance greater than 2%, a total of 29 families were studied in the polluted swamp, while the top with 3 week, 14 week and 50 week spill history had 23, 22 and 5 families respectively. The top with 3 week spill history showed the presence of Unknown (61.5%), Mycobacteriaceae (31.58%), Rhodobacteraceae (19.52%), Alkanivoraceae (6.68%), Bacteroidaceae (4.5%), Physciaceae (3.42%), Pseudomonadaceae (3.21%). While the top with 14 week spill showed the presence of Unknown (61.54%), Acetobacteraceae (28.5%), Eubacteriaceae (16.24%), Pleosporaceae (3.85%), Chloroflexaceae (3.85%), Piscirickettsiaceae (2.86%), Flexibacteraceae (2.14%), Sphingomonadaceae and Desulfovabaceae (2.14%), while the top with 50 week spill showed the presence of Oxalobactereaceae (85.34%), Acidaminococcaceae (8.3%) > Unknown (6.83%). The oldest spill site had the least value of specie richness and diversity. Mycobacterium mageritense and Paracoccus carotinifaciens were dominant species in the 3 week spill. Hierarchical clustering analysis using UPGMA based on overall patterns of variation showed distinct microbial communities in relation to geographical locations and this spatial species distribution were attributed to the monitored geochemical parameters of polluted swamps. The findings of this study have established variations in microbial compositions and diversity among crude oil contaminated sites as well pollution history. Effective bioremediation strategies in the mangrove ecosystem will depend on the knowledge of the ecology of microorganisms in the individual swamps.
Abstract Title:
Comparative Analysis of the Bacterial Communities of Planktonic and Benthic Environments in the West Branch of the Susquehanna River

Primary Author Block:
E. Stowe, J. Yankauskas; Bucknell Univ., Lewisburg, PA

Abstract Body:
While many scientists have completed metagenomics analyses on the Chesapeake Bay, little work has been done on the Susquehanna River. The Susquehanna River is one of the largest rivers in the Chesapeake watershed. It is the longest non-navigable river in the eastern United States. Two branches contribute to form the Susquehanna, the North Branch originates in Cooperstown, NY and the West Branch originates in western Pennsylvania. The branches merge in central Pennsylvania before emptying into the Chesapeake Bay. While there is currently no commercial boat traffic, both branches and the main river were important in timber and coal transport in the past and are currently impacted by agriculture, oil and gas drilling and fracking. While the Pennsylvania Department of Environmental Protection frequently assesses the river and outbreaks of smallmouth bass diseases are investigated, to my knowledge, no assessment of the microbial flora of the river has been undertaken. Our previous work in the Susquehanna using sequencing of cloned 16S rRNA PCR products established a shallow assessment of the river community. Our analysis indicated a high abundance of betaproteobacteria, though this is possibly the result of primer bias. When we specifically assessed the cyanobacterial community in the river, we saw five abundant families, all filamentous cyanobacteria. In 2016, we collected water and sediment samples from the West Branch of the Susquehanna in Lewisburg, PA. Total environmental DNA was isolated using MoBio Power Water and Power Soil extraction kits. The DNA was then sent to ZymoResearch for 16s rRNA amplification and analysis. Preliminary analysis by Zymo research bioinformaticians indicated that sample diversity clustered based on planktonic versus benthic point of collection as opposed to date of collection or location within the river (samples were collected at both banks and the middle of the river). All cyanobacterial sequences identified by Zymo clustered within unicellular strains. Since this did not corroborate our previous findings we used Qiime2 to assess the data ourselves. Here we present reanalysis and comparison of this data using Qiime2.
Abstract Title:
Microbial Changes in Gene Expression Level in Response to Environmental Conditions in the Delaware Bay

Primary Author Block:
M. I. Patel, B. J. Campbell; Clemson Univ., Clemson, SC

Abstract Body:
Background: Bacteria dominate in abundance, diversity and potentially metabolic activity in many environments. Our current knowledge on the influence of specific individual taxa on these processes is largely lacking. To bridge these gaps, we chose three metagenome assembled genomes (MAGs) from the Delaware Bay, phylogenetically associated with the Roseobacter clade, to examine the levels of select gene transcripts and their associated genes under different environmental conditions. Methods: Delaware Bay samples were collected and their metagenomes sequenced from various conditions including: season, salinity, time and size fraction, and were assembled into MAGs. These MAGs were annotated via RAST and used for further differential gene expression analysis with DESeq2. A phylogenomic tree was constructed with the RAxML and FastTree packages. Results: The three MAGs were phylogenetically associated with the Roseobacter clade. The closest relatives, based on NCBI and RAST databases, were Planktomarina temperata RCA23 and Rhodobacteraceae bacterium HIMB11. In general, there was a higher abundance of gene transcripts mapping to the MAGs from the summer season compared to the fall. The greatest differential expression was seen between two different seasons (summer versus fall) and time (night versus day). In addition, the different size fractions had differential expression for genes associated with motility. The highest differential expression was also seen in categories such as chemotaxis, membrane transport, amino acids, carbohydrates and photosynthesis. Conclusions: The differential expression of gene transcripts from all three MAGs was correlated to the various environmental parameters, especially season and time of day. The differential abundance of transcripts by season indicates a higher relative metabolic activity during the summer than the fall. Nutrient differences, such as carbon source, likely play an important role in seasonal variability. The MAGs of interest have genes to accommodate phototrophic metabolism, and the differences in gene expression of photosynthetic genes between night and day are likely a reflection of light availability. Understanding how expression levels of specific genes vary in relation to environmental conditions provides insights into how these microbes may affect biogeochemical cycling, especially during different seasons.
Abstract Title:
Microbial Community Structure and Biogeochemistry of Three Small Eutrophic Lakes

Primary Author Block:
J. B. James1, J. E. Rogers1, J. A. Lisa2, K. A. Houghton1, R. D. Devereux1; 1US Environmental Protection Agency, Gulf Breeze, FL, 2Rutgers Univ., New Brunswick, NJ

Abstract Body:
Background: The three Jackson Lakes within the Bayou Chico Watershed in NW FL, USA, were formed at different times from abandoned sand pits. The lakes experienced inundation with marine water during Hurricane Ivan 2004 and, despite their proximity and similar dimensions, have developed different biogeochemical profiles over time. While the NE lake is fully freshwater, the SE and SW lakes have freshwater above the oxycline with salinities below the oxycline ranging between 2.3 and 4.5. This study was undertaken to investigate the microbial communities of the lakes and how microbial communities are shaped over redox gradients.

Methods: The lakes were sampled quarterly during September of 2016 through October 2017. Water column chemical profiles were obtained for salinity, O2, NH4+, NO3-/NO2-, Fe2+, PO43-, SO42-, H2S, CO2, CH4, N2O, dissolved organic carbon (DOC), and particulate carbon and nitrogen. Continuous dissolved oxygen and temperature sensors were deployed 14 days prior to sampling. Water was collected at ten depths, from surface to bottom, with focus around the oxycline. The water was filtered using 0.22 µm filters, the DNA extracted from the filters, and the V4 region of the 16S rRNA gene amplified and sequenced. The sequences were used to determine community structures for comparison with environmental data.

Results: The communities were more similar within a lake, and between sampling times with depth being a major determinant within lakes, than between lakes. In agreement with the chemical data, the sulfidic SW and SE lakes were more like one another than either was to the NE lake. Continuous data monitors demonstrated that these lakes are polymictic. The community structure data suggests that after a mixing event, the SW and SE lakes established redox gradients more rapidly than in the NE lake, where DOC concentrations were lowest. The community structures of the lakes correlated to different environmental drivers. However, as expected, the highest single correlation to community structure was with salinity. Organisms were identified as common across lakes, as well as being abundant only in a particular lake, or during single sampling events.

Conclusion: The putative identification of the organisms in the different depths and seasons within the lakes, along with the water column chemical profiles, provides insights into the biogeochemical cycling within the lakes. Microbial community structures returned to near their initial states following mixing in re-establishing the biogeochemical gradients in the lakes.
Abstract Title:
Bacterial and Protozoan Dynamics Upon Thawing and Freezing of An Active Layer Permafrost Soil

Primary Author Block:
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Abstract Body:
Background: The active layer of soil overlaying permafrost in the Arctic is subjected to drastic annual changes in temperature and soil chemistry, which we hypothesize to affect the overall soil community.

Methods: We investigated the changes in the soil microorganisms at different temperatures during warming and freezing of active layer soil from Svalbard. Soil community data were obtained by direct shotgun sequencing the total RNA.

Results: We observed changes when warming from -2°C to +2°C with drop in fungal rRNA and increase in some OTUs belonging to Gemmatimonadetes, Bacteroidetes and betaproteobacteria. Even more substantial changes occurred when incubating at 2°C for 16 days, featuring a drop in total fungi potential activity and decline in oligotrophic members from Actinobacteria and Acidobacteria. Additionally we detected an increase in bacterial phyla Bacteriodetes, Firmicutes and the Beta-Gamma-proteobacteria - collectively presumed to be copiotrophic. Furthermore, we detected an increase in bacterivorous heterotrophic flagellates, likely due to predation upon the bacterial community via grazing. While this grazing activity may explain the large changes in the bacterial community composition, no changes in total 16S rRNA gene copy number were observed and total RNA level remained stable during incubation.

Conclusions: Thus, combination of a decrease in potential fungal activity, decline in oligotrophic bacteria and grazing of bacteria by protozoa may delay the degradation of more complex organic matter upon thawing of active layer soil.
Abstract Title:
Evaluating Metaproteomics Software Tools for Functional Microbiome Analysis

Primary Author Block:
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Abstract Body:
Background: For a full appreciation of microbiome dynamics, it is important to understand the functional role of expressed microbial genes/proteins. Metaproteomics, which characterizes the protein complement of the microbiome, has enabled such functional characterization (1). There are several functional analysis software tools that measure the combined response of microbial communities to perturbations in their environment. In this work, we evaluate the performance of selected software tools, so that metaproteomics researchers can make informed decisions based on research goals. Methods: Plaque samples from children at high-risk for dental caries were grown in paired biofilm reactors, in the presence and absence of sucrose (2). The samples were subjected to protein extraction, sample processing, and mass spectrometry (MS) measurements. The resultant mass spectral data were searched against Human Oral Microbiome database (HOMD) to identify microbial peptides. The outputs from this dataset were subjected to functional analysis using software platforms such as MEGAN6 (3), EggNOG Mapper (4), Unipept (5), MetaProteomeAnalyzer (6) and MetaGOmics (7).

Results: Functional analysis of plaque microcosm paired metaproteomic data had previously shown sucrose-induced changes in protein relative abundance patterns for several metabolic pathways (2). Spectral count measurements were used as a quantitative analysis metric for this study. After subjecting these microbial peptides to functional analysis tools, the following qualitative and quantitative features were assessed: a) number of peptides that were mapped to functional groups and gene ontology terms; b) ability to normalize and assign spectral counts to functional groups; c) ability to visualize datasets and d) biological conclusions drawn from data. A customized script was used to benchmark the results of the software tools being tested. As an outcome from this benchmarking study, detailed feedback is given to the upstream software developers for algorithm optimization. Results from this analysis and guidelines for software selection will be presented. Conclusions: Our study evaluates outputs and features from functional analysis software tools enabled by metaproteomics analysis. Our observations will aid researchers in making informed decisions on the choice of software platform for advanced functional analysis of complex microbiomes.
Abstract Title:
Effects of 30°C Temperature Incubation on the Bacterial Microbiome of Ixodes Scapularis

Primary Author Block:
S. Thapa, Y. Zhang, M. Allen; Univ. of North Texas Hlth.Sci. Ctr., Graduate Sch. of BioMed. Sci., Fort Worth, TX

Abstract Body:
Background: The blacklegged tick (Ixodes scapularis), widely distributed throughout the eastern United States, is the primary vector of Lyme disease and several other zoonotic pathogens. The bacterial microbiome of the vector has been shown to influence carriage and transmission of a variety of pathogens, however, the environmental drivers of variation in the bacterial community are poorly understood. Methods: With an objective to understand the effect of temperature on the tick microbial community, we performed Illumina MiSeq sequencing of the bacterial 16S V4 rRNA gene of colony-reared I. scapularis adults (n=20) incubated at 30°C for ten days at a constant humidity (~85%), and that of baseline controls (n=10). Bacterial composition was characterized by the relative abundance of bacterial taxa, and diversity was estimated with observed operational taxonomic units (OTUs), Shannon index, and principal coordinate analysis (PCoA) plots. Results: Pseudomonas, the most abundant genus in the male ticks held at 30°C for 5-7 days (mean=37.9%), showed a proportional decrease after eight days of incubation, with a concomitant increase in the relative proportion of Brevibacterium (from a baseline average of 11.2% to 48.8% by day 9). In addition, abundance of the genus Streptomyces increase from 0.5% at baseline to 72.3% in males incubated at 30°C for 10 days. The overall bacterial richness and diversity of the male ticks was also decreased at 30°C in comparison to the baseline. On the other hand, Rickettsia, the most dominant genus in baseline female samples (mean=97.2%), had a slight decrease in abundance (92.1%) upon incubation at 30°C for 8-10 days. Conclusions: Our data provide evidence that the bacterial community of I. scapularis change upon prolonged incubation at 30°C, and is dependent on sex-specific attributes and length of exposure, suggesting potential roles of environmental temperature on tick bacterial community variation. Our findings, when combined with existing knowledge on vector distribution and disease transmission dynamics, may help inform effective interventions to control the regional and seasonal incidence of Lyme disease and other tick-borne diseases.
Abstract Title:
Comparative metagenomic study of mangrove microbiome: Prevalence of heavy metal and antibiotic resistome across different ecosystems irrespective of anthropogenic activities

Primary Author Block:
R. Kumavath; Central Univ. of Kerala, Kasragod, India

Abstract Body:
In addition the continuous destruction of mangroves, the preservation of mangroves is critical in the current age of global warming. In this study, we analyzed mangrove sediments from India using de novo whole metagenome NGS and compared it to mangrove sediments from Brazil and Saudi Arabia. Proteobacteria and Euryarchaeota are the most profuse phyla within and between the samples for bacterial and archaeal domains. The samples exhibited 593 bacterial and 61 archaeal genera. The core bacterial microbiome comprised of 97.9% of the overall bacterial diversity, covering over 99.97% ± 0.01 of the bacterial abundance, indicating the commonness of microbial diversity in mangrove environment irrespective of geographical location. Functionally, 1942 genes were shared in all the mangrove sediments. All of the mangrove resistome consistently showed high abundance of fluoroquinolone and acriflavine resistance. The mangrove and terrestrial samples were clearly enriched in heavy metal resistance, while the ocean samples were enriched in drug resistance genes. The ocean had fluoroquinolone and methicillin resistance gene as high as 28.178%±3.619 and 10.776% ± 1.823. In contrast, Cobalt-zinc-cadmium resistance genes were higher in the mangrove (23.495% ±4.701) and terrestrial (27.479%±4.605) ecosystems. Our results asserted the wide spread heavy metal and antibiotic resistance genes in nature irrespective of ecosystems. Keywords: mangroves, whole metagenome analysis (NGS), microbial community, acriflavine, fluoroquinolone, methicillin, beta-lactamase
Intermittent Hypoxia and Hypercapnia, A Hallmark of Obstructive Sleep Apnea, Alters the Gut Microbiome and Metabolome

Primary Author Block:
A. Tripathi, A. Melnik, J. Xue, O. Poulsen, M. Meehan, G. Humphrey, L. Jiang, G. Ackermann, D. Zhou, R. Knight, P. Dorrestein, G. Haddad; Univ. of California San Diego, La Jolla, CA

Abstract Body:
Obstructive sleep apnea (OSA) is a common disorder characterized by episodic obstruction to breathing due to upper airway collapse during sleep. OSA has been associated with adverse cardiovascular and metabolic outcomes, although data regarding potential causal pathways are still evolving. Because O2 and CO2 intake affect the ecology of the gut microbiota and the microbiota has been shown to contribute to various cardio-metabolic disorders, we hypothesized that the downstream physiological consequences of OSA are linked to functional alterations in the gut ecosystem. Here, we model human OSA and its cardiovascular consequence using atherosclerosis-prone (Ldlr -/-) adult mice fed high-fat diet (resembling western dietary practices). To mimic the changes in blood gases that occur in OSA, these mice were longitudinally exposed to intermittent hypoxia and hypercapnia (IHH; analogous to chronic OSA) in a computer-controlled atmosphere chamber system (treatment group; n=8) or housed in room air (control group; n=8), and examined for 6 weeks. We had previously shown that IHH exacerbates atherosclerosis plaque formation in this model system.2 Fecal samples, a representative of the gut ecosystem, were collected at baseline and twice each week thereafter, and microbiome and metabolome were profiled using 16S rRNA amplicon sequencing and LC-MS/MS-based untargeted mass-spectrometry, respectively. We estimated relative abundances of microbial features (using QIIME2 tools: qiime.org) and molecular features (using GNPS tools: gnps.ucsd.edu) per sample and compared OSA-mimicking and control mice using multivariate statistical models. Starting from a highly congruent gut composition at baseline, both microbiome and metabolome of IHH-exposed mice cumulatively diverged from controls and the changes in '-omics were correlated over time of IHH-exposure. We noted marked compositional changes in both microbial (>10%, mostly increases in Clostridia) and molecular (>22%) species in the gut. Furthermore, top significantly altered molecules included microbe-derived secondary bile acids, enterolignans and fatty acids (identified to the highest level of annotation per metabolomics standards), highlighting the impact of IHH on host-commensal co-metabolism in the gut. Thus, we present the first evidence that IHH functionally perturbs the gut ecosystem, setting the stage for understanding its involvement in associated cardio-metabolic disorders.
Abstract Title:
Longitudinal Impact of Prophylactic Antibiotic Use on the Gut Microbiota and Antimicrobial Resistance Genes

Primary Author Block:
A. M. Seekatz, S. Weiner, C. Bassis, L. O'Donohue, A. Penkevich, V. B. Young, K. Rao; Univ. of Michigan, Ann Arbor, MI

Abstract Body:
The gut microbiota maintains health and helps prevent infection. Antibiotic exposure is known to disrupt the gut microbiota, allowing for potential colonization with resistant microbes. Currently, there is a need for prospective, longitudinal studies in healthy individuals assessing the impact of antibiotics on the gut microbiota's structure and function. To assess the impact of antibiotics on the gut microbiota, we recruited healthy adults undergoing elective orthopaedic or urologic procedures where one or more doses of perioperative antibiotics are routinely given. Up to seven stool samples from each subject were collected starting 1 month before out to 3 months after antibiotics. We used 16S rRNA gene sequencing to assess the microbiota and targeted qPCR to identify the presence of selected antibiotic resistance genes. A total of 42 subjects were enrolled (229 samples; 69.4% male; mean age 45.1 years). The majority of patients received one dose of cefazolin (29) or clindamycin (9). Principal coordinates analysis of the microbiota communities revealed that individual subjects' samples clustered together independent from antibiotic use. Mixed models accounting for subject-specific trends were more revealing than groupwise analyses in differentiating the microbiota pre- and post-antibiotics. After antibiotic exposure, Shannon diversity decreased (~0.14, P =.045), total %Firmicutes decreased (~5.7%, P =.007), and total %Bacteroidetes increased (+4.5%, P =.047). Several individual operational taxonomic units (OTUs) classified as Lachnospiraceae decreased following antibiotic use. After receiving cefazolin, vanB levels increased 168-fold in a subject, and qnrS increased 22 to 27-fold in two subjects. Our data suggest that even single doses of select perioperative antibiotics can impact the microbiota, particularly individual Firmicutes members, and resistance gene reservoirs.
Abstract Title:
Cross-Feeding Changes the Rate and Mechanisms of Antibiotic Resistance Evolution

Primary Author Block:
B. Adamowicz, M. Muza, W. Harcombe; Univ. of Minnesota, St. Paul, MN

Abstract Body:
Background: Despite an increasing awareness of the multi-species nature of many bacterial infections, our understanding of how antibiotics influence microbial communities remains limited. Mutualistic partners, wherein the survival of each species is dependent on the survival of the other, are common in nature, but there is conflicting evidence as to whether mutualists are better or worse at adapting to environmental stresses. We test this phenomenon in a microbial mutualism facing an antibiotic selection pressure. We predict that an obligate mutualist system will evolve antibiotic resistance more slowly than a single-species system, and that the mechanisms of resistance will differ due to the need to maintain cross-feeding in the mutualist system. Methods: We used a previously engineered Escherichia coli - Salmonella enterica obligate cross-feeding system wherein each species requires a metabolite secreted by the other to grow. We grew six replicates of the co-culture, as well as each species in monoculture, along two antibiotic gradients (ampicillin and rifampicin). We passaged these cultures for 20 passages (~140 generations), doubling the antibiotic concentration and tracking the minimum inhibitory concentration (MIC) at each passage. We then sequenced the most resistant population from each replicate, and phenotyped three isolates from each population for growth rate, yield, and MIC in mono- and co-culture. Results: Phenotyping data suggest that cross-feeding changed the rate and mechanisms by which antibiotic resistance evolved. Resistance to both ampicillin and rifampicin increased more slowly in cross-feeding populations than in monocultures; population MICs were also lower for co-cultures. When we compared isolate MICs, monoculture isolates had higher MICs than co-culture isolates, in accordance with our population results. The relative cost of resistance also differed for isolates evolved in monoculture and co-culture, as measured by growth rate and yield. Sequencing of whole genomes to investigate the genetic mechanisms underlying resistance evolution are currently underway. Conclusions: Investigations into the true multispecies ecological context in which antibiotic resistance evolves are critical for tracking and halting resistance spread in polymicrobial infections. Our work highlights how ecological interactions between bacteria alter the dynamics and mechanisms of evolution of resistance. More broadly, this work provides insight into how mutualisms constrain adaptation to environmental stress.
Session Number: 91
Session Type: Poster
Session Number: 91
Session Type: Poster
Session Title: MEE08 - Patterns, Mechanisms, and Experimental Approaches in Molecular Evolution
Session Start Date Time: 6/8/2018 11:00:00 AM
Session End Date Time: 6/8/2018 1:00:00 PM
Session Primary Track: Microbial Ecology and Evolution
Abstract Control Number: 3649
Poster Board Number: FRIDAY - 1001

Abstract Title:
Lipid Droplets Stabilize Genomic Dna to Survive A Bacterium In Extreme Environments

Primary Author Block:
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Abstract Body:
Background: The lipid droplet (LD) is a multi-functional organelle that exists in organisms ranging from bacteria to humans. LD research has focused on lipid metabolism while other LD functions remain to be explored. Methods: Using EMSA, single molecular binding, in vitro assays with adiposome, Results: we found two functions of LDs in the bacterium Rhodococcus jostii RHA1 (RHA1). 1) The bacterial LDs bound and protected genomic DNA through the major LD protein, microorganism lipid droplet small (MLDS), which increased survival rate of RHA1 during ultraviolet exposure or extremely low nitrogen environments. MLDS expression was regulated by a transcriptional regulator, MLDS regulator (MLDSR) that bound the operator and promoter of the operon of mlds and mldsr. 2) LDs sequestered MLDSR to control its availability for transcriptional regulation. Conclusions: Together, this study suggests that the LD is a unique endomembrane organelle that can function in a manner analogous to the eukaryotic nucleus to facilitate bacterial survival and adaption to extreme environments. Based on the extensive distribution, as well as the conservation of structure, composition, and functions of LDs from almost all living organisms, we propose a hypothesis that the LD is a conserved organelle from bacteria to humans.
Abstract Title:
Outstanding Abstract Award: Investigating Chemical and Genetic Mechanisms of Antimicrobial Production and Resistance in A Co-Evolutionary Arms Race
Primary Author Block:
K. B. O'Brien, J. L. Chodkowski, A. Shade; Michigan State Univ., East Lansing, MI
Abstract Body:
Though research on antibiotics and antibiotic resistance mechanisms has been predominantly focused on human pathogens in the clinical setting, environmental microorganisms also evolve resistances to antibiotics. Preliminary observations in our lab suggested that evolved strains of Burkholderia thailandensis had increased inhibition towards Flavobacterium johnsoniae as compared to its ancestor strain, while evolved strains of F. johnsoniae had increased resistance as compared to its ancestor. Thus, we hypothesized that there was a co-evolutionary arms race occurring between these two environmental bacteria. We plated five replicates of B. thailandensis and F. johnsoniae on agar media that allowed for interspecies interactions to be mediated by small molecule diffusion. After two weeks of growth, colonies were transferred to fresh plates, for a total of 10 transfers for each of the five replicate lineages. Ancestor strains and evolved strains (from the tenth plates) were re-plated in all four combinations and in isolation. Colony phenotypes were imaged for quantitative and qualitative analyses. Changes in colony size over subsequent transfers suggested that the ancestor B. thailandensis strain inhibited the evolved F. johnsoniae strains, but the evolved B. thailandensis strains did not (ANOVA, F = 4.259, p = 0.04). We also observed that all evolved F. johnsoniae strains had increased resistance as compared to their ancestor. However, one of the replicate lineages revealed an outcome consistent with initial findings: we observed a potential arm’s race of resistance and inhibition between the competing strains. Taken together, our results suggest that multiple evolutionary outcomes of antibiotic resistance and production can result from measurably identical conditions of consistent competition pressure. Furthermore, we hypothesized the inhibitory molecule made by B. thailandensis was bactobolin, a characterized broad-spectrum antibiotic. However, when a B. thailandensis mutant for bactobolin production was plated with F. johnsoniae, there was no change in inhibition. Thus, we are isolating and characterizing the structure of the potentially new inhibitory molecule using liquid and solid extraction methods, HPLC bioassay-guided fractionation, and mass spectrometry. The results of this work provide insights into the conditions under which bacterial interactions underpinned by antibiotic resistance and production can co-evolve, as well as the variability in the outcomes of these interactions.
Microbes are robust organisms capable of persisting in the face of extreme environments. In nature, access to nutrients is not always consistent and populations commonly experience cycles of feast and famine. To further understand how microbes persist and eventually thrive in environments where nutrient availability is sporadic, we performed an evolution experiment varying intervals between feeding times in laboratory Escherichia coli populations. Over 1000-days, 48 populations of E. coli K-12 str. MG1655 were challenged with three different starvation-interval treatments, with either 1-, 10-, or 100-days between feedings. Using whole-genome population-level sequencing, we identified 19 genes enriched for nonsynonymous mutations in starved populations, eight of which are associated with transcription (crp, hns, rho, and rpoC) or translation (fusA, infB, rplF, and rpsL). Further investigation reveals that for many of these genes, loss-of-function mutations result in lethal phenotypes. As such, mutations affecting these genes are likely adaptive. Two mutations in particular: 1) a R109H substitution in the RNA binding 1 domain of Rho, one of three transcription termination factors in E. coli, has fixed in five-independent starved populations; and 2) a R93H substitution in the DNA binding domain of H-NS, a histone-like transcriptional regulator, has also fixed in these populations. Previous studies have suggested interactions exist between Rho and H-NS, repressing antisense RNA transcription. As many E. coli antisense RNAs are involved in environmental stress-responses, reduced binding activity of Rho and H-NS, resulting in de-repression of antisense RNA, may be beneficial in populations enduring long-term starvation. In contrast, we observe overexpression of H-NS in daily-fed populations, where environmental stress is less severe.
Abstract Title:
Dynamics of Evolution of the Mar- Sox-Rob Regulatory Network in E. coli

Primary Author Block:
K. Jain, S. Saini; Indian Inst. of Technology Bombay, Mumbai, India

Abstract Body:
Background: Enteric bacteria use the Mar- Sox-Rob regulon to control gene expression of a large number of targets which control cellular physiology. Each of the three systems responds to an overlapping set of cellular and environmental cues, and in turn, regulates an overlapping set of target genes. In addition, the Mar, Sox, and Rob systems regulate each other's expression as well. In this work, we rewire the regulatory network between the mar-sox-rob genes and evolve them in different environments.

Methods: The rewiring of the regulatory network was done by scarless chromosomal mutations. Evolution experiments were performed in series of batch experiments (in test tubes). Results: After evolution for 2000 generations, several themes regarding the evolutionary dynamics of the regulatory network emerge. Our experiments show that while many solutions emerge within the loci, in many experiments the "solution" to the environmental challenge is arrived at by mutation outside the mar-sox-rob loci. Despite variations in the genetic origins of the mutations, parallel experiments exhibit a strong similarity in the rate of fitness increase between different lines in identical environments.

Conclusions: Through our experiments we demonstrate the similarity in rate of phenotypic changes despite a large variation in genetic changes facilitating increase in fitness.
Abstract Title:
The Emergence and Evolutionary Refinement of Glycolytic Metabolism in Shewanella Oneidensis

Primary Author Block:
L. M. Chubiz; Univ. of Missouri - St. Louis, St. Louis, MO

Abstract Body:
Background: Carbon metabolism is a defining feature of many bacterial species and can be altered by acquisition of genetic material or mutations in existing genes. Given the range of reduced carbon many bacteria experience how does a bacterial species engage in novel carbon metabolism to exploit new niches and what tradeoffs accompany these changes? Using an evolved glucose metabolism in Shewanella oneidensis, we look to explore these questions. Despite its remarkable respiratory capacity, S. oneidensis grows primarily on oxidized carbon substrates like lactate. Under laboratory conditions, S. oneidensis will rapidly evolve to consume glucose, an otherwise unusable substrate, providing an ideal model to understand the benefits of forgoing a glucose diet. Methods: We used whole genome resequencing to identify putative mutations resulting in adaptive glucose metabolism and its refinement through serial transfer laboratory evolution. Traditional genetic and biochemical approaches were used to validate phenotypic effects of these mutations. Metabolic flux measurements were performed by measuring 13C incorporation into protein-bound amino acids. Results: We discovered that adaptive glucose metabolism is due to a deletion of N-acetylglucosamine (GlcNAc) regulator, nagR. Loss of nagR resulted in constitutive expression of a GlcNAc transporter (nagP) and kinase (nagK), both display promiscuous activity towards glucose. 13C-based metabolic flux analysis demonstrated that this novel glucose metabolism relies entirely on the Entner-Doudoroff pathway for substrate oxidation, confirming several flux-based models. We also observed decreases in both rate and yield on lactate after emergence of glucose metabolism, posing a possible reason why many bacteria opt to forgo a glucose diet. Laboratory evolution was used to improve growth on glucose. Adaptive mutations were identified in putative glucose transporters, including nagP. In some populations, increased fitness costs on lactate were observed. Conclusion: Glucose utilizing S. oneidensis is a tractable model to understand how adaptation to consume complex carbon sources occurs, with possible biotechnology applications.
Abstract Title:
Long-Term Exposure to Pesticides Changes Function of Microbiota, Accelerating Selection on Host Populations
Primary Author Block:
G-H. Wang, R. M. Brucker; Harvard Univ., Cambridge, MA
Abstract Body:
Background: The microbiota of a host animal plays a crucial role in animal physiology, behavior, nutrition, and speciation. The composition of gut microbiota is highly plastic under different diets and antibiotic treatments, and the disturbed microbial community can have harmful effects on the host. However, there are no studies to determine the gut bacteria composition and function due to long-term exposure of environmental stress across generations. Materials: To test this, we are using the parasitic wasp animal model - Nasonia vitripennis - to determine how the gut bacterial community composition changes and responses across multiple host generations when exposed to low levels of a commonly used herbicide that is bioavailable to bacteria. The exposed and control populations' host microbiota and immune responses are determined by next generation sequencing using 16s amplicons and transcriptomics, respectively. The function of single bacterial strain isolated from wasp gut is explored in vivo, in vitro, and in germ-free wasps as well. Results: We observe differences in host physiology (toxicity, body weight, mating behavior) and gut bacterial structure through 16s amplicon sequencing, qPCR, and selective bacterial isolation between exposed and control populations. Using mass spectrometry and metabolic growth assays, we have determined that the gut bacterial metabolism is heritable across generations. However, the differences in phenotypes between exposed and control populations are eliminated under germ-free rearing. Furthermore, by screening for microsatellite markers in exposed and control populations' host genotype, we observe host genome topology congruent to the composition of their microbiota - known as phylosymbiosis. Conclusion: Our findings highlight the significance of the microbiota’s function in driving resistance to environmental stress and its potential role in driving genomic selection in their host. This is the first case of experimental phylosymbiosis.
Abstract Title:
Mobility of Antibiotic Resistance in Salmonella Typhimurium Under Natural and Simulated Farm Environments

Primary Author Block:
M. Peng, S. Salaheen, D. Biswas; Univ. of Maryland - Coll. Park, College Park, MD

Abstract Body:
Background: Microbial horizontal gene transfer is a continuous process which shapes bacterial genomic adaptation and composition of con-current microbial ecology. The role of human employed synthetic antibiotics/chemicals in genomic adaptation and their influence on the evolution of biological network are most critical as important concerns. Though attention has been paid on contributions of antibiotic utilization in farm animal production to overall antibiotic resistance issue, the mechanism behind the evolution isn't fully understood yet. The purpose of this study is to investigate mechanisms of sub-therapeutic/therapeutic dosages of synthetic antibiotic that foster the exchange of genetic materials in microbial community in farming environments.

Methods: Both antibiotic resistant and sensitive Salmonella enterica serovar Typhimurium (ST) isolates were allowed to survive in natural soil environment containing with or without sub-lethal tetracycline (4 mg/kg) to evaluate the genetic adaptation. Tetracycline MIC of ST isolates was determined by standard agar dilution method on MH agar. Quantitative PCR was performed to confirm genetic adaption, and the genetic configuration relatedness among ST isolates was investigated based on genome-wide SNP comparisons.

Results: The tetracycline-resistant ST retained their resistance (MIC>64µg/mL) over 6 months, whereas sensitive ST gradually (from 2 weeks to 6 months) acquired higher MICs and evolved tetracycline resistance (MIC>64µg/mL). Differential expression of antibiotic-resistance genes in ST artificially inoculated under antibiotic pressure was detected. Specifically, tetA and vapB were up-regulated by 650-3300 and 2-5 folds. Besides, based on genome sequences, antibiotic-resistant ST were clustered with pathogenic ST, indicating a positive relevancy between drug resistance and pathogenicity.

Conclusions: Although resistant ST isolates barely ceased their tetracycline resisnacy, the tetracycline-susceptible ST isolates managed to develop their resistancy quickly in natural soil environments. Based on the whole genome sequencing and quantitative PCR analysis, we identified the relative bacterial genetic alterations withstanding the rapid physical buildup of antibiotic resistance. By tracking the phenotypical transition of antibiotic-resistance in microbial ecosystem, this data offers us directed strategies in combating Salmonella antibiotic-resistance dissemination and minimization of their virulence.
Abstract Title:
A Machine Learning Approach for Predictive and Explanatory Microbial Ecology
Primary Author Block:
D. DiMucci, D. Segre; Boston Univ., Boston, MA
Abstract Body:
Pairwise co-culture experiments of all microbial species in a community are emerging as powerful tools for understanding the interactions that determine function and dynamics in microbial communities. These studies are performed with the goals of predicting the outcomes of new unobserved interactions and developing plausible explanations regarding mechanism but are often limited in scope by the experimental logistics of handling large communities. We have combined a trait-based representation of microbial interactions with machine learning to develop a model for predicting yet to be seen interactions and the guided generation of mechanistic hypotheses. All pairwise combinations between 100 genome-scale metabolic models of human gut associated bacteria [1] were performed in silico and the relative yield of each model in co-culture with each other model was recorded. Models were negatively affected by their partner 5563 times and unaffected by the presence of an interaction partner 4337 times. Interactions between microbial models were represented as a binary vector where each element corresponds to one of 388 unique metabolic reactions; vectors were subsequently used as predictors for machine learning. Evaluation on the full experimental space with five fold cross validation yielded a balanced accuracy of ~90% when predicting the qualitative outcome of a held out interaction. Furthermore, a learning curve analysis revealed that a balanced accuracy of 78.56% could be attained using as few as 5% (248) of possible interactions to predict the remaining 95% (4702) pairwise interactions. Finally, the application of feature selection methods to generate ranked lists of potential mechanisms results in the discovery of mechanisms of competition in interactions with negative interactions at rates as high as 97 times faster than what would be expected by chance for this data set. Our results indicate that the goals of predictive microbial ecology can be greatly facilitated by combining trait-level representations of microbes with machine learning. Feature selection methods allow us to generate ranked lists of potential mechanisms that are specific to an interaction of interest and expedite the discovery of molecular mechanisms of interaction. This approach should prove useful as a guide for the construction of synthetic microbial communities and for lightening the experimental burden associated with mechanistic inquiries.
Abstract Title:
Gi-Scanner: An Algorithm to Predict Genomic Islands by Comparative Genomics
Primary Author Block:
Y. Hur, J. Chun; Seoul Natl. Univ., Seoul, Korea, Republic of
Abstract Body:
Background: Genomic islands (GIs) are clusters of genes that are mobile via the mechanisms of lateral gene transfer. Since GIs are known to play important roles in spreading phenotypes such as pathogenicity and antibiotic resistance, detecting them is critical in modern microbiology. As the number of genome sequences in the database increases exponentially, the accurate and efficient method to predict GIs on a large scale is of premium need. Methods: Three sets of Vibrio cholerae genomes were selected and grouped as 29 complete genomes, 23 genomes with high N50, and 17 genomes from the previous study. We generated pairwise reciprocal BLAST hit (RBH) ortholog matrix for each genome sets, and detect clusters of genes not aligned to other genomes among the group. Non-aligned cluster sharing their synteny and orthologs grouped again as possible GI clusters, and the clusters were finally determined as GI following maximum parsimony algorithm. Hidden Markov Model (HMM) profiles were generated for each GI in order to perform a quick search for new query genome. Moreover, to add a new genome into the result instead of the quick search feature, this program calculates only the new genome over the core genome set such as complete genome set, or optionally whole genomes in the result. Results: The GI prediction for 69 genome set ran for about 6 hours on a single server computer, resulting 94 predicted GIs. Out of 74 GIs predicted in the previous study based on manual curation, 72 GIs were successfully recovered. The 94 GIs predicted by our method were compared with those predicted by IslandViewer, a database contains the results of IslandPick, IslandPath-DIMOB, SIGI-HMM, and Islander. Each predicted GI was visualized on web page either single GI view or single genome view with phylogenetic tree and pairwise matrix. As a result, we created an up-to-date database for V. cholerae GIs. Conclusions: The prediction result of Vibrio cholerae shows the impact of GI on microbe and the importance of GI prediction. GI-Scanner provides relevant GI predictions according to the suitable phylogenetic tree for a genome set, and modifying the genome set is easy to apply on the result since the program reuse previous calculation. GI-Scanner is a fast and accurate GI predictor in comparative genomics approach, and it helps understanding how microbe rapidly obtains a trait and diversity.
Abstract Title:
From Raw Reads to Trees: Whole Genome Single Nucleotide Polymorphisms Phylogenetics Across the Tree of Life

Primary Author Block:

Abstract Body:
Next generation sequencing is increasingly being used to examine both genetic diversity and relationships among closely related organisms or cell lines. However, while genome-wide single nucleotide polymorphisms (SNPs) provide an excellent resource for phylogenetic reconstruction, to date, evolutionary analyses have been performed using different ad hoc methods that are not often widely applicable to organisms across tree of life. While some groups may use methods not widely applicable to other organisms/datasets, other groups routinely fall back on older methods, selecting certain genomic regions corresponding to genes (as with Multi Locus Sequence Typing trees), to accomplish a less refined tree. To facilitate the construction of robust phylogenies using all discernable SNPs among a suite of genomic datasets from organisms across tree of life, we have developed a comprehensive tool for genome-wide identification/characterization of SNPs directly from raw reads, genome assemblies, and complete genomes. Our phylogenetic and molecular evolutionary (PhaME) analysis software is unique in its ability to take raw reads and draft/complete genome(s) as input, derive core genome alignments, identify SNPs, construct phylogenies, and perform evolutionary analyses. Here, we show several examples using genomes, contigs, metagenomes, and raw reads of bacterial, eukaryotic and viral origin to demonstrate the broad and robust functionality of PhaME.
Abstract Title:
MiGA - the Microbial Genomes Atlas: Expanding the Catalogued Diversity of Archaea and Bacteria

Primary Author Block:
L. M. Rodriguez-R1, S. Gunturu2, C. Jain1, J. Tiedje2, J. Cole2, K. T. Konstantinidis1; 1Georgia Inst. of Technology, Atlanta, GA, 2Michigan State Univ., East Lansing, MI

Abstract Body:
The use of small subunit ribosomal RNA gene (16S rRNA) sequencing has successfully enabled the large-scale cataloguing of prokaryotic species diversity but offers limited resolution typically at the genus or species level, and information on gene content and other genomic features can be predicted only when representative genomes are available in the databases, and not always reliably so. These limitations have been circumvented with advances on metagenomics techniques but it remains computationally challenging to explore the newly uncovered diversity of the geometrically increasing number of recovered prokaryotic genomes. Here, we present The Microbial Genomes Atlas (MiGA), a genomic data management and processing tool integrating best practices in genomic analyses with recent and novel developments in whole-genome-based taxonomy and classification. MiGA features an indexing system based on medoid clustering over sparse matrices of Average Nucleotide and Amino Acid Identity (ANI-AAI) guided by heuristic approximations, enabling the fast classification of query genomes. The recent development of FastANI, a k-mer based estimator of ANI now integrated in MiGA (Jain et al., 2018), has allowed us to explore the distribution of ANI values among more than 90 thousand complete and draft genomes. This distribution revealed a pronounced valley in the ANI range 83-95%, indicating that the genetic discontinuity previously observed with smaller sets of genomes, reflective of the existence of discrete species, is maintained in larger scales regardless of taxonomic diversity or historic sequencing trends. Moreover, the taxonomy utilities of MiGA allowed us to compare the ANI values with the existing taxonomic assignments. The upper boundary of this range (ANI 95%) consistently reflected the standing nomenclature of species in this large-scale collection (accuracy > 98%). The availability of this indexed set of genomes, as well as other sets including metagenome-assembled genomes from various large-scale projects, and high-quality curated collections like RefSeq and RefSoil allows the rapid search for close relatives of any complete or draft query genomes as well as a robust AAI-based taxonomic classification. MiGA has a web interface easy to use available through http://microbial-genomes.org/.
Session Number: 113
Session Type: Poster Talk
Session Title: New Bioinformatic Tools and Discoveries
Session Start Date Time: 6/8/2018 12:15:00 PM
Session End Date Time: 6/8/2018 1:15:00 PM
Session Primary Track: Microbial Ecology and Evolution
Abstract Control Number: 9460
Poster Board Number:

Abstract Title:
Moderator
Primary Author Block:
Jon Jongsik Chun; Seoul Natl. Univ., Seoul, Korea, Republic of
Abstract Body:
Evaluating Metaproteomics Software Tools for Functional Microbiome Analysis

Background: For a full appreciation of microbiome dynamics, it is important to understand the functional role of expressed microbial genes / proteins. Metaproteomics, which characterizes the protein complement of the microbiome, has enabled such functional characterization (1). There are several functional analysis software tools that measure the combined response of microbial communities to perturbations in their environment. In this work, we evaluate the performance of selected software tools, so that metaproteomics researchers can make informed decisions based on research goals. Methods: Plaque samples from children at high-risk for dental caries were grown in paired biofilm reactors, in the presence and absence of sucrose (2). The samples were subjected to protein extraction, sample processing, and mass spectrometry (MS) measurements. The resultant mass spectral data were searched against Human Oral Microbiome database (HOMD) to identify microbial peptides. The outputs from this dataset were subjected to functional analysis using software platforms such as MEGAN6 (3), EggNOG Mapper (4), Unipept (5), MetaProteomeAnalyzer (6) and MetaGOmics (7). Results: Functional analysis of plaque microcosm paired metaproteomic data had previously shown sucrose-induced changes in protein relative abundance patterns for several metabolic pathways (2). Spectral count measurements were used as a quantitative analysis metric for this study. After subjecting these microbial peptides to functional analysis tools, the following qualitative and quantitative features were assessed: a) number of peptides that were mapped to functional groups and gene ontology terms; b) ability to normalize and assign spectral counts to functional groups; c) ability to visualize datasets and d) biological conclusions drawn from data. A customized script was used to benchmark the results of the software tools being tested. As an outcome from this benchmarking study, detailed feedback is given to the upstream software developers for algorithm optimization. Results from this analysis and guidelines for software selection will be presented. Conclusions: Our study evaluates outputs and features from functional analysis software tools enabled by metaproteomics analysis. Our observations will aid researchers in making informed decisions on the choice of software platform for advanced functional analysis of complex microbiomes.
How Nitrifiers Drive Nitrification and Redox Metabolism in Soil?

S. R. Mohanty, B. Kollah, M. Nagarjuna, A. K. Patra; Indian Inst. of Soil Sci., Bhopal, India

Background: Nitrification is a key biogeochemical process for the global nitrogen balance. In the first step of nitrification \( \text{NH}_4 \) binds to the membrane bound nitrifying enzymes and is oxidized to \( \text{NO}_3 \). The product \( \text{NO}_3 \) may bind to various cationic molecules including the extracellular organic compounds forming a biogenic nitrate. So far there is no information on the role of biogenic nitrate on the redox metabolism.

Materials and methods: The effect of both biogenic nitrate and chemical nitrate on redox metabolism was evaluated. Nitrification activity was observed under repeated \( \text{NH}_4 \)-N amendment. Nitrate was estimated by phenol disulphonic acid method, Abundance genes estimated by real time PCR, and characteristic of nitrified soil was analyzed by raman spectroscopy.

Results: Repeated \( \text{NH}_4 \)-N amendment increased nitrification rate (mM \( \text{NO}_3 \) produced g\(^{-1}\) soil d\(^{-1}\)) from 0.49 to 0.65. Nitrification stimulated (\( p<0.01 \)) the abundances of 16S rRNA gene of eubacteria (43.67±4.510 x 10⁶), amoA gene of nitrifying bacteria (102.33±8.50 x 10⁴) and nitrifying archaea (94.33±7.77 x 10⁴). The rate of reduction of terminal electron acceptors (mM reduced g\(^{-1}\) soil d\(^{-1}\)) due to the biogenic nitrate followed as : \( \text{NO}_3^- \) reduction 4.01±0.229, \( \text{Fe}^{3+} \) reduction 5.37±0.122, \( \text{SO}_4^{2-} \) reduction 9.56±0.165 and \( \text{CH}_4 \) production (µg g\(^{-1}\) soil) 0.46±0.051. Nitrification inhibited denitrification by a factor of 1.4. Similarly it inhibited the \( \text{Fe}^{3+} \) reduction, \( \text{SO}_4^{2-} \) reduction, and \( \text{CH}_4 \) production by factors of 1.8, 1.13, and 1.66 respectively. Raman spectra of the nitrified soils indicated build up of aliphatics in soil. Probably, these aliphatics bind to \( \text{NO}_3 \) and form biogenic nitrate. The microbial volatile organic compounds (mVOCs) produced from nitrifiers enhanced (\( p<0.05 \)) nitrification. The mVOCs stimulated (\( p<0.05 \)) abundances of nitrifying bacteria than the eubacteria and nitrifying archaea.

Conclusion: Nitrification modulated soil redox metabolism through biogenic nitrate and microbial volatiles. Biogenic nitrate may hold keys for sustainable agriculture and climate change mitigation. 1
Abstract Title:
Composition of Expressed Fungal Class II Peroxidases is Altered by Anthropogenic N Deposition

Primary Author Block:
E. M. Entwistle1, K. J. Romanowicz2, W. A. Argiroff2, Z. B. Freedman3, J. J. Morris1, D. R. Zak2; 1Univ. of Alabama at Birmingham, Birmingham, AL, 2Univ. of Michigan, Ann Arbor, MI, 3West Virginia Univ., Morgantown, WV

Abstract Body:
Background: Anthropogenic nitrogen (N) deposition, an important and widespread agent of global change, has the potential to increase soil carbon (C) storage by altering the expression of fungal oxidative enzymes and, thereby, the process of plant-litter decomposition. In long-term field experiments, anthropogenic N deposition retards lignin decomposition, which is the rate-limiting step in plant litter decay, and increases soil C storage in forests. Fungal class II peroxidases are important enzymes in this process because they mineralize the lignin in plant detritus and the lignin-derived polyphenolic compounds in soil to carbon dioxide. Anthropogenic N deposition reduces both peroxidase enzyme activity in soil and the relative abundance of fungi which produce these oxidative enzymes. In light of this, it is plausible that anthropogenic N deposition has reduced plant litter decay and increased soil C content by altering the composition of peroxidases expressed by the fungal community.

Methods: To test this, we compared the composition and richness of fungal class II peroxidase transcripts occurring in forest floor material collected from the ambient and experimental N deposition treatments in a long-term field experiment encompassing a series of northern hardwood stands in which the amount of N deposition has been experimentally increased to simulate rates of anthropogenic N deposition predicted for midcentury (30 kg N ha\(^{-1}\) y\(^{-1}\)).

Results: Our results indicate that ca. 20 yrs of experimental N deposition altered the phylogenetic composition of lignin-decaying class II peroxidases expressed by forest floor fungi, a response which has occurred concurrently with reductions in plant-litter decomposition and a rapid accumulation of SOM. This observation indicates that fungal taxa are expressing peroxidases differentially in response to anthropogenic N deposition, a result which likely has important functional consequences for the biological decay of lignin and polyphenolics in SOM.

Conclusions: The changes in expressed peroxidase composition we observed may, in part, explain previously documented increases in soil C storage under long-term, anthropogenic N deposition.
Abstract Title: Tile Drainage and Anthropogenic Land Use Contribute to Harmful Algal Blooms and Microbiota Shift in Inland Water Bodies

Primary Author Block:
I. Mrdjen1, J. Lee1, S. Fennessy2, J. Slonczewski2, A. Schaal2, R. Dennis2; 1The Ohio State Univ., Columbus, OH, 2Kenyon Coll., Gambier, OH

Abstract Body:
Background: Freshwater harmful algal blooms (HABs), resulting from high nutrient inputs from human use of developed lands, have been shown to pose unique risks to human and ecological health worldwide. A major contributor of nutrient content in affected water bodies is agricultural land use, specifically effluent from tile drainage. Small lakes and ponds (SLaPs) are at elevated risk for HAB appearance, as they are uniquely sensitive to nutrient input, and rarely monitored by state agencies. HABs introduce exposure risk to cyanotoxins, specifically microcystins (MC), which are suspected carcinogens and competent liver toxins. Methods: To investigate the impact of anthropogenic land use on SLaPs, 24 sites were sampled over a 3-month period in the summer of 2015. MC concentrations and water chemistry characteristics were determined using ELISA and chemical assays, respectively. Microbial source tracking was utilized to study sources of nutrient intrusion, while microbial community analysis was used to study composition of SLaP microbial communities. Results: The presence of tile drainage systems or animals was significantly correlated with concentrations of microcystin-producing Microcystis aeruginosa throughout the sample set (p= 0.03). The relative abundance of HAB-forming genera was correlated with elevated concentrations of nitrate and soluble reactive phosphate. One location (FC) showed MC concentrations exceeding 875 µg/L and large community shifts in ciliates. FC’s prokaryotic community was dominated by Planktothrix spp. and contained detectable levels of Microcystis spp. at the July 28th sampling point. Conclusions: These results demonstrate the impact of HABs in SLaPs and that prevailing issues extend beyond cyanotoxins, and that further investigation of microbial communities impacted by HABs is necessary.
Zinc oxide (ZnO) nanoparticles (NPs) have a vast range of applications, release of these NPs into waste streams and further into the environment may adversely impact non-target organisms. Natural variations in tolerance to nano ZnO may exist among various microalgae, and the availability of tolerant and sensitive species may lead to better understanding of tolerance mechanism and potential applications in bioremediation and ecotoxicology. To elucidate the effects of nano and bulk zinc oxides on the growth of microalgae, three marine green microalgae species, and four marine cyanobacteria species, were used in a replicated study. The algal strains were incubated in three different growth media: media containing standard BG-11 media, and media with various concentrations of either nanoparticle ZnO or bulk ZnO at concentrations up to 1 ppm. The culture flasks were incubated on a shaker at 25°C under continuous fluorescent lighting. Changes in terms of growth and morphology of the species to the treatments were assessed by measuring optical density and microscopic observations for a total of 7 days. Results showed marked difference among species tested to varying ZnO concentrations. Most of the cyanobacteria showed reduced growth as a result of exposure to ZnO, which included decreased cell size and discoloration. On day 7, among cyanobacterial species tested Leptolyngbya boryana showed the most growth inhibition (90%) and loss of pigmentation even at 0.125 ppm nano-Zn concentration (OD 0.159). At all levels of nano-Zn tested, the three-marine green microalgal species showed marked tolerance (OD more than 0.8618). These findings provide a strong impetus for screening a larger collection of diverse indigenous microalgae for tolerance to NPs and which may yield potential candidates for bioremediation of NPs and also sensitive indicator species for ecotoxicological testing.
Abstract Title:
Outstanding Abstract Award: Seeing the Trees Through the Forest: Measuring Microbial Community Biomass Stoichiometry One Cell At A Time

Primary Author Block:
M. P. Manzella, E. K. Hall; Colorado State Univ., Fort Collins, CO

Abstract Body:
Microbial biomass stoichiometry, typically the ratio of the biologically-important elements carbon, nitrogen, and phosphorus (C:N:P), has historically been examined at a course resolution where the contribution of the individual is masked by the reported community or population average. Examining the distribution of individuals within a population, and populations within a community has the potential to improve our understanding of the drivers of microbial community stoichiometry. We measured biomass ratios (C:N:P) of individual microbial cells using energy dispersive spectroscopy (EDS). We report the distribution and average cellular phenotype within both cultured and uncultured communities. First, we analyzed three microbial cultures (a Pseudomonas, Flavobacterium, and a Brevundimonas isolate) by both bulk measures and EDS to compare the two approaches. Next, we examined biomass C:N:P of these same strains during batch growth in both high-P and low-P media. Finally, we analyzed microbial cells taken from three Colorado lakes to examine the stoichiometric distribution within each community. Biomass C:N:P obtained by EDS (34:8:1, 53:11:1, and 69:14:1) were similar to those obtained by bulk measures (31:8:1, 57:12:1, and 74:15:1, respectively) for the test strains. Within the growth experiment, biomass C:N was relatively conserved across growth stages for each strain, whereas N:P and C:P changed in response to changing P availability. Cultures grown in the low-P medium exhibited greater stoichiometric plasticity than those in the high-P medium. In general, the distribution of biomass ratios (C:N, N:P, and C:P) within these monoculture experiments followed a unimodal distribution. However, within the lake communities, the distribution of N:P and C:P was multimodal, containing local maxima across the phenotypic landscape. This study illustrates that community-level stoichiometric measures have masked differences in microbial biomass phenotypes within populations and communities. We show that monocultures exhibit a unimodal stoichiometric distribution and that both phylogeny and physiological state affect cellular response to P limitation. These traits become clear when the individual, rather than the community, is the subject of investigation. Within the lake communities, microbial phenotypic clusters exist with stoichiometries that vary from the community average. It is currently unknown what drives the development of these local maxima or the consequences of divergent phenotypes within the sampled community.
Abstract Title:
Metaproteomics Reveals A Novel Betaproteobacterium with Roles in Metal and Nitrogen Cycling in the Deep Subsurface

Primary Author Block:
N. Szeinbaum1, C. Henny2, S. A. Crowe3, F. J. Stewart1, T. J. DiChristina1, C. T. Reinhard1, B. L. Nunn4, J. B. Glass1; 1Georgia Inst. of Technology, Atlanta, GA, 2Indonesian Inst. of Sci., Cibinong, Indonesia, 3Univ. of British Columbia, Vancouver, BC, Canada, 4Univ. of Washington, Atlanta, GA

Abstract Body:
Background: Microbial genomes assembled from deep subsurface environments are revealing novel respiratory pathways distinct from those of surface-dwelling species. The advent of metaproteomics has enabled detection of novel genes expressed under diverse conditions from microbes that resist cultivation attempts. We evaluated the metaproteomic profile of a sediment microbial community enriched over ~1 year with Mn3+ as electron acceptor. Methods: Deep sediments from Lake Matano, Indonesia were sampled under anoxic conditions, and inoculated into minimal media containing soluble Mn3+-pyrophosphate as sole electron acceptor, methane as sole electron donor, and ammonium as sole nitrogen source (0.2 mM). Cultures were incubated in the dark at 30°C, and diluted 1:10 for three transfers. Changes in community taxonomic composition were monitored by 16S rRNA gene amplicon sequencing. Metagenomic and metaproteomic sequencing was performed on total DNA and protein recovered from the third transfer on day 335. Shotgun proteomics were completed in technical triplicates on a high accuracy tandem mass spectrometer, and spectral counts were used to determine relative quantification of proteins (Nunn, Slattery et al. 2015). Results: Relative quantification of shotgun metaproteomic data revealed that cultures were dominated by protein sequences affiliated with a new species of Dechloromonas (Betaproteobacteria), for which we propose the name “Candidatus Dechloromonas occultata” [occultata (L. adj.), hidden]. “Ca. D. occultata” expressed an uncharacterized cytochrome-c-rich operon containing NHL-repeat proteins and an extracellular undecaheme protein also present in other Betaproteobacteria from metal-rich ecosystems, including uncultivated Gallionellales and Rhodocyclales from the deep subsurface, and the Fe2+- and Mn2+-oxidizer Leptothrix cholodnii SP-6. Paradoxically, the enzymatic machinery for denitrification was among the most expressed proteins despite the lack of nitrogen oxides in the media. A hemolysin-type Ca2+-binding domain protein was also highly expressed. Conclusions: The metabolic potential recovered from metaproteomes thus implicates “Ca. D. occultata” and by extension possibly other Betaproteobacteria like deep subsurface Gallionellales and Rhodocyclales, in oxidative metal and reductive nitrogen metabolisms. Delineating the extent to which these metabolisms can be coupled within “Ca. D. occultata” and other Betaproteobacteria represents an important opportunity for future research.
A Quantitative View of the Biosphere: from the Most Abundant Taxa to the Most Abundant Proteins

Primary Author Block:
Y. M. Bar-On; Weizmann Inst. of Sci., Rehovot, Israel

Abstract Body:
Background: A census of the biomass on Earth is key for understanding the structure and dynamics of the biosphere. Similarly, at the protein level, a census of the mass of proteins that drive global biogeochemical cycles can help us better understand the constraints that they experience in the wild. Yet, a quantitative, global view of how the biomass of different taxa compare with each other, and which proteins are most abundant in nature is still lacking. Methods: We perform a comprehensive meta-analysis, mining data on the biomass of about 20 different taxa. We establish the first census of the biomass of all the kingdoms of life. Using this census of biomass, we estimate the global abundance of ubiquitous proteins throughout the kingdoms of life. Our survey encompasses proteins such as Rubisco, previously suggested to be the most abundant protein in the biosphere, as well as proteins essential to key biological processes including protein translation, metabolism and the structural proteins actin, myosin and histones. Results: From this global and quantitative view, we find key take-home messages. We highlight the overall dominance of plant biomass, which account for 80% of the total biomass on Earth - far above all microbial biomass. We find that different kingdoms of life concentrate in different environments - plants and fungi are mostly terrestrial, whereas animals are mostly marine. We show that 90% of the biomass of bacteria is located in deep subsurface environments such as aquifers and subseafloor sediments. In addition to describing current biomass stocks, we also quantify the impact of humans on plant and vertebrate biomass. Using our biomass data, we find that in terms of mass, histones rival Rubisco as the most abundant protein. Based on our estimates for the total abundance of Rubisco, we quantify the effective rate of carbon fixation of Rubisco in the wild, and estimate it works at only 1% of its maximal capacity in terrestrial environments. We explore the different factors that contribute to this decrease in effective rate. Conclusions: In this study we assembled the first census of biomass distribution of Earth. We leverage this census to look at the way in which biomass is distributed across taxa, environments and trophic modes. We also use this census to estimate what is the most abundant protein on Earth, and quantify the effective rate of Rubisco in the wild.
Session Number: 153
Session Type: Rapid Fire
Session Title: Microbes and Planetary Processes-Think Big or Go Home
Session Start Date Time: 6/8/2018 3:00:00 PM
Session End Date Time: 6/8/2018 3:45:00 PM
Session Primary Track: Microbial Ecology and Evolution
Abstract Control Number: 9463
Poster Board Number:

Abstract Title:
Moderator
Primary Author Block:
Joan L. Slonczewski; Kenyon Coll., Gambier, OH
Abstract Body:
Session Number: 153
Session Type: Rapid Fire
Session Title: Microbes and Planetary Processes-Think Big or Go Home
Session Start Date Time: 6/8/2018 3:00:00 PM
Session End Date Time: 6/8/2018 3:45:00 PM
Session Primary Track: Microbial Ecology and Evolution
Abstract Control Number: 9588
Poster Board Number:

Abstract Title:
Sequential Reduction of Terminal Electron Acceptors Modulates Nitrification and Dynamics of Nitrifying Bacteria and Archaea

Primary Author Block:

Abstract Body:
Background: Microbially mediated nitrification is a key biogeochemical process in the global nitrogen balance. However, it is not clearly understood how the sequential reduction of soil due to submerging will influence nitrification. Materials and Methods: Experiments were carried out using the soil samples collected from the experimental fields of the Indian Institute of Soil Science, Bhopal, Madhya Pradesh, India (23.30 N, 77.40 E, 485 m above mean sea level). Incubation experiment was carried out in 20 g portion of soil placed in 130 ml pre-sterilized serum bottles and closed with neoprene septa. Soils were incubated to undergo sequential reduction of terminal electron acceptors. Nitrification potential of a tropical vertisol saturated with water was estimated during sequential reduction of NO3-, Fe3+, SO42- and CO2 in terminal electron accepting processes (TEAPs). Concentration of electron acceptors were estimated by wet chemical methods. Nitrifying bacteria and archaea were enumerated by real time PCR targeting amoA genes. Results: In general the terminal electron accepting processes (TEAPs) enhanced the potential nitrification rate (PNR) of the soil. Nitrification was highest at Fe3+ reduction followed by SO42- reduction, NO3- reduction and lowest in unreduced control soil. Observed PNR (µg NO3- produced per g soil day-1) varied from 0.23 to 3.12. Multiple regression models predicted PNR as 0.303 µM NO3- + 0.661 µM Fe2+ - 0.629 µM SO42- - 0.074 µg CH4. Predicted PNR correlated significantly with the observed PNR. Electron donor Fe2+ stimulated PNR, while S2- inhibited it significantly (p < 0.05). Terminal restriction fragment length polymorphism (TRFLP) targeting amoA gene of ammonia oxidizing bacteria (AOB) and ammonia oxidizing archaea (AOA) highlighted population dynamics during the sequential reduction of terminal electron acceptors. Relative abundance of AOA varied significantly with the TEAPs. However, the relative abundance of AOB did not vary significantly with TEAPs. Relative abundance of AOB correlated with NO3- and Fe2+. Conclusions: Based on this study, the anoxic-oxic condition of soil as predicted by IPCC is likely to enhance nitrification in the tropical vertisol. High nitrification may aggravate nitrogen loss through N2O emission which may complicate the problems affecting agriculture, climate and environment.
Abstract Title:
Nutrient Level Does Not Determines the Ecological Strategies in Marine Bacterial Communities

Primary Author Block:
Y. Gao, M. Wu; Univ. of Virginia, Charlottesville, VA

Abstract Body:
The copy number of ribosomal RNA operon (rrn) in a bacterial genome is an important functional trait. Studies on isolated bacterial strains have found that the rRNA copy number correlated positively with the cell's maximum growth rate and negatively with its growth efficiency [1]. Therefore, it is hypothesized that nutrient level dictates the ecological strategies of bacteria in nature in that nutrient-rich environments select for bacteria with high rrn copy numbers (high growth rate) while nutrient-poor ones select for bacteria with low rrn copy numbers (high growth efficiency) [2]. It follows that bacterial communities in copiotrophic environments should have higher mean rrn copy numbers than those in oligotrophic environments. We tested this hypothesis using bacterial community profiling data from two large-scale microbiome studies, the Earth Microbiome Project (EMP, 16S rRNA amplicon sequencing) [3] and the Tara Oceans expedition (TARA, shotgun metagenomics) [4]. Mean rrn copy numbers were estimated using phylogenetics-based method [5] for samples in EMP 16S rRNA dataset, and as the proportion of 16S rRNA gene reads for samples in TARA metagenomic dataset. Correlation between the estimated mean rrn copy number and the environmental factors was tested using generalized linear regression. We found significant correlation between the mean copy number and environmental factors such as temperature and salinity. Surprisingly, we found no correlation between the mean copy number and concentrations of inorganic nutrients (phosphate, nitrate, nitrite and ammonium) (Figure 1). Our results suggest that in ocean, nutrient level does not play an important role in determining the ecological strategies of bacteria as hypothesized previously. The lack of selection on rrn copy number by nutrients also suggests that the actual growth rate of bacteria in ocean is determined by environmental factors like temperature and salinity, but not the nutrients.
Abstract Title:
High Prokaryotic Metabolic Activities in the Northern South China Sea
Primary Author Block:
X. Li, L. Lu, H. Dang, N. Jiao; State Key Lab. of Marine Environmental Sci., Coll. of Ocean and Earth Sci., Xiamen Univ., Xiamen, China, Xiamen, China
Abstract Body:
Microbiological processes are believed to be critical in marine carbon cycling. Although marine particles provide many benefits for microbes, there is still a lack of research on microbial processes of particle-attached (PA) microbes. During the past decades, main microbial processes involving community respiration (CR), prokaryotic production (PP) and dark carbon fixation (DCF) are measured in many ocean waters. However, investigations on all these processes simultaneously, particularly for PA microbial communities, are scarce. In our current study, CR, PP and DCF were determined respectively by in vivo electron transport system activity assays, [3H]-leucine incorporation assays and dark [14C]-bicarbonate assimilation assays, for both PA and free-living (FL) microbial communities along a transect from the Pearl River estuary to the basin SEATS station in the northern South China Sea (nSCS) in November 2016. All CR, PP and DCF rates show a nearshore-to-offshore and surface water-to-bottom water decreasing trend. PA communities accounted for 33.05%, 15.55% and 19.37% of the bulk CR, PP and DCF, respectively. We find that the contributions of PA community to metabolic activities became more important with the increase of water depth (ranging from 9.73% to 73.94%, 9.58% to 68.11%, and 9.45% to 83.14% for water column CR, PP, and DCF, respectively), indicating that particles are more important for microbiological processes in deep waters of the nSCS. The averages of prokaryotic growth efficiencies (PGE) were ~5.98%, with higher values occurring in nearshore and surface waters. DCF rates (~0.63 mmol C m⁻³ d⁻¹) were largely higher (~543.82%) than PP rates throughout the water column, and represented an important source of organic matter in the water columns. Results of 16S rRNA gene MiSeq high-throughput sequencing demonstrated that the bacterial community compositions were statistically different between the offshore and the nearshore waters, between the shallow layers and the deep layers of the water columns, and between the FL and the PA habitats. Our data illustrated that dark carbon fixation was widespread and potentially important in the nSCS, especially in nearshore and shallow waters. DCF might be an important source of primary production in this marginal sea. The metabolic activities and community compositions differed between PA and FL assemblages, suggesting their different roles in marine carbon cycling. PA microorganisms seem to play a more significant role in community metabolic activities in deeper waters of the nSCS.
Session Number: 177  
Session Type: Rapid Fire  
Session Title: Some like it Salty: Marine Bacteria in Coastal and Deep-sea Environments  
Session Start Date Time: 6/8/2018 4:30:00 PM  
Session End Date Time: 6/8/2018 5:00:00 PM  
Session Primary Track: Microbial Ecology and Evolution  
Abstract Control Number: 8809  
Poster Board Number: 

Abstract Title: Characterization of Cyanobacteria Diversity in Shallow Water Shipwrecks  
Primary Author Block: C. G. Daokoru-Olukole; Niger Delta Univ., Yenagoa, Nigeria  
Abstract Body: The diversity of shipwrecks cyanobacteria in shallow water of New Calabar River, in River State - Nigeria was examined. The 16S rRNA gene sequences from brown rusticles (braided structures attached on the wreck surfaces) and bio-concretions (layered coatings of different concretions) were investigated using Illumina High Thorough-put. The visual examination revealed 3 types of rusticles: brown rusticles, dendritic concretion, biofilm and also diverseness in the cyanobacteria distribution patterns. The diazotrophic filamentous genus Trichodesmium sp., dominated the bio-concretion, having in abundance 4 of its species; T. erythraeum (8.75%), T. hildebrandtii (1.08%), T. contortum (1.04%) and T. tenue (1.02%). The availability of iron on the bioconcretions could explain the reason for the presence of the Trichodesmium clades present. Phormidiaceae, Cyanobacteriacea, Nostocaceace and the nonheterocystous filaments members of the Pseudanabaenaceae were dominant on the rusticle samples. We can associate the formation of rusticles by Cyanobacteria as one of their eroding characteristics on shipwrecks. This study gives insight that the presence of mat-matrix forming cyanobacteria can actually aid aerobic corrosion on shallow water shipwrecks.
Abstract Title: Hierarchical Substrate Preferences in the Marine Bacterium Sagittula Stellata E-37 During Growth on Mixtures of Plant-Derived Aromatic Compounds

Primary Author Block: M. Chua, A. Buchan; Univ. of Tennessee, Knoxville, TN

Abstract Body:
As a component of vascular plants, lignin is the most abundant aromatic biopolymer on Earth. Lignin and its derivatives serve as substrates for heterotrophic microbes in salt marsh ecosystems, where the dissolved organic carbon pool is rich in aromatic compounds. Members of the Roseobacter lineage of marine bacteria are abundant in these salt marshes and play a major role in transforming lignin-derived aromatic compounds. When bacterial strains are presented with a mixture of aromatic compounds, substrate utilization usually proceeds according to a hierarchy of preferences. Contrary to this paradigm, it has been previously demonstrated that Roseobacter lineage member Sagittula stellata E-37 simultaneously catabolizes two aromatic compounds that result from lignin biodegradation (benzoate [BEN] and p-hydroxybenzoate). When doing so, growth rates are faster relative to when either substrate is presented alone, suggesting an ecological growth advantage in lignin-rich environments. Here, we extended prior studies to examine the universality of this catabolic synergy by E-37 by monitoring growth dynamics and extracellular aromatic concentrations in mixed substrate experiments. Co-mixtures of BEN with p-coumarate (COU), ferulate (FER), or vanillate (VAN) revealed a preference for the non-benzoate substrate. BEN + FER and BEN + VAN cultures had significantly lower (at least 2-fold) growth rates compared to single substrate cultures. Growth rates on BEN + COU were indistinguishable from the single COU cultures. Analysis of E-37 mutants with disruptions in the BEN degradation pathway (box) revealed diminished growth rates on VAN and FER, providing genetic support for cross-regulation between the pathways required for degradation of these compounds. These results provide the mechanistic underpinnings for understanding how microbes transform these compounds in the complex mixtures found in nature.
Session Title: Some like it Salty: Marine Bacteria in Coastal and Deep-sea Environments
Session Start Date Time: 6/8/2018 4:30:00 PM
Session End Date Time: 6/8/2018 5:00:00 PM
Session Primary Track: Microbial Ecology and Evolution
Abstract Control Number: 8851
Poster Board Number:

Abstract Title:
Ecological and Evolutionary Genomics of the Globally-Abundant Phylum Marinimicrobia

Primary Author Block:
E. W. Getz, F. O. Aylward; Virginia Tech, Blacksburg, VA

Abstract Body:
Background: The phylum “candidatus Marinimicrobia” represents a diverse group of uncultivated bacteria that has been identified in marine environments across the globe, and ‘omic insights have implicated members of this phylum as prominent contributors to global nitrogen, carbon, and sulfur cycling. Despite the importance of this group to marine environments, the factors shaping the diversity and genomic repertoires of Marinimicrobia remain obscure and are hampered by difficulties in recovering complete genomes from environmental samples and the lack of representative genomes from prominent clades. Methods: To provide insight into the evolutionary genomics of the Marinimicrobia we performed a phylogenomic analysis on a set of >200 available draft genomes from this phylum. Using a multi-locus phylogenetic approach we generated a high-resolution phylogeny of the Marinimicrobia and defined the major clades for which multiple genomic representatives were available. We analyzed trends associated with genome size, %GC content, and proteome nitrogen content and interpreted the results in light of genomic streamlining theories. Moreover, using pan-genomic methods we defined core- and lineage-specific genes for the prominent clades of Marinimicrobia, with an emphasis on functional genes that are markers for biogeochemically-important processes. Results and Conclusions: We found that that fundamental characteristics of Marinimicrobia genomes varied by clade and appeared to be the result of selection due to differing nutrient regimes experienced by these groups. Our pan-genomic analysis supported these findings and pointed to specific forms of metabolism as being associated with streamlined genomes, consistent with previous findings from other marine bacterial clades. Overall, our findings demonstrate that Marinimicrobia are an excellent system for assessing the relationship between ecological factors and genome evolution, and suggest that common selective pressures shape the genomic architectures of prominent bacterial groups throughout the ocean.
Session Number: 177
Session Type: Rapid Fire
Session Title: Some like it Salty: Marine Bacteria in Coastal and Deep-sea Environments
Session Start Date Time: 6/8/2018 4:30:00 PM
Session End Date Time: 6/8/2018 5:00:00 PM
Session Primary Track: Microbial Ecology and Evolution
Abstract Control Number: 9464
Poster Board Number:

Abstract Title:
Moderator
Primary Author Block:
Frank Aylward; Virginia Tech Univ., Blacksburg, VA
Abstract Body:
Abstract Title:
Virulence-Associated Elements in the Accessory Genome of Pseudomonas Aeruginosa Clin. Respiratory Isolates

Primary Author Block:
K. R. Murphy, A. R. Hauser, E. A. Ozer; Northwestern Univ., Chicago, IL

Abstract Body:

Background: Pseudomonas aeruginosa (PA) is an opportunistic gram-negative pathogen and major cause of hospital-acquired pneumonia. Though there are several well-defined virulence factors in PA, variation in strain-to-strain virulence remains poorly understood. Comparative genomics may be a useful tool to identify novel factors in the accessory genome contributing to PA virulence.

Methods: We sequenced the genomes of 83 clinical respiratory isolates using Illumina MiSeq. Core genome phylogeny identified distinct clonal groups from which 1 representative strain was randomly chosen from each group for further analysis. Shared accessory genomic elements (AGEs) were identified using Spine and ClustAGE, and the relative virulence for each isolate was assessed in a mouse model of acute pneumonia. Putative virulence-associated AGEs (vAGEs) significantly correlated with virulence (p<0.01) and containing 1 or more genes with similarity to known virulence factors in the MVirDB database were selected for further study. Each of the selected vAGEs was deleted from representative strains using Gibson Assembly and mutants were tested for virulence attenuation in the mouse model of infection.

Results: The core genome phylogenetic analysis identified 34 distinct clonal groups within the 83 isolates. Among the 34 isolates randomly selected to represent each clonal group, the relative virulence in the mouse model ranged between 5 – 8 log CFU/mouse of bacteria to induce severe disease symptoms in ≥ 50% infected animals. Of the more than 5000 unique AGEs ≥ 100 bp in length identified by ClustAGE, 151 had a statistically significant correlation with virulence and 46 were highly correlated with p-values <0.01. Five vAGEs (vAGE01 – 05) were chosen for further analysis, ranging in size from 0.9 – 5.2 kB and containing sequence with similarity to known virulence factors in other species. Deletion mutants for each AGE were generated in two strain backgrounds (PS1788, PS2055) representing the most virulent isolates tested in this collection. Testing of the mutants for virulence attenuation in the mouse model is currently ongoing, though early studies indicate that deletion of vAGE04 may attenuate virulence.

Conclusions: We successfully identified putative vAGEs that have not previously been linked to virulence in PA and have successfully created deletion mutants for each vAGE. Further in vivo characterization of the mutants will be used to identify novel virulence factors in PA contributing to pathogenesis in acute pneumonia.
Abstract Title:
Chromosome Rearrangement, Gene Amplification, and Insertion Sequence Elements in the Genome Evolution of Bordetella pertussis And the Genus Bordetella

Primary Author Block:
M. R. Weigand1, Y. Peng1, D. Batra1, M. Burroughs1, J. K. Davis1, K. Knipe1, V. N. Loparev1, T. Johnson1, P. Jueng1, L. A. Rowe1, M. Sheth1, K. Tang1, K. Xavier2, M. M. Williams1, M. L. Tondella1; 1CDC, Atlanta, GA, 2Colorado Dept. of Publ. Hlth and Environment, Denver, CO

Abstract Body:
Background: Whooping cough, primarily caused by Bordetella pertussis, has surged in the United States even though coverage with pertussis-containing vaccines remains high. There is evidence to suggest that increased disease results, in part, from genetic divergence of circulating strain populations away from vaccine references. Recent genomic analyses have revealed considerable chromosome structural fluidity, such as rearrangement and deletion, among clinical isolates despite limited gene nucleotide sequence variation. Methods: To investigate the contribution of gene order rearrangement towards pertussis disease resurgence, we have studied differences in chromosome structure among 400 B. pertussis isolates as well as 50 isolates of related Bordetella species. Genome sequencing was performed using the combined technologies of PacBio RSII, Illumina HiSeq/MiSeq, and OpGen or Nabsys enzyme mapping to produce complete, reference-quality assemblies for analysis. Results: Most changes in gene order were flanked by insertion sequence elements (ISEs) and specific rearrangements differentiated B. pertussis vaccine reference strains and clinical isolates of various genotypes. Select, unrelated strains also included amplification of large genomic regions, varying in size and gene content but primarily occurring at two discrete loci, which were also flanked by ISEs. Genomes of B. pertussis have >240 copies of IS481 and closely-related isolates with differing rearrangements, amplifications, and insertion content were observed. Within the broader genus Bordetella, which includes additional human and animal pathogens, chromosome rearrangements were only detected in species harboring other, multi-copy ISEs. Conclusions: These results illustrate the immense chromosome structural diversity among circulating B. pertussis and reveal that such variation also exists in related Bordetella species colonized by ISEs. Specific gene order changes appear phylogenetically conserved, suggesting rearrangement contributes to genome evolution and thus perhaps pertussis disease resurgence. However, rapidly detecting such structural variation for sequence-based molecular epidemiology requires further development of sequencing, mapping, and bioinformatics methods.
Abstract Title: Large Tandem Duplications are Frequent in Microbial Genomes But Go Undetected in Genome Assemblies

Abstract Body: Large, multi-gene tandem duplications within chromosomes of prokaryotic cells occur frequently but are generally unstable. Homologous recombination between the two copies would either result in a loss of the duplication or further gene amplification. Unless this increased copy number of genes provides a fitness benefit that allows the cell to outgrow the rest of the population, it is not maintained. Thus, these gene amplifications are considered rare at the population level. However, gene copy number is a critical consideration during strain construction by genetic manipulation and their identification within published genome sequences may be underestimated because these tandem copies would be problematic for genome assembly programs. The only unique sequence that differentiates a tandem array of identical genes from a single copy of genes is at the junction of the repeated segment that may be interpreted as sequence errors or a chimera. Analysis of coverage discrepancies across the genome would identify regions of increased coverage that could be due to gene amplification and further analysis would identify the junction sequence, but this is not traditionally described in post-assembly processing methods. For 1489 unique bacterial and archaeal taxa in the NCBI Database, raw sequences were mapped to their corresponding genome sequence and the resulting assembly met the quality criteria of an average coverage >50x and a standard deviation less than 1.5x the average. Of these genomes, 67% contained regions >1500 bp with >1.5x the average coverage. These discrepancies could be due to gene amplification, a lysogenic virus becoming lytic, or complications in sequencing and are being explored. For one organism, Desulfovibrio vulgaris Hildenborough (DvH), a 7.7 Kb duplication was identified and verified by PCR. Of five laboratories that have contributed to the 28 available raw sequence datasets, two have lost and three have maintained the duplication. Within this duplication is cytochrome d ubiquinol oxidase (DVU3270-1). These genes have previously been shown in DvH to provide resistance to exposures to low concentration of oxygen. Strains containing two (wild-type), one, and no copies of these genes have been generated to determine whether gene copy number further contributes to resistance to oxygen. The frequency of these discrepancies in genomes exposes a problem in the current pipeline for genome assembly that could easily be remedied with minor post-assembly assessment.
Background: Complexity constitutes a fundamental attribute of life. Complex systems are made of parts that together perform functions that a single component, or most subsets containing individual components, cannot. Examples of complex molecular systems in bacteria include protein structures such as the F1F0-ATPase, the ribosome, or the flagellar motor: each one of these structures requires most or all of its components to function properly. At the molecular level, operons are a classic example of a complex system. An operon’s genes are co-transcribed under the control of a single promoter to a polycistronic mRNA molecule, with its gene products forming molecular complexes or metabolic pathways. With the large number of complete bacterial genomes available, we now have the opportunity to examine the evolution of operons and identify possible intermediate states. Methods: In this work, we develop a simple vertical evolution model of how operons evolve from individual component genes and orthologous gene blocks or orthoblocks. Utilizing this model, we present two algorithms to reconstruct ancestral operon states using a maximum parsimony approach. Results & Conclusions: We describe several ancestral states that are plausible functional intermediate forms leading to the full operon. A few interesting observations emerge regarding conservation and ancestry of operons. It appears that essentiality (the trait of being essential to life) and the formation of a protein complex are the main drivers for gene block conservation. Figure caption: Orthologous gene blocks from species A-E are arranged in a species phylogenetic tree. Species C has an experimentally-determined operon (Black arrows), and serves as the reference taxon. The tree’s inner nodes show proposed intermediate states in the operon’s evolution using maximum parsimony.
**Abstract Title:**
A New Activation Mechanism of Silent Genomic Islands Mediated by Integrative and Conjugative Elements

**Primary Author Block:**
P. Wang; South China Sea Inst. of Oceanology, Guangzhou, China

**Abstract Body:**
Genomic islands (GIs) are very abundant on prokaryotic genome and represent one of the most intraspecies diversity; however, many of them are silent or have a low expression in planktonic cells. How and when these GIs are activated remains largely unknown. The GIs integrated into the yicC locus can be classified into at least three subclasses according to the conserved core combined with the evolutionary relationship of the integrase. In this study, GIPsp11900 and GIPspSM9913 from two Pseudoalteromonas strains isolated from two distinctive marine environments were selected as the representative GIs of two extensively existed subclasses to investigate the mobilization of GI. We revealed that the subclass of GIs represented by GIPsp11900 could be activated by integrative and conjugative elements (ICEs). Furthermore, in addition to the excision and transfer of GIPsp11900, SXT/R391 ICE-encoded transcriptional regulator SetCD also can upregulate the replication of GIPsp11900, thereby increasing the doses of GIPsp11900-encoded genes. These GIs and SXT/R391 ICEs are widely spread in marine Gammaproteobacteria and the transfer of GI by SXT/R391 ICEs could be triggered by environmental cues during stressed conditions. We also demonstrated that GIPsp11900-encoded genes can defense against the intrusion of foreign DNA. Such action suggested that transcriptional activator SetCD encoded by SXT/R391 ICEs could function as a signal to activate the GIs to mediate horizontal gene transfer. In contrast, SXT/R391 ICE could not activate the GI integrated in yicC in SM9913, another subclass of GIs represented by GIPspSM9913. This study illustrates that genomic islands accessorize the core genome and play important roles in shaping bacterial genome structure, and bacteria can utilize mobile genetic element such as ICE to mediate the expression of genes carried by these genomic islands.
Session Number: 207
Session Type: Poster Talk

Session Title: Evolutionary Histories of Microbial Genomes
Session Start Date Time: 6/9/2018 11:00:00 AM
Session End Date Time: 6/9/2018 12:00:00 PM
Session Primary Track: Microbial Ecology and Evolution
Abstract Control Number: 9461
Poster Board Number:

Abstract Title:
Moderator
Primary Author Block:
Jizhong Zhou; Univ. of Oklahoma, Norman, OK
Abstract Body:
Abstract Title:
Pathogens from the Arctic - What is the Real Risk?

Primary Author Block:
D. C. Mogrovejo; Dr. Brill + Partner GmbH, Inst. für Hygiene und Mikrobiologie, Hamburg, Germany

Abstract Body:
Background: Earth is full of places challenging our understanding of physiology and evolution. Of these, cold environments are of high interest to study how bacteria survive in extreme conditions. Thus, many studies are oriented towards the diversity of the microflora in these habitats, which, for a long time, were considered lifeless. Climate change is globally increasing the risks of infectious diseases with favorable conditions allowing expansion of vector habitats and destructive cryogenic processes facilitating infiltration of disease agents from previously inaccessible areas, causing the emergence of new natural infection loci [1]. Despite these scenarios, few studies have investigated cold-adapted pathogenic bacteria and the possibility of them settling into somewhat warmer environments. This work aims at providing information on potentially pathogenic bacteria in Arctic samples and the risks they could pose in the light of global warming and increasingly higher Arctic temperatures. Methods: For this purpose, snow and water samples from Norway and Iceland were cultured in non-selective media at a range of temperatures for a prolonged period of time (4+months) in aerobic & microaerophilic conditions. Isolates were selected based on morphology, incubated in blood agar and their antimicrobial resistance phenotypes were tested (EUCAST V6.0) against 7 antibiotics. Results: More than 100 isolates have been obtained. The majority are pigmented, Gram negative species, psychrotolerant or mesophilic, including the hemolytic isolates, all obtained from cultivation at or over 17°C. About 30% of aerobic isolates show some degree of hemolysis, compared to 60% of microaerophilic isolates. In addition, 60% of the isolates tested are resistant to at least one antibiotic and, of those, the majority resists nalidixic acid. The “most effective” antibiotics were chloramphenicol, ciprofloxacin and tetracycline with streptomycin being the “least effective” Conclusions: These preliminary results illustrate morphological and virulence phenotypes commonly used to categorize microorganisms as pathogens. In addition, the observed antibiotic resistance, even for bacteria with none or minimal exposure to antibiotics, is unsurprising [2] for antagonistic behaviors are common adaptations within communities [3]. Future steps in this research include the identification of the isolates via MALDI-TOF, antimicrobial resistance profiles specific to the species identified as well as sequencing and screening for pathogenicity genes.
Abstract Title: Denitrifying Anaerobic Methane Oxidation: a Previously Overlooked Methane Sink in Intertidal Zone

Primary Author Block: J. Wang, B. Hu; Zhejiang Univ., Hangzhou, China

Abstract Body:
Background: The intertidal zone is an open ecosystem rich in organic matter and plays an important role in global geochemical cycle. It was previously considered that methane was mainly removed by sulfate-dependent anaerobic methane oxidation (SAMO) process in marine ecosystems while other anaerobic methane oxidation processes were ignored. Recent researches have demonstrated that denitrifying anaerobic methane oxidation (DAMO), consisting of nitrite-dependent anaerobic methane oxidation (Nitrite-AOM) and nitrate-dependent anaerobic methane oxidation (Nitrate-AOM), can also oxidize methane. Methods: In this work, the community structure, quantity and potential methane oxidizing rate of DAMO archaea and bacteria in the intertidal zone were studied by high-throughput sequencing, qPCR and stable isotope tracing method. Results: The results showed that the potential activity of Nitrate-AOM and Nitrite-AOM were $0 \sim 1.57$ nmol $13CO_2$ g$^{-1}$ (dry sediment) day$^{-1}$ and $0 \sim 1.51$ nmol $13CO_2$ g$^{-1}$ (dry sediment) day$^{-1}$ respectively. The copy number of 16S rRNA gene of ANME-2d archaea and NC10 bacteria were $4.34 \times 10^4 \sim 1.18 \times 10^5$ copies g$^{-1}$ (dry sediment) and $4.90 \times 10^4 \sim 3.27 \times 10^5$ copies g$^{-1}$ (dry sediment) respectively. The contribution rate of DAMO process to total anaerobic methane removal in the intertidal zone reached 65.6% ~ 100%, which indicates that DAMO process is an important methane sink in intertidal ecosystem. The simulated column experiments also indicated that DAMO archaea were more sensitive to oxygen and preferred a more anoxic environment in coastal wetlands. Conclusions: DAMO process is an important methane sink in intertidal ecosystem. And DAMO archaea were more sensitive to oxygen and preferred a more anoxic environment in coastal wetlands.

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Abstract Title:
Dramatic Expansion of the Translation Apparatus of Giant Viruses

Primary Author Block:
P. Colson, B. La Scola, D. Raoult; IHU Méditerranée Infection, Marseille, France

Abstract Body:
Background: Giant viruses of amoebae are an expanding group of viruses whose remarkable characteristics have been unveiled since 2003. Among features that differentiate them from classical viruses are genes unique among viruses including some encoding translation components. We describe here the recent boom of the set of translation components in giant viruses of amoebae as well as data on its functionality.

Methods: Giant virus genomes/gene contents were from GenBank and our institution sequence database. Sequence similarity searches used BLAST. Gene silencing used RNA interference. Proteomic analyses included proteins detection in Mimivirus virions and their quantification with the Hi3 technique. Codon usage was analyzed using the CAIcal server.

Results: Initially, Mimivirus was found to encode 16 translation components with 3 initiation factors. We detected 2 of these factors (R458, R464) in purified virions, which suggests their early requirement for replication. R458 gene product was the most abundant. R458 gene silencing deregulated 32 proteins, mostly encoded by genes lately transcribed, and lengthened the replicative cycle. These data suggest that reduced R458 protein synthesis may lead Mimivirus to enroll amoebal substitutes. They are in line with previous description of an increased adaptation during the replicative cycle of expressed viral genes to Mimivirus codon usage, and they suggest an increased requirement of the Mimivirus machinery.

Most recently, two new giant viruses named tupanviruses, which are tailed mimiviruses, were described. Their set of translation components is the most complete among known viruses. It includes 20 aminocyl-tRNA synthetases (the full set), 11 translation factors, and 67-70 tRNA. In addition, 2 distinct copies of an 18S rRNA intronic region were detected.

Conclusions: Even if giant viruses are still ‘microbes devoid of ribosomes’, the recent enlargement of their translation machinery and evidence of the functionality of some of its components strongly suggest that these viruses may at least supplement the host translation as other intracellular microbes, and, eventually may employ an alternative translation process.
Session Title: SATURDAY - MEE Late-breakers
Session Start Date Time: 6/9/2018 11:00:00 AM
Session End Date Time: 6/9/2018 1:00:00 PM
Session Primary Track: Microbial Ecology and Evolution
Abstract Control Number: 7610
Poster Board Number: SATURDAY - MEE LB5

Abstract Title:
Two Novel Swarming Flavobacterium Species Isolated from Antarctica

Primary Author Block:
S. Kralova1, H. J. Busse2, P. Svec1, E. Staňkova1, I. Sedaľaček1; 1Masaryk Univ., Brno, Czech Republic,
2Univ. of Vet. Med., Vienna, Austria

Abstract Body:
Background: Polar microbiology is fast growing field within research on polar regions, which drove increased scientific attention due to their uniqueness, importance in biosphere and unquestionable vulnerability to anthropogenic changes. Microorganisms present in polar regions represent rich source of novel knowledge as they are unique by their cold-adaptation mechanisms, physiology and ecological roles in polar ecosystems and/or biogeochemical cycling. Material: A group of 16 psychrotrophic bacterial strains was isolated in the frame of CzechPolar research project partially focused on the diversity of cultivable bacteria from Antarctica. This project has been performed at the Johann Gregor Mendel Antarctic station situated on the James Ross Island near the Antarctic Peninsula. Analyzed strains were isolated from environmental materials and this sampling has been conducted yearly since 2007 during expeditions realized in Antarctic summer seasons. Methods: A polyphasic taxonomic approach was applied to clarify taxonomic position of isolated Antarctic bacteria. Molecular analyses, i.e. 16S rRNA sequencing, DNA-DNA hybridization (DDH) and (GTG)5-PCR were followed by extensive biotyping to describe physiological and biochemical properties of these bacteria. Finally, chemotaxonomic approaches including analysis of fatty acid methyl esters, menaquinones, polyamines and polar lipids was applied for further characterization of investigated strains. Results: All isolates were rod-shaped, Gram-stain-negative, swarming, catalase positive bacteria that produced yellowish and opalescent colonies on R2A agar. The 16S rRNA gene analysis revealed two separated clusters among the genus Flavobacterium. Flavobacterium aquidurense (98.3 %) and Flavobacterium hydatis (98.9%) represented the closest phylogenetic neighbors to cluster I and II respectively, with DDH similarity values reaching 23% and 13%. Common to representatives of both groups were presence of major menaquinone MK-6, sym-homospermidine as the major polyamine and phosphatidylethanolamine as the major identified lipid. Major fatty acids were C15:0 iso, C15:1 iso G, C15:0 iso 3OH, C17:0 iso 3OH and Summed Feature 3 (C16:1 ω7c/ C16:1 ω6c). Conclusion: All obtained results showed that the analyzed Antarctic strains represent two novel species of the genus Flavobacterium, for which the names Flavobacterium circumlabens sp. nov. (type strain CCM 8828T) and Flavobacterium cupreum sp. nov. (type strain CCM 8825T) are proposed.
Session Title: MEE02 - Biogeochemical Processes and Systems Biology: Biogeochemical Processes
Session Start Date Time: 6/9/2018 11:00:00 AM
Session End Date Time: 6/9/2018 1:00:00 PM
Session Primary Track: Microbial Ecology and Evolution
Abstract Control Number: 3499
Poster Board Number: SATURDAY - 948

Abstract Title: How Nitrifiers Drive Nitrification and Redox Metabolism in Soil?
Primary Author Block:
S. R. Mohanty, B. Kollah, M. Nagarjuna, A. K. Patra; Indian Inst. of Soil Sci., Bhopal, India

Abstract Body:
Background: Nitrification is a key biogeochemical process for the global nitrogen balance. In the first step of nitrification NH4 binds to the membrane bound nitrifying enzymes and is oxidized to NO3. The product NO3 may bind to various cationic molecules including the extracellular organic compounds forming a biogenic nitrate. So far there is no information on the role of biogenic nitrate on the redox metabolism. Materials and Methods: The effect of both biogenic nitrate and chemical nitrate on redox metabolism was evaluated. Nitrification activity was observed under repeated NH4-N amendment. Nitrate was estimated by phenol disulphonic acid method, Abundance genes estimated by real time PCR, and characteristic of nitrified soil was analyzed by raman spectroscopy. Results: Repeated NH4-N amendment increased nitrification rate (mM NO3 produced g-1 soil d-1) from 0.49 to 0.65. Nitrification stimulated (p<0.01) the abundances of 16S rRNA gene of eubacteria (43.67±4.510 x 106), amoA gene of nitrifying bacteria (102.33±8.50 x 104) and nitrifying archaea (94.33±7.77 x 104). The rate of reduction of terminal electron acceptors (mM reduced g-1 soil d-1) due to the biogenic nitrate followed as : NO3- reduction 4.01±0.229, Fe3+ reduction 5.37±0.122, SO42- reduction 9.56±0.165 and CH4 production (µg g-1 soil) 0.46±0.051. Nitrification inhibited denitrificaton by a factor of 1.4. Similarly it inhibited the Fe3+ reduction, SO42- reduction, and CH4 production by factors of 1.8, 1.13, and 1.66 respectively. Raman spectra of the nitrified soils indicated build up of aliphatics in soil. Probably, these aliphatics bind to NO3 and form biogenic nitrate. The microbial volatile organic compounds (mVOCs) produced from nitrifiers enhanced (p<0.05) nitrification. The mVOCs stimulated (p<0.05) abundances of nitrifying bacteria than the eubacteria and nitrifying archaea. Conclusion: Nitrification modulated soil redox metabolism through biogenic nitrate and microbial volatiles. Biogenic nitrate may hold keys for sustainable agriculture and climate change mitigation. 1
Abstract: Composition of Expressed Fungal Class II Peroxidases is Altered by Anthropogenic N Deposition

Primary Author Block:
E. M. Entwistle1, K. J. Romanowicz2, W. A. Argiroff2, Z. B. Freedman3, J. J. Morris1, D. R. Zak2; 1Univ. of Alabama at Birmingham, Birmingham, AL, 2Univ. of Michigan, Ann Arbor, MI, 3West Virginia Univ., Morgantown, WV

Abstract Body:
Background: Anthropogenic nitrogen (N) deposition, an important and widespread agent of global change, has the potential to increase soil carbon (C) storage by altering the expression of fungal oxidative enzymes and, thereby, the process of plant-litter decomposition. In long-term field experiments, anthropogenic N deposition retards lignin decomposition, which is the rate-limiting step in plant litter decay, and increases soil C storage in forests. Fungal class II peroxidases are important enzymes in this process because they mineralize the lignin in plant detritus and the lignin-derived polyphenolic compounds in soil to carbon dioxide. Anthropogenic N deposition reduces both peroxidase enzyme activity in soil and the relative abundance of fungi which produce these oxidative enzymes. In light of this, it is plausible that anthropogenic N deposition has reduced plant litter decay and increased soil C content by altering the composition of peroxidases expressed by the fungal community.

Methods: To test this, we compared the composition and richness of fungal class II peroxidase transcripts occurring in forest floor material collected from the ambient and experimental N deposition treatments in a long-term field experiment encompassing a series of northern hardwood stands in which the amount of N deposition has been experimentally increased to simulate rates of anthropogenic N deposition predicted for midcentury (30 kg N ha-1 y-1). Results: Our results indicate that ca. 20 yrs of experimental N deposition altered the phylogenetic composition of lignin-decaying class II peroxidases expressed by forest floor fungi, a response which has occurred concurrently with reductions in plant-litter decomposition and a rapid accumulation of SOM. This observation indicates that fungal taxa are expressing peroxidases differentially in response to anthropogenic N deposition, a result which likely has important functional consequences for the biological decay of lignin and polyphenolics in SOM.

Conclusions: The changes in expressed peroxidase composition we observed may, in part, explain previously documented increases in soil C storage under long-term, anthropogenic N deposition.
Abstract Title:
Outstanding Abstract Award: Seeing the Trees Through the Forest: Measuring Microbial Community Biomass Stoichiometry One Cell At A Time
Primary Author Block:
M. P. Manzella, E. K. Hall; Colorado State Univ., Fort Collins, CO
Abstract Body:
Microbial biomass stoichiometry, typically the ratio of the biologically-important elements carbon, nitrogen, and phosphorus (C:N:P), has historically been examined at a course resolution where the contribution of the individual is masked by the reported community or population average. Examining the distribution of individuals within a population, and populations within a community has the potential to improve our understanding of the drivers of microbial community stoichiometry. We measured biomass ratios (C:N:P) of individual microbial cells using energy dispersive spectroscopy (EDS). We report the distribution and average cellular phenotype within both cultured and uncultured communities. First, we analyzed three microbial cultures (a Pseudomonas, Flavobacterium, and a Brevundimonas isolate) by both bulk measures and EDS to compare the two approaches. Next, we examined biomass C:N:P of these same strains during batch growth in both high-P and low-P media. Finally, we analyzed microbial cells taken from three Colorado lakes to examine the stoichiometric distribution within each community. Biomass C:N:P obtained by EDS (34:8:1, 53:11:1, and 69:14:1) were similar to those obtained by bulk measures (31:8:1, 57:12:1, and 74:15:1, respectively) for the test strains. Within the growth experiment, biomass C:N was relatively conserved across growth stages for each strain, whereas N:P and C:P changed in response to changing P availability. Cultures grown in the low-P medium exhibited greater stoichiometric plasticity than those in the high-P medium. In general, the distribution of biomass ratios (C:N, N:P, and C:P) within these monoculture experiments followed a unimodal distribution. However, within the lake communities, the distribution of N:P and C:P was multimodal, containing local maxima across the phenotypic landscape. This study illustrates that community-level stoichiometric measures have masked differences in microbial biomass phenotypes within populations and communities. We show that monocultures exhibit a unimodal stoichiometric distribution and that both phylogeny and physiological state affect cellular response to P limitation. These traits become clear when the individual, rather than the community, is the subject of investigation. Within the lake communities, microbial phenotypic clusters exist with stoichiometries that vary from the community average. It is currently unknown what drives the development of these local maxima or the consequences of divergent phenotypes within the sampled community.
Abstract Title:
High Prokaryotic Metabolic Activities in the Northern South China Sea

Primary Author Block:
X. Li, L. Lu, H. Dang, N. Jiao; State Key Lab. of Marine Environmental Sci., Coll. of Ocean and Earth Sci., Xiamen Univ., Xiamen, China, Xiamen, China

Abstract Body:
Microbiological processes are believed to be critical in marine carbon cycling. Although marine particles provide many benefits for microbes, there is still a lack of research on microbial processes of particle-attached (PA) microbes. During the past decades, main microbial processes involving community respiration (CR), prokaryotic production (PP) and dark carbon fixation (DCF) are measured in many ocean waters. However, investigations on all these processes simultaneously, particularly for PA microbial communities, are scarce. In our current study, CR, PP and DCF were determined respectively by in vivo electron transport system activity assays, [3H]-leucine incorporation assays and dark [14C]-bicarbonate assimilation assays, for both PA and free-living (FL) microbial communities along a transect from the Pearl River estuary to the basin SEATS station in the northern South China Sea (nSCS) in November 2016. All CR, PP and DCF rates show a nearshore-to-offshore and surface water-to-bottom water decreasing trend. PA communities accounted for 33.05%, 15.55% and 19.37% of the bulk CR, PP and DCF, respectively. We find that the contributions of PA community to metabolic activities became more important with the increase of water depth (ranging from 9.73% to 73.94%, 9.58% to 68.11%, and 9.45% to 83.14% for water column CR, PP, and DCF, respectively), indicating that particles are more important for microbiological processes in deep waters of the nSCS. The averages of prokaryotic growth efficiencies (PGE) were ~5.98%, with higher values occurring in nearshore and surface waters. DCF rates (~0.63 mmol C m-3d-1) were largely higher (~543.82%) than PP rates throughout the water column, and represented an important source of organic matter in the water columns. Results of 16S rRNA gene MiSeq high-throughput sequencing demonstrated that the bacterial community compositions were statistically different between the offshore and the nearshore waters, between the shallow layers and the deep layers of the water columns, and between the FL and the PA habitats. Our data illustrated that dark carbon fixation was widespread and potentially important in the nSCS, especially in nearshore and shallow waters. DCF might be an important source of primary production in this marginal sea. The metabolic activities and community compositions differed between PA and FL assemblages, suggesting their different roles in marine carbon cycling. PA microorganisms seem to play a more significant role in community metabolic activities in deeper waters of the nSCS.
Biotic and Abiotic Effects on Microbial and Nitrogen Functional Genes Within Loblolly Pine and Eucalypt Tree Plantations At the Extreme Ends of A Groundwater Depth Gradient

Primary Author Block:
T. Wu1, A. Gray1, G. Liu1, H. Kaminski1, B. Osi Efa1, D. Aubrey2; 1Georgia Southern Univ., Statesboro, GA, 2Univ. of Georgia, Aiken, SC

Abstract Body:
Microbial communities found in soil ecosystems have long been recognized in decomposing organic materials and recycling nutrients thus affecting plant growth in terrestrial ecosystems. Both biotic and abiotic factors of soil affect the diversity and function of soil microbial communities. A clear understanding on how biotic and abiotic factors influence the microbial community and its functional role in ecosystems is fundamental to terrestrial biogeochemistry and plant production. The purpose of this experiment was to investigate microbial communities and functional genes involved in nutrient cycling amongst varying groundwater depth plots and tree species: Eucalypt and Pine, and their potential for plant production. The soil bacterial and archaeal 16 rRNA gene, nitrifying ammonia oxidation archaeal gene amoA (AOA) and bacterial gene amoA (AOB), nitrite oxidoreductase gene nrxA, and denitrifying bacterial narG, nirK, nosZ and nirS genes were detected using Polymerase Chain Reaction (PCR) and Denaturing Gradient Gel Electrophoresis (DGGE) for the soil samples of Spring and Fall of 2015. Fungal communities and ectomycorrhizal colonization rates of eucalypt and pine were determined by length heterogeneity PCR (LH-PCR) and routine root staining techniques respectively. Soil bacterial and archaeal community structure remained similar between the forest plantation types, but differed between groundwater depths in samples of Spring, and also in Fall for bacterial community. The abundant archaeal amoA for nitrification were detected; whereas, the bacterial amoA, nrxA for nitrification were only produced little to no bands. The bacterial nirK and nosZ genes for nitrification were detected; whereas, the bacterial narG and nirS genes were not detected. The detected nitrification/denitrification community, archaeal amoA and bacterial nosZ, varied significantly with respect to water levels in Spring, but not in Fall. There was no difference of overall nitrifying archael or denitrifying bacterial community between different forest plantation types in Spring, neither in Fall. Both total fungal community and ectomycorrhizal colonization rates are affected by forest plantation types. The different microbial community observed within two forest plantations and at shallow and deep groundwater depths may provide important information for the intensity of management and planting a woody crop eucalypt species with even higher production potential than loblolly pine.
Abstract Title:
Hierarchical Substrate Preferences in the Marine Bacterium Sagittula Stellata E-37 During Growth on Mixtures of Plant-Derived Aromatic Compounds

Primary Author Block:
M. Chua, A. Buchan; Univ. of Tennessee, Knoxville, TN

Abstract Body:
As a component of vascular plants, lignin is the most abundant aromatic biopolymer on Earth. Lignin and its derivatives serve as substrates for heterotrophic microbes in salt marsh ecosystems, where the dissolved organic carbon pool is rich in aromatic compounds. Members of the Roseobacter lineage of marine bacteria are abundant in these salt marshes and play a major role in transforming lignin-derived aromatic compounds. When bacterial strains are presented with a mixture of aromatic compounds, substrate utilization usually proceeds according to a hierarchy of preferences. Contrary to this paradigm, it has been previously demonstrated that Roseobacter lineage member Sagittula stellata E-37 simultaneously catabolizes two aromatic compounds that result from lignin biodegradation (benzoate [BEN] and p-hydroxybenzoate). When doing so, growth rates are faster relative to when either substrate is presented alone, suggesting an ecological growth advantage in lignin-rich environments. Here, we extended prior studies to examine the universality of this catabolic synergy by E-37 by monitoring growth dynamics and extracellular aromatic concentrations in mixed substrate experiments. Co-mixtures of BEN with p-coumarate (COU), ferulate (FER), or vanillate (VAN) revealed a preference for the non-benzoate substrate. BEN + FER and BEN + VAN cultures had significantly lower (at least 2-fold) growth rates compared to single substrate cultures. Growth rates on BEN + COU were indistinguishable from the single COU cultures. Analysis of E-37 mutants with disruptions in the BEN degradation pathway (box) revealed diminished growth rates on VAN and FER, providing genetic support for cross-regulation between the pathways required for degradation of these compounds. These results provide the mechanistic underpinnings for understanding how microbes transform these compounds in the complex mixtures found in nature.
Abstract Title:
Sensitivity of Marine Cyanobacteria and Green Microalgae to Nano and Bulk Zinc Oxides
Primary Author Block:
J. Gil Acevedo, M. Gantar, K. Shetty, K. Jayachandran; Florida Intl. Univ., Miami, FL
Abstract Body:
Zinc oxide (ZnO) nanoparticles (NPs) have a vast range of applications, release of these NPs into waste streams and further into the environment may adversely impact non-target organisms. Natural variations in tolerance to nano ZnO may exist among various microalgae, and the availability of tolerant and sensitive species may lead to better understanding of tolerance mechanism and potential applications in bioremediation and ecotoxicology. To elucidate the effects of nano and bulk zinc oxides on the growth of microalgae, three marine green microalgae, and four marine cyanobacteria species, were used in a replicated study. The algal strains were incubated in three different growth media: media containing standard BG-11 media, and media with various concentrations of either nanoparticle ZnO or bulk ZnO at concentrations up to 1 ppm. The culture flasks were incubated on a shaker at 25°C under continuous fluorescent lighting. Changes in terms of growth and morphology of the species to the treatments were assessed by measuring optical density and microscopic observations for a total of 7 days. Results showed marked difference among species tested to varying ZnO concentrations. Most of the cyanobacteria showed reduced growth as a result of exposure to ZnO, which included decreased cell size and discoloration. On day 7, among cyanobacterial species tested Leptolyngbya boryana showed the most growth inhibition (90%) and loss of pigmentation even at 0.125 ppm nano-Zn concentration (OD 0.159). At all levels of nano-Zn tested, the three-marine green microalgal species showed marked tolerance (OD more than 0.8618). These findings provide a strong impetus for screening a larger collection of diverse indigenous microalgae for tolerance to NPs and which may yield potential candidates for bioremediation of NPs and also sensitive indicator species for ecotoxicological testing.
Abstract Title:
Diversity and Gene Neighborhoods of Microbial Rhodopsins from A Tropical Freshwater Reservoir

Primary Author Block:
R. Kaushik1, P. Vijayakumar1, L. Loh2, S. Te2, K. Y. H. Gin2, J. R. Thompson1; 1Singapore-MIT Alliance for Res. and Technology, Singapore, Singapore, 2Natl. Univ. of Singapore, Singapore, Singapore

Abstract Body:
Solar energy can be harvested by two major mechanisms in biological systems: chlorophyll-based photosynthesis and light-driven ion-pumping by membrane-bound rhodopsins. Rhodopsins have been found in bacteria, archaea, viruses and dinoflagellate algae. Metagenomic analysis of surface waters reveals a nearly ubiquitous occurrence of rhodopsin genes including Proteorhodopsin (PR), Xanthorhodopsin (XR) and Bacteriorhodopsin (BR). Although estimates suggest that up to 70% of cells in surface waters may contain a rhodopsin, the physiological role of these systems remain largely unknown as clear-cut demonstration of rhodopsin-mediated phototrophy are sparse. Rhodopsins require the cofactor retinal for activity and some may associate with additional cofactors such as salinixanthin for light capture. While rhodopsin genes tend to occur in clusters with retinal biosynthesis pathway genes, the absense of cofactor genes in some organisms with functional rhodopsins has led to the hypothesis that scavenging may supply retinal and possibly other cofactors via commensal interactions in the plankton. Here, we studied the prevalence and diversity of microbial rhodopsin genes in metagenomic datasets of size-separated water samples collected monthly over two years from a eutrophic tropical freshwater reservoir. Rhodopsin genes were found to have the highest prevalence in the less than 1.5 μm size fraction relative to the smaller (<0.22 μm) and larger (>1.5 μm) size fractions. Phylogenetic analysis indicated Xanthorhodopsin to be the dominant type of rhodopsin in all size fractions. Rhodopsin genes were primarily associated with metagenomic sequence contigs from Actinobacteria, FCB group, and Terrabacteria. Network analysis indicated that gene neighborhoods for predicted rhodopsins (±3500 bp) contained genes annotated as hypothetical proteins and glutamate racemase (essential for peptidoglycan biosynthesis) as the first and second most frequent associations. Genes for beta-carotene and retinal biosynthesis (CrtEBYI and blh) and various integral membrane proteins including cytochrome C associated proteins and ABC- and TonB transporters also co-occurred in the rhodopsin gene neighborhoods. Our findings enrich evidence towards diverse and widespread microbial rhodopsins in surface aquatic habitats where conservation of gene neighborhoods for different rhodopsin types may shed light on their physiological roles.
Abstract Title:
Organic Carbon Source Shape Sediment Bacterial Composition in Two China Marginal Seas and their Tributaries

Primary Author Block:
K. Wang; Kent State Univ., Kent, OH

Abstract Body:
Background: Marginal sea sediments receive organic substrates of different origins, but whether and to what extent sediment microbial communities are reflective of the different sources of organic substrates remain unclear. Methods: To address these questions, sediment samples were collected in two connected China marginal seas, i.e., Bohai Sea and Yellow Sea, and their two major tributaries (Yellow River and Liao River). Sediment bacterial community composition (BCC) was examined using 16S rRNA gene pyrosequencing. In addition, physicochemical variables that describe environmental conditions and sediment features were measured. Results: Our results revealed in BCC changes with salinity and organic carbon (OC, terrestrial- and phytoplankton-derived) variations such as members of Gaiellaceae and Comamonadaceae showed a rapid decrease as salinity and phytoplankton-derived OC increase, while Piscirickettsiaceae and Desulfobulbaceae exhibited an opposite distribution pattern. Differences of riverine vs. marginal sea sediment BCCs could be mostly explained by salinity. However, within the marginal seas, sediment BCC variations were mainly explained by OC-related variables, including terrestrial-derived fatty acids (Terr_FA), phytoplankton-derived polyunsaturated fatty acids (Phyto_PUFA), stable carbon isotopes (δ13C), and carbon to nitrogen ratio (C/N). In addition to environmental variables, interactions among individual bacterial taxa were also found important in shaping sediment BCCs in the studied areas as indicated by network analysis. Conclusions: In conclusion, our results suggested that OC source might be an important factor in regulating bacterial communities in Bohai and Yellow Seas and more specifically phytoplankton-derived OC.
Abstract Title:
Thirteen Years of Environmental Dynamics in Laguna Alalay Lake, Bolivia, An Eutrophic Lake

Primary Author Block:
C. A. Perez-Fernandez1, A. M. Romero-Jaldin2, G. Toranzos1; 1Univ. of Puerto Rico, San Juan, PR, 2Univ. Mayor de San Simon, Cochabamba, Bolivia, Plurinational State of

Abstract Body:
Background: Since industrial revolution, water bodies became rich in nitrogen and phosphorus resulting in algal blooms by human activity in urban areas. In 2016, an event of mass death of fish at Laguna Alalay was apparently caused by a bloom. Eutrophication reached a critical point as a result of urban growth. Actions taken for the lake restoration were the removal of sediments and macrophytes in 1997 and 2004 respectively. Influent water quality was not taken in consideration since there exists an absence of systematic environmental information results for the lake management. This work illustrates how the compilation of long-term data from non-systematic monitoring could be used to provide a technical document for the management of contaminated water bodies. Methods: We organized different reports of environmental monitoring from Alalay provided by the CASA, UMSS. Data comprises a series of measurements from 2003 to 2016. However, parameters were incomplete and the values were not systematically selected. For data pre-treatment, parameters containing less than 75% of the number of observations were eliminated; similarly, observations containing less than 50% of the parameters were also eliminated. Missing data was filled by multiple imputations using mice R package. Filled data was complemented with rain precipitation values obtained from a public database (SENAMHI). Finally, exploratory analysis was done to describe environmental dynamics. Results: Total phosphorus (TP), Total reactive phosphorus (TRP), nitrates (NO3), nitrites (NO2), ammonia (NH3), organic nitrogen (ON), dissolved oxygen (DO), conductivity (C), and precipitation (Pr) were used. We detected the existence of correlated parameters as well as independent ones (Bartlet test (p < 0.05) and MSA = 0.56). These variability in data didn’t allow us to use multivariate statistics. Correlated parameters were TP-TRP-C, and Pr-C. Independent ones were ON and DO. Environmental dynamics reflected a constant state of hyper-eutrophication despite some punctual events such as the increase in rain precipitation in 2008, dissolved oxygen in 2009, and conductivity in 2014. Conclusions: Our analysis shows that an appropriate treatment of incomplete would be useful for preliminary analysis in water bodies management. Our results suggest that Alalay’ nutrient levels remain constant through time. Finally, we recommend the systematic monitoring of the lake, the pretreatment of influent water, and the use of the “citizen scientist” approach for a more proactive restitution of Alalay.
Abstract Title:
Evaluation of Native Bacterial Consortium from Crude Oil-Impacted Tropical Environment for Integration Into Bioremediation Process

Primary Author Block:
O. A. Ojo-Omoniyi, R. O. Abegunrin; Lagos State Univ., Lagos, Nigeria

Abstract Body:
One of the most important organic pollutants is petroleum hydrocarbons from petroleum product. Petroleum-based products are the major source of energy for industry and daily life. Leaks and accidental spills occur regularly during the exploration, production, refining, transport, and storage of petroleum and petroleum products. Tropical soil and natural water samples (0 - 30cm depth) obtained from crude oil mining area of Niger / Delta, southern Nigeria were evaluated for petroleum-hydrocarbon utilizers. A total of twenty seven bacterial species of relevance in bioremediation were isolated and characterized using standard and conventional methods. The predominant species belong to the genera Pseudomonas and Proteus. The ability of Pseudomonas aeruginosa, Pseudomonas stutzeri, Proteus mirabilis, Serratia marcescens and Bacillus subtilis to degrade diesel oil was studied. The results showed maximal increase in optical densities and total viable counts, however, with decrease in pH of the culture media after 8 - day incubation period. Typical generation times varied between 0.89 and 4.0d for Pseudomonas aeruginosa, 0.85 and 0.32d for Pseudomonas stutzeri, 0.8 and 3.9d for Proteus mirabilis.

All the isolates utilized petroleum hydrocarbon as sole carbon and energy sources for growth on Minimal Salt Medium (MSM) supplemented with (0.2%) diesel oil. The isolates were tested against 7 different antibiotics and were found to be resistant to 5 antibiotics. Consequently, Twenty four isolates were randomly selected for plasmid DNA isolation. The presence of plasmid DNA was confirmed in 22 isolates where the molecular weight size ranged between 7 – 23.1kb. Although, Pseudomonas aeruginosa, Proteus mirabilis and Bacillus subtilis had low specific growth rate on Diesel, they were the best choice for bioremediation since they had the highest mean generation time respectively. The knowledge of the potentials of these isolates to degrade hydrocarbons will increase the possibilities of developing novel strains and strategies for removing hydrocarbon pollutants from the natural environment.
Abstract Title:
Distinct Distribution Patterns of Prokaryotes and Nitrogen Related Microbes in the Yellow River Estuary, China

Primary Author Block:
Z. Gao; Shandong Agricultural Univ., Taian, China

Abstract Body:
The Yellow River is the largest turbid river in the world. The Yellow River estuary (YRE), is suffering a series of pollutants, such as nitrogen and organic matter, due to the urbanization and anthropogenic activities. Microbes are often involved in transformation and degradation of pollutants. The research on the microbiome dynamics, structure, composition and function in YRE will be helpful for understanding of environment suffering and protection. Surface water and sediments were collected from the YRE. The prokaryotes communities were elucidated by qPCR and highthroughput sequencing. Notably higher prokaryotic abundance and diversity were detected in the sediment than in water, and bacterial abundance and diversity were remarkably higher than those of archaea. Bacterial communities at different taxonomic levels were apparently distinct between the sediment and water, but archaeal communities were not. Although distinct prokaryotic distribution patterns were observed, most of the dominant bacteria and archaea present were related to carbon, nitrogen, and sulfur cycling processes. Ammonia oxidation is the rate limiting step of nitrification. Quantitative PCR revealed that AOA showed significant higher abundance than AOB both in sediment and water samples. Clone library analyses showed that Nitrososphaera was predominant AOA in sediment, while Nitrosopumilus and Nitrosotalea dominated in water column. Nitrosospira was dominating AOB in both habitats. Principal coordinate analysis indicated AOA community structures exhibited significant differences between two habitats, while AOB were not. Ammonium and carbon contents were the potential key factors to influence AOMs' abundance and compositions in sediment. Denitrification is the critical in eliminating nitrogen in estuarine ecosystems. Clone library showed that nirK and nirS denitrifiers exhibited different distribution patterns in sediments samples. Canonical correlation analysis demonstrated that the community structure of nirK was more responsive to environmental factors than that of nirS. Interestingly, only nirK denitrifiers were successfully amplified in the water samples, and the community compositions of nirK were related to salinity and nitrate concentration. In all, this study briefly elucidated the distribution patterns of prokaryotes and nitrogen related microbes in YRE. Much more researches on the function, activities and interaction among microbes will be performed in the future.
Abstract Title:
A Quantitative View of the Biosphere: from the Most Abundant Taxa to the Most Abundant Proteins
Primary Author Block:
Y. M. Bar-On; Weizmann Inst. of Sci., Rehovot, Israel
Abstract Body:
Background: A census of the biomass on Earth is key for understanding the structure and dynamics of the biosphere. Similarly, at the protein level, a census of the mass of proteins that drive global biogeochemical cycles can help us better understand the constraints that they experience in the wild. Yet, a quantitative, global view of how the biomass of different taxa compare with each other, and which proteins are most abundant in nature is still lacking. Methods: We perform a comprehensive meta-analysis, mining data on the biomass of about 20 different taxa. We establish the first census of the biomass of all the kingdoms of life. Using this census of biomass, we estimate the global abundance of ubiquitous proteins throughout the kingdoms of life. Our survey encompasses proteins such as Rubisco, previously suggested to be the most abundant protein in the biosphere, as well as proteins essential to key biological processes including protein translation, metabolism and the structural proteins actin, myosin and histones. Results: From this global and quantitative view, we find key take-home messages. We highlight the overall dominance of plant biomass, which account for 80% of the total biomass on Earth - far above all microbial biomass. We find that different kingdoms of life concentrate in different environments - plants and fungi are mostly terrestrial, whereas animals are mostly marine. We show that 90% of the biomass of bacteria is located in deep subsurface environments such as aquifers and subseafloor sediments. In addition to describing current biomass stocks, we also quantify the impact of humans on plant and vertebrate biomass. Using our biomass data, we find that in terms of mass, histones rival Rubisco as the most abundant protein. Based on our estimates for the total abundance of Rubisco, we quantify the effective rate of carbon fixation of Rubisco in the wild, and estimate it works at only 1% of its maximal capacity in terrestrial environments. We explore the different factors that contribute to this decrease in effective rate. Conclusions: In this study we assembled the first census of biomass distribution of Earth. We leverage this census to look at the way in which biomass is distributed across taxa, environments and trophic modes. We also use this census to estimate what is the most abundant protein on Earth, and quantify the effective rate of Rubisco in the wild.
Abstract Title:
Phosphate Addition Increases Tropical Soil Respiration Primarily by Deconstraining Microbial Population Growth

Primary Author Block:
E. R. Johnston1, M. Kim1, J. K. Hatt1, J. R. Phillips2, Q. Yao2, Y. Song2, C. Pan2, T. C. Hazen3, M. A. Mayes2, K. T. Konstantinidis1; 1Georgia Inst. of Technology, Atlanta, GA, 2Oak Ridge Natl. Lab., Oak Ridge, TN, 3Univ. of Tennessee, Knoxville, Knoxville, TN

Abstract Body:
Tropical ecosystems are an important sink for anthropogenic CO2 emissions; however, sustained uptake is increasingly restricted by phosphorus (P) availability. Soil microbiota facilitate the turnover of organic P and carbon (C) compounds and scavenge P from inorganic forms, but their role in tropical C-P-coupled biogeochemistry remains poorly understood. To advance this topic, soils collected from four sites in the El Yunque National Forest in Puerto Rico were incubated with exogenous PO43-. Phosphorus amendment increased CO2 respiration by 13.6-23.3% relative to control incubations for soils taken from all sites except the most P-rich one soil (no difference compared to its control). RNA-seq analysis revealed the increased relative transcription of genes involved in the biosynthesis of essential cell infrastructure components (purines, pyrimidines, phospholipids), cell division, and nutrient uptake and assimilation. A novel methodology assessing population-level gene expression by integrating companion metagenomic and metatranscriptomic datasets revealed that the trend of enhanced growth/respiration was community-wide. P-limited microbial communities possessed a greater abundance of genes for the biosynthesis of α-glucosyl polysaccharides, reflecting a community-wide adaptation to store excess organic carbon substrates under poor growth conditions (e.g., limiting phosphorus). Phosphorolysis genes governing the degradation of α-glucosyl polymers were more abundant in low-P soils and also had increased relative expression with P-amendment; these functions likely regulate the metabolism of stored organic substrates in soils with scarce P-availability. Collectively, our results provide quantitative estimates of increased CO2 respiration upon an alleviation of P-constraints and elucidated the underlying ecological and molecular mechanisms involved.
Abstract Title:
High Interspecific & Intraspecific Diversity of Dominant Cyanobacteria Throughout A Bloom in Lake Harsha (Ohio, USA) High Interspecific and Intraspecific Diversity of Dominant Cyanobacteria Throughout A Bloom in Lake Harsha (Ohio, USA)

Primary Author Block:
B. Zhu1, H. Cao2, J. Lu3; 1Jinan Univ., Cincinnati, OH, 2US EPA, Tempe, AZ, 3US EPA, Cincinnati, OH

Abstract Body:
Cyanobacterial blooms are global harmful hazards, and a long-suspected question is whether high abundance of dominant species is paralleled by high intraspecific and/or interspecific diversity. Using high throughput 16S rRNA gene sequencing of samples from a complete bloom cycle in Harsha Lake (Ohio, USA), we found main phyla Cyanobacteria, Proteobacteria, Bacteroidetes, Actinobacteria, and Verrucomicrobia were more abundant in blooming than pre-bloom stage, while Planctomycetes showed an opposite change at five locations. Cyanobacteria had five main genera, Planktothrix, Aphanizomenon, Dolichospermum, Microcystis and Cylindrospermopsis, along with three minor genera Anabaena, Oscillatoria, and Leptolyngbya. Moreover, 25% - 42% of the genera are positively or negatively correlated. On an intergenic level, Planktothrix, Dolichospermum, Aphanizomenon, and Microcystis each had at least two species, giving 6 to 13 total cyanobacterial species. Most importantly, dominant species had the highest intraspecific diversity. Planktothrix rubescens, Dolichospermum flos-aquae, and Aphanizomenon issatschenkoi each had at least 10 strains, while non-dominant species only has one or several strains. Most strains behave differently throughout the bloom cycle, which are partially accounted by nitrogen and phosphorus nutrients. These novel findings that dominant cyanobacteria have high inter- and intra-specific diversity and intraspecific strains behave differently provides a foundation for precision bloom control.
Sequential Reduction of Terminal Electron Acceptors Modulates Nitrification and Dynamics of Nitrifying Bacteria and Archaea

Abstract Body:
Background: Microbially mediated nitrification is a key biogeochemical process in the global nitrogen balance. However, it is not clearly understood how the sequential reduction of soil due to submerging will influence nitrification. Materials and Methods: Experiments were carried out using the soil samples collected from the experimental fields of the Indian Institute of Soil Science, Bhopal, Madhya Pradesh, India (23.30 N, 77.40 E, 485 m above mean sea level). Incubation experiment was carried out in 20 g portion of soil placed in 130 ml pre-sterilized serum bottles and closed with neoprene septa. Soils were incubated to undergo sequential reduction of terminal electron acceptors. Nitrification potential of a tropical vertisol saturated with water was estimated during sequential reduction of NO3-, Fe3+, SO42- and CO2 in terminal electron accepting processes (TEAPs). Concentration of electron acceptors were estimated by wet chemical methods. Nitrifying bacteria and archaea were enumerated by real time PCR targeting amoA genes. Results: In general the terminal electron accepting processes (TEAPs) enhanced the potential nitrification rate (PNR) of the soil. Nitrification was highest at Fe3+ reduction followed by SO42- reduction, NO3- reduction and lowest in unreduced control soil. Observed PNR (µg NO3- produced per g soil day-1) varied from 0.23 to 3.12. Multiple regression models predicted PNR as 0.303 µM NO3- + 0.661 µM Fe2+ - 0.629 µM SO42- - 0.074 µg CH4. Predicted PNR correlated significantly with the observed PNR. Electron donor Fe2+ stimulated PNR, while S2- inhibited it significantly (p < 0.05). Terminal restriction fragment length polymorphism (TRFLP) targeting amoA gene of ammonia oxidizing bacteria (AOB) and ammonia oxidizing archaea (AOA) highlighted population dynamics during the sequential reduction of terminal electron acceptors. Relative abundance of AOA varied significantly with the TEAPs. However, the relative abundance of AOB did not vary significantly with TEAPs. Relative abundance of AOB correlated with NO3- and Fe2+. Conclusions: Based on this study, the anoxic-oxic condition of soil as predicted by IPCC is likely to enhance nitrification in the tropical vertisol. High nitrification may aggravate nitrogen loss through N2O emission which may complicate the problems affecting agriculture, climate and environment.
Abstract Title:
Flocculating Performance of A Bioflocculant Produced by “Arthrobacter Humicola” in Sewage Wastewater Treatment

Primary Author Block:
M. O. Agunbiade1, C. P. Albertyn1, E. V. Heerden1, A. O. T. Ashafa2; 1Univ. of the Free State, Bloemfontein, South Africa, 2Univ. of the Free State, Puthaditjabha, South Africa

Abstract Body:
Background: The discharge of poorly treated effluents into the environment has far reaching, consequential impacts on human and aquatic life forms. We report for the first time the ability of a biopolymeric flocculant produced by Arthrobacter humicola in the treatment of sewage wastewater.

Methods: This strain was isolated from sediment soil sample at Sterkfontein Dam in the Eastern Free State province of South Africa. Kaolin clay suspension was used to validate the flocculating efficiency of the test organism and 16S rDNA gene sequencing was done to confirm the identity of the positive bioflocculant. The evaluation of the functional group present in the purified bioflocculant was performed using Fourier transform infrared spectroscopy (FTIR) while Thermogravimetric analyzer (TGA) was used to monitor the pyrolysis profile of the positive bioflocculant. Results: Basic Local Alignment Search Tool (BLAST) analysis of the nucleotide sequence of the 16S rDNA revealed the bacteria to have 99% similarity to Arthrobacter humicola strain R1 and the sequence was deposited in the Gene bank as Arthrobacter humicola with accession number KC816574.1. Flocculating activity was enhanced with the aid of divalent cations, pH 12, at a dosage concentration of 0.8 mg/mL. The purified bioflocculant was heat stable and could retain more than 78% of its flocculating activity after heating at 90°C for 25 min. Fourier transform infrared spectroscopy analysis demonstrated the presence of hydroxyl and carboxyl moieties as the functional groups. The thermogravimetric analysis confirmed the stability of the purified bioflocculant and elemental composition analysis revealed C: O: P: Na: K with 13.90: 41.96: 26.79: 16.61: 0.74 weight percentage respectively. The purified bioflocculant was able to remove chemical oxygen demand, biological oxygen demand, suspended solids, nitrate and turbidity from sewage wastewater at efficiencies of 65.7%, 63.5%, 55.7%, 71.4% and 81.3% respectively. Conclusions: The results of this study indicate the possibility of using the bioflocculant produced by Arthrobacter humicola in sewage wastewater treatment and other industrial wastewater.
Abstract Title:
Detection and Quantification of Comammox Bacteria in Nitrogen Removal Sys. from Wastewater Treatment Plants
Primary Author Block:
I. Cotto1, K. J. Vilardi1, W. O. Khunjar2, C. Wilson3, H. De Clippeleir4, A. J. Pinto1; 1Northeastern Univ., Boston, MA, 2Hazen and Sawyer, Fairfax, VA, 3Hampton Roads Sanitation District, Virginia Beach, VA, 4District of Columbia Water and Sewer Authority, Washington, DC
Abstract Body:
Until recently, it was thought that aerobic nitrification was an obligate two-step process carried out by two microorganisms, i.e. ammonia-oxidizing archaea (AOA) or bacteria (AOB) and nitrite- oxidizing bacteria (NOB). Nevertheless, recent studies have discovered the existence of microorganisms able to accomplish complete ammonia oxidation to nitrate, i.e. complete ammonia oxidizing (comammox - CMX) bacteria. Findings from these studies indicate CMX bacteria are adapted to low ammonia dissolved oxygen environments and prefer the biofilm niche. This suggests that CMX bacteria can exhibit a significant impact on the process performance and economic feasibility of nitrogen removal systems in the wastewater industry. The overall goal of this project is to develop methods to allow for their rapid and reliable quantification, and to utilize these methods to monitor their temporal dynamics in a range of nitrogen removal systems. Samples are being collected on a monthly basis at wastewater treatment plants that rely on three fundamentally different nitrification approaches, i.e. two-step nitrification-denitrification, simultaneous nitrification-denitrification, and partial nitritation-annamox. DNA extractions are being performed using the DNAeasy PowerSoil Kit by Qiagen, automated with the QIAcube instrument. Currently available primers are being tested for CMX bacteria quantification by targeting the ammonia monooxygenase subunit A (amoA) gene. PCR assays using publically available primers have been optimized to quantify total bacteria (16S rRNA gene), AOB (16S rRNA and amoA genes) and Nitrospira (16S rRNA gene) abundance from fifteen full-scale nitrogen removal systems. While protocols for detection of total bacteria, AOB and Nitrospira targeting the 16S rRNA gene, and AOB targeting the amoA gene are completely optimized, unspecific product formation has been the major issue in the PCR optimization effort of the currently available CMX bacteria primers. Preliminary results, using currently available primer sets, suggest the presence of CMX bacteria in at least two nitrogen removal systems. Since CMX bacteria belong to the Nitrospira genus, the abundance of Nitrospira over AOB suggests that CMX bacteria could be present in that system. Consequently, samples with low AOB:NOB ratio will be considered for metagenomics analyses to obtain a primer bias-free analyses of CMX bacterial population in sampled systems, followed by design of new primers for the qPCR based quantification.
Abstract Title:
Bacterial Diversity Impacts As A Result of Combined Sewer Overflow in A Polluted Waterway

Primary Author Block:
O. Calderón1, H. Porter-Morgan1, J. Jacob1, W. Elkins2; 1LaGuardia Community Coll., Long Island City, NY, 2Newtown Creek Alliance, Sunnyside, NY

Abstract Body:
Newtown Creek is an industrial waterway and former tidal wetland in New York City. It is one of the most polluted water bodies in the United States and was designated as a Superfund site in 2010. For over a century, petroleum, heavy metals, and other forms of industrial pollution have disrupted the creek’s environment. The creek also is impacted by discharges from twenty combined sewer overflow pipes, which may deposit raw sewage or partially treated wastewater directly into the creek during heavy or sustained rain events. Combined sewer overflow events and associated nutrient over-enrichment at the creek drive eutrophication and subsequent hypoxia. Three sites were sampled one week apart during a dry period and a wet period, where indication of a combined sewage overflow event could be detected. 16s rRNA high-throughput sequencing from these three sites collectively yielded over 1000 species of bacteria belonging to twenty-two classes. Based on these data, it is hypothesized that differences identified in the microbiome on wet versus dry days are as a result of combined sewage overflow, street runoff, and additional fluctuations in the creek’s environment associated with rain. Species found in Newtown Creek include pelagic, marine, human and animal pathogens, hydrocarbonoclastic, and other environmental microbes.
Session Number: 261
Session Type: Poster
Session Number: 261
Session Type: Poster
Session Title: MEE02 - Biogeochemical Processes and Systems Biology: Biogeochemical Processes
Session Start Date Time: 6/9/2018 11:00:00 AM
Session End Date Time: 6/9/2018 1:00:00 PM
Session Primary Track: Microbial Ecology and Evolution
Abstract Control Number: 7219
Poster Board Number: SATURDAY - 968

Abstract Title:
Is Heavy Metal Bad for Your Biomass? Consequences of Metal Exposure to Bacterial Stoichiometry Upstream and Downstream from A Superfund Site

Primary Author Block:
K. Harrington, B. A. Wolff, M. P. Manzella, E. K. Hall; Colorado State Univ., Fort Collins, CO

Abstract Body:
Metal contamination is the most prevalent stress to freshwater ecosystems in Colorado and in large parts of the North American Mountain West. The Upper Arkansas River near Leadville CO, a high elevation headwater ecosystem, has been a site of heavy metal contamination since the mid-1800s and the site of concerted environmental restoration for the past 20 years. Due to intensive restoration efforts the benthic invertebrate communities upstream and downstream of the metal contamination now have similar levels of taxonomic richness, however the composition of both communities remain markedly different. Traditional analyses of metal-contaminated streams have relied exclusively on measuring direct exposure of benthic invertebrates to dissolved metals in the laboratory. However, multiple lines of evidence indicate that dietary exposure is more important for determining toxicity of metals to primary consumers. To address the potential for differences in microbial biomass to affect the invertebrate community composition we sampled benthic microbial communities at multiple sites upstream and downstream of the point source for metal contamination. We analyzed bacterial community and biomass composition from each location. From each site we isolated ~ 300 bacterial strains on a minimal media containing no metals, with copper (Cu), with zinc (Zn), and with Cu and Zn. We chose a subset of bacterial isolates that were phylogenetically similar, but grew on media both with and without metals. For each isolate x media combination we compared differences in biomass phenotypes (i.e. biomass C:N:P stoichiometry and metal content). Environmental communities upstream and downstream of the metal contamination site differed in community composition, as determined by 16S amplicon sequencing. In addition, downstream microbial biofilms were enriched in Zn and Cu relative to upstream microbial biofilms. Bacterial isolates grown in the presence and absence of Cu and Zn differed in both biomass stoichiometry and metal content. Our experiment suggests that microbial biomass composition (both C:N:P stoichiometry and heavy metal content) changes in response to metal exposure. This change in biomass composition alters the quality of the microbial biomass as a food resource for benthic invertebrates. Changes in microbial biomass phenotype in response to environmental stress have the potential to alter important aspects of ecosystem structure through previously unconsidered pathways.
Session Title: MEE02 - Biogeochemical Processes and Systems Biology: Biogeochemical Processes
Session Start Date Time: 6/9/2018 11:00:00 AM
Session End Date Time: 6/9/2018 1:00:00 PM
Session Primary Track: Microbial Ecology and Evolution
Abstract Control Number: 4625
Poster Board Number: SATURDAY - 969

Abstract Title:
Metaproteomics Reveals A Novel Betaproteobacterium with Roles in Metal and Nitrogen Cycling in the Deep Subsurface

Primary Author Block:
N. Szeinbaum1, C. Henny2, S. A. Crowe3, F. J. Stewart1, T. J. DiChristina1, C. T. Reinhard1, B. L. Nunn4, J. B. Glass1
1Georgia Inst. of Technology, Atlanta, GA, 2Indonesian Inst. of Sci., Cibinong, Indonesia, 3Univ. of British Columbia, Vancouver, BC, Canada, 4Univ. of Washington, Atlanta, GA

Abstract Body:
Background: Microbial genomes assembled from deep subsurface environments are revealing novel respiratory pathways distinct from those of surface-dwelling species. The advent of metaproteomics has enabled detection of novel genes expressed under diverse conditions from microbes that resist cultivation attempts. We evaluated the metaproteomic profile of a sediment microbial community enriched over ~1 year with Mn3+ as electron acceptor. Methods: Deep sediments from Lake Matano, Indonesia were sampled under anoxic conditions, and inoculated into minimal media containing soluble Mn3+-pyrophosphate as sole electron acceptor, methane as sole electron donor, and ammonium as sole nitrogen source (0.2 mM). Cultures were incubated in the dark at 30°C, and diluted 1:10 for three transfers. Changes in community taxonomic composition were monitored by 16S rRNA gene amplicon sequencing. Metagenomic and metaproteomic sequencing was performed on total DNA and protein recovered from the third transfer on day 335. Shotgun proteomics were completed in technical triplicates on a high accuracy tandem mass spectrometer, and spectral counts were used to determine relative quantification of proteins (Nunn, Slattery et al. 2015). Results: Relative quantification of shotgun metaproteomic data revealed that cultures were dominated by protein sequences affiliated with a new species of Dechloromonas (Betaproteobacteria), for which we propose the name “Candidatus Dechloromonas occultata” [occultata (L. adj.), hidden]. “Ca. D. occultata” expressed an uncharacterized cytochrome-c-rich operon containing NHL-repeat proteins and an extracellular undecaheme protein also present in other Betaproteobacteria from metal-rich ecosystems, including uncultivated Gallionellales and Rhodocyclales from the deep subsurface, and the Fe2+- and Mn2+-oxidizer Leptothrix cholodnii SP-6. Paradoxically, the enzymatic machinery for denitrification was among the most expressed proteins despite the lack of nitrogen oxides in the media. A hemolysin-type Ca2+-binding domain protein was also highly expressed. Conclusions: The metabolic potential recovered from metaproteomes thus implicates “Ca. D. occultata” and by extension possibly other Betaproteobacteria like deep subsurface Gallionellales and Rhodocyclales, in oxidative metal and reductive nitrogen metabolisms. Delineating the extent to which these metabolisms can be coupled within “Ca. D. occultata” and other Betaproteobacteria represents an important opportunity for future research.
Abstract Title:
Developing Engineered Membrane Transporters to Capture Radioactive Cs from Water

Primary Author Block:
S. Hakim Elahi, O. Conroy-Ben; Arizona State Univ., Tempe, AZ

Abstract Body:
Background: One of the most significant radioactive isotopes of cesium is 137Cs. 137Cs has been identified in ground water, surface water and in many types of food including breast milk and pasteurized milk causing serious health problems. Current methods for removing 137Cs include coagulation–flocculation–sedimentation, however, turbidity can significantly affect their efficient removal. Our newly developed method uses bacterial membrane transporters to capture 137Cs. It has been reported that the low affinity potassium transport system, known as Kup encoded by the trkD gene in E. coli, is capable of high affinity cesium transport. In this work, we present our easy, efficient methods developed to purify the intended membrane proteins and insert them into the liposomes to make an impeccable engineered vesicle called proteoliposomes. Methods: Extraction and purification methodologies were optimized to obtain pure protein from E. coli. For this, two different methods, one including PMSF, DNase and lysozyme and the other including no chemicals but cell sonication and membrane solubilization were compared; all the pellets and supernatants obtained from each step of centrifugation, were also purified and run on the gel to check for the sharpest bands. Large unilamellar vesicles (~200 nm) were then synthesized and proteins were inserted into the liposome, creating a proteoliposome as a tool for capturing 137 Cs. Verification of the reconstitution was done using sucrose density gradient centrifugation (9%-37%) as well as Cryo-transmission electron microscopy imaging.

Results: Supernatant samples obtained from the second extraction method formed less unspecific bands according to SDS-PAGE images. Optimized concentrations of imidazole to elute Kup from the nickel column found to be 50 & 1000 mM in wash and elution buffer, respectively. SDS-PAGE indicated that over-expressed trkD was present at the expected molecular weight of 69.3 kDa. Using HEPES-KOH, DTT buffers and Triton X-100, total 3-hr 30-min of sample incubation and 1-hr centrifugation at about 450,000 rpm at 4 0C, proved to give the best proteoliposome formation. Cryo-TEM imaging also confirmed the presence of the target protein in the liposome vesicles. Conclusion: By using these engineered vesicles, we learn more about the behavior and uptake mechanism of bacteria membrane transporters in the presence of nuclear waste contaminants. Subsequently emerging scientific contribution to water and wastewater treatment industry can be made.
Abstract Title:
Bacterial Leaching of Low Grade Manganese Ore

Primary Author Block:
A. P. Das; Tripura Univ., Agartala, India

Abstract Body:
Background: Bioleaching technology aims on accomplishing efficient recovery of minerals and metals from their solid waste residues using microbial techniques. Bacterial bioleaching of low grade manganese ores was investigated in this present investigation. Methods: Three bacterial strains were screened for their bioleaching efficiency of low grade Mn ore collected from ferromanganese mines of Keonjhar, Odisha, India. A single bacterial strain, Bacillus species (GenBank Accession Number: JQ936966) was selected which demonstrate elevated efficiency of Mn leaching. Results: The selected bacterium was used for bioleaching of low grade manganese ore under the optimized effect of Particle size (-150μm size), Pulp density (2%), Sucrose concentration (10g/100ml), Initial pH 6, Incubation temperature 320C and duration of 20days. The maximum bioleaching efficiency of manganese obtained was 68 % with Bacillus species. Conclusions: This result proposes the potential application of economic recovering of Mn from mining waste residues.
Tile Drainage and Anthropogenic Land Use Contribute to Harmful Algal Blooms and Microbiota Shift in Inland Water Bodies

Primary Author Block:
I. Mrdjen1, J. Lee1, S. Fennessy2, J. Slonczewski2, A. Schaal2, R. Dennis2; 1The Ohio State Univ., Columbus, OH, 2Kenyon Coll., Gambier, OH

Abstract Body:
Background: Freshwater harmful algal blooms (HABs), resulting from high nutrient inputs from human use of developed lands, have been shown to pose unique risks to human and ecological health worldwide. A major contributor of nutrient content in affected water bodies is agricultural land use, specifically effluent from tile drainage. Small lakes and ponds (SLaPs) are at elevated risk for HAB appearance, as they are uniquely sensitive to nutrient input, and rarely monitored by state agencies. HABs introduce exposure risk to cyanotoxins, specifically microcystins (MC), which are suspected carcinogens and competent liver toxins. Methods: To investigate the impact of anthropogenic land use on SLaPs, 24 sites were sampled over a 3-month period in the summer of 2015. MC concentrations and water chemistry characteristics were determined using ELISA and chemical assays, respectively. Microbial source tracking was utilized to study sources of nutrient intrusion, while microbial community analysis was used to study composition of SLaP microbial communities. Results: The presence of tile drainage systems or animals was significantly correlated with concentrations of microcystin-producing Microcystis aeruginosa throughout the sample set (p = 0.03). The relative abundance of HAB-forming genera was correlated with elevated concentrations of nitrate and soluble reactive phosphate. One location (FC) showed MC concentrations exceeding 875 µg/L and large community shifts in ciliates. FC’s prokaryotic community was dominated by Planktothrix spp. and contained detectable levels of Microcystis spp. at the July 28th sampling point. Conclusions: These results demonstrate the impact of HABs in SLaPs and that prevailing issues extend beyond cyanotoxins, and that further investigation of microbial communities impacted by HABs is necessary.
Estimations of Ghg Fluxes Reveal A Reduction in Methane Consumption During Transformation of Marginal Soils Into Biofuel Crops (Switchgrass, Panicum Virgatum)

Primary Author Block:
C. T. Bates1, J. Kuang1, A. Escalas2, L. Wu1, M. Firestone3, J. Zhou1; 1The Univ. of Oklahoma, Norman, OK, 2Museum Natl. d'Historie Naturelle, Paris, France, 3The Univ. of California, Berkeley, Norman, CA

Abstract Body:
Understanding microbial mediated soil processes in agronomical systems has long been a goal in microbial ecology, but little is known on the dynamics of these processes during the conversion of unproductive marginal soils using auspicious bioenergy crops like Switchgrass (SG; Panicum virgatum L.). This crop is expected to improve soil quality through the net input of carbon during its establishment and continual cultivation, but exactly how SG establishment affects key belowground ecosystem functions is not known. In this study, we hypothesize that the conversion of low yielding grassland into SG fields will impact nutrient cycling and belowground microbial communities, leading to differences in GHG fluxes relative to a native grassland fallow (FL). More precisely, we investigated greenhouse gases (GHG: carbon dioxide, methane, and nitrous oxide) fluxes, soil chemistry and microbial communities structure. Over an 18-month period at 2 sites in southern Oklahoma considered ‘marginal’ for N and P nutrients availability, GHG concentrations were measured, using cavity ring down spectrometry (i.e. Picarro G2038/G2508 analyzer) while bacterial and fungal communities were assessed using amplicon sequencing. Our results indicate significant changes in available phosphate levels, organic matter content, and % soil carbon in the SG treatment. Unexpectedly, SG cultivation induced a significant reduction in methane consumption in both sites indicating a dramatic transformation of the microbial ecology relative to the native fallow. Further investigations are underway to elucidate the link between the microbial communities and the various GHG emissions. Connecting GHG fluxes, soil chemistry, and microbial succession appears promising to improve our understanding of the potential of SG to be not only a viable bioenergy crop but also its ability to positively impact the quality of marginal soils across the southern U.S.
Abstract Title:
Gi-Scanner: An Algorithm to Predict Genomic Islands by Comparative Genomics

Primary Author Block:
Y. Hur, J. Chun; Seoul Natl. Univ., Seoul, Korea, Republic of

Abstract Body:
Background: Genomic islands (GIs) are clusters of genes that are mobile via the mechanisms of lateral gene transfer. Since GIs are known to play important roles in spreading phenotypes such as pathogenicity and antibiotic resistance, detecting them is critical in modern microbiology. As the number of genome sequences in the database increases exponentially, the accurate and efficient method to predict GIs on a large scale is of premium need. Methods: Three sets of Vibrio cholerae genomes were selected and grouped as 29 complete genomes, 23 genomes with high N50, and 17 genomes from the previous study. We generated pairwise reciprocal BLAST hit (RBH) ortholog matrix for each genome sets, and detect clusters of genes not aligned to other genomes among the group. Non-aligned cluster sharing their synteny and orthologs grouped again as possible GI clusters, and the clusters were finally determined as GI following maximum parsimony algorithm. Hidden Markov Model (HMM) profiles were generated for each GI in order to perform a quick search for new query genome. Moreover, to add a new genome into the result instead of the quick search feature, this program calculates only the new genome over the core genome set such as complete genome set, or optionally whole genomes in the result. Results: The GI prediction for 69 genome set ran for about 6 hours on a single server computer, resulting 94 predicted GIs. Out of 74 GIs predicted in the previous study based on manual curation, 72 GIs were successfully recovered. The 94 GIs predicted by our method were compared with those predicted by IslandViwer, a database contains the results of IslandPick, IslandPath-DIMOB, SIGI-HMM, and Islander. Each predicted GI was visualized on web page either single GI view or single genome view with phylogenetic tree and pairwise matrix. As a result, we created an up-to-date database for V. cholerae GIs. Conclusions: The prediction result of Vibrio cholerae shows the impact of GI on microbe and the importance of GI prediction. Gi-Scanner provides relevant GI predictions according to the suitable phylogenetic tree for a genome set, and modifying the genome set is easy to apply on the result since the program reuse previous calculation. Gi-Scanner is a fast and accurate GI predictor in comparative genomics approach, and it helps understanding how microbe rapidly obtains a trait and diversity.
Abstract Title:
Microbial Voltage-Gated Ion Channels
Primary Author Block:
K. Bennett, T-T. Tseng; Kennesaw State Univ., Kennesaw, GA
Abstract Body:
Voltage-gated ion channels (VIC) form a superfamily that is well known for transporting potassium, sodium, and calcium ions in excitable tissues. Ancestral members of this superfamily were thought to transport only potassium ions, unlike modern homologs from eukaryotes, which also transport sodium and calcium ions. The discovery of a sodium channel from Bacillus halodurans had significantly renewed the interest of physiological roles for ion transport in bacterial and archaeal species. Structurally, these bacterial channels shared the same topology with their eukaryotic counterparts while their shared history of evolution remained unresolved. The selectivity filter for ion specificity had a markedly different sequence composition that contradicted the canonical understanding of sodium-selectivity development, despite a highly conserved topology. This study sought out to address the inclusion of novel homologs into the overall evolution of ion-selectivity. An exhaustive similarity search was conducted as the first step on all domains of life to include all sequences that showed similarity to functionally characterized voltage-gated ion channels. Bacterial sequences were compared against other characterized sequences with multiple alignment to confirm the observed similarity to eukaryotic calcium selectivity pores. Over 300 novel homologs were identified from a wide variety of genera, such as Geminocystis, Xanthomonas, and Pseudomonas. Phylogenetic trees were also built from these alignments to show that potassium-selective channels from ancestral bacteria gave rise to a potential calcium-selective precursor in addition to potential functional assignments of putative homologs. This precursor then likely gave rise to sodium selectivity when passed to bacteria and calcium selectivity in eukaryotes. These findings would suggest that sodium specificity arose at least twice in VIC evolution, which provided the explanation for a sodium-selective channel from Bacillus halodurans.
Background: The phylum “candidatus Marinimicrobia” represents a diverse group of uncultivated bacteria that has been identified in marine environments across the globe, and 'omic insights have implicated members of this phylum as prominent contributors to global nitrogen, carbon, and sulfur cycling. Despite the importance of this group to marine environments, the factors shaping the diversity and genomic repertoires of Marinimicrobia remain obscure and are hampered by difficulties in recovering complete genomes from environmental samples and the lack of representative genomes from prominent clades. Methods: To provide insight into the evolutionary genomics of the Marinimicrobia we performed a phylogenomic analysis on a set of >200 available draft genomes from this phylum. Using a multi-locus phylogenetic approach we generated a high-resolution phylogeny of the Marinimicrobia and defined the major clades for which multiple genomic representatives were available. We analyzed trends associated with genome size, %GC content, and proteome nitrogen content and interpreted the results in light of genomic streamlining theories. Moreover, using pan-genomic methods we defined core- and lineage-specific genes for the prominent clades of Marinimicrobia, with an emphasis on functional genes that are markers for biogeochemically-important processes. Results and Conclusions: We found that that fundamental characteristics of Marinimicrobia genomes varied by clade and appeared to be the result of selection due to differing nutrient regimes experienced by these groups. Our pan-genomic analysis supported these findings and pointed to specific forms of metabolism as being associated with streamlined genomes, consistent with previous findings from other marine bacterial clades. Overall, our findings demonstrate that Marinimicrobia are an excellent system for assessing the relationship between ecological factors and genome evolution, and suggest that common selective pressures shape the genomic architectures of prominent bacterial groups throughout the ocean.
Abstract Title:
Whole-Genome Sequencing and Phylogenomic Analysis of “Cryptic” Escherichia Strains Reveals Novel Species- and Subspecies-Level Clades

Primary Author Block:
M. A. Riojas, B. J. Cantwell, M. H. Hazbón; ATCC, Manassas, VA

Abstract Body:
Background: Previously described environmental, animal, and human Escherichia isolates were found to be monophyletic and form distinct clades but were referred to as “cryptic” because they were found to be phenotypically indistinguishable from representative E. coli strains. Methods: The whole-genome sequence of 89 Escherichia strains deposited with BEI Resources was obtained via Illumina MiSeq (v2 2x250 cycle flow cell) and de novo assembled using CLC Genomics Workbench. These genomes were compared to 11 type/reference strains from Escherichia and closely related species in GenBank using digital DNA-DNA hybridization (dDDH) via the Genome-to-Genome Distance Calculator (GGDC) 2.1. Results: A phylogenomic analysis of these 100 samples shows the existence of distinct clades that indicate both multiple novel subspecies of E. coli and novel species of Escherichia. Of the 89 strains, 21 clade at the subspecies level with the type strain of E. coli, 45 clade at the subspecies level with both E. coli K-12 and the type strain of Shigella flexneri, 10 form a distinct subspecies-level clade, and two strains each form an additional single-strain clade. Four strains (which had previously been suggested to represent a subspecies-level clade using multilocus sequence typing) show that they form a species-level clade that likely represents a novel species very closely related to but distinct from E. coli. Two strains clade into two different subspecies of the same novel species. The remaining five strains clade with the type strains of existing species, E. albertii (2), E. marmotae (2), and E. fergusonii (1). Conclusion: This work shows that at the genomic level, these cryptic strains form cohesive and distinct phylogenomic clusters. Thus, these strains are sufficiently distinguishable from existing taxa that they warrant possible formal reclassification into novel species and/or subspecies.
Abstract Title:
A New Activation Mechanism of Silent Genomic Islands Mediated by Integrative and Conjugative Elements

Primary Author Block:
P. Wang; South China Sea Inst. of Oceanology, Guangzhou, China

Abstract Body:
Genomic islands (GIs) are very abundant on prokaryotic genome and represent one of the most intraspecies diversity; however, many of them are silent or have a low expression in planktonic cells. How and when these GIs are activated remains largely unknown. The GIs integrated into the yicC locus can be classified into at least three subclasses according to the conserved core combined with the evolutionary relationship of the integrase. In this study, GIPsp11900 and GIPspSM9913 from two Pseudoalteromonas strains isolated from two distinctive marine environments were selected as the representative GIs of two extensively existed subclasses to investigate the mobilization of GI. We revealed that the subclass of GIs represented by GIPsp11900 could be activated by integrative and conjugative elements (ICEs). Furthermore, in addition to the excision and transfer of GIPsp11900, SXT/R391 ICE-encoded transcriptional regulator SetCD also can upregulate the replication of GIPsp11900, thereby increasing the doses of GIPsp11900-encoded genes. These GIs and SXT/R391 ICEs are widely spread in marine Gammaproteobacteria and the transfer of GI by SXT/R391 ICEs could be triggered by environmental cues during stressed conditions. We also demonstrated that GIPsp11900-encoded genes can defense against the intrusion of foreign DNA. Such action suggested that transcriptional activator SetCD encoded by SXT/R391 ICEs could function as a signal to activate the GIs to mediate horizontal gene transfer. In contrast, SXT/R391 ICE could not activate the GI integrated in yicC in SM9913, another subclass of GIs represented by GIPspSM9913. This study illustrates that genomic islands accessorize the core genome and play important roles in shaping bacterial genome structure, and bacteria can utilize mobile genetic element such as ICE to mediate the expression of genes carried by these genomic islands.
Abstract Title:
Title: Reconstructing the History of Genomic Island Insertions in Clades of Microbes Using Xenogi
Primary Author Block:
E. C. Bush, D. M. Stoebel; Harvey Mudd Coll., Claremont, CA
Abstract Body:
Microbes have acquired many important traits through the horizontal transfer of genomic islands. Understanding these traits often requires us to understand their history. Existing methods for detecting genomic islands are most suited to recognizing islands that are present in one strain and absent in others. Thus they are not ideal for studying the history of genomic island insertions in an entire clade of microbes. Here we describe xenoGI, a software package that is able to reconstruct this history. Taking a set set of sequenced genomes and a phylogenetic tree as input, it identifies genomic islands and automatically determines the branch on which they inserted. To do this, the package creates gene families in a way that takes account of both the species tree and synteny information. It then identifies families whose members are adjacent and whose most recent common ancestor is shared, and merges them into islands reflecting a common origin. We demonstrate the capabilities of the package with an example from enteric bacteria. gadB is a glutamate decarboxylase enzyme known to be involved in acid tolerance in E. coli. In an analysis of eleven enteric species, xenoGI finds that gadB is part of an island of eight genes that inserted on the branch leading to Escherichia, before the divergence of E. fergusonii.<br />
In addition we use output from simulations and a set of known genomic islands from the literature to show that xenoGI can accurately identify genomic islands and place them on a phylogenetic tree. The package can be downloaded for free and is also available as a web service.
Abstract Title:
Large Tandem Duplications are Frequent in Microbial Genomes But Go Undetected in Genome Assemblies

Primary Author Block:
K. B. De Leon1, T. R. Juba1, G. M. Zane1, V. V. Trotter2, D. Ning3, J. Z. Zhou3, G. P. Butland2, J. D. Wall1;
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Abstract Body:
Large, multi-gene tandem duplications within chromosomes of prokaryotic cells occur frequently but are generally unstable. Homologous recombination between the two copies would either result in a loss of the duplication or further gene amplification. Unless this increased copy number of genes provides a fitness benefit that allows the cell to outgrow the rest of the population, it is not maintained. Thus, these gene amplifications are considered rare at the population level. However, gene copy number is a critical consideration during strain construction by genetic manipulation and their identification within published genome sequences may be underestimated because these tandem copies would be problematic for genome assembly programs. The only unique sequence that differentiates a tandem array of identical genes from a single copy of genes is at the junction of the repeated segment that may be interpreted as sequence errors or a chimera. Analysis of coverage discrepancies across the genome would identify regions of increased coverage that could be due to gene amplification and further analysis would identify the junction sequence, but this is not traditionally described in post-assembly processing methods. For 1489 unique bacterial and archaeal taxa in the NCBI Database, raw sequences were mapped to their corresponding genome sequence and the resulting assembly met the quality criteria of an average coverage >50x and a standard deviation less than 1.5x the average. Of these genomes, 67% contained regions >1500 bp with >1.5x the average coverage. These discrepancies could be due to gene amplification, a lysogenic virus becoming lytic, or complications in sequencing and are being explored. For one organism, Desulfovibrio vulgaris Hildenborough (DvH), a 7.7 Kb duplication was identified and verified by PCR. Of five laboratories that have contributed to the 28 available raw sequence datasets, two have lost and three have maintained the duplication. Within this duplication is cytochrome d ubiquinol oxidase (DVU3270-1). These genes have previously been shown in DvH to provide resistance to exposures to low concentration of oxygen. Strains containing two (wild-type), one, and no copies of these genes have been generated to determine whether gene copy number further contributes to resistance to oxygen. The frequency of these discrepancies in genomes exposes a problem in the current pipeline for genome assembly that could easily be remedied with minor post-assembly assessment.
Large Scale Analysis and Visualization of the Diversity of Bacteria

Abstract Body:
The threat of food-borne illness has widespread effect on every part of the supply chain as well as government and healthcare agencies. Even with the industry’s best practices, today’s testing methods remain limited and outbreaks of foodborne illness still regularly occur, costing the US economy billions of dollars every year. We are currently developing technology in order to harvest and sequence the DNA and RNA of simple food samples to determine hazards based on the organisms present and their metabolic activity. However, bacteria are the most diverse organisms on the planet and capturing and analyzing that diversity is essential towards these goals. With the decrease in cost and increase in accessibility of high-throughput sequencing, the number of bacterial genomes that have been sequenced has escalated rapidly. The sheer amount of data (there are >100,000 genomes in GenBank as of Nov 2017) presents two major problems: many methods of comparison do not scale with the data and genomes from various sources of various qualities can be difficult to compare fairly. To overcome these difficulties, we have developed new pipelines that assemble genomes, measure whole genome-genome distance, and created new tools to visualize the results. The power of these methods allow us to analyze many hundreds of thousands of raw sequencing datasets.
Abstract Title:
From Raw Reads to Trees: Whole Genome Single Nucleotide Polymorphisms Phylogenetics Across the Tree of Life

Primary Author Block:

Abstract Body:
Next generation sequencing is increasingly being used to examine both genetic diversity and relationships among closely related organisms or cell lines. However, while genome-wide single nucleotide polymorphisms (SNPs) provide an excellent resource for phylogenetic reconstruction, to date, evolutionary analyses have been performed using different ad hoc methods that are not often widely applicable to organisms across tree of life. While some groups may use methods not widely applicable to other organisms/datasets, other groups routinely fall back on older methods, selecting certain genomic regions corresponding to genes (as with Multi Locus Sequence Typing trees), to accomplish a less refined tree. To facilitate the construction of robust phylogenies using all discernable SNPs among a suite of genomic datasets from organisms across tree of life, we have developed a comprehensive tool for genome-wide identification/characterization of SNPs directly from raw reads, genome assemblies, and complete genomes. Our phylogenetic and molecular evolutionary (PhaME) analysis software is unique in its ability to take raw reads and draft/complete genome(s) as input, derive core genome alignments, identify SNPs, construct phylogenies, and perform evolutionary analyses. Here, we show several examples using genomes, contigs, metagenomes, and raw reads of bacterial, eukaryotic and viral origin to demonstrate the broad and robust functionality of PhaME.
Abstract Title:
MiGA - the Microbial Genomes Atlas: Expanding the Catalogued Diversity of Archaea and Bacteria

Primary Author Block:
L. M. Rodriguez-R1, S. Gunturu2, C. Jain1, J. Tiedje2, J. Cole2, K. T. Konstantinidis1; 1Georgia Inst. of Technology, Atlanta, GA, 2Michigan State Univ., East Lansing, MI

Abstract Body:
The use of small subunit ribosomal RNA gene (16S rRNA) sequencing has successfully enabled the large-scale cataloguing of prokaryotic species diversity but offers limited resolution typically at the genus or species level, and information on gene content and other genomic features can be predicted only when representative genomes are available in the databases, and not always reliably so. These limitations have been circumvented with advances on metagenomics techniques but it remains computationally challenging to explore the newly uncovered diversity of the geometrically increasing number of recovered prokaryotic genomes. Here, we present The Microbial Genomes Atlas (MiGA), a genomic data management and processing tool integrating best practices in genomic analyses with recent and novel developments in whole-genome-based taxonomy and classification. MiGA features an indexing system based on medoid clustering over sparse matrices of Average Nucleotide and Amino Acid Identity (ANI/AAI) guided by heuristic approximations, enabling the fast classification of query genomes. The recent development of FastANI, a k-mer based estimator of ANI now integrated in MiGA (Jain et al., 2018), has allowed us to explore the distribution of ANI values among more than 90 thousand complete and draft genomes. This distribution revealed a pronounced valley in the ANI range 83-95%, indicating that the genetic discontinuity previously observed with smaller sets of genomes, reflective of the existence of discrete species, is maintained in larger scales regardless of taxonomic diversity or historic sequencing trends. Moreover, the taxonomy utilities of MiGA allowed us to compare the ANI values with the existing taxonomic assignments. The upper boundary of this range (ANI 95%) consistently reflected the standing nomenclature of species in this large-scale collection (accuracy > 98%). The availability of this indexed set of genomes, as well as other sets including metagenome-assembled genomes from various large-scale projects, and high-quality curated collections like RefSeq and RefSoil allows the rapid search for close relatives of any complete or draft query genomes as well as a robust AAI-based taxonomic classification. MiGA has a web interface easy to use available through http://microbial-genomes.org/.
Abstract Title:
A Reference Phylogeny of More Than 10,000 Microbial Genomes

Primary Author Block:
Q. Zhu1, U. Mai1, S. Janssen1, W. Pfeiffer2, J. G. Sanders1, F. Asnicar3, G. A. Al-ghalith4, E. Kopylova1, D. McDonald1, Z. Z. Xu1, T. Koscielak1, S. Podell5, J. T. Morton1, D. Knights4, N. Segata3, L. Smarr1, S. Mirarab1, R. Knight1; 1Univ. of California, San Diego, La Jolla, CA, 2Univ. of California, La Jolla, CA, 3Univ. of Trento, Povo, Italy, 4Univ. of Minnesota, Minneapolis, MN, 5Scripps Inst. of Oceanography, Univ. of California, San Diego, La Jolla, CA

Abstract Body:
Exponential growth of microbial genome and metagenome data availability imposes both demands and challenges on defining and resolving evolutionary relationships among microbes. Taxonomy is a commonly used but inaccurate approximation of these relationships, due to limited hierarchies, biased representation of living species, and frequent inconsistencies with the actual evolutionary patterns. A reference phylogeny that fully and impartially covers known biodiversity is essential to overcome these issues. In this study, we expanded the “tree of life” to over 10,000 bacterial and archaeal genomes, covering more than 140 phyla, many of which represent poorly studied organisms. The genomes were systematically sampled from 86,200 available reference genomes, using a novel approach that statistically maximizes the diversity of the selected subset. Up to 400 universal marker genes were sampled from across the genomes for phylogenetic reconstruction. Importantly, in building the phylogeny, we were able to use a gene tree summarization approach called ASTRAL, which accounts for gene tree discordance, as well as the traditional concatenation approach. The use of summary methods at this scale is unprecedented. We further inferred putative horizontal gene transfer events among clades of the tree using complementary approaches. The resulting phylogeny was then systematically evaluated against existing classification systems and phylogenetic trees, yielding new knowledge about microbial evolution. The workflow is made automatic without the need for manual curation, permitting easy updating along with future expansion of microbial genome data. We developed protocols that allow this phylogeny and its associated genome catalog to be easily incorporated into popular metagenomics applications, including the assessments of community composition and ecology and the comparison of genome contents. We further demonstrated that improved resolution and novel insights can be achieved when using phylogeny instead of taxonomy. This study significantly expands the knowledge of microbial evolution, and adds a comprehensive and generally useful resource to the toolkit of the microbial research community.
Session Number: 262
Session Type: Poster
Session Number: 262
Session Type: Poster
Session Title: MEE04 - Comparative and Evolutionary Genomics: Methods and Resources
Session Start Date Time: 6/9/2018 11:00:00 AM
Session End Date Time: 6/9/2018 1:00:00 PM
Session Primary Track: Microbial Ecology and Evolution
Abstract Control Number: 7163
Poster Board Number: SATURDAY - 986

Abstract Title:
Adaptive Lab. Evolution: An Example of Reproducibility of the Genotype-Phenotype Relationship
Primary Author Block:
S. B. Carmona, A. Escalante; Inst. de Biotecnología, Cuernavaca, Morelos, Mexico
Abstract Body:
A short-time evolution experiment to improve growth on glucose of an Escherichia coli strain lacking phosphotransferase system (PB11, µ=0.17 h⁻¹) was performed, obtaining in barely 100 h a mutant (PB12), which recovered in 250% the growth rate. To determine how reproducible the genotype-phenotype relationship is, and to study the evolutive landscape of the PB11 strain, three independent ALE experiments from this strain were performed with interesting results. The occurrence of spontaneous mutants was monitored, and those with higher growth rate were characterized and sequencing. Variant calling was performed and singles and doubles mutants of identified mutated genes were constructed in the PB11 strain, to evaluate its role in the adaptive process. Selected strains showed growth increases among 172% and 253%. We know that PB12 strain has a 10 Kb chromosomal deletion that include galR and rppH genes and we observe again such deletion in one of the triplicates; however, the resulting fitness is lower compared to strains that conserve the fragment. Mutations in galR gene were observed in all experiments, indicating the action of a selection pressure over this gene. galR gene does not have a direct effect on the growth rate, however has a contribution on the fast growth in combination with others mutations. New beneficial mutations in genes as rng and gatC, that contribute greatly to recovery the fast growth on glucose were found. rng gene is involved in the mRNA degradation; to know more about the role of this gene in the metabolism, we are working in RTqPCR experiments and at this time, we had identified overexpression of genes that could be transporting glucose. Fast evolution of the PB11 strain was confirmed. We detected mutations in galR and rng genes and the loss of the 10 kb fragment in different experiments at 54 h, this is after 14 generations; from this time we observed an enrichment of this mutations on the population. We are studying why or how, this occurred. This study show the adaptive capacity of PB11 strain, this strain can follow several strategies to reach certain objective. We observed a degree of reproducibility in the adaptive process, which indicates the action of natural selection as result of a selective pressure. The characterization of selected strains allow us to identify relevant genes involved in the adaptive process to grow in glucose, what in turn raises the possibility to select new strains with better capabilities for growth and with relevant characteristics for metabolites production.
Abstract Title:
Libra: Massive Comparative Metagenomics Using Hadoop Mapreduce

Primary Author Block:
B. Hurwitz, I. Choi, J. Hartman, A. Ponsero, M. Bomhoff; Univ. of Arizona, Tucson, AZ

Abstract Body:
Background: Technological innovations in ‘omics sequencing technologies are changing the way scientific research is done. In particular, reductions in cost and increases in sequencing capacity make it possible to generate, aggregate, and share ‘omics datasets that are terabytes and even petabytes in size. This new capacity permits a complete analysis of microbes (from dominant to rare) to examine microbial interactions from the Earth to Human Microbiome. These methods are fundamental to understanding microbial populations given that >99% of environmental microbes fail to culture. Yet, despite capabilities to sequence uncover novel microbes, most bioinformatics methods focus on the mapping sequences to known genomes, neglecting the uncultured majority. To overcome these issues, comparative metagenomic approaches that examine the genetic composition between samples are being developed. The challenge is that all-vs-all sequence comparison to elucidate these relationships remains difficult and computationally intractable.

Methods: Here we describe a scalable algorithm for comparative metagenomics called Libra that is capable of performing an all-vs-all sequence analysis on hundreds of metagenomes in a Hadoop big data framework. Libra also employs a vector space model to consider genetic distance and microbial abundance simultaneously that is fundamental to differentiating communities of microorganisms.

Results: We demonstrate Libra’s capabilities using simulated metagenomic datasets and compare to similar tools including Mash and SIMKA. We then examine Libra’s sensitivity in distinguishing mixed microbiome using a dataset comprised of controlled binary mixtures of bacteria that vary by phylogenetic distance and abundance. We compare these results to Mash and SIMKA to examine the effect of data reduction techniques and distance metrics. Next, we show Libra’s ability to distinguish metagenomes by both community composition and abundance using 48 samples (16S rRNA and WGS) from the human microbiome project (HMP) across diverse body sites, and compare the results to Mash. Finally, we show that Libra can scale to massive global-scale datasets by examining viral diversity in 43 Tara Ocean Viromes (TOV) from the 2009-2011 Expedition that represent 26 sites containing about 4.2 billion reads.

Conclusions: The resulting data demonstrate that Libra provides accurate, efficient, and scalable compute for comparative metagenomics that can be used to discern global patterns in microbial ecology.
Abstract Title:
The Effect of Personal Care Ingredients on the Attachment of Viruses to A Surface
Primary Author Block:
Abstract Body:
Non-enveloped viruses, such as Norovirus, Adenovirus, Coxsackievirus, and Rotavirus are causative agents of serious disease in humans, however they can be difficult to study due to the need to culture in mammalian cell lines. However, bacteriophages PhiX 174 and MS2 are widely accepted as surrogates for the mammalian viruses and they can be cultured with relative ease. Previous work has demonstrated that PhiX 174 and MS2 are useful models for detecting the attachment of viruses to surfaces. This study describes the effect of 34 personal care ingredients on the attachment of bacteriophage to a surface. A 96-well microplate-based assay was used to quantitate the attachment of the bacteriophage to untreated and treated MBECTM (Innovotech, Edmonton, Canada) polystyrene pegs. Bacteriophage were then harvested from the MBECTM pegs and quantified via plaque assays. PhiX 174 and MS2 were propagated in the appropriate host (Escherichia coli ATCC 13706 and Escherichia coli ATCC 15597, respectively). Of the 34 ingredients screened, 31 ingredients significantly decreased the attachment of PhiX 174, whereas only 19 significantly decreased the attachment of MS2. Seventeen ingredients significantly decreased the attachment of both bacteriophage tested and one ingredient (methyl hydroxyethyl cellulose) significantly increased the attachment of both bacteriophage. None of the compounds tested had an antimicrobial effect on the bacterial hosts. This study demonstrates that personal care ingredients have an effect on how viruses interact with and attach to surfaces.
Session Number: 263
Session Type: Poster
Session Number: 263
Session Type: Poster
Session Title: MEE05 - Ecology and Evolution of Viruses and Phage
Session Start Date Time: 6/9/2018 11:00:00 AM
Session End Date Time: 6/9/2018 1:00:00 PM
Session Primary Track: Microbial Ecology and Evolution
Abstract Control Number: 6228
Poster Board Number: SATURDAY - 989

Abstract Title:
Carriage and Exchange of Temperate Bacteriophages Genomes Within the Chronic Respiratory Disease Lung Environment between Non-Clonal Populations of Pseudomonas Aeruginosa

Primary Author Block:
L. Duignan1, M. Tariq1, G. S. Holt1, J. Fothergill2, A. de Soyza3, C. Winstanley2, D. L. Smith1;
1Northumbria Univ. at Newcastle, Newcastle upon Tyne, United Kingdom, 2Univ. of Liverpool, Liverpool, United Kingdom, 3Freeman Hosp., Newcastle upon Tyne, United Kingdom

Abstract Body:
Background: The complexity of the accessory genome of Pseudomonas aeruginosa (PA) is frequently over looked compared to the core bacterial genome. They regularly contain temperate bacteriophages (phage) and other transposable genetic elements. PA’s presence in the chronically infected lung correlates with loss of lung function particularly in cystic fibrosis (CF) and non-CF bronchiectasis (nCFBR) patients. This study focuses on comparing the carriage and diversity of intact and remnant phages carried in PA variants between both individual patients and how this compares between patient isolates.

Methods: The temperate phages were identified from the 13 bacterial sequences from patient isolates taken at same time-point, showing two or three with different clonal lineages and morphologies using PHASTER. Comparisons thereafter were made using Gepard and MAUVE bioinformatic tools then finally comparing phages using MUMmer and visualising with Circos

Results: Prophages identified in PA from the same patients were compared, and illustrated different complements of intact and remnant prophages and using Circos allows the visualisation of genome similarities between prophages firstly of non-clonal isolates from the BR lung which may suggest transfer of phages within the lung environment.

Conclusions: Most isolates showed very similar phage regions and in some cases almost complete homogeneity to the other phages found in the comparator isolate from the same patient. This illustrates conservation in the lung and possible phage transfer between isolates. In the remnant phages conserved regions may show selection of these phage regions as important for colonisation of the lung. The possibility that there is transfer of phages within the lung environment is significant as it would lead to the any advantages that phage gives to the host i.e. increase bacterial fitness or increase antibiotic-resistance.
Abstract Title:
Puppet Masters of the Microbiome? Widespread & Beneficial Phages of the Human Gut Symbiont Bacteroides

Primary Author Block:
D. E. Campbell1, P. H. Degnan2; 1Univ. of Illinois at Urbana-Champaign, Urbana, IL, 2Univ. of California Riverside, Riverside, CA

Abstract Body:
Background: The human gut microbiome is a diverse and dynamic microbial community that plays a significant role in human health and development. Within this highly competitive community, the Bacteroides is one of the most common and abundant bacterial genera, playing vital roles in complex carbohydrate digestion and immune modulation. Despite this, little is known about the bacteriophages that infect it; these phages likely play important roles in modulating bacterial host fitness, altering gut community dynamics, and maintaining microbial diversity within the gut microbiome. Results: Phage BVΦ01 was first identified as a prophage on the chromosome of its host B. vulgatus ATCC 8482. Outside of the host, BVΦ01 exists as a 59-kb protein-protected dsDNA molecule, as detected by PCR and next generation sequencing. Although no new lytic or lysogenic infections have been detected in vitro, BVΦ01 is constitutively released from the cell at ratios of approximately 1 free phage to 5x10^5 bacterial host cells. Genetic manipulation of BVΦ01 is accomplished through a counter-selectable allelic exchange system in the bacterial host. BVΦ01 release is abolished with the deletion of one of three putative phage-encoded integrases; this activity can be complemented in trans. Barcoded pairwise competition assays demonstrate that BVΦ01 lysogens are more fit than their cured lysogen, integrase-deletion, or integrase-complement counterparts, suggesting that free BVΦ01 plays a role in modulating bacterial fitness. This fitness difference is not attributable to differences in growth rate or antagonism. The bacterial attachment site (attB) utilized by phage BVΦ01 is in the putative terminator of an efflux operon and is conserved across gut isolates of the family Bacteroidales. Bacteroides efflux operon-integrating (BEOI) phages utilize this attB in divergent Bacteroides isolates. The nineteen BEOI phages identified so far represent a closely related group of phages infecting several Bacteroides species, potentially playing far-reaching roles in gut community modulation. Conclusions: Phage BVΦ01 and its BEOI relatives likely represent a larger group of active lysogenic phages in the human gut microbiome. These results emphasize that apparently small phenotypes, such as low-level phage release, can have large impacts on bacterial fitness in competitive environments. Although the mechanisms underlying the fitness advantage of free BVΦ01 production remain unknown, these results highlight the importance of bacteriophages in the human gut microbiome.
Who Coinfects: Viral Coinfection Dynamics in Microbial Hosts

S. L. Diaz-Munoz; Univ. of California, Davis, Davis, CA

Background: Viral coinfection occurs when more than one virus infects a host. A first step in deciphering virus-virus interactions is determining the identity of participants and their patterns of co-occurrence in hosts. Superinfection exclusion mechanisms suggest that closely related viruses will be excluded from infections and distantly related viruses can sometimes complement each other leading to increased viral fitness or mutualistic relationships. This leads to an expectation that coinfection partners should be distantly related. Yet determining whether there is a general pattern of viral identity in the composition of coinfections has been technically challenging. Methods: I performed statistical analysis of a microbial data sets of virus-host interactions that record active and latent viral infections recorded in the genome sequences of bacterial and archaeal hosts (n=8,770). This data set is composed by sequence-based infection detection of cultured and uncultured strains. I used linear models to test whether infections in shared the same viral cluster (roughly equivalent to genus). I also examined the influence of viral infection mode and nucleic acid composition on the identity of coinfecting viruses. Results: Coinfections varied in the number of viral clusters (~genera) according to whether they were composed by prophages, extrachromosomal viruses, or a mixture of the two (15.60% of variance, ANOVA p < 2e-16), after controlling for number of viruses in the coinfection. All coinfections had few coinfections (3-10%) composed of viruses in the same viral cluster. While extrachromosomal and mixed coinfections commonly had multiple coinfecting viral clusters (>8), coinfections of prophages with many viruses were rare with >64% of coinfections composed of 2 viral clusters. The nucleic acid composition of viruses in a coinfection did not explain a large proportion of the variance in the number of viral clusters. Conclusions: These results suggest that viruses of the same virus cluster (~genus) rarely find each other in coinfection. Extrachromosomal viruses do not appear to have an upper limit to the number of viral genera in a coinfection. In contrast, prophages appear to additionally limit coinfection of more than two viral genera. Collectively, these results suggest that patterns of viral coinfection can be affected by the identity of coinfecting viruses and likely have a strong role in shaping viral ecology and evolution.
Abstract Title: Bacteriophage Attack Breaks Mutualisms and Has Indirect Effects on Non-Host Species

Primary Author Block: L. Fazzi, W. R. Harcombe; Univ. of Minnesota, St. Paul, MN

Abstract Body:
Bacteriophage (phage) are an understudied member of microbial communities, but can have significant impact on community composition and dynamics (1). Phage often alter community composition by suppressing competitively dominant bacteria (2), and competition between species influences evolution of phage resistance (3). However, quantitative studies of responses to phage in mutualistic communities are lacking. We tested how obligate mutualistic microbial communities respond to phage attack. We predicted that phage attack of a species in an obligate mutualism would limit growth of all community members that rely on the attacked strain. This hypothesis was tested with an engineered community of Escherichia coli and Salmonella enterica in which the species can be switched from obligate metabolic mutualists to competitors by changing the media (Fig 1) (4). We tested the impact of E. coli-specific phage (T7) when the bacteria were cooperating or competing. We tracked species-specific population dynamics with fluorescent tags, and plated for PFUs and CFUs. Unlike in competition, metabolic mutualism had little impact on final abundance of phage resistance compared to no-phage controls. However, we found that the impact of phage on non-hosts was opposite what we expected in the mutualism. Rather than constraining the growth of an obligate mutualist, killing E. coli with phage led to 2-3 fold increases in S. enterica population size. Our resource-explicit mathematical models support the hypothesis that the increased growth was the result of consumption of nutrients released by lysis. In conclusion, phage attack in a two-member microbial community can break constraints on species ratios and modulate non-host population sizes. Determining the ecological and evolutionary effects that phage have on microbial communities will allow us to more accurately predict microbial community-phage interactions.
Abstract Title:
Viral Mediated Antagonistic Mutualism Promotes Vertical Transmission in Crispr-Immune Populations
Primary Author Block:
S. DeWerff, R. Whitaker; Univ. of Illinois Urbana-Champaign, Urbana, IL
Abstract Body:
It is generally believed that host-virus coevolution should select for viruses to become more benevolent to their host. However, this may not always be the case as virulence evolution depends on selection at multiple points throughout the infection cycle and can be dependent on host spatial structure, timing and mode of transmission. With horizontal transmission, selection may favor a more virulent virus that will harm its host compared to a virus that uses a vertical transmission strategy where negative outcomes of the host are directly linked to negative outcomes of the virus. While multiple studies exist of the trade-off between vertical and horizontal transmission modes, relatively few look at the effects of mixed-mode transmission in virus-host coevolution. To study how mixed-mode transmission influences coevolution we are studying chronic infection of Sulfolobus spindle-shaped virus 9 (SSV9) of the host Sulfolobus islandicus M.16.4. This is an ideal model system because we can study different host-virus phenotypes in the same system, including immunity through CRISPR-Cas systems as well as chronic infection. In chronic infection, the virus exhibits both vertical transmission within the host as an episome, but continues to produce viral particles allowing for horizontal transmission. Initial characterization of chronic infection compared to an isogenic uninfected strain through growth curves showed that there is a cost to the host when infected. However, in competition with an immune host the chronically infected strain can outcompete the immune host whereas the uninfected cannot. This competitive advantage allows the chronically infected strain to fix in the population, even when initially rare, but does not require active infection by the virus. Chronic infection with other SSVs has shown that this phenotype is not limited to SSV9 infection. These results would suggest that in this mixed-mode transmission model, the virus is becoming a mutualistic virus for its host.
Abstract Title:
Ancestrality and Mosaicism of Giant Viruses Supporting the Definition of the Fourth Truc of Microbes

Primary Author Block:
P. Colson1, A. Levasseur1, B. La Scola1, A. Nasir2, V. Sharma1, P. Pontarotti1, G. Caetano-Anolles2, D. Raoult1; 1IHU Méditerranée Infection, Marseille, France, 2Evolutionary Bioinformatics Lab., Dept. of Crop Sci., Univ. of Illinois, Urbana, IL

Abstract Body:
Background: Giant viruses of amoebae have been discovered in 2003. Their diversity has greatly expanded since then. They are visible by optical microscopy but devoid of ribosomal genes. They were found to form a fourth branch of life, named a ‘TRUC’ (for “Things Resisting Uncompleted Classifications”) alongside Bacteria, Archaea and Eukarya. Their origin and ancestrality remain controversial. Here, we specify the evolution and definition of giant viruses. Methods: Both phylogenetic and phenoetic analyses of informational gene repertoires of giant viruses and selected cellular organisms among Bacteria, Archaea and Eukarya were performed, which included structural phylogenomics based on protein structural domains grouped into 289 fold superfamilies (FSFs). Hierarchical clustering analysis was performed based on a binary presence/absence matrix constructed using 727 informational COGs. The presence/absence of ‘universal’ FSF domains was used to generate an unrooted maximum parsimony phylogenomic tree. The gene contents of a giant virus and a bacteria, an archaea and an eukaryota with small genomes were compared. Results: Giant viruses appears as a basal group in the tree of all proteomes; including when exploring the temporal space of ages of individual structural domains in proteomes. A pangenome and core genome determined for Rickettsia bellii (bacteria), Methanomassiliicoccus luminyensis (archaea), Encephalitozoon intestinalis (eukaryota), and Tupanvirus (giant virus) showed a substantial proportion of Tupanvirus genes that overlap with those of the cellular microbes. In addition, a substantial genome mosaicism was observed, with 51%, 11%, 8% and 0.2% of Tupanvirus genes best matching with viruses, eukaryota, bacteria and archaea. Finally, we found that genes may be subject to lateral sequence transfer rearrangements. Conclusion: Our data highlight the quantum lip between classical and giant viruses. They confirm previous evidence of the existence of a fourth TRUC of life that includes giant viruses, and highlight its ancestrality and mosaicism. They also point out that best representations for microorganisms evolution are rhizomes, and that sequence rather than gene transfers have to be considered.
Abstract Title:
Photosynthesis Auxiliary Metabolic Genes in Viral Genomes Across A Freshwater-Saltwater Gradient in Southeast Us

Primary Author Block:
C. A. Ruiz Perez; Georgia Inst. of Technology, Atlanta, GA

Abstract Body:
Phages infecting cyanobacteria play an important role in controlling host population dynamics and modifying host genome. Some of these phages encode host-acquired functional genes, known as auxiliary metabolic genes (AMGs) that are thought to confer a fitness advantage because the expression of the corresponding proteins increases progeny yield during infection. AMGs involved in photosynthesis are widespread among marine phages, especially those belonging to the Myoviridae and Podoviridae families infecting Prochlorococcus and Synechococcus. Although photosynthesis-related AMGs have been extensively studied in oceans, their distribution, prevalence, diversity, and genomic context in freshwater habitats remain poorly understood. To advance understanding of these issues, we surveyed five freshwater lake systems and two estuarine locations in the Southeast US interconnected by the Chattahoochee River, for four years, using metagenomic sequencing of viral populations. Our results revealed the prevalence of photosynthesis-related AMGs in viral genomes recovered in all five lakes, albeit at relative abundances about 10 times lower compared to ocean or estuarine locations, due presumably to different infection strategies and viral latent periods. The freshwater version of the most widespread photosynthesis AMG (psbA) showed a distinct evolutionary history compared to the marine counterpart also clustering separately from the freshwater cyanobacterial host version of the gene. Classification strategies using shared protein content with VConTACT and average nucleotide identity (ANI) of the genomes recovered in our survey against the viral RefSeq database indicated that there is high unreported diversity at the levels of viral genera and species suggesting that encoding photosynthesis-related AMGs in viral genomes is a much more widespread infection and replication strategy than previously thought.
Abstract Title:
Broad Host-Range Lysogenic Enterophages Isolated from Soil and Water from Puerto Rico: Possible Role in Genetic Evolution

Primary Author Block:
A. A. Gonzalez, Y. A. Serrano, A. R. Rivera, G. A. Toranzos; Univ. of Puerto Rico, San Juan, Puerto Rico

Abstract Body:
Enterococci spp. are part of the gut microbiota of humans; constituting up to 1% in adults. The mechanisms used by the microorganism to initially colonize the gastrointestinal track are not well understood, but it is known that exposure to antibiotics facilitates colonization of the GI track by multi-drug resistant enterococci, causing nosocomial diseases. Phage therapy has been proposed as an alternative treatment solution to battle diseases produced by Enterococci spp and reducing the use of antibiotics. Bacteriophages are known to be vital in controlling bacterial population dynamics. Enterococci spp. are currently used as indicators of fecal contamination in recreational waters. However, the presence of this organism has been demonstrated in pristine zones outside of their expected habitat. The survival of this organism could be as a result of the acquisition of traits coming from bacteriophages present in the environment. To study the presence of prophages in Enterococci spp. and their ability to lyse the bacteria cells, resident Enterococci were isolated from soil and water in a pristine area of Puerto Rico. The expression of phages in these Enterococcus isolates was induced using antibiotics. Supernatant was tested by the spot test against 8 different Enterococcus type strains. Preliminary data demonstrate the presence of prophages in environmental isolates, with a prevalence of 41% and 27%, in water and soil respectively. Prophage induction indicated the presence of specific range as follows: 24.8% E. faecalis, 11.3% E. faecium, 6.4% E. hirae, 5.7 % E. durans, 3.5% E. dispar, 5 % E. casseliflavus, 1.4% E. pseudoavium and 1.4% E. gallinarum. Some resulting supernatants had a wide host range, infecting all 8 Enterococcus type strains used in our laboratory. Some could infect up to 7 type strains. Our data suggest that environmental lysogenic enterophages have a wide host range. The presence of these lysogenic enterophages supports the hypothesis that phages, and more specifically lysogenic phages, may be the most important vectors of horizontal gene transmission (HGT) at least amongst members of the same bacterial genera. It remains to be seen if these lysogenic enterophages are even more promiscuous, infecting members of different genera and thus HGT may be a more dynamic process than previously thought. Although, we need to focus on the whole genome of lysogenic phages in order to better understand genetic evolution. Our results are the first step in determining how phages are the "movers and shakers" of evolution in a given ecosystem.
Abstract:
Microbial and viral contamination of critical water sources is an important issue facing many communities. In New York State alone, 792 waterbodies are on the “Impaired/TMDL” list, which identifies bodies of water that might require remediation through establishment of a total maximum daily load. In the lower Hudson River drainage basin, the Sparkill Creek, placed on the list first in 2010, faces issues with urban/stormwater runoff, causing elevated levels of pathogens in the creek and decreased oxygen availability. According to published data, the levels of the indicator bacteria, Enterococcus, are 24 times higher than EPA standards for issuing a beach advisory. The Sparkill Creek was examined for microbial and coliphage loads and diversity as related to weather events, in addition to correlation with soil communities. Coliform and E. coli levels were measured using Petrifilm®, with a significant increase found with a rainfall event for both sampling locations. The coliphage numbers were also significantly higher with a rain event. Family typing of these coliphage by PCR, following one round of purification, was attempted on 36 isolates. Of these, 19 were found to be Microviridae (5%), Siphoviridae JK (26%), Siphoviridae Lambda (16%), and Myoviridae T4 (58%). DNA from Gram negative cultured bacteria was isolated, and subjected to 16S rDNA sequencing. Of note was the presence of Stenotrophomonas retroflexus in dry weather, seen less often after a rain event, and an increased prevalence of Enterobacter and Escherichia at a site approximately 3 miles from the mouth of the creek. Analysis of data along the creek from its headwaters to the point it empties into the Hudson River is expected help pinpoint the location where the contamination is most prominent. Future studies include an examination into the prevalence of beta-lactamase genes within the coliphage, purportedly leading to transfer of antibiotic resistance among their respective hosts.
Community Metabolic Modeling Reveals Common Auxotrophies Across A Variety of Microbiome Systems

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While the collection of microorganisms present define the potential metabolic behavior of a microbiome, it is the interactions among them that determine their realized metabolic activity. However, it is often not feasible to characterize both individual organisms and their interactions through detailed laboratory study. Thus, community models derived from metagenomic data may prove to be valuable tools for improving our understanding of the molecular interactions that guide the behavior of microbial communities, with numerous applications in health, the environment, and industry. The DOE’s Systems Biology Knowledgebase (KBase) includes a pipeline for the development and analysis of community metabolic models starting from raw sequence data. This pipeline integrates 11 analysis steps, including: metagenome assembly; binning of assembled contigs by species; assessment of assembly and genome quality and completeness; genome annotation; genome metabolic model reconstruction; generation of a community model; mapping RNA-seq reads to individual species; model gapfilling; flux balance analysis; comparison of flux and expression profiles; and simulation of growth phenotypes. All steps are available within a user-friendly interface at http://narrative.kbase.us. We applied this pipeline to understand the trophic interactions occurring within four microbiome-based datasets: (i) a lab-constructed community comprised of the cyanobacterium Thermosynechococcus elongatus and the heterotroph Meiothermus ruber; (ii) 120 soil cultures, many of which are comprised of 2-3 tightly coupled species; (iii) 3 dominant members of a 13-species electrosynthetic community; and (iv) an 18-species epsomitic phototrophic mat in Hot Lake, Washington. We demonstrate how the tools in KBase can integrate community Biolog profiles and metatranscriptomic profiles in our community models of these systems. Using this approach, we find potential trophic interactions in all communities. Across these diverse systems, we see emerging patterns in common auxotrophies; with vitamins being the most common, followed by lysine and aromatic amino acids. We also see distributed auxotrophy across genomes within a microbiome, suggesting these dependencies may constrain community composition. This modeling approach provides insight into the potential metabolic transfers supporting interactions within these communities and provides the basis for developing hypotheses regarding when these species may interact within more complex communities.
Abstract Title:
In Silico Microbial Metabolic Interaction Network Reveals the Key Influencers of the Colon Cancer-Associated Microbiome

Primary Author Block:
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Abstract Body:
One of the crowning achievements of the microbiome sciences and modern ‘omics technologies has been hypothesis generation through observation of populations with and without disease. Each new association adds a new possibility for progress and cures. Evaluating the impact of one microbial species at a time on host health is a valuable but time-consuming and costly process, and without accounting for microbial community dynamics, results often have limited applicability in “real-world” systems. In silico modeling offers a way to overcome many of these limitations and combine our relatively sparse observations with mechanistically-based predictive modeling coupled with targeted experimental validation. Here, we present our work on microbial metabolic influence networks on colorectal cancer (CRC). In our approach, we show how next-generation sequencing and community genome-scale metabolic modeling using the Microbial Modeling Interactions (MMInte) framework, enable in silico modeling of microbial interaction networks the GI tract and how these networks provide information about which microbes are the most metabolically influential to the community, and how these influencers are associated to disease state. Special focus is given to the multi-scale nature of the modeling approach, which steps from the use of 16S rDNA amplicon sequencing of more than 900 stool samples to community metabolic models of the GI microbiota. The latter is then be analyzed to predict pairwise microbial interactions, which allows us to weigh the relative influence of one microbe onto another.
Abstract Title:
Determination of Intracellular Allolactose Concentrations During Induction and Catabolite Repression of the Lac Operon of Escherichia coli: Evidence of Positive Feedback in the Regulation

Primary Author Block:
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Abstract Body:
The lac operon of Escherichia coli is repressed 600-fold in the presence of glucose. This repression is usually attributed to two molecular mechanisms, namely activation by 3′,5′-cyclic adenosine monophosphate (cAMP) and inducer exclusion. However, since these mechanisms, by themselves, repress lac expression only 2-3 fold, they cannot account for the observed 600-fold repression. To bridge this repression deficit, we have hypothesized that lac expression is subject to positive feedback which amplifies the small effects of cAMP-mediated regulation and inducer exclusion; moreover, the positive feedback occurs because intracellular allolactose stimulates the synthesis of Lac permease and β-galactosidase, which in turn promote the accumulation of even more allolactose (1). It is widely accepted that during growth of E. coli in the presence of gratuitous inducers (e.g. TMG), positive feedback exists and arises from the above mentioned mechanism (2). However, this is not the case for growth on lactose. Indeed, based on mathematical models, some have argued that there is no positive feedback in this case because Lac permease and β-galactosidase do not promote the accumulation of intracellular allolactose — in fact, the intracellular allolactose levels are completely independent of these enzyme levels (3). To resolve this debate, we developed a simple method for measuring the intracellular allolactose concentration. Specifically, we found that the concentration of intracellular allolactose is directly proportional to its rate of expulsion from the cell. With the help of this method, we measured the evolution of the intracellular allolactose levels during the course of induction in the presence of lactose, and repression in the presence of lactose + glucose. In the first case, the intracellular allolactose level increased with the specific β-galactosidase activity, which contradicts the conclusions derived from mathematical models. It follows that lactose enzymes do promote allolactose accumulation, and there is positive feedback in the regulation of the lac operon. In the second case, the intracellular allolactose level and specific β-galactosidase activity decreased in tandem, which suggests that catabolite repression occurs because the positive feedback loop turns in the reverse direction.
Abstract Title:
Metabolic Mechanisms of Interaction Within A Defined Gut Microbiota

Primary Author Block:
G. Medlock1, M. Carey1, D. McDuffie1, N. Giallourou2, J. Swann2, G. Kolling1, J. Papin1; 1Univ. of Virginia, Charlottesville, VA, 2Imperial Coll., London, United Kingdom

Abstract Body:
Metabolic interactions between bacterial species govern important processes ranging from global biogeochemical cycles to colonization resistance to pathogens. However, identifying interacting species within a microbial community and the associated metabolic mechanism of interaction is a daunting task. Here, we present a co-culture resource allocation model (CRAM) that infers metabolites that may govern growth-modulating interactions between species grown in vitro from untargeted supernatant metabolomics data. To develop and test this method, we performed pairwise growth experiments between each of 6 strains from the altered Schaedler flora (ASF), a synthetic community of 8 bacterial strains used to standardize the microbiota of mice in breeding facilities. While co-culture of pairs of strains generally led to negative growth interactions (i.e. strains grew to lower abundance than in monoculture), it also led to higher total biomass production (i.e. together, strains grew to higher density than either in monoculture). We applied the CRAM to untargeted supernatant metabolomics data from these experiments to compare co-culture metabolic profiles to expected profiles based on monoculture behavior. We found striking increases in efficiency in co-culture, as well as differential behavior in co-culture suggestive of cross-feeding. We used the CRAM results to interrogate a putative cross-feeding interaction in which one strain produces amino acids that another strain consumes through Stickland fermentation. We coupled the CRAM with genome-scale metabolic network reconstructions for each of the ASF species to provide parallel support for putative metabolic interactions, then validated these predictions in vitro. This framework for inferring mechanisms of metabolic interaction between species is generalizable to communities of any size, and can easily be modified to interrogate interactions that modulate phenotypes other than growth.
Session Number: 286
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Session Number: 286
Session Type: Poster Talk
Session Title: Antibiotics and the Evolution of Resistance
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Session Primary Track: Microbial Ecology and Evolution
Abstract Control Number: 8945
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Abstract Title:
Outstanding Abstract Award: Investigating Chemical and Genetic Mechanisms of Antimicrobial Production and Resistance in A Co-Evolutionary Arms Race

Primary Author Block:
K. B. O'Brien, J. L. Chodkowski, A. Shade; Michigan State Univ., East Lansing, MI

Abstract Body:
Though research on antibiotics and antibiotic resistance mechanisms has been predominantly focused on human pathogens in the clinical setting, environmental microorganisms also evolve resistances to antibiotics. Preliminary observations in our lab suggested that evolved strains of Burkholderia thailandensis had increased inhibition towards Flavobacterium johnsoniae as compared to its ancestor strain, while evolved strains of F. johnsoniae had increased resistance as compared to its ancestor. Thus, we hypothesized that there was a co-evolutionary arms race occurring between these two environmental bacteria. We plated five replicates of B. thailandensis and F. johnsoniae on agar media that allowed for interspecies interactions to be mediated by small molecule diffusion. After two weeks of growth, colonies were transferred to fresh plates, for a total of 10 transfers for each of the five replicate lineages. Ancestor strains and evolved strains (from the tenth plates) were re-plated in all four combinations and in isolation. Colony phenotypes were imaged for quantitative and qualitative analyses. Changes in colony size over subsequent transfers suggested that the ancestor B. thailandensis strain inhibited the evolved F. johnsoniae strains, but the evolved B. thailandensis strains did not (ANOVA, F = 4.259, p = 0.04). We also observed that all evolved F. johnsoniae strains had increased resistance as compared to their ancestor. However, one of the replicate lineages revealed an outcome consistent with initial findings: we observed a potential arm’s race of resistance and inhibition between the competing strains. Taken together, our results suggest that multiple evolutionary outcomes of antibiotic resistance and production can result from measurably identical conditions of consistent competition pressure. Furthermore, we hypothesized the inhibitory molecule made by B. thailandensis was bactobolin, a characterized broad-spectrum antibiotic. However, when a B. thailandensis mutant for bactobolin production was plated with F. johnsoniae, there was no change in inhibition. Thus, we are isolating and characterizing the structure of the potentially new inhibitory molecule using liquid and solid extraction methods, HPLC bioassay-guided fractionation, and mass spectrometry. The results of this work provide insights into the conditions under which bacterial interactions underpinned by antibiotic resistance and production can co-evolve, as well as the variability in the outcomes of these interactions.
Abstract Title:
Longitudinal Impact of Prophylactic Antibiotic Use on the Gut Microbiota and Antimicrobial Resistance Genes

Primary Author Block:
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Abstract Body:
The gut microbiota maintains health and helps prevent infection. Antibiotic exposure is known to disrupt the gut microbiota, allowing for potential colonization with resistant microbes. Currently, there is a need for prospective, longitudinal studies in healthy individuals assessing the impact of antibiotics on the gut microbiota’s structure and function. To assess the impact of antibiotics on the gut microbiota, we recruited healthy adults undergoing elective orthopaedic or urologic procedures where one or more doses of perioperative antibiotics are routinely given. Up to seven stool samples from each subject were collected starting 1 month before out to 3 months after antibiotics. We used 16S rRNA gene sequencing to assess the microbiota and targeted qPCR to identify the presence of selected antibiotic resistance genes. A total of 42 subjects were enrolled (229 samples; 69.4% male; mean age 45.1 years). The majority of patients received one dose of cefazolin (29) or clindamycin (9). Principal coordinates analysis of the microbiota communities revealed that individual subjects’ samples clustered together independent from antibiotic use. Mixed models accounting for subject-specific trends were more revealing than groupwise analyses in differentiating the microbiota pre- and post-antibiotics. After antibiotic exposure, Shannon diversity decreased (−0.14, P =.045), total %Firmicutes decreased (−5.7%, P =.007), and total %Bacteroidetes increased (+4.5%, P =.047). Several individual operational taxonomic units (OTUs) classified as Lachnospiraceae decreased following antibiotic use. After receiving cefazolin, vanB levels increased 168-fold in a subject, and qnrS increased 22 to 27-fold in two subjects. Our data suggest that even single doses of select perioperative antibiotics can impact the microbiota, particularly individual Firmicutes members, and resistance gene reservoirs.
Abstract Title: 
Development of A Microfluidics Platform to Uncover Novel Antibiotics from Actinomycetes

Primary Author Block: 
P. Buenbrazo, D. J. McClung, M. F. Traxler; Univ. of California Berkeley, Berkeley, CA

Abstract Body:
Developing new tactics to battle multi-drug resistant bacteria poses a serious challenge for public health. We have missed discovering many natural products from actinomycetes due to our controlled conditions of monocultures, which keeps many of their natural product biosynthesis gene clusters silent (Wiemann & Keller, 2014). We posit that replicating naturally occurring interactions from the soil will stimulate antibiotic production in rare actinomycetes. Actinomycetes produce more metabolites that are unique to individual interactions than common to two or more unique interactions (Traxler, et al., 2013), suggesting that we need to analyze many pairwise interactions to elucidate the true wealth of antibiotic potential of this group of bacteria. I aim to develop a microfluidics device to screen approximately 10,000 pairwise interspecies interactions of rare, soil actinomycetes for synthesis of novel antibiotics. Initial PDMS-based prototypes were made up of four chambers, each separated by a semi-permeable barrier, which physically separates species but allows chemical communication to occur. Each of the inner two chambers houses a different actinomycete species. The outer two chambers house an indicator strain: a naturally multi-drug resistant actinomycete, Amycolatopsis sp. AA4. If the interaction between the inner two actinomycetes stimulates antibiotic production, then it will diffuse into the outer two chambers, potentially inhibiting the growth of the indicator. We are optimizing devices for the most robust barrier that fulfills the requirements of the screen without compromising manufacturing efficiency. From preliminary designs, colored dyes and an unknown antibiotic produced by a streptomycete diffuse well through various barriers. However, simple mazes did not prevent filamentous actinomycetes from growing into the next chamber, demonstrating that the current maze designs are not sufficient to physically separate bacterial species. Our work will set up a high-throughput screening method for discovery of natural products (i.e. antibiotics) from actinomycete interspecies interactions, increasing the efficiency in identifying biologically active small molecules that can be used for future therapeutics.
Background: Despite an increasing awareness of the multi-species nature of many bacterial infections, our understanding of how antibiotics influence microbial communities remains limited. Mutualistic partners, wherein the survival of each species is dependent on the survival of the other, are common in nature, but there is conflicting evidence as to whether mutualists are better or worse at adapting to environmental stresses. We test this phenomenon in a microbial mutualism facing an antibiotic selection pressure. We predict that an obligate mutualist system will evolve antibiotic resistance more slowly than a single-species system, and that the mechanisms of resistance will differ due to the need to maintain cross-feeding in the mutualist system. Methods: We used a previously engineered Escherichia coli - Salmonella enterica obligate cross-feeding system wherein each species requires a metabolite secreted by the other to grow. We grew six replicates of the co-culture, as well as each species in monoculture, along two antibiotic gradients (ampicillin and rifampicin). We passaged these cultures for 20 passages (~140 generations), doubling the antibiotic concentration and tracking the minimum inhibitory concentration (MIC) at each passage. We then sequenced the most resistant population from each replicate, and phenotyped three isolates from each population for growth rate, yield, and MIC in mono- and co-culture. Results: Phenotyping data suggest that cross-feeding changed the rate and mechanisms by which antibiotic resistance evolved. Resistance to both ampicillin and rifampicin increased more slowly in cross-feeding populations than in monocultures; population MICs were also lower for co-cultures. When we compared isolate MICs, monoculture isolates had higher MICs than co-culture isolates, in accordance with our population results. The relative cost of resistance also differed for isolates evolved in monoculture and co-culture, as measured by growth rate and yield. Sequencing of whole genomes to investigate the genetic mechanisms underlying resistance evolution are currently underway. Conclusions: Investigations into the true multispecies ecological context in which antibiotic resistance evolves are critical for tracking and halting resistance spread in polymicrobial infections. Our work highlights how ecological interactions between bacteria alter the dynamics and mechanisms of evolution of resistance. More broadly, this work provides insight into how mutualisms constrain adaptation to environmental stress.
Session Number: 286
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Session Title: Antibiotics and the Evolution of Resistance
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Abstract Control Number: 9467
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Abstract Title:
Moderator
Primary Author Block:
Jeremiah Johnson; Univ. of Tennessee, Knoxville, TN

Abstract Body:
Session Number: 286  
Session Type: Poster Talk  
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Session Title: Antibiotics and the Evolution of Resistance  
Session Start Date Time: 6/9/2018 12:15:00 PM  
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Session Primary Track: Microbial Ecology and Evolution  
Abstract Control Number: 9595  
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Abstract Title:  
Genome Analysis of A Multidrug-Resistant (Mdr) Salmonella enterica Serovar 4,[5],12,i:- Isolate Associated with A 2015 Foodborne Outbreak from Pork  
Primary Author Block:  

Abstract Body:  
Salmonella enterica serovar 4,[5],12,i:- has emerged as a frequent cause of salmonellosis in humans and, based on 2014 National Antimicrobial Resistance Monitoring System (NARMS) data, is ranked as the fifth most common serovar isolated from patients in the U.S. Approximately 50% of serovar 4,[5],12,i:- isolates in the U.S. are considered multidrug-resistant (MDR) which is defined by resistance to 3 or more antimicrobial classes. Over the last 20 years, the prevalence of serovar 4,[5],12,i:- has increased globally and its incidence is primarily associated with food animals. In 2015, a foodborne outbreak due to serovar 4,[5],12,i:- was linked to pork with 188 human infections and 30 hospitalizations. Investigation of 10 serovar 4,[5],12,i:- isolates from the pork outbreak indicated that all the strains were MDR, conferring resistance to the antimicrobials ampicillin, streptomycin, sulfisoxazole, and tetracycline (R-type ASSuT). To facilitate characterization of a MDR serovar 4,[5],12,i:- isolate associated with the pork outbreak, we sequenced the FSIS 1503788 genome using the PacBio and Illumina platforms. Deletion of the fljB region in FSIS 1503788 is due to insertion of a module containing genes that provide resistance to mercury and multiple antimicrobials (ASSuT). The deletion of the fljB gene results in this isolate being monophasic because it only expresses one (FliC) of the two flagellar antigens. Furthermore, FSIS 1503788 contains Salmonella Genomic Island 4 (SGI-4), an ~80 kb element that harbors genes for resistance to additional heavy metals (copper, zinc, and arsenic). The FSIS 1503788 genome is predicted by Phaster to have up to 6 intact prophage. Some Salmonella isolates have been previously shown to contain prophage that encode virulence genes or participate in horizontal gene transfer. Thus, our genome analysis indicates that MDR FSIS 1503788, associated with a 2015 pork outbreak, contains two genomic insertions previously described in other serovar 4,[5],12,i:- isolates that confer resistance to antimicrobials and heavy metals. The presence of SGI-4 and the MDR module in Salmonella serovar 4,[5],12,i:- may provide colonization or virulence benefits that have helped promote the increased prevalence of this serovar globally.
Abstract Title:
Photosynthesis Auxiliary Metabolic Genes in Viral Genomes Across A Freshwater-Saltwater Gradient in Southeast Us

Primary Author Block:
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Abstract Body:
Phages infecting cyanobacteria play an important role in controlling host population dynamics and modifying host genome. Some of these phages encode host-acquired functional genes, known as auxiliary metabolic genes (AMGs) that are thought to confer a fitness advantage because the expression of the corresponding proteins increases progeny yield during infection. AMGs involved in photosynthesis are widespread among marine phages, especially those belonging to the Myoviridae and Podoviridae families infecting Prochlorococcus and Synechococcus. Although photosynthesis-related AMGs have been extensively studied in oceans, their distribution, prevalence, diversity, and genomic context in freshwater habitats remain poorly understood. To advance understanding of these issues, we surveyed five freshwater lake systems and two estuarine locations in the Southeast US interconnected by the Chattahoochee River, for four years, using metagenomic sequencing of viral populations. Our results revealed the prevalence of photosynthesis-related AMGs in viral genomes recovered in all five lakes, albeit at relative abundances about 10 times lower compared to ocean or estuarine locations, due presumably to different infection strategies and viral latent periods. The freshwater version of the most widespread photosynthesis AMG (psbA) showed a distinct evolutionary history compared to the marine counterpart also clustering separately from the freshwater cyanobacterial host version of the gene. Classification strategies using shared protein content with VConTACT and average nucleotide identity (ANI) of the genomes recovered in our survey against the viral RefSeq database indicated that there is high unreported diversity at the levels of viral genera and species suggesting that encoding photosynthesis-related AMGs in viral genomes is a much more widespread infection and replication strategy than previously thought.
Session Number: 327
Session Title: Phages: Good Things Come in Small Packages
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Session Primary Track: Microbial Ecology and Evolution
Abstract Control Number: 8825
Poster Board Number:

Abstract Title:
Broad Host-Range Lysogenic Enterophages Isolated from Soil and Water from Puerto Rico: Possible Role in Genetic Evolution

Primary Author Block:
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Abstract Body:
Enterococci spp. are part of the gut microbiota of humans; constituting up to 1% in adults. The mechanisms used by the microorganism to initially colonize the gastrointestinal track are not well understood, but it is known that exposure to antibiotics facilitates colonization of the GI track by multi-drug resistant enterococci, causing nosocomial diseases. Phage therapy has been proposed as an alternative treatment solution to battle diseases produced by Enterococci spp and reducing the use of antibiotics. Bacteriophages are known to be vital in controlling bacterial population dynamics. Enterococci spp. are currently used as indicators of fecal contamination in recreational waters. However, the presence of this organism has been demonstrated in pristine zones outside of their expected habitat. The survival of this organism could be as a result of the acquisition of traits coming from bacteriophages present in the environment. To study the presence of prophages in Enterococci spp. and their ability to lyse the bacteria cells, resident Enterococci were isolated from soil and water in a pristine area of Puerto Rico. The expression of phages in these Enterococcus isolates was induced using antibiotics. Supernatant was tested by the spot test against 8 different Enterococcus type strains. Preliminary data demonstrate the presence of prophages in environmental isolates, with a prevalence of 41% and 27%, in water and soil respectively. Prophage induction indicated the presence of specific range as follows: 24.8% E. faecalis, 11.3% E. faecium, 6.4% E. hirae, 5.7 % E. durans, 3.5% E. dispar, 5 % E. casseliflavus, 1.4% E. pseudoavium and 1.4% E. gallinarum. Some resulting supernatants had a wide host range, infecting all 8 Enterococcus type strains used in our laboratory. Some could infect up to 7 type strains. Our data suggest that environmental lysogenic enterophages have a wide host range. The presence of these lysogenic enterophages supports the hypothesis that phages, and more specifically lysogenic phages, may be the most important vectors of horizontal gene transmission (HGT) at least amongst members of the same bacterial genera. It remains to be seen if these lysogenic enterophages are even more promiscuous, infecting members of different genera and thus HGT may be a more dynamic process than previously thought. Although, we need to focus on the whole genome of lysogenic phages in order to better understand genetic evolution. Our results are the first step in determining how phages are the "movers and shakers" of evolution in a given ecosystem.
Abstract Title:
Who Coinfects: Viral Coinfection Dynamics in Microbial Hosts
Primary Author Block:
S. L. Diaz-Munoz;  Univ. of California, Davis, Davis, CA
Abstract Body:
Background: Viral coinfection occurs when more than one virus infects a host. A first step in deciphering virus-virus interactions is determining the identity of participants and their patterns of co-occurrence in hosts. Superinfection exclusion mechanisms suggest that closely related viruses will be excluded from infections and distantly related viruses can sometimes complement each other leading to increased viral fitness or mutualistic relationships. This leads to an expectation that coinfection partners should be distantly related. Yet determining whether there is a general pattern of viral identity in the composition of coinfections has been technically challenging. Methods: I performed statistical analysis of a microbial data sets of virus-host interactions that record active and latent viral infections recorded in the genome sequences of bacterial and archaeal hosts (n=8,770). This data set is composed by sequence-based infection detection of cultured and uncultured strains. I used linear models to test whether infections in shared the same viral cluster (roughly equivalent to genus). I also examined the influence of viral infection mode and nucleic acid composition on the identity of coinfecting viruses. Results: Coinfections varied in the number of viral clusters (~genera) according to whether they were composed by prophages, extrachromosomal viruses, or a mixture of the two (15.60% of variance, ANOVA p < 2e-16), after controlling for number of viruses in the coinfection. All coinfections had few coinfections (3-10%) composed of viruses in the same viral cluster. While extrachromosomal and mixed coinfections commonly had multiple coinfecting viral clusters (>8), coinfections of prophages with many viruses were rare with >64% of coinfections composed of 2 viral clusters. The nucleic acid composition of viruses in a coinfection did not explain a large proportion of the variance in the number of viral clusters. Conclusions: These results suggest that viruses of the same virus cluster (~genus) rarely find each other in coinfection. Extrachromosomal viruses do not appear to have an upper limit to the number of viral genera in a coinfection. In contrast, prophages appear to additionally limit coinfection of more than two viral genera. Collectively, these results suggest that patterns of viral coinfection can be affected by the identity of coinfecting viruses and likely have a strong role in shaping viral ecology and evolution.
Session Number: 327  
Session Type: Rapid Fire
Session Number: 327  
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Session Title: Phages: Good Things Come in Small Packages
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Session Primary Track: Microbial Ecology and Evolution
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Abstract Title:
Puppet Masters of the Microbiome? Widespread & Beneficial Phages of the Human Gut Symbiont Bacteroides

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Abstract Body:
Background: The human gut microbiome is a diverse and dynamic microbial community that plays a significant role in human health and development. Within this highly competitive community, the Bacteroides is one of the most common and abundant bacterial genera, playing vital roles in complex carbohydrate digestion and immune modulation. Despite this, little is known about the bacteriophages that infect it; these phages likely play important roles in modulating bacterial host fitness, altering gut community dynamics, and maintaining microbial diversity within the gut microbiome. Results: Phage BVΦ01 was first identified as a prophage on the chromosome of its host B. vulgatus ATCC 8482. Outside of the host, BVΦ01 exists as a 59-kb protein-protected dsDNA molecule, as detected by PCR and next generation sequencing. Although no new lytic or lysogenic infections have been detected in vitro, BVΦ01 is constitutively released from the cell at ratios of approximately 1 free phage to 5x105 bacterial host cells. Genetic manipulation of BVΦ01 is accomplished through a counter-selectable allelic exchange system in the bacterial host. BVΦ01 release is abolished with the deletion of one of three putative phage-encoded integrases; this activity can be complemented in trans. Barcoded pairwise competition assays demonstrate that BVΦ01 lysogens are more fit than their cured lysogen, integrase-deletion, or integrase-complement counterparts, suggesting that free BVΦ01 plays a role in modulating bacterial fitness. This fitness difference is not attributable to differences in growth rate or antagonism. The bacterial attachment site (attB) utilized by phage BVΦ01 is in the putative terminator of an efflux operon and is conserved across gut isolates of the family Bacteroidales. Bacteroides efflux operon-integrating (BEOI) phages utilize this attB in divergent Bacteroides isolates. The nineteen BEOI phages identified so far represent a closely related group of phages infecting several Bacteroides species, potentially playing far-reaching roles in gut community modulation. Conclusions: Phage BVΦ01 and its BEOI relatives likely represent a larger group of active lysogenic phages in the human gut microbiome. These results emphasize that apparently small phenotypes, such as low-level phage release, can have large impacts on bacterial fitness in competitive environments. Although the mechanisms underlying the fitness advantage of free BVΦ01 production remain unknown, these results highlight the importance of bacteriophages in the human gut microbiome.
Session Number: 327
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Session Title: Phages: Good Things Come in Small Packages
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Session Primary Track: Microbial Ecology and Evolution
Abstract Control Number: 9367
Poster Board Number:

Abstract Title:
Moderator
Primary Author Block:
Samuel Diaz-Munoz; Univ. of California, San Diego, CA
Abstract Body:
Abstract Title:
Viral Mediated Antagonistic Mutualism Promotes Vertical Transmission in Crispr-Immune Populations

Primary Author Block:
S. DeWerff, R. Whitaker; Univ. of Illinois Urbana-Champaign, Urbana, IL

Abstract Body:
It is generally believed that host-virus coevolution should select for viruses to become more benevolent to their host. However, this may not always be the case as virulence evolution depends on selection at multiple points throughout the infection cycle and can be dependent on host spatial structure, timing and mode of transmission. With horizontal transmission, selection may favor a more virulent virus that will harm its host compared to a virus that uses a vertical transmission strategy where negative outcomes of the host are directly linked to negative outcomes of the virus. While multiple studies exist of the trade-off between vertical and horizontal transmission modes, relatively few look at the effects of mixed-mode transmission in virus-host coevolution. To study how mixed-mode transmission influences coevolution we are studying chronic infection of Sulfolobus spindle-shaped virus 9 (SSV9) of the host Sulfolobus islandicus M.16.4. This is an ideal model system because we can study different host-virus phenotypes in the same system, including immunity through CRISPR-Cas systems as well as chronic infection. In chronic infection, the virus exhibits both vertical transmission within the host as an episome, but continues to produce viral particles allowing for horizontal transmission. Initial characterization of chronic infection compared to an isogenic uninfected strain through growth curves showed that there is a cost to the host when infected. However, in competition with an immune host the chronically infected strain can outcompete the immune host whereas the uninfected cannot. This competitive advantage allows the chronically infected strain to fix in the population, even when initially rare, but does not require active infection by the virus. Chronic infection with other SSVs has shown that this phenotype is not limited to SSV9 infection. These results would suggest that in this mixed-mode transmission model, the virus is becoming a mutualistic virus for its host.
Abstract Title:
Networks Reveal Patterns of Gene Sharing across Bacterial, Archaeal, and Eukaryotic Viruses
Primary Author Block:
J. W. Shapiro, C. Putonti; Loyola Univ. Chicago, Chicago, IL
Abstract Body:
Background: While phylogenetic methods are valuable for understanding the evolution of specific virus groups, no single gene is shared among all viruses, and in many cases, horizontal gene transfer complicates phylogenetic inference further. These issues make it difficult to address broader questions in viral ecology and evolution. In recent years, networks have emerged as an alternative for depicting relationships among bacteriophage (phage) genomes and also between phages and their hosts.
Methods: Here, we build on our previous work (Shapiro & Putonti, in press) that used a subset of available RefSeq phage genomes to identify gene clusters shared among phages. In this network, each node represented all homologs of a particular gene, and two nodes were connected if the two genes are ever found in the same genome. In the present work, we extend this network to include all available RefSeq virus genomes (including also eukaryotic and archaeal viruses), as well as all metaviromic contigs identified by IMG/VR from the Human Microbiome Project (HMP). Results: New isolate genomes and metaviromic contigs fill in the gaps between previously-identified gene clusters; metaviromic contigs also form a large set of unique groups, emphasizing how little of viral diversity has been cultured in the lab. Overall, the expanded gene network is dominated by one large connected component, which includes nearly all bacteriophages, most archaeal viruses, and a subset of eukaryotic viruses. Interestingly, not all archaeal viruses cluster together, and two subclusters contain genes with homologs found in both bacterial and archaeal viruses. In one case, homologs related to zot, a common virulence factor associated with pathogenic bacteria, appear to act as a hub bridging viruses that infect archaea and bacteria. Conclusion: This network approach can be updated readily as new sequences are made available. Moreover, metadata for each genome or metagenome can be associated with each node in the network, enabling statistical analysis of associations between gene sharing and viral ecology. Networks, therefore, provide a flexible framework for exploring viral ecology and evolution when phylogenetic methods would otherwise fall short.
Abstract Title:
Estimations of GhG Fluxes Reveal A Reduction in Methane Consumption During Transformation of Marginal Soils Into Biofuel Crops (Switchgrass, Panicum Virgatum)

Primary Author Block:
C. T. Bates1, J. Kuang1, A. Escalas2, L. Wu1, M. Firestone3, J. Zhou1; 1The Univ. of Oklahoma, Norman, OK, 2Museum Natl. d'Historie Naturelle, Paris, France, 3The Univ. of California, Berkeley, Norman, CA

Abstract Body:
Understanding microbial mediated soil processes in agronomical systems has long been a goal in microbial ecology, but little is known on the dynamics of these processes during the conversion of unproductive marginal soils using auspicious bioenergy crops like Switchgrass (SG; Panicum virgatum L.). This crop is expected to improve soil quality through the net input of carbon during its establishment and continual cultivation, but exactly how SG establishment affects key belowground ecosystem functions is not known. In this study, we hypothesize that the conversion of low yielding grassland into SG fields will impact nutrient cycling and belowground microbial communities, leading to differences in GHG fluxes relative to a native grassland fallow (FL). More precisely, we investigated greenhouse gases (GHG: carbon dioxide, methane, and nitrous oxide) fluxes, soil chemistry and microbial communities structure. Over an 18-month period at 2 sites in southern Oklahoma considered ‘marginal’ for N and P nutrients availability, GHG concentrations were measured, using cavity ring down spectrometry (i.e. Picarro G2038/G2508 analyzer) while bacterial and fungal communities were assessed using amplicon sequencing. Our results indicate significant changes in available phosphate levels, organic matter content, and % soil carbon in the SG treatment. Unexpectedly, SG cultivation induced a significant reduction in methane consumption in both sites indicating a dramatic transformation of the microbial ecology relative to the native fallow. Further investigations are underway to elucidate the link between the microbial communities and the various GHG emissions. Connecting GHG fluxes, soil chemistry, and microbial succession appears promising to improve our understanding of the potential of SG to be not only a viable bioenergy crop but also its ability to positively impact the quality of marginal soils across the southern U.S.
Abstract Title:
Outstanding Abstract Award: Cas1-Targeted Metagenome Assembly Reveals the Response of Crispr/Cas to Continuous Soil Warming Treatment

Primary Author Block:
R. Wu1, B. Chai2, E. R. Johnston3, G. Xue4, J. R. Cole2, J. M. Tiedje2; 1The Univ. of Hong Kong, Hong Kong, Hong Kong, 2Michigan State Univ., East Lansing, MI, 3Georgia Inst. of Technology, Atlanta, GA, 4Univ. of Oklahoma, Oklahoma, OK

Abstract Body:
The intriguing ability of bacterial and archaeal CRISPR/CAS systems to excise exogenic elements and integrate them into a collection of CRISPR spacers has been repurposed for more programmable biology. The universal and most conserved Cas protein, Cas1 would be an ideal marker to elucidate the ecology of CRISPR/CAS for study beyond the limited cultured organisms. Therefore, we used the Xander assembler with 8 Cas1 Hidden Markov models (HMM) for more gene-targeted mining of soil metagenomes. Read simulation on 91 genomes from 11 phyla was used to assess the specificity and sensitivity of the models. All 17 subtypes of Cas1, including the newly discovered Archaeal II, CasX and CasY, IA-F, IU, IIA-C, IIIA-D were captured with >88.96% identity. The phylogenetic analysis indicates that Cas1 with same subtype are clustered together allowing emphasis on subtypes of Cas1 using the corresponding model. The Cas1 subtypes, however, do not correspond to the organism’s taxonomy. We then applied HMM-guided assembly method (Xander) to detect the Cas1 in Alaska tundra soils (AK) and Oklahoma temperate soils (OK) both with soil warming treatments. The results revealed that there was a lower Cas1 diversity and abundance in OK which has a more diverse microbiome. To further dissect the response of CRISPR/CAS to soil warming, we detect Cas1 in OK soil over an eight-year continuous warming treatment. The relative abundance of Cas1 adversely changed with increased temperature. Actinobacteria, one of the dominant phyla, had the most positive response to soil warming but less counts of Cas1 compared to Euryarchaeota and Thermotogae, the two less abundant phyla. The distribution of CRISPR/CAS significantly varies among phyla and microbial communities in different environments. This research can provide an alternative method to study Cas1 in metagenomes and enhance the understanding of CRISPR/CAS’ ecological role and phylogenetic distribution.
Abstract Title:
Different Relationships between Climatic Factors and West Nile Virus Disease Incidence in Humans and Mosquitoes among California Counties

Primary Author Block:
L. Beltz, C. Edwards, L. Muriuki1, A. Klemann; Malone Univ., Canton, OH

Abstract Body:
The role of several climatic factors on West Nile virus (WNV) cases in California was examined in order to better predict and respond to infections. This is particularly important since WNV is transmitted by mosquitoes, whose life-cycle might be affected by such factors. In this study, correlations between human and mosquito infections was compared to precipitation and temperature data on a per county or state-wide basis from 2008-2015 (includes only more recent and relevant data). Correlations between human and mosquito infections per county were also examined. Methods: Reported cases of human and mosquito infections were obtained from ArboNet. Climatic data and numbers of human infections were acquired from a SQL database under development by Malone University that will soon open for public use. Climate data for this database was retrieved from NOAA’s climate FTP climate division server. Census data were from the Census Bureau’s American Community Survey Application Programming Interface. Results: Correlation coefficients between reported infection in humans or mosquitoes and average, minimum, and maximum temperature exceeded 0.75 in only 5 CA counties: Butte, Glenn, and Yolo in northern-central California and Los Angeles and Orange County in the southern, coastal region. Northern counties also had high correlations between numbers of human and mosquito infections (>0.75), with much lower human/mosquito correlations in the southern region. No such correlation was found state-wide. Maximum and average precipitation did not correlate with numbers of human or mosquito infections per county or state. The northern counties are in the same climate division and the southern counties, in a separate division. Differences between precipitation and temperatures in these climate divisions differed significantly (p<0.0001), indicating that other factors must also affect human infection. Conclusions: Precipitation did not correlate with human WNV infection in CA by state or by county, while temperature strongly correlated with human and mosquito infections in 5 counties, but not state-wide. Strong correlations between human and mosquito infections are seen in the northern, but not the southern, counties. Predicting human WNV infection using climatic data, therefore, is not useful in CA, except in 5 counties.
Abstract Title:
Acclimation of Soil Fungal Community to Soil Transplantation Simulating Climate Changes
Primary Author Block:
M. Zhao1, B. Sun2, J. Zhou3, Y. Yang1; 1Tsinghua Univ., Beijing, China, 2Chinese Academy of Sci.,
Nanjing, China, 3Univ. of Oklahoma, Norman, OK
Abstract Body:
Although the fungal biomass is less abundant in soils than that of bacteria, they are considered to play
more substantial roles in mediating belowground carbon cycling in terrestrial ecosystems. To predict
future carbon stability, it is thus imperative to understand their responses to environmental changes,
which remains challenging owing to our limited knowledge. As current global warming causes range
shifts toward higher latitudes, we conducted three reciprocal soil transplantation experiments over
large transects in 2005 to simulate climate changes. In 2011, we collected soil samples to examine
microbial biomass, community composition and functional gene inventories. Six years after soil
transplantation, fungal biomass of transplanted soils showed a general pattern of changes from donor
sites to destination, which were more obvious in bare fallow soils than maize cropped soils. Strikingly,
fungal community compositions were clustered by sites, demonstrating that fungi of transplanted soils
acclimated to the destination environment. Several fungal taxa displayed sharp changes in relative
abundance, including Podospora, Chaetomium, Mortierella and Phialemonium. In contrast, bacterial
communities remained resistant to environmental changes. Consistent with the important role of fungi
in affecting soil carbon cycling, 8.1-10.0% of fungal genes encoding carbon-decomposing enzymes were
significantly (P < 0.01) increased and larger than those from bacteria (5.7-8.4%). To explain those
observations, we found that fungal occupancy across samples was mainly determined by annual average
air temperature and rainfall, whereas bacterial occupancy was more related to soil conditions, which
remained stable six years after soil transfer. Together, these results demonstrate that soil fungal
community is highly sensitive to climatic drivers, which may have large consequences for ecosystem-
scale carbon cycling.
Session Number: 350
Session Type: Rapid Fire
Session Title: Climate Change and Microbes
Session Start Date Time: 6/9/2018 4:30:00 PM
Session End Date Time: 6/9/2018 5:00:00 PM
Session Primary Track: Microbial Ecology and Evolution
Abstract Control Number: 9366
Poster Board Number:

Abstract Title:
Moderator
Primary Author Block:
James M. Tiedje; Michigan State Univ., East Lansing, MI
Abstract Body:
Session Title: The Secret Life of Microbes: Monitoring Microbial Activities in Diverse Environments
Session Start Date Time: 6/10/2018 12:45:00 PM
Session End Date Time: 6/10/2018 1:35:00 PM
Session Primary Track: Microbial Ecology and Evolution
Abstract Control Number: 8920
Poster Board Number:

Abstract Title:
Unraveling the Microbial and Functional Diversity of Clara Cave and Empalme Sinkhole, Puerto Rico Using Cultivable and Uncultivable Methods

Primary Author Block:
L. E. Rodriguez-Ramos, C. Rios-Velazquez; Univ. of Puerto Rico at Mayaguez, Mayaguez, Puerto Rico

Abstract Body:
Camuy River Cave Park (CRCP) is an underground cave system located at a karst in the subtropical moist forest of northern Puerto Rico. Clara Cave and Empalme Sinkhole are part of CRCP that have not been extensively studied by microbiology and metagenomics. The focus of our research was to explore the cultivable bacterial communities and molecularly, the functional diversity of Clara Cave and Empalme Sinkhole soil and water samples. Thirty-six (36) bacteria were isolated from a total of nine samples, whose pH ranged from 7.45 to 9.93, using LBA incubated at 25°C. It was found that most of them were Gram negative bacteria of variable sizes. In addition, we generated two metagenomic datasets of four water samples between Clara Cave and Empalme Sinkhole using uncultivable methods and DNA shotgun sequencing. The environmental DNA (eDNA) was extracted using Metagenomic DNA Isolation Kit for Water (Epicentre) for 200mL of water sample. Next-Generation-Sequencing technology (Illumina MiSeq) was used to process the isolated eDNA, generating two genomics libraries. Subsequently, the libraries were submitted to MG-RAST online server for taxonomic profile and functional in silico description of the samples. Our analyses showed an abundance of Proteobacteria (96.0%) at the phylum level, followed by unclassified for viruses (1.0%), Bacteroidetes (1.0%), Actinobacteria (1.0%), and Firmicutes (0.3%). At the genus level, Acinetobacter (24.0%) predominate, followed by Pseudomonas (16.0%), Bordetella (7.0%), Acidovorax (6.0%), and Achromobacter (5.0%). Meanwhile, subsystem functional analysis demonstrated that 11.0% of genes belong to clustering-based subsystems, 11.0% to amino acids and derivatives, 10.0% to carbohydrates, and 6.0% to protein metabolism. In addition, the in silico analysis suggest the presence of genes associated with resistance to erythromycin, fosfomycin, methillicin, vancomycin, streptothricin, and fluoroquinolones. Further, gene sequences related to multidrug resistance efflux pumps and anaerobic degradation of aromatic compounds were also found. In silico functional analysis using Non-Supervised Orthologous Groups (NOG) annotation showed that most proteins are poorly characterized, indicating the possibility of potential reservoirs of biomedical, industrial, or biotechnological applications. The datasets will provide a source not only for comparison with other caves studied in Puerto Rico, but also develop strategies to identify bioprospects with biomedical and biotechnological applications.
Abstract Title:
Microbial Changes in Gene Expression Level in Response to Environmental Conditions in the Delaware Bay

Primary Author Block:
M. I. Patel, B. J. Campbell; Clemson Univ., Clemson, SC

Abstract Body:
Background: Bacteria dominate in abundance, diversity and potentially metabolic activity in many environments. Our current knowledge on the influence of specific individual taxa on these processes is largely lacking. To bridge these gaps, we chose three metagenome assembled genomes (MAGs) from the Delaware Bay, phylogenetically associated with the Roseobacter clade, to examine the levels of select gene transcripts and their associated genes under different environmental conditions. Methods: Delaware Bay samples were collected and their metagenomes sequenced from various conditions including: season, salinity, time and size fraction, and were assembled into MAGs. These MAGs were annotated via RAST and used for further differential gene expression analysis with DESeq2. A phylogenomic tree was constructed with the RAxML and FastTree packages. Results: The three MAGs were phylogenetically associated with the Roseobacter clade. The closest relatives, based on NCBI and RAST databases, were Planktomarina temperata RCA23 and Rhodobacteraceae bacterium HIMB11. In general, there was a higher abundance of gene transcripts mapping to the MAGs from the summer season compared to the fall. The greatest differential expression was seen between two different seasons (summer versus fall) and time (night versus day). In addition, the different size fractions had differential expression for genes associated with motility. The highest differential expression was also seen in categories such as chemotaxis, membrane transport, amino acids, carbohydrates and photosynthesis. Conclusions: The differential expression of gene transcripts from all three MAGs was correlated to the various environmental parameters, especially season and time of day. The differential abundance of transcripts by season indicates a higher relative metabolic activity during the summer than the fall. Nutrient differences, such as carbon source, likely play an important role in seasonal variability. The MAGs of interest have genes to accommodate photoheterotrophy, and the differences in gene expression of photosynthetic genes between night and day are likely a reflection of light availability. Understanding how expression levels of specific genes vary in relation to environmental conditions provides insights into how these microbes may affect biogeochemical cycling, especially during different seasons.
Abstract Title:
Drought Conditions Decrease Respiration Rates of Most Abundant Bacterial Taxa in the Southern California Grassland Litter

Primary Author Block:
Z. Gomez-Lunar, A. B. Chase, J. B. H. Martiny, A. C. Martiny; Univ. of California, Irvine, CA

Abstract Body:
Background: Different microbial communities can carry out litter decomposition at different rates; however, little is known about when and how the composition and physiological traits of litter microbial community affect decomposition. Evidence suggests that drought conditions in southern California reduce grassland litter decomposition rate, change bacterial community composition, and also reduce bacteria population size. However, there is a knowledge gap regarding the mechanisms that regulate microbial drought tolerance as well as their impact on carbon metabolism. In this sense, if bacterial cells require large amounts of energy to drought tolerance traits, then a trade-off against growth traits and resource acquisition may result. The aim of this research is to recognize the mechanisms that regulate drought tolerance, as well as its impact on carbon metabolism in bacteria from litter communities.

Methods: Curtobacterium spp. will be used, as study model given that is the most abundant bacterial taxa in the southern California grassland litter community. Besides, Curtobacterium spp. are cellulolytic bacteria that may contribute to litter decomposition. A microcosm system will be established using grassland litter as carbon substrate in a gradient of water potential. Fifteen Curtobacterium spp. strains from five different sites will be used during two weeks of the experiment. To estimate litter decomposition, respiration measurements will be performed every 24 h, and cell abundance will be measured every 48 h by flow cytometry. To identify drought tolerance and carbon metabolism traits, samples will be analyzed via transcriptomics at two different times (48 and 336 h). Results: Preliminary results indicated that the addition of Curtobacterium spp. to microcosms increase respiration rates in all water potential level tested in comparison to negative control (no inoculated). Curtobacterium respiration rates decrease in drought conditions (water potential from -54 to -12 MPa) in comparison to higher water potential from -6 to -2.5 MPa. The highest respiration rates in all water potential levels were observed after 48 h of experiment and gradually decrease over time. Differences in cell abundance between water potential levels were not observed during the time. Conclusions: Curtobacterium spp. is an important member of litter community that contributes to carbon metabolism by breaking down structural carbon sources of litter. Additionally, drought conditions reduce Curtobacterium spp. metabolism and resource acquisition.
Effects of Long-Term Elevated CO2 On the Taxonomic and Functional Compositions of Soil Microbial Community in A Californian Annual Grassland

Primary Author Block:
S. Yang1, Q. Zheng1, M. Yuan2, Z. Shi2, N. Chiariello3, K. Docherty4, C. Field3, Y. Gu2, J. Gutzknecht5, B. Hungate6, X. L. Roux7, X. Ma1, A. Niboyet8, T. Yuan2, J. Zhou1, Y. Yang1; 1Tsinghua Univ., Beijing, China, 2Univ. of Oklahoma, Norman, OK, 3Carnegie Inst. for Sci., Stanford, CA, 4Western Michigan Univ., Kalamazoo, MI, 5Helmholtz Ctr. for Environmental Res. - UFZ, Beijng, Germany, 6Univ. of Minnesota, Twin Cities, MN, 7Univ. of Lyon, Beijng, France, 8UMR 7618 Université Pierre et Marie Curie / CNRS / AgroParisTech, AgroParisTech, France

Abstract Body:
The continuously increasing concentration of atmospheric CO2 has considerably altered ecosystems. However, there is only a limited number of studies to examine the long-term (i.e. over a decade) effect of elevated CO2 on soil microbial community. Using 16S rRNA gene amplicons and a GeoChip 4.6 microarray, we investigated soil microbial communities from a Californian annual grassland after 14 years of experimentally elevated CO2 treatment (300 ppm higher than the ambient level). Both taxonomic and functional gene compositions of the soil microbial community were affected. Taxa with higher ribosomal RNA operon (rrn) copy number (e.g. Ferruginibacter) decreased but those with lower rrn copy number (e.g. Acidobacteria Group 1) increased. As a consequence, the abundance-weighted average rrn copy number was significantly (P<0.01??) decreased from 2.46 in ambient CO2 plots to 2.36 in elevated CO2 plots. In addition, a large number of microbial genes related to carbon (C) degradation were affected by elevated CO2, while those related to C fixation remained largely unchanged. The nifH nitrogen (N) fixation gene and the amoA nitrification gene significantly decreased in elevated CO2 plots by -12.6% and -6.1%, respectively. Concomitantly, nitifying enzyme activity decreased by -48.3%. There was also notable N reallocation from soil to plants as revealed by increased annual grass biomass (+29.3%), perennial forbs biomass (+77.3%) and total root biomass (ca. +30%). The overall changes of microbial communities, plant and soil N pools induced by long-term elevated CO2 treatment were consistent with the N limitation theory.
Abstract Title:
Microbial Communities At Varying Depths of Siberian Permafrost

Primary Author Block:
A. Almatari1, D. Williams1, E. Spirina2, S. Pfiffner1, E. Rivkina2, K. G. Lloyd1, T. Vishnivetskaya1; 1Univ. of Tennessee Knoxville, Knoxville, TN, 2Inst. of Physicochemical and Biological Problems in Soil Sci., Pushchino, Russian Federation

Abstract Body:
This research seeks to explore the differences in microbial communities with respects to geological age and depth. Samples were collected from Northern Siberia at the Alazeya River in boreholes from the surface to a maximum depth of 24.6 meters. We hypothesize that sediments whose DNA was isolated from shallower depths may show more diversity than deeper sediments due to the age of the sediments and time microbial cells stay frozen. Cores were collected with a slow rotary drill using quality control procedures. Collected cores were subsampled with sterile knife into sterile Whirl-Pak bags and were transported to the University of Tennessee in a frozen state. DNA was isolated from these samples using Fast Spin DNA Kit for Soil®. The 16S rRNA gene was amplified using 27F and 1492R with a further nested amplification using 515F and 806R primers. All samples were cleaned and concentrated using DNA Clean & Concentrator® Kit (Zymo Research). DNA concentration was measured ranging from 1.2-83 ng/µL. After confirming DNA presence without contamination using Agilent Automatic Gel Electrophoresis, correct DNA concentration for MiSeq was determined using qPCR. Sequencing was completed using Illumina MiSeq and comparative analyses were achieved by using the CLC Genomics Workbench. The Greengenes 16S rRNA gene database was used to cluster sequences into OTUs at 95% sequence similarity threshold. Data were normalized through CLC. We see that the samples in bore hole AL1 at depths 1.75-1.8 m and 2.95-3.0 m have a greater alpha diversity than samples from depths of 22.9 m and 24.5-24.6 m. Actinobacteria predominated in the shallow depths of 1-3 m at 47-60 % compared to the 1-6% seen in the deeper sediments (23-25 m). Likewise Firmicutes were more prominent in the deeper sediments at 46-87% compared to the 5-35% shown in the shallower depths. From shallow permafrost sediments to deeper we see changes in bacterial community from aerobic non-spore-forming high GC Gram-positive Actinobacteria to anaerobic spore-forming low GC Gram-positive Firmicutes. The deepest permafrost sample was different and we see a lot of Betaproteobacteria, Burkholderia, which are Gram-negative aerobic bacteria that often found in decaying organic matter. Microbial diversity data from the permafrost strata will add to our understanding of polar microbial ecology.
Session Number: 382
Session Type: Poster Talk

Session Title: The Secret Life of Microbes: Monitoring Microbial Activities in Diverse Environments
Session Start Date Time: 6/10/2018 12:45:00 PM
Session End Date Time: 6/10/2018 1:35:00 PM
Session Primary Track: Microbial Ecology and Evolution
Abstract Control Number: 9365
Poster Board Number:

Abstract Title:
Moderator
Primary Author Block:
Karen Lloyd; Univ. of Tennessee, Knoxville, TN
Abstract Body:
Abstract Title:
Plant and the Tibetan Plateau Uplifting Jointly Influence Altitudinal Zonation of Soil Microbes

Primary Author Block:
G. Zhang1, A. Hu1, J. Wang2, H. Sun3, J. Zhou4; 1Inst. of Tibetan Plateau Res. CAS, Beijing, China, 2Nanjing Inst. of Geography & limnology CAS, Nanjing, China, 3Kunming Inst. of Botany CAS, Kunming, China, 4Tsinghua Univ., Beijing, China

Abstract Body:
Altitudinal zonation is a basic ecological pattern in distribution of plants and animals, community assemblages and species interactions, and the causes thereof in mountain research. It is unknown whether soil microbial assemblages will organize themselves into altitudinal zones. Here, a range of over 3,000 m altitudinal gradient on the Tibetan Plateau, from a tropical monsoon rain forest to frigid shrub meadow ecosystem, was studied to compare the composition and function of soil microbial communities. We found altitudinal zonation patterns among microbial habitats and communities. Microbial phospholipid fatty acids, enzyme activities, alpha-diversity, beta-diversity, phylogenetic relatedness and microbial functional groups (e.g., N2-fixation and phototrophic CO2-fixation bacteria) showed discontinuous transitions from tropical and subtropical broadleaved to temperate and frigid coniferous forests. Altitudinal zonation patterns among microbial communities were further confirmed with a forest, wetland and grassland altitudinal gradient on the Tibetan plateau. Plant and the Tibetan plateau uplifting jointly influence altitudinal zonation of soil microbes. The stronger effect of plant on microbial community composition and function in temperate and frigid coniferous region might benefit microbes adapting to alpine environment. These different coupling patterns between plants and microbes may structure microbial assemblages into zones. Our findings may provide deeper insights on mechanistic interpretation of altitudinal zonation of soil microbes and speculations on zonation patterns across ecosystems and altitudes in the tectonic plates.
Abstract Title:
Multidrug resistant IncA/C plasmids shaped the evolution of major Salmonella Newport food-animal clone ST45

Primary Author Block:
M. Yue1, H. Pan1, S. Rankin2, D. Schifferli2, W. Fang1; 1Zhejiang Univ., Hangzhou, China, 2Univ. of Pennsylvania, Philadelphia, PA

Abstract Body:
Background: Antibiotic use in livestock production is considered to play an important role in the selection of antibiotic-resistant bacteria. And the genetic and/or evolutionary pressure for selection of antibiotic-resistant pathogens in food-animals remains unclear. Salmonella enterica serovar Newport (S. Newport) is an important foodborne pathogen which can serve as a model to address this question.

Methods: This study was a global investigation of the population diversity of S. Newport isolates by studying the genomic evolution and antibiotic resistance of 1,855 isolates. Results: MLST identified three clades that correlated with niches/origins of isolation (human, animal, environment). Genomic analysis of 1,855 isolates identified Sequence Type 45 (ST45) as the predominant clone among the animal isolates (87%), but only in 9% for human infections. ST45 isolates carried multiple plasmids, the majority (> 90%) had a unique IncA/C plasmid (from 80 to 200 kb). The plasmid carried genes responsible for multidrug resistance, including floR, tetAR, strAB, sul, and bla. Importantly, three Chinese strains carried the mcr-1 gene, that confers plasmid-mediated resistance to colistin. A genome-wide association study (GWAS) correlated chromosome regions or genetic variations with maintenance of IncA/C plasmid in ST45 isolates. An additional investigation of MIC of 27 antibiotics in isolates (food-animals, retail-meat, humans) suggested that Antibiotic-resistant strains from humans have multiple but distinct origins. Animal and retail-meat isolates are distinct from > 92% of the human isolates by their antibiotic-resistant patterns. Finally, we have identified SNP markers that correlate with antibiotic-resistant pattern in the available strains, which can be used as novel diagnostic tool to predict antibiotic-resistance.

Conclusions: S. Newport ST45 is the dominant clone in food-animals across countries studied. The GWAS will serve to investigate genetic determinants that contribute to the maintenance of this clone in food-animals.
Abstract Title:
Metagenome-Assembled Genomes Reveal Previously Unrecognized Levels of Bacterial Diversity in the Oral Microbiome

Primary Author Block:
D. R. Utter1, G. G. Borisy2, C. M. Cavanaugh1; 1Harvard Univ., Cambridge, MA, 2The Forsyth Inst., Cambridge, MA

Abstract Body:
Humans and their microbes are highly integrated, with microbiome composition an essential aspect of human health. Thus, it is crucial to study the composition, ecology, and evolution of healthy microbiomes. Here we leverage the application of a new approach at the pangenome level, providing unprecedented insights into microbial diversity in a complex community. The human tongue microbiome represents a good test bed for such inquiries given its accessibility, stable community, broad phylogenetic diversity, and clinical relevance. Previous genomic investigations in any system have been limited by cultivability biases. However, metagenome-assembled genomes (MAGs) generated from whole-community sequencing now allow direct investigation of the environment. Short-read metagenomic data can also be recruited back to a pangenome of cultured isolates or MAGs. This revolutionary analysis reveals the environmental abundance of every genome and highlights the genes establishing that abundance. We studied the genomic composition of the tongue microbiome by sequencing bulk community DNA from the tongues of 19 individuals sampled 4-7 times over a 2 week time course. Using Anvi’o, an interactive metagenomic analysis platform, 400 high-quality bacterial MAGs were obtained in total. Pangenomic analysis demonstrated that MAGs generally clustered into species groups, and revealed significant differences between the tongue MAGs and cultured representatives. Based on short-read data from our metagenomes and the Human Microbiome Project (HMP), cultured genotypes were environmentally rare in either dataset, if at all present, while MAGs were prevalent and abundant in both datasets. Oral genera which had both well-defined MAGs and sufficient genomes in existing databases were analyzed, including Rothia, Prevotella, and Capnocytophaga. Each MAG contributed tens to hundreds of novel genes. Phage and other mobile genetic elements were abundant in the MAG-specific gene set. Other environmentally relevant genes coding for surface receptors, iron uptake and utilization, multidrug efflux pumps, and carbon metabolism were also well represented, suggesting that the uncultured microbiome may employ different ecological strategies than their cultured relatives. The oral microbiome clearly harbors a vast amount of environmental diversity, indicating previously underappreciated ecological and evolutionary processes at work.
Session Title: SUNDAY - MEE Late-breakers

Abstract Title: Plasmid-mediated Salt Tolerance in Halophyte Rhizosphere Microbiome

Primary Author Block: S. Mukhtar1, S. Mehnaz1, M. S. Mirza2, K. A. Malik1; 1Forman Christian Coll., Lahore, Pakistan, 2Forman Christian Coll., Faisalabad, Pakistan

Abstract Body:
Background: Soil salinity has been negatively impacting plant productivity for centuries, limiting their growth, thus decreasing the crop yield. The rhizosphere of halophytes harbours a variety of microorganisms (microbiome) that have ability to promote plant growth. Main purpose of this research was to study plasmid-conferring salt tolerance in rhizospheric bacteria isolated from Salsola stocksii and Atriplex amnicola. Methods: Twenty two strains were characterized morphologically, biochemically and identified by PCR amplification of specific 16S rRNA gene sequences. Plasmid curing of isolates was done by heat shock method to study the effect of plasmid conferring salt tolerance. These plasmids were isolated and transformed into E. coli and growth response of original strains and transformed E. coli was compared at 1.5-4M NaCl concentration. Selected plasmids were sequenced by using NGS and characterised osmoregulatory genes. Results: Almost all strains showed optimum growth at 1-3.5M NaCl. These strains were related to Bacillus spp., Nesterenkonia sp., Oceanobacillus sp., Halomonas spp., Citricoccus sp., and Kocuria sp. Results of plasmid curing showed that some halophilic bacterial strains such as Bacillus strain HL2HP6, Oceanobacillus strain AT3HP15, Nesterenkonia strain NRS3HaP12 and Oceanobacillus strain LK3HaP7 lost their ability to grow in halophilic medium but they grew well on LB medium. Some plasmid cured strains also showed a change in sensitivity to specific antibiotics. Conclusions: Functional analysis of plasmid sequences showed different proteins and enzymes which are known to be involved in genetic information processing, carbohydrate metabolism, amino acid metabolism, xenobiotics biodegradation and metabolism, glycan biosynthesis and metabolism and human diseases. Some unclassified and uncharacterized proteins were also identified.
Abstract Title:
Starting Small, Getting Smaller to Survive: Evolutionary Rescue and Genomic Adaptation of Small Colony Variant Gram-Negative Bacteria from Chronically Infected Pediatric Patients

Primary Author Block:
A. Greninger1, Y. Tao2, A. Addetia3, X. Qin3;  1Univ. of Washington, Seattle, WA, 2Shanghai Children's Translational Inst., Shanghai, China, 3Seattle Children's Hosp., Seattle, WA

Abstract Body:
Background: The emergence of bacterial small-colony-variants (SCVs) from the initial isogenic normal colony phenotype (NCV) parental isolates has been described in various clinical settings, usually as an adaptive response to chronic antimicrobial treatment (1). Bacterial SCV phenotypes are characterized by slow growth rate, auxotrophism, and reduced response to antibiotics in vivo (1, 2). Other common SCV nutritional deficiencies include growth requirements for thymidine and methionine whose final product depend on an intact folic acid biosynthetic pathway (3, 4). SCV strains have long been noted to be associated with Staphylococcus aureus, Escherichia coli, Neisseria gonorrhoeae, Stenotrophomonas maltophilia, Enterococcus, Salmonella, but have not been investigated in other species of bacteria, nor is the SCV mechanism genomically characterized. Methods: Three pairs of NCV and SCV co-isolates of Citrobacter freundii, Enterobacter cloacea, and Escherichia coli from 3 affected pediatric patients were chosen for whole genome sequencing. SCV-specific variants were called by mapping reads to the paired NCV genome and calling variants with >10X coverage and 70% variant allele frequency. Genetic complementation was performed using electroporation of the SCV strain with plasmids expressing the wild type Escherichia coli homolog. Results: The three corresponding SCV Enterobacteriaceae isolates were phenotypically auxotrophic for heme. The responsible mutations in the SCVs were immediately apparent showing genetic lesions in hemL of C. freundii, hemN of E. cloacea, and both hemF and hemL of E. coli which were intact in their corresponding NCV genomes. The growth of E. coli SCV containing a W59R in hemL and ΔER232 in hemF was completely restored by complementation in trans using a pAC24N plasmid carrying a wildtype E. coli hemF, while a partial growth restoration in SCV E. cloaeae containing a L366Q in hemN was complemented by the E. coli K-12 hemN. Other genetic lesions including a truncation in fes encoding the enterochelin esterase and yusV encoding a putative siderophore transport system ATP-binding protein were found in the SCV C. freundii and SCV E. coli, which suggested a novel finding of bacterial growth and virulence attenuation by limiting of iron availability. Conclusions: Adaptive lesions in heme and iron homeostasis genes are repeatedly recovered as a form of evolutionary rescue in Enterobacteriaceae in response to chronic antimicrobial administration in pediatric patients.
Abstract Title:
Multiple Sequence Alignment of Ndm-1 Dna Sequence of Different Carbapenemase Producing Gram-Negatives

Abstract Body:
Background: NDM-1 is an enzyme that confers resistance to bacteria against a broad range of β-lactam antibiotics. It is one of the most effective Carbapenemases in terms of carbapenem hydrolysis and geographical spread. Therefore, learn how its DNA sequence evolved through testing its sequence in some organisms might give an idea about the effect of geographical distribution and other environmental effects. In our work, we are trying to find out how the wide spreading of DNM-1 among different Gram-negatives could affect the conservatively of its DNA sequence i.e. the percentage of similarity and differences among NDM-1 DNA sequence. This might give possible insights into the development of new resistance against current effective antibiotics which in turns might help to the precedent event for a possible treatment Methods: From GenBank, the complete DNA coding sequences of NDM-1 of Acinetobacter (johnsonii NF114, sp. NF111, sp. NF116), Klebsiella pneumoniae (Res2011-182, K.P-UC-13, K.P-UC-14, K.P-UC-15), Citrobacter freundii NF109, Escherichia coli NF113, Providencia vermicola NF115 and Pseudomonas sp. NF117 were obtained (813 bp for each). DNA sequence analysis was carried out by means of multiple sequence alignment using EMBOSS Clustal Omega and aligned using default setting with all mentioned strains Results: Percent identity matrix showed a 100% of similarity between all Klebsiella strains, it also showed a 100% similarity between Acinetobacter strains, C. freundii NF109, E. coli NF113, P.vermicola NF115 and Pseudomonas sp. NF117. But showed only 44.12% of similarity between all the above-mentioned strains with K. pneumoniae strains. The cladogram showed unexpected evolutionary pathway of NDM-1 between Klebsiella strains and A. johnsonii and A. sp. NF111, compared with A. sp. NF116 and other tested strains Conclusions: NDM-1 sequence evolved dramatically when transferred to some species while still conserved with others. The evolution might arise as a result of a mutation in the resistance sequence while being conserved among different species could be attributed to the convergent evolution
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Session Primary Track: Microbial Ecology and Evolution
Abstract Control Number: 5013
Poster Board Number: SUNDAY - 950

Abstract Title:
Reconstruction and of Health-Impact Pathways in the Human Gut Microbiome
Primary Author Block:
D. A. Ravcheev, L. Moussu, I. Thiele; Univ. of Luxembourg, Esch-sur-Alzette, Luxembourg

Abstract Body:
Background: The human gut microbiome (HGM) have been shown to play a crucial role in human physiology, nutrition, health, and disease. Thus, to date, more than 50 human diseases have been demonstrated to be associated with HGM alterations and more than a dozen HGM-produced metabolites are known to be associated with human health state. Here we present results of a computational comparative-genomic analysis of pathways for biosynthesis or catabolism of such health-impact compounds in the HGM genomes. Methods: We applied genomic analysis to 632 HGM genomes microorganisms found in the human gut belonging to the phyla of Actinobacteria, Bacteroidetes, Euryarchaeota, Firmicutes, Fusobacteria, Proteobacteria, Synergistetes, Tenericutes, and Verrucomicrobia. For the annotation of gene functions, the PubSEED platform (http://pubseed.theseed.org) was used. The gene function annotation was done using available literature data, protein sequence similarity, protein domain structure, and genome-context based approaches, including gene chromosomal clustering and phyletic patterns. Results: We analyzed six types metabolic pathways associated with production, catabolism, or transformation of health impact compounds, as follows, (1) biosynthesis of vitamin K), (2) biosynthesis of butyrate, (3) biosynthesis of propionate, (4) utilization of ethanolamine, (5) utilization of propanediol, and (6) biotransformations of bile acids. First, for each of health-impact compound, more than one pathway was found in the HGM genomes. Second, distribution of these health-impact pathways in the HGM genomes is a taxonomy-specific, but a distribution of no one pathway is limited by a single microbial taxon. Third, distribution of the analyzed pathways points to an intensive exchange of health-impact compounds or their precursors in the HGM. Finally, comparison of a taxonomic composition of healthy and diseased HGMs demonstrated the different distribution of the analyzed pathways in healthy and diseased microbiomes. For example, genes for bile acid biotransformation were significantly underrepresented in Crohn’s Disease microbiomes, in comparison with healthy HGMs. Conclusions: We demonstrate that pathways for metabolism of six different health-impact compounds are broadly distributed in HGM organisms. Data on the distribution of these pathways can improve an understanding of a role of the HGM and provide a future prediction of the HGM genes associated with the host health state.
Abstract Title:
Chromosome Rearrangement, Gene Amplification, and Insertion Sequence Elements in the Genome Evolution of Bordetella pertussis And the Genus Bordetella

Primary Author Block:
M. R. Weigand1, Y. Peng1, D. Batra1, M. Burroughs1, J. K. Davis1, K. Knipe1, V. N. Loparev1, T. Johnson1, P. Juieng1, L. A. Rowe1, M. Sheth1, K. Tang1, K. Xavier2, M. M. Williams1, M. L. Tondella1; 1CDC, Atlanta, GA, 2Colorado Dept. of Publ. Hlth.and Environment, Denver, CO

Abstract Body:
Background: Whooping cough, primarily caused by Bordetella pertussis, has resurfaced in the United States even though coverage with pertussis-containing vaccines remains high. There is evidence to suggest that increased disease results, in part, from genetic divergence of circulating strain populations away from vaccine references. Recent genomic analyses have revealed considerable chromosome structural fluidity, such as rearrangement and deletion, among clinical isolates despite limited gene nucleotide sequence variation. Methods: To investigate the contribution of gene order rearrangement towards pertussis disease resurgence, we have studied differences in chromosome structure among 400 B. pertussis isolates as well as 50 isolates of related Bordetella species. Genome sequencing was performed using the combined technologies of PacBio RSII, Illumina HiSeq/MiSeq, and OpGen or Nabsys enzyme mapping to produce complete, reference-quality assemblies for analysis. Results: Most changes in gene order were flanked by insertion sequence elements (ISEs) and specific rearrangements differentiated B. pertussis vaccine reference strains and clinical isolates of various genotypes. Select, unrelated strains also included amplification of large genomic regions, varying in size and gene content but primarily occurring at two discrete loci, which were also flanked by ISEs. Genomes of B. pertussis have >240 copies of IS481 and closely-related isolates with differing rearrangements, amplifications, and insertion content were observed. Within the broader genus Bordetella, which includes additional human and animal pathogens, chromosome rearrangements were only detected in species harboring other, multi-copy ISEs. Conclusions: These results illustrate the immense chromosome structural diversity among circulating B. pertussis and reveal that such variation also exists in related Bordetella species colonized by ISEs. Specific gene order changes appear phylogenetically conserved, suggesting rearrangement contributes to genome evolution and thus perhaps pertussis disease resurgence. However, rapidly detecting such structural variation for sequence-based molecular epidemiology requires further development of sequencing, mapping, and bioinformatics methods.
Abstract Title:
Comparative Sequence Analysis Revealed Altered Chromosomal Organization and A Novel Insertion Sequence Encoding Dna Modification and Potentially Stress-Related Functions in An Escherichia coli O157:H7 Foodborne Isolate

Primary Author Block:

Abstract Body:
We recently described the complete genome of enterohemorrhagic Escherichia coli (EHEC) O157:H7 strain NADC 6564, an isolate of strain 86-24 linked to the 1986 disease outbreak. In the current study, we compared the chromosomal sequence of NADC 6564 to the well-characterized chromosomal sequences of EHEC O157:H7 strains EDL933 and Sakai in order to identify differences in chromosomal organization and determine the presence or absence of specific sequences indicative directly or indirectly of differential host or environmental adaptability of NADC 6564. The comparative analysis revealed that the NADC 6564 chromosome (5466 kbp) was 32 kb and 81 kb smaller than Sakai (5498 kbp) and EDL933 (5547 kbp) chromosomes, respectively. Aligned chromosomal sequences of these strains contained identical Linear Conserved Blocks (LCB) but some of these LCBs had undergone rearrangement in NADC 6564. All three strains contained 18 bacteriophage-like elements at similar chromosomal sites except that NADC 6564 contained an additional phage-like element in a 33 kb DNA segment flanked by direct repeats. The 33 kb region contained genes encoding integrases, transposases, a transcriptional regulator, a DNA binding protein, an adhesion, and a phosphoethanolamine (PEA) transferase. The PEA gene showed 32-33% homology (nucleotide and amino acid) to four other PEA transferase-encoding genes present in the chromosome of NADC 6564 and other EHEC strains, but greater than 95% homology to a PEA transferase-encoding gene of uropathogenic and avian pathogenic E. coli and EHEC O104:H21. The five PEA genes of NADC 6564 showed varying degrees of homology to mcr1 and mcr2 encoding colistin resistance. Although NADC 6564 showed higher minimum inhibitory concentration for colistin compared to EDL933 and Sakai harboring four PEA genes in their chromosomes, NADC 6564 remained colistin-sensitive. The 33 kb region also contained two oppositely transcribed operons encoding for DNA backbone S-modification via phosphorothioation and DNA restriction pathways, respectively. These operons conferred S-modification on DNA, which has been linked to enhanced bacterial tolerance to oxidative stressors. In summary, the altered physical organization of the conserved regions of NADC 6564 chromosome could be attributed to recombination events and the acquisition of genes for DNA S-modification could potentially enhance NADC 6564 adaptability to host and/or environment under conditions of oxidative stress as has been demonstrated for bacterial strains capable of S-modification of DNA.
Radiation Oncology: A New Way to Study Clin. Microbial Genomic Streamlining in Vivo?

A. Greninger;  Univ. of Washington, Seattle, WA

Abstract Body:
Background: Microbial genome evolution is known to be extraordinarily rapid with promiscuous gene acquisition/loss and swift generation of single nucleotide variants. Bioinformatic analyses of microbial genomes require discussion of both core- and pan-genomes, but understanding of whether a given gene is truly required or adaptive is limited in such analysis. Gene essentiality screens using randomly integrating transposon mutagenesis and/or deep mutational scans have been used to experimentally assess the genomic requirements for replication, but they are limited to cell culture and animal models. There is currently no simple method of experimentally studying large-scale microbial genomic evolution in the human host. Methods: We describe a proof-of-concept study on the use of radiation oncology to understand in vivo genomic streamlining. We prospectively sequenced genomes from microbial strains associated with recent hematopoietic cell transplantation (HCT). Results: We describe metagenomic sequencing of a human coronavirus 229E from a patient with AML and persistent upper respiratory symptoms, who underwent HCT. Sequencing revealed a 548-nucleotide deletion, which comprised the entire ORF4 gene. No minor allele variants were detected to suggest a mixed infection. As part of her pre-HCT conditioning regimen, the patient received myeloablative treatment with cyclophosphamide and 12 Gy total body irradiation. Iterative sequencing and RT-PCR confirmation of 4 respiratory samples over the 4-week peritransplant period revealed that the pre-conditioning strain contained an intact ORF4 gene, while the deletion strain appeared after conditioning and persisted over a 2.5-week period. This sequence represents one of the largest genomic deletions detected in a human RNA virus and is the first description of large-scale viral mutation associated with myeloablation. Previous in vitro studies have hinted at the dispensability of ORF4 in human alphacoronaviruses, but no function has been elucidated for this mysterious gene. Conclusions: Radiation oncology treatments provide an intriguing opportunity for the study of microbial gene essentiality and genomic streamlining in humans. We hypothesize that radiation oncology may prove more fruitful in understanding prokaryotic genomic evolution where gene dispensability is greater than in RNA viruses. We will discuss opportunities for understanding genomic streamlining in real-time in the human host in the setting of different radiation oncology treatments.
Abstract Title:
Phylogenomic Analysis of Fusobacterium Necrophorum Based on Whole Genome Sequencing and its Assoc. with Disease and Host

Primary Author Block:
A. Jensen; Aarhus Univeristy, Aarhus, Denmark

Abstract Body:
Objective: Fusobacterium necrophorum (FN) is the main cause of Lemierre’s syndrome and FN has also been associated with tonsillitis and peritonsillar abscesses, otitis media and colorectal cancer. FN may also be found in asymptomatic carriers. Virtually nothing is known about this bacterium at the molecular and genetic level. The aim of this study was by whole genome sequencing of a large volume of strains isolated from patients with different diseases and hosts to determinate the phylogenetic diversity of FN and to identify whether certain phylogenetic lineages of FN may be associated with specific diseases.

Material and Methods: In total, genomes of 85 strains were analyzed. Of these, 70 were from our own strain collection while the genomes of 15 strains were extracted from Genbank. Phylogenomic analysis were based on single nucleotide polymorphism (SNP) analysis using the software program parsnp while pairwise average nucleotide identity (ANI) was calculated in the JSpecies program.

Results: Phylogenomic analysis based on SNPs of the whole genome sequences revealed that the FN strains grouped into three distinctive clades, corresponding to the two subspecies of FN. The third clade consisted of two penicillin-resistant human isolates of FN. The third clade identity were around 95% supporting separation of FN into subspecies. Strains of F. necrophorum subsp. funduliforme clustered into two distinct clusters. Within both cluster a clonal relationship between most of the strains were observed. Most interestingly, no separation of the strains based on the host or disease could be found. Conclusions: No lineages of FN were associated with specific diseases in humans and invasive strains were phylogenetically similar to strains from local infections and strains isolated from healthy carriers. Also, human isolates of FN were similar to animal strains. Our results indicate that virulence and invasiveness of FN is not associated with a specific phylogenetic lineage. Therefore, FN infections might involve additional factors to cause infections (host specific and other external factors (e.g. viruses)). Preliminary data on the intracluster virulence potential and its relationship to disease will be presented at the congress.
Abstract Title:
The Role of Recombination-Driven Gene-Specific Sweeps in Phenotypic Differentiation of Pathogenic Bacteria with Streptococcus Pyogenes As A Paradigm

Primary Author Block:
Y. Bao; Univ. of Notre Dame, Notre Dame, IN

Abstract Body:
Background: The robust characterization of phenotype-genotype correlations in diseases is essential for physiological study and precision therapeutics. The correlations are often complicated in infectious diseases caused by pathogenic bacteria due to rapid adaptation of pathogens to host environments through gaining diverse genetic variations. Recombination is one of the most important mechanisms employed by pathogens to gain variations and influence disease phenotypes. In this study, we aim to characterize the role of recombination and its mechanisms in shaping phenotypic differentiation in pathogenic bacteria.

Methods: Using the highly ranked infectious agent, Group A Streptococcus pyogenes (GAS), as a paradigm, we performed a population genomic study on natural GAS isolates. We first used genome-wide association framework to identify genetic variations associated with distinct disease phenotypes. We subsequently performed statistical modeling and phylogenetic congruence test to elucidate the evolutionary mechanisms underlying the differentiated phenotypes. The contribution of recombination to the differentiation was assessed by relative rate of recombination vs. point mutation.

Results: The population genomic study based on genome-wide single-nucleotide polymorphisms (SNPs) demonstrates a phenotypic differentiation, represented by separate clustering of isolates into two sublineages infecting distinct human tissue sites, i.e., skin and pharynx. Genome-wide association study identified 895 and 1,638 SNPs associated with infections at the two sites, respectively (p-value ≤ 10^{-3}). We modeled the SNP distribution against a null hypothesis of random independent occurrence across the genome. The chi-square goodness-of-fit test reveals a pattern of SNP distribution caused by gene-specific sweeps, whereby multiple SNPs are derived by individual recombination events. Phylogenetic congruence test also confirms the acquisition of the SNPs by recombination at specific gene regions. We also assessed the relative contribution of recombination vs. point mutation r/m. It shows that more than 90% of the SNPs associated with phenotypic differentiation may have been brought about by recombination-driven gene-specific sweeps.

Conclusions: The present study provides a paradigm of phenotypic differentiation in the human pathogen causing clinical complications. It demonstrates the remarkable impact of genomic recombination on shaping the phenotypic traits and accelerating adaptive evolution.
Comparative Genomics of the Emerging Pathogen Streptococcus Pseudopneumoniae

G. Garriss, P. Nannapaneni, B. Henriques-Normark; Karolinska Inst., Stockholm, Sweden

Background: Streptococcus pseudopneumoniae (Sppn) is a gram positive bacterium closely related to the major human pathogen Streptococcus pneumoniae (Spn). While initially considered as a commensal, there is increasing evidence that Sppn is a pathogen (1, 2); however the lack of a specific test for identifying Sppn severely hampers the understanding of its pathogenic potential. Current typing methods discriminate Spn from commensal α-hemolytic streptococci based on optochin susceptibility, bile solubility and capsular serotyping. Atypical phenotypes, lack of a pneumococcal capsule and variations in optochin susceptibility testing conditions leads to common misidentification of Sppn isolates as non-typable (NT) Spn or other species such as S. mitis (1, 3). Our objectives were to gain understanding of the genetic characteristics of Sppn clinical isolates and to identify species-specific markers allowing their precise identification.

Methods: Whole-genome sequencing of 25 presumed Sppn strains from lower respiratory tract infections and core genome phylogenetic analysis including publicly available Sppn, Spn, NT Spn and S. mitis genomes were used to identify the Sppn clade. Collectively these genomes encompass isolates from carriage and invasive disease from children and adults. The defined Sppn clade was further investigated in silico to identify known and potential new virulence genes, acquired antimicrobial resistances (AMR), mobile genetic elements (MGEs) and unique genes. Results: 43 strains, including 22/25 of our clinical isolates and 21 NCBI genomes were found to belong to the Sppn clade, including 8 currently misclassified as NT Spn. We found that a large number of pneumococcal virulence genes are part of the core Sppn genome. MGEs encoding resistance to tetracycline and macrolides, as well as plasmids, were widely distributed and all strains carried at least one full-length prophage. Sppn strains clustered in 3 different sub-clades characterized by different accessory virulence genes, MGEs and AMR genes. Genetic loci found exclusively in Sppn strains were used for the development of a PCR-based assay that allowed the precise identification of Sppn isolates from other α-hemolytic streptococci.

Conclusions: Our study reveals the large number of pneumococcal virulence genes that are part of core genome of Sppn as well as the composite scenario of MGEs and their association with AMR genes. Our novel typing assay provides a simple tool for better understanding the epidemiology and clinical significance of Sppn.
Abstract Title: OutstandingAbstract Award: Two Faces of Janus: Group A Streptococcal (Gas) Upper Respiratory Tract Colonization and Invasive Infection: Molecular Diversity and Transcriptomics

Primary Author Block:
T. Abraham, S. Sistla; Jawaharlal Inst. of Postgraduate Med. Ed. and Res. (JIPMER), Puducherry, India

Abstract Body:
Background: GAS is a strict human pathogen causing a broad spectrum of diseases. The throat and skin epithelia form the primary ecological niches of GAS as well as the sites for tissue intrusion in case of invasive disease. Pathogenic bacteria employ diverse mechanisms to fine-tune the expression of virulence factors and metabolic processes in response to growth conditions encountered in different milieu and stages of infection. Understanding the evolution of niche specialization can reveal new insights about the critical factors essential for the disease development. The present study was undertaken to determine the molecular diversity of colonizing and invasive GAS isolates and the basis of such transition with transcriptome analysis of select isolates.

Methods: The study comprised of 107 GAS isolates, (59 from invasive diseases, 48 from asymptomatic throat colonizers). To determine molecular diversity, the isolates were characterized by emm genotyping and MLST. Whole transcriptome analysis was performed on select isolates using Illumina Hiseq 2500 platform, and the gene expressions were estimated using String_Tie program. Results: A total of 41 emm types were identified from clinical isolates with emm82, emm183, emm44, emm66 and emm63 in decreasing order. Six of the emm typable community isolates belonged to emm66, emm80, emm77 and emm75.1. MLST analysis of the randomly selected 36 isolates demonstrated a considerable genetic diversity, with twenty different STs and some new STs (969, 970, 971, 972, 973). More than 400 transcripts were highly expressed, mainly belonging to functional categories involved in the adaptive metabolic shift (ska, RelA, CodY, arcCBA, Sdh, Idh, etc) and virulence gene transcription (Isp, hasABC, hyl, smeZ, saga, crg, scpA, etc). About 49% of the expressed genes are hypothetical ORFs of unknown function. Genes with significant expression changes (DEGs) were identified by FDR<0.10 and log2FC ≥1 and considering the community isolate as wild type. Thirteen transcripts were up-regulated (clpL, int3, speI, speH, hylP2) and one was down-regulated in the blood isolate. In the tissue isolate, twenty transcripts were up-regulated (salA, scpA, mga, emm1, lys, hylP2) and sixteen were down-regulated (srt, ddh, sat, hsdS, Spy_1075). Conclusions: Our study provides insights into the changes associated with the plasticity of GAS transcriptome. Increased understanding of how GAS strains from various habitats respond during ex vivo culture could be helpful in developing novel preventive or therapeutic strategies.
Abstract Title:
Genomic Characterization of Urethritis-Associated Neisseria Meningitidis Shows that A Wide Range of N. Meningitidis Strains Can Cause Urethritis

Primary Author Block:
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Abstract Body:
Background: N. meningitidis, typically a resident of the oropharynx and the causative agent of meningococcal meningitis and meningococcemia, is capable of invading and colonizing the urogenital tract. This can result in urethritis, akin to its sister species N. gonorrhoeae, the etiologic agent of gonorrhea. Recently, meningococcal strains associated with outbreaks of urethritis were reported to share genetic characteristics with gonococcus, raising the question of the extent to which these strains contain features that promote adaptation to the genitourinary niche, making them “gonococcus-like” and distinguishing them from other N. meningitidis. Methods: A total of 39 urethritis-associated N. meningitidis, representing multiple serogroups and independently collected over a decade and 3 continents, underwent genome sequencing and analysis. The genomes were compared with serogroup-matched N. meningitidis strains isolated from carriage and invasive disease and N. gonorrhoeae strains isolated from men with urethritis. Results: Intact nitrite reductase (AniA), disrupted factor-H binding protein (fHbp), and the lack of capsule are features previously speculated to promote urogenital colonization. However, we found that a considerable number (n=11) of meningococcal urethritis isolates harbor mutations in AniA predicted to result in truncated peptides and a minority (n=7) of these isolates contained alleles associated with frameshifted fHbp. We noted substantial diversity in the capsule biosynthetic locus, including intact, disrupted, and absent capsules, indicating urogenital colonization is possible across a range of capsular phenotypes. In an ST-11 lineage of N. meningitidis previously associated with outbreaks of nongonococcal urethritis, we observed novel signatures of horizontal gene transfer that may have contributed to the relative success of this clade. Conclusions: The meningococcal urethritis strains in this study overall do not share the allelic patterns of AniA, fHbp, or the capsule locus previously reported for urethritis-associated N. meningitidis; however, particular lineages may have undergone further adaptation to the urogenital environment via recombination. Genomic analysis therefore might provide insight into the biological basis for both sporadic and sustained cases of urethritis caused by N. meningitidis.
Abstract Title:
Biomarker Traits Exclusive to Neonatal Meningitic Cronobacter Sakazakii Clonal Complex 4

Primary Author Block:
N. Masood, F. Al Madani, A. Albastaki, R. Poria, S. Forsythe, S. Bagheri; Nottingham Trent Univ., Nottingham, United Kingdom

Abstract Body:
Background: Cronobacter sakazakii is a member of the Cronobacter genus and is associated with serious infections such as meningitis, septicaemia and necrotizing enterocolitis, especially among infants and immunocompromised adults. A multilocus sequence typing (MLST) scheme has now been established for the genus and has revealed a strong association between C. sakazakii clonal complex 4 (CC4) strains with neonatal meningitis. This study was aimed at the identification of the CC4 specific traits using the genome sequencing data of 30 C. sakazakii isolates. These isolates included 18 C. sakazakii CC4 strains collected from 11 different countries for over 6 decades. This was achieved by whole genome comparative studies of 30 C. sakazakii strains; 18 C. sakazakii CC4 and 12 C. sakazakii non-CC4 strains.

Methods: Comparative genomics of the sequenced isolates, downloadable from Genbank, was performed using Gegenees (v2.0.3) to carry out Fragmented All Against All Comparison (biomarker score 1.0) of 30 genome sequenced isolates of C. sakazakii, and Artemis Comparison Tool (ACT). Results: Fragmented comparative genomics identified a gene Hyp-2 encoding for uncharacterised hypothetical protein, a homologue of the membrane protein (BLASTx), unique to C. sakazakii CC4. These findings were validated by PCR amplification of the selected C. sakazakii CC4 and non-CC4 isolates. The results indicated that outer membrane protein homologue Hyp-2 was unique to C. sakazakii CC4. Conclusions: Comparative genomic analysis of neonatal meningitic C. sakazakii CC4 showed a hypothetical protein specific to C. sakazakii CC4, a clonal complex linked predominantly with neonatal meningitis. None of the previous studies have identified the genetic basis of C. sakazakii CC4 being a unique lineage within Cronobacter. The present study aimed to investigate the uniqueness of this lineage. The identification of nucleotide sequence specific to C. sakazakii CC4 could potentially be used as a biomarker for its quick identification in powdered infant formula (PIF). The homology of Hyp-2 with a membrane protein could mean a potential role in virulence. The characterisation of this uncharacterised protein is warranted to elucidate its significance towards the virulence and/or environmental fitness of C. sakazakii CC4.
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Session Primary Track: Microbial Ecology and Evolution
Abstract Control Number: 2002
Poster Board Number: SUNDAY - 961

Abstract Title:
Whole Genome Characteristics of Helicobacter Pylori From Native Arab Kuwaitis

Primary Author Block:
M. Albert1, N. Kumar2, H. Alabkal1, I. Siddique1, N. Ahmed2; 1Kuwait Univ. Faculty of Med., Jabiya, Kuwait, 2Univ. of Hyderabad, Hyderabad, India

Abstract Body:
Background: As information on the genotype(s) of Helicobacter pylori causing disease in Kuwait is scarce, we carried out whole-genome sequencing and comparative analysis of three H. pylori strains isolated from native, Arab, Kuwaiti patients. Methods: Three H. pylori strains-HP22, HP45, HP59-were cultured from gastric biopsies of three patients with active chronic gastritis and intestinal metaplasia (origin of HP22 & HP45) or follicular gastritis (origin of HP59) treated at Al Amiri Hospital, Kuwait, during Dec 2012 to Feb 2013. Paired-end sequencing of DNA was done on a MiSeq Illumina platform. High-quality reads were assembled using velvet assembler into contigs. Contig Layout Authenticator (CLA) was used to assemble draft genome with H. pylori 26695 genome as the reference. Genomes were analyzed using Artemis, tRNAscan-SE, RNAmmer, ISfinder and PHAST and RAST. Whole-genome SNP-based and MLST-based phylogenetic trees were constructed along with 31 other H. pylori representing several genotypes from NCBI database, using Harvest and visualized with FigTree. cagA gene-based phylogenetic tree was constructed using neighbor-joining algorithm. Protein sequences were compared using OrthoMCL and functional categories identified using CDD database. Virulence genes were identified using VFDB database. Results: The Kuwaiti H. pylori chromosomes had 73 to 59 contigs with approximately 1600 genes. The strains possessed cagA and vacA genes, oipA gene in “on” position (contributing to inflammation), up to seven phage-associated regions, a plasmid and a complete cag-PAI and a plasticity zone. They had a cagA AB-C EPIYA motif. HP22 and HP45 encoded cagA with an EPIY(T) B-type motif (associated with mild inflammation) and HP59 encoded a EPIY(A) B type motif (associated with severe inflammation). These correlated with biopsy histologies. vacA gene type combinations for HP45, HP59 and HP22 were s1/m1, s1/m2 and s2/m2 respectively. Another 58 virulence-associated genes were conserved. Strain-specific genes encoding hypothetical proteins were present. In the phylogenetic analyses, there were six clusters. Kuwaiti strains formed a single group and were placed with HpEurope strains. The EPIYA-AB-C type motif of CagA reaffirmed the European ancestry of Kuwaiti strains. This phylogenetic association correlates with a high incidence of gastritis and duodenal ulcer and a low incidence of gastric cancer in Kuwait. Conclusions: The comparative genomic analysis of Kuwaiti H. pylori strains revealed a European ancestry and a high pathogenic potential.
Abstract Title:
Dengue Virus (Denv): Can Conserved Regions Reveal the Secret for Successful Vaccine Development?

Primary Author Block:
M. Verma; Sri Venkateswara Coll., Univ. of Delhi, Delhi, India

Abstract Body:
Background: Dengue is a severe emerging arthropod borne viral disease occurring globally. Around two fifths of the world's population, or up to 3.9 billion people, are at a risk of dengue infection. There are five different serotypes of virus namely DENV-1 to DENV-5. Infection induces a life-long protective immunity to the homologous serotype but confers only partial and transient protection against subsequent infection against other serotypes. Thus, there is a need for a vaccine which is capable of providing a life-long protection against all the serotypes of dengue virus. In our study, comparative genomics of Dengue virus was conducted to explore potential candidates for novel vaccine targets.

Methods: All ten proteins of four serotypes of dengue virus were downloaded and analysed for conserved motifs using MEME software. Several B-cell epitopes were predicted using Immune Epitope Database B-cell prediction tool and Bepipred. Overlapping epitopes sequences were analysed for surface accessibility and conservancy. Results: Using comparative genomics & immunoinformatics approach, we could successfully found conserved epitopes in Capsid protein, Envelope protein, NS1, NS3, NS4B and NS5 proteins in DENV serotypes. Conclusions: The conservancy of the epitopes can be explored as peptide vaccine candidates to combat dengue worldwide.
Abstract Title:
Comparative Genomic Analysis Provides Insights Into Molecular Basis of Pastereulla Multocida Host Predilection and Phylogeny
Primary Author Block:
Z. Peng1, W. Liang2, L. Hua1, R. Zhou1, H. Chen1, B. Wu1; 1Huazhong Agricultural Univ., Wuhan, China, 2Hubei Academy of Agricultural Sci., Wuhan, China
Abstract Body:
Background: Pasteurella multocida is a leading cause of porcine respiratory disorders. This Gram-negative bacterium has been assigned in to five capsular genotypes (A, B, D, E, F) and/or eight lipopolysaccharide (LPS) genotypes (L1-L8), respectively. It is widely documented that there was a certain correlation between P. multocida genotypes/serotypes and its hosts/specific types of diseases (called “host predilection”). However, the molecular basis for this “host predilection” it is still completely unknown. In addition, the phylogeny of P. multocida recovered from different hosts/diseases remains an unsolved problem. The phylogenetic determinants of P. multocida remains unclear. Methods: In this study, a total of 47 P. multocida isolates from pigs were sequenced. Together with the other 62 sequences of P. multocida from different hosts (Avian, bovine, rabbit and porcine), comparative genomics analyses were performed between these 109 P. multocida genomes. Results: Genotyping determination showed the a capsular: LPS: MLST genotype A: L1: ST129 (43.75%) was predominate among the avian isolates; and B: L2: ST122 (60.00%) as well as A: L3: ST79 (30.00%) for the bovine isolates. For the porcine isolates, D: L6: ST50 (37.50%) was the predominate genotype; and A: L3: ST9 (76.47%) for the rabbit isolates. Although genotyping revealed a certain level of “host predilection”, there was no “host specific gene” identified via comparative genomic analysis. In particular, there was also no difference on the distribution of virulence factor-associated genes among P. multocida strains from different hosts. In addition, we also found that the P. multocida strains discussed in this study were phylogenetically classified according to their LPS/MLST genotypes. Conclusions: These findings suggest that the so-called “host predilection” might be only associated in part with the bacterial capsule and LPS, and the LPS genotype plus the MLST genotype were the phylogenetic determinants of P. multocida.
Session Title: MEE04 - Comparative and Evolutionary Genomics: Pathogens and Human-associated Microbes

Abstract Title: Col Plasmids Contribute to the Fitness of Salmonella Heidelberg in Poultry Litter Evolution Experiment

Abstract Body:
Salmonella enterica subsp. enterica serovar Heidelberg (S. Heidelberg) is a clinically-important serovar, linked to food-borne illness, and commonly isolated from poultry. Investigations of a large, multistate outbreak in the USA in 2013 identified poultry litter as an important extra-intestinal environment that may have selected for specific S. Heidelberg strains. Poultry litter (PL) is a mixture of bedding materials and chicken excreta that contain chicken GI bacteria, undigested feed, feathers, and other materials of host origin. In this study, we investigated the role of PL on the fitness of two S. Heidelberg strains (SH-2813 and SH-116) recovered from chicken carcass during routine US Department of Agriculture monitoring in 2013. The strains are closely related at the chromosome level, differing by only 69 single-nucleotide variants. We performed a series of controlled laboratory experiments which assessed the evolution of these strains in PL previously used to raise 3 flocks of broiler chickens. Whole genome sequencing was performed on 86 strains recovered after 0, 1, 7 and 14 days of evolution in PL. Using plasmid-specific loci, we conducted plasmid typing, as well as quantitative and reverse-transcription PCR to determine plasmid copy numbers and expressed transcripts. We also performed a disk diffusion susceptibility test for selected aminoglycosides and fosfomycin. The population of SH-2813 in PL decreased by 4-logs after 1 day and increased by 1.5-logs after 7 days, with a final population of log 2.5 CFU g^-1 at day 14. This rebound in population after 7 days, coincided with the acquisition of 3 col plasmids in all SH-2813 strains recovered at days 7 and 14 (n=27). Inspection of the assembly graph indicated that the majority (269/301) of the plasmid contigs represented complete circular plasmids. For SH-116, we observed a gradual decline in population and the final population was below the limit of quantification. The IncX1 (38kb) and colE-like (4 and 6kb) plasmids belonged to MOBQ and MOBP families respectively. They were determined to be low copy number plasmids (1-12 copies/cell) and were maintained at a ratio of 1 copy of MOBQ to 2-12 copies of MOBP per cell. The 6kb MOBp plasmids carry two putative proteins encoding a AbiEii type IV toxin and a fragment of aminoglycoside 6-N-acetyltransferase. The evolved SH-2813 isolates exhibited a significant decrease in their zone of inhibition to tobramycin, gentamicin, neomycin, kanamycin, and fosfomycin. These findings suggest that col plasmids may increase S. Heidelberg fitness in PL.
Abstract Title:
Genome Analysis of A Multidrug-Resistant (Mdr) Salmonella enterica Serovar I 4,[5],12,i:- Isolate Associated with A 2015 Foodborne Outbreak from Pork

Primary Author Block:

Abstract Body:
Salmonella enterica serovar I 4,[5],12,i:- has emerged as a frequent cause of salmonellosis in humans and, based on 2014 National Antimicrobial Resistance Monitoring System (NARMS) data, is ranked as the fifth most common serovar isolated from patients in the U.S. Approximately 50% of serovar I 4,[5],12,i:- isolates in the U.S. are considered multidrug-resistant (MDR) which is defined by resistance to 3 or more antimicrobial classes. Over the last 20 years, the prevalence of serovar I 4,[5],12,i:- has increased globally and its incidence is primarily associated with food animals. In 2015, a foodborne outbreak due to serovar I 4,[5],12,i:- was linked to pork with 188 human infections and 30 hospitalizations. Investigation of 10 serovar I 4,[5],12,i:- isolates from the pork outbreak indicated that all the strains were MDR, conferring resistance to the antimicrobials ampicillin, streptomycin, sulfisoxazole, and tetracycline (R-type ASSuT). To facilitate characterization of a MDR serovar I 4,[5],12,i:- isolate associated with the pork outbreak, we sequenced the FSIS 1503788 genome using the PacBio and Illumina platforms. Deletion of the fljB region in FSIS 1503788 is due to insertion of a module containing genes that provide resistance to mercury and multiple antimicrobials (ASSuT). The deletion of the fljB gene results in this isolate being monophasic because it only expresses one (FliC) of the two flagellar antigens. Furthermore, FSIS 1503788 contains Salmonella Genomic Island 4 (SGI-4), an ~80 kb element that harbors genes for resistance to additional heavy metals (copper, zinc, and arsenic). The FSIS 1503788 genome is predicted by Phaster to have up to 6 intact prophage. Some Salmonella isolates have been previously shown to contain prophage that encode virulence genes or participate in horizontal gene transfer. Thus, our genome analysis indicates that MDR FSIS 1503788, associated with a 2015 pork outbreak, contains two genomic insertions previously described in other serovar I 4,[5],12,i:- isolates that confer resistance to antimicrobials and heavy metals. The presence of SGI-4 and the MDR module in Salmonella serovar I 4,[5],12,i:- may provide colonization or virulence benefits that have helped promote the increased prevalence of this serovar globally.
Abstract Title: Nucleotide Variation and Geographic Heterogeneity Define the Legionella pneumophila Sequence Type 1 Population


Abstract Body: Background: Legionella spp. are the cause of a severe bacterial pneumonia known as Legionnaires’ disease (LD). In some cases, current genetic subtyping methods cannot resolve LD outbreaks caused by common and potentially endemic L. pneumophila (Lp) sequence types (ST), which complicates laboratory investigations and environmental source attribution. In the United States (US), ST1 is the most prevalent clinical and environmental Lp sequence type. Methods: Recent reports demonstrate the value of whole-genome sequencing (WGS) for improving discrimination of Legionella isolates during outbreak investigations. Therefore, we sequenced 288 outbreak and non-outbreak associated clinical and environmental ST1 and ST1-variant Lp strains from the US. Together with international isolate sequences, we explored their genetic and geographic diversity, and tested methods for increasing LD cluster resolution. Results: The ST1 population was highly conserved at the nucleotide level; 98% of core nucleotide positions were invariant. However, environmental isolates unassociated with human disease contained ~65% more nucleotide diversity compared to clinical-sporeadic or outbreak-associated ST1 subgroups. The accessory pangenome of environmental isolates was also ~30-60% larger than other subgroups and was enriched for transposition and conjugative transfer-associated elements. By analysis of molecular variance (AMOVA), up to 10% of US ST1 genetic structure could be explained by geographic origin, but considerable genetic conservation existed among strains isolated from geographically distant states and across multiple decades. Conclusions: In summary, we determined that WGS combined with one or more powerful bioinformatic methods, such as phylogenetic clustering, can help resolve LD outbreaks due to a widely distributed Legionella sequence type. These results also suggest that intrinsic genetic differences between Legionella populations could be exploited to allow the development of improved, rapid tools for L. pneumophila source attribution during outbreak investigations.
Abstract Title:
Multidrug Resistant Organisms on ICU Surfaces in the U.S. and Pakistan

Primary Author Block:
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Abstract Body:
Background: Multidrug resistant organisms (MDROs) can survive for extended periods of time on environmental surfaces. We longitudinally sampled ICU surfaces in the US and Pakistan to identify reservoirs of MDROs. Analysis of hospital surface microbes may inform improved infection prevention strategies. Methods: 5 high contact surfaces were sampled using the ESewab system in 4 rooms at a tertiary care hospital in St. Louis, MO (USA) and Islamabad (Pakistan) over the course of a year. Swabs were enriched for MDROs using a variety of selective and differential agars. Isolated bacteria had putative species assigned by MALDI-TOF and were whole genome sequenced using Illumina technology.

Results: Acinetobacter baumannii and Escherichia coli were isolated from US ICU surfaces (n=18 isolates). However, 1,052 isolates from 36 genomospecies were isolated from Pakistan ICU surfaces. A. baumannii was the most prevalent species in both countries. 84% (67/80) A. baumannii from Pakistan tested were extensively antibiotic resistant. 91% and 82% of A. baumannii isolates had the carbapenemase blaOXA-23 and blaOXA-66, respectively (fig. 1). A. baumannii sequence type 208 isolates were found in both the US and Pakistan. However, single nucleotide polymorphism (SNP) analysis indicates that they are separate clonal groups. Conclusions: There was a high burden of MDROs on ICU surfaces in Pakistan, primarily by known nosocomial pathogens. Antibiotic resistance gene annotation of the isolated bacteria identified a high prevalence of clinically important resistance genes within certain taxa. SNP based analysis revealed that multiple lineages may exist within the abundant sequence types of some species. These results have informed infection control protocols in the Pakistani Hospital. Figure 1: A. A. baumannii susceptibility testing results. B. A. baumannii Resfinder results. <p><a href="http://files.abstractsonline.com/CTRL/b0/d/b2c/2ff/d9b/469/db3/8be/f84/182/600/36/g6496_3.jpg" target='_blank' address=no >img src="http://files.abstractsonline.com/CTRL/b0/d/b2c/2ff/d9b/469/db3/8be/f84/182/600/36/g6496_3.jpg" alt="" border="0" width="600" height="688" /></a></p>
Abstract Title:
Virulence-Associated Elements in the Accessory Genome of Pseudomonas Aeruginosa Clin. Respiratory Isolates

Primary Author Block:
K. R. Murphy, A. R. Hauser, E. A. Ozer; Northwestern Univ., Chicago, IL

Abstract Body:
Background: Pseudomonas aeruginosa (PA) is an opportunistic gram-negative pathogen and major cause of hospital-acquired pneumonia. Though there are several well-defined virulence factors in PA, variation in strain-to-strain virulence remains poorly understood. Comparative genomics may be a useful tool to identify novel factors in the accessory genome contributing to PA virulence. Methods: We sequenced the genomes of 83 clinical respiratory isolates using Illumina MiSeq. Core genome phylogeny identified distinct clonal groups from which 1 representative strain was randomly chosen from each group for further analysis. Shared accessory genomic elements (AGEs) were identified using Spine and ClustAGE, and the relative virulence for each isolate was assessed in a mouse model of acute pneumonia. Putative virulence-associated AGEs (vAGEs) significantly correlated with virulence (p<0.01) and containing 1 or more genes with similarity to known virulence factors in the MVirDB database were selected for further study. Each of the selected vAGEs was deleted from representative strains using Gibson Assembly and mutants were tested for virulence attenuation in the mouse model of infection. Results: The core genome phylogenetic analysis identified 34 distinct clonal groups within the 83 isolates. Among the 34 isolates randomly selected to represent each clonal group, the relative virulence in the mouse model ranged between 5 – 8 log CFU/mouse of bacteria to induce severe disease symptoms in ≥ 50% infected animals. Of the more than 5000 unique AGEs ≥ 100 bp in length identified by ClustAGE, 151 had a statistically significant correlation with virulence and 46 were highly correlated with p-values <0.01. Five vAGEs (vAGE01 – 05) were chosen for further analysis, ranging in size from 0.9 – 5.2 kB and containing sequence with similarity to known virulence factors in other species. Deletion mutants for each AGE were generated in two strain backgrounds (PS1788, PS2055) representing the most virulent isolates tested in this collection. Testing of the mutants for virulence attenuation in the mouse model is currently ongoing, though early studies indicate that deletion of vAGE04 may attenuate virulence. Conclusions: We successfully identified putative vAGEs that have not previously been linked to virulence in PA and have successfully created deletion mutants for each vAGE. Further in vivo characterization of the mutants will be used to identify novel virulence factors in PA contributing to pathogenesis in acute pneumonia.
Abstract Title: Comparative Genomic Characterization of Brucella Canis From Humans & I t Dogs from Zhejiang, China

Abstract Body: Background: Brucellosis caused by various species of the Gram-negative Brucella bacteria continues to be one of the most serious zoonotic diseases for humans worldwide, especially in China, human brucellosis cases have dramatically increased in recent years. The primary pathogenic agents in human brucellosis are B. melitensis, B. abortus, and B. suis. B. canis can also infect human but clinical cases are very rare with most cases just showing stealth infection. Recently, one B. canis strain was isolated and identified from a 54-year-old woman in Zhejiang province, China. The typical symptoms of brucellosis were observed including recurrent fever, fatigue, arthrodynia and myalgia. Subsequent MLVA analysis shown that the newly isolated B. canis strain (named ZJ-1) was closely clustered into local strains from dogs together while they were very distinct in genetic to other previously identified B. canis strains in China. Methods: Whole Genome Sequencing (WGS) and draft genome comparative analysis were then carried out to feature the genetic characteristics of ZJ-1 and ZJ-2, meanwhile, virulence related genes was also screened against B. canis referential strains ATCC 23365, HSK A52141 and SVA13 to identify potential virulence factors which could be responsible for the relatively high virulence observed in ZJ-1 and ZJ-2 strains. Results: In comparison to other strains, ZJ-1 and ZJ-2 are phylogenetically closed and share more unique genes, among which many are virulence related and involving several metabolism pathways including lipopolysaccharide biosynthesis, iron acquisition and transport, specific saccharide utilization and cycling, etc. Glucose/Galactose transporter, one predicted virulence factor unique for ZJ-1 and ZJ-2 strains, was also identified in B. suis strain 92/29 implying that ZJ-1 and ZJ-2 may horizontally acquire some virulence genes from relatively high virulent species probably through recombination in the same host. Conclusions: Although B. canis is traditionally recognized as a low pathogenic agent for human, recent whole genome comparative studies in this paper suggested a clue how this mild bacteria could evolve to be highly virulent to human and more attention should be paid for this specific Brucellosis in China.
Abstract Title:
Pathogenomic Characterization of Bacillus anthracis Plasmids Pxo1 and Pxo2 and Copy Number Variation

Primary Author Block:
C. A. Gulvik1, A. Pena-Gonzalez2, L. M. Rodriguez-R2, C. K. Marston1, J. E. Gee1, C. B. Kolton1, E. Saile1, M. Frace1, A. R. Hoffmaster1, K. K. Konstantinidis2; 1Ctr. for Disease Control and Prevention (CDC), Atlanta, GA, 2Georgia Inst. of Technology, Atlanta, GA

Abstract Body:
Bacillus anthracis plasmids pXO1 and pXO2 carry the main virulence factors responsible for anthrax disease. However, the extent of copy number variation within the species and how the plasmids are related to pXO1/pXO2-like plasmids in other species of the Bacillus cereus sensu lato group remain unclear. To gain new insights into these issues, we sequenced >400 B. anthracis strains composing the CDC historical collection which represents the phylogenetic and ecological diversity of the species around the world and developed a bioinformatic pipeline to assemble and annotate libraries and characterize the complete set of draft genomes including more than 100 reference B. cereus sensu lato genomes obtained from public databases. Our results revealed that B. anthracis genomes carried, on average, 3.86 and 2.29 copies of pXO1 and pXO2, respectively, and a positive linear correlation between the copy numbers of pXO1 and pXO2. No correlation between the plasmid copy number and phylogenetic relatedness of the strains was observed. However, strains isolated from animal tissues generally maintained a higher plasmid copy number than genomes of strains from environmental sources (p<0.05, Welch two sample t-test). Comparisons against B. cereus genomes carrying complete or partial pXO1 and pXO2-like plasmids showed that plasmid-based phylogeny recapitulated that of the main chromosome, indicating limited plasmid horizontal transfer between these species. Comparison of gene content revealed a closed pXO1 and pXO2 pangenome, e.g., plasmids encode <8 unique genes, on average, and a single large fragment deletion of pXO1 in one B. anthracis strain (2000031682). Collectively, our results provide a more complete view of the genomic diversity of B. anthracis plasmids and the virulence potential of other Bacillus species carrying pXO1/pXO2-like plasmids.
Abstract Title:
Comparative Clustering of V. Parahaemolyticus And V. Vulnificus Isolates Using Phylogenetics and Protein Motif Fingerprints

Primary Author Block:
K. J. Jesser1, B. A. Froelich1, W. Valdivia-Granda2, R. T. Noble1; 1UNC Chapel Hill Inst. of Marine Sci., Morehead City, NC, 2Orion Integrated BioSci.s, New Rochelle, NY

Abstract Body:
Background: Vibrio parahaemolyticus (Vp) and Vibrio vulnificus (Vv) are ubiquitous and abundant members of native microbial assemblages in coastal ecosystems. Though both species are predominantly environmental, some strains have been shown to opportunistically infect human hosts and their prevalence in densely populated coastal areas as well as increasing human infection rates underscore the importance of understanding the evolution of virulent phenotypes in these organisms.

Methods: We utilized high quality sequence data to compare the genomes of both Vv (n=46) and Vp (n=47) strains isolated from a variety of geographic locations and clinical and environmental matrices using a range of phylogenetic analyses, including multilocus sequence typing and whole-genome alignments, as well as a novel classification approach which relies on protein motif fingerprint (MF) patterns. Results: Both phylogenetic methods effectively differentiated Vv strains based on previously defined genotypes which correlate with clinical v. environmental origins. For Vp, the phylogenetic analyses did not cluster organisms based on their pathogenicity, as determined by the widely used virulence genes trh and tdh, even within defined MLST-types which have been associated with pathogenic strains. The MF clusters were different from the phylogenetically-derived clusters and permitted greater insights into isolation source and potential indicators of virulence. Conclusions: Together with phylogenetic clustering, MF analyses represent a promising new tool for better understanding of Vp and Vv genetic similarities and differences which can provide new insights into the molecular evolution of virulence.
Abstract Title:
Genes Significantly Associated with Human Virulence Revealed Through Comparative Genomics of Vibrio Vulnificus Strains

Primary Author Block:
K. J. Jesser1, R. Nowell2, B. A. Froelich1, R. T. Noble1; 1UNC Chapel Hill Inst. of Marine Sci., Morehead City, NC, 2Imperial Coll. London, Berks, United Kingdom

Abstract Body:
Background: Vibrio vulnificus (Vv), a native and ubiquitous member of bacterial assemblages in marine waters and shellfish, is of interest from a human health perspective because of its potential to infect human hosts. Despite concerns over the increasing number of Vv cases worldwide, Vv infections are relatively rare due to the fact that only a small subset of strains appear to be disease-causing. Methods: To identify genomic indicators of virulence in Vv strains, we have utilized high-quality genomic sequencing data and a statistical method that accounts for phylogenetic relatedness to investigate significant associations between gene presence and isolation source (i.e. clinical or environmental) across 27 Vv genomes isolated in the United States between 2006 and 2012 from blood and oyster matrices. Results and Conclusions: This statistical approach is an improvement over other comparative methods, which are often based only on gene presence or absence and do not consider strain relatedness, and has allowed us to identify genomic regions that are significantly associated with either human hosts or the environment. We have evidence for a common set of genes associated with human disease, which includes previously identified indicators of virulence as well as a suite of new genotypic markers linked to disease causing strains.
Abstract Title:
Comparative Genomic Analysis of Host-Adapted Cryptosporidium Hominis Subtypes

Primary Author Block:
Y. Wang1, L. Chen1, D. M. Roellig2, A. Kelley2, Y. Feng3, L. Xiao2; 1East China Univ. of Sci. and Technology, Shanghai, China, 2CDC, Atlanta, GA, 3South China Agricultural Univ., Guangzhou, China

Abstract Body:
Although Cryptosporidium hominis is widely considered a human-specific Cryptosporidium species, it is now increasingly reported in animals. Nonhuman primates, horses, and donkeys appear to be normal hosts of C. hominis. Recent genetic characterizations of C. hominis isolates at the small subunit (SSU) rRNA and 60 kDa glycoprotein (gp60) loci have shown genetic differences between C. hominis isolates from nonhuman primates or equine animals and those from humans. In the present study, we sequenced the genome of eight C. hominis isolates from crab eating macaques (Macaca fascicularis) in Hainan, China and conducted a comparative genomic analysis of the data. At the SSU rRNA locus, C. hominis isolates from these animals had 9 instead of 11 consecutive T in the hypervariable region of the gene and an A-to-T nucleotide substitution upstream from the region, whereas at the gp60 locus, they belonged to two new subtype families that were genetically related to the Ia and Id subtype families. At the whole genome level, isolates sequenced in the study had some gene gains and losses only seen in C. parvum, such as the loss of the only C. hominis-specific gene Chro.50011 in chromosome 3 and the gains of two genes each encoding the MEDLE and insulinase-like secretory proteins in chromosome 5. There were ~16,000 single nucleotide polymorphism (SNPs) between the two new subtype families and common subtype families seen in humans, compared with fewer than 4,000 SNPs among human isolates. The highly polymorphic genes among the subtype families mostly included those encoding secretory proteins located in the sub-telomeric regions of the eight chromosomes, especially mucin-like glycoproteins. These data suggest the existence of host-adapted C. hominis subtypes in nonhuman primates, and potential involvement of subtelemereic genes encoding secretory proteins in host adaptation within C. hominis.
Outstanding Abstract Award: Application of A 17th Century Mycobacterium Tuberculosis Genome to Mycobacterial Dating and Phylogeography

Primary Author Block:

Abstract Body:
Ancient pathogen genomics has opened a new avenue for directly studying the temporal and spatial history of certain infectious diseases. Tuberculosis occupies an intense focus of modern medical and microbiological research, but questions persist regarding its origin and earliest association with its human host. Based on the phylogeography of modern Mycobacterium tuberculosis complex (MTBC) genomes, one hypothesis posits that the pathogen followed human Pleistocene migrations out of Africa, yielding an extrapolated date for the most recent common ancestor (MRCA) of ~70,000 years before present. However, the two studies that have incorporated ancient genomes to calibrate the molecular clock have generated much younger MRCA dates of less than 6,000 years. This discrepancy can only be addressed by continued sampling of diverse, high-quality, ancient tuberculosis genomes that provide numerous calibration points to account for potential rate heterogeneity between different MTBC lineages and over time. Using DNA extraction and next-generation library construction methods designed specifically to accommodate ancient and degraded genetic material, we were able to identify DNA belonging to Mycobacterium tuberculosis in a calcified lung nodule from the mummified remains of Bishop Peder Winstrup of Lund (b. 1605 - d. 1679). With a custom-designed in-solution capture method we were able to reconstruct a 141-fold coverage genome of Mycobacterium tuberculosis from a library for which only 0.045% of the metagenomic DNA had been identified as belonging to members of the MTBC. Phylogenetic analysis revealed this sample to be part of the recently defined L4.10/PGG3 sublineage of the Mycobacterium tuberculosis complex. This high-quality, high-coverage 17th century Mycobacterium tuberculosis genome provides an opportunity to apply temporally diverse data to a phylogeographic analysis of the L4.10/PGG3 sublineage, and adds a reliable calibration point for dating the MTBC via Bayesian methods. This ancient genome provides better resolution to the genetic history of this critical pathogen.
Abstract Title: Comparative Genomic Analysis Reveals Divergent Evolution of Two Methylobacterium Species
Primary Author Block: C. Park1, S-y. Park2, W. Park1; 1Korea Univ., Seoul, Korea, Republic of, 2Hyundai Motor Group, Euiwang, Korea, Republic of
Abstract Body:
Two Methylobacterium species, designated as Methylobacterium sp. TX0642 and Methylobacterium sp. PR1016A, were isolated from the air-conditioning system of automobiles. Genome analysis and average nucleotide identity (ANI) showed that TX0642 strain is close to M. branchiatum 111MFTsu3 by 96.2%, while PR1016A strain has 89.9% identity with M. aquaticum GR16, suggesting that PR1016A strain is a novel Methylobacterium species. High copies of genomic island (GI), insertion sequence (IS), and phage integrase contribute to the large size of the PR1016A genome (approximately 1,405 kb larger than that of TX0642). Interestingly, alignments of each 5S rRNAs and 16S rRNAs revealed that one rRNA operon is highly variable among others, implying the presence of another synteny. Largely distinctive phenotypic characteristics in biofilm formation and growth characteristics between two species were shown in rich media (TYG). In addition, comparative genome analysis showed loss of mxal gene, encoding a methanol dehydrogenase small subunit, in the genome of PR1016A, indicating that divergent evolution between PR1016A and TX0642 in terms of methanol metabolism. Colanic acid synthesis genes (wca ABCFJL, wza, and wzc) are scattered in the PR1016A genome, however, TX0642 possesses genes encoding succinoglycans (exo genes), which is supported by high-performance anion-exchange chromatography (HPAEC) analysis of exopolysaccharides (EPS). High concentration of mannose from EPS and gum homologues in the genomes of two species also suggest the synthesis of xanthan in two species. Although PR1016A harbors only two copies of luxR and luxI genes, higher amounts of quorum sensing (QS) molecules were observed using Agrobacterium indicator, which might be linked to its more enhanced biofilm formation. Furthermore, increased biofilm of PR1016A might result in its stronger resistance against heat-, and surfactants (sodium dodecyl sulfate, SDS). In conclusion, two methylobacterium species originated from similar niches, yet, showed genotypical and phenotypical differences.
Abstract Title:
Lactobacillus Acidophilus Membrane Vesicles As A Vehicle for Bacteriocin Delivery

Primary Author Block:

Abstract Body:
Background: The membrane vesicles (MVs) shed by bacteria are known to have highly diverse functionality including roles in community interaction, inter-species transfer of biomolecules, in host defense mechanisms, and for delivery of antimicrobial factors, and the production and release of MV is known in many bacteria, including those relevant to human health. A recent report has characterized the production on MVs by the probiotic lactic acid bacteria Lactobacillus acidophilus, demonstrating that certain protein components were significantly enriched in the vesicles. Identified, enriched proteins included components of the lab operon, specifically bacteriocin peptide and peptide involved in the regulation of bacteriocin production. This study further characterizes the bacteriocin-MV relationship.

Methods: L. acidophilus ATCC 53544 and Lactobacillus delbrueckii were grown in de Man, Rogosa, Sharpe (MRS) broth under anaerobic or 5% CO2. MVs were purified using filtration and ultracentrifugation. Synthetic IP1800 signaling peptide (Genscript) was used for induction of bacteriocin production. Bacteria used for atomic force microscopy (AFM) were fixed with 0.25% glutaraldehyde. LIVE/DEAD analysis was performed using confocal microscopy, flow cytometry, and fluorescence methods. Growth inhibition assays were performed using L. delbrueckii as previously described. Results: Our results showing that growth inhibition of the indicator strain L. delbrueckii by MV-associated bacteriocin occurs in an IP1800-dependent manner. Protease treatment of L. acidophilus MVs and MV-free supernatant suggests specific packaging of bacteriocin peptide in or anchoring on secreted vesicles. LIVE/DEAD stain experiments demonstrate L. delbrueckii membrane disruption by purified L. acidophilus MVs, confirmed by AFM imaging. Conclusions: Our results show that L. acidophilus MVs are conduits of both signaling and community-control peptides, and are involved in the delivery antimicrobials to competing cells in the environment, and suggest that MVs may play a role in the gut microbiome niche as a mechanism for killing of competing Lactobacillus species.
Bacteriocin-Mediated Interactions Increase with Spatial Scale in Household Pseudomonas aeruginosa Isolates

Primary Author Block:
A. Mojesky, E. Miller, J. Bara, S. Remold; Univ. of Louisville, Louisville, KY

Abstract Body:
Pseudomonas aeruginosa is a Gram-negative bacterium known to abundantly produce a class of narrow spectrum antimicrobial toxins called bacteriocins. While bacteriocins have traditionally been studied in laboratory settings, an emerging field of research is exploring the particular role of these antibiotics in natural microbial assemblages. Recent studies have shown that bacteriocins are important in the spatial structuring of natural microbial populations. Little inhibition has been observed in coexisting environmental isolates of Pseudomonas fluorescens and in species of the Xenorhabdus genus; yet, as spatial scale increases, bacteriocin-mediated interactions among these isolates will increase as well. However, it is not well understood how spatial isolation between isolates of P. aeruginosa may influence the outcome of bacteriocin-mediated interactions, nor whether isolates living in different habitats display differences in bacteriocin-mediated interactions. We collected isolates of P. aeruginosa from bathroom and kitchen sink drains in 70 households located in Kentucky. A 54 x 54 pairwise-inhibition assay was used to assess bacteriocin-mediated interactions among isolates collected from the same drain, isolates collected from a different drain in the same home, and isolates collected from different homes. We hypothesize that bacteriocin-mediated inhibition will increase in household isolates of P. aeruginosa as spatial scale increases, and that isolates from bathroom sink drains and kitchen sink drains would differ in their patterns of inhibition. The results of this study identified the lowest probability of inhibition to be among isolates collected from the same drain and the highest probability of inhibition to be among isolates collected from different homes. Additionally, bathroom sink drain isolates were found have a significantly higher probability of inhibition than kitchen sink drain isolates. These results are consistent with previous findings in other systems and indicate that bacteriocins play an important role in shaping patterns diversity in household isolates of P. aeruginosa.
Abstract Title:
Effects of Mycobacterium Ulcerans Lipid Toxin, Mycolactone on Staphylococcus aureus Growth and Quorum Sensing

Primary Author Block:
L. Dhungel, H. Jordan; Mississippi State Univ., Starkville, MS

Abstract Body:
Background: Buruli ulcer disease (BUD) remains a ‘mysterious disease’ due to its unknown mode of transmission and pathogenesis. The disease is caused by the environmental pathogen, Mycobacterium ulcerans (MU) whose major virulence factor is mycolactone, a lipid cytotoxic molecule. BUD has been found to be colonized by various quorum sensing bacteria such as Staphylococcus aureus, S. epidermidis, and Pseudomonas aeruginosa, but without typical pathology associated with those pathogens’ colonization. Microbial pathogenesis may not only be an individual act, but may also depend on synergistic or antagonistic approach it exhibits within a polymicrobial network. Hence, it is important to understand the interaction of MU with other bacteria encountered during skin infection or within its natural environment. The central hypothesis of the study is that MU attenuates virulence of colonizing pathogens via mycolactone. Methods: In order to understand the interaction of MU with S. aureus, we cultured S. aureus in the presence of mycolactone and measured resulting S. aureus growth, hemolytic activity and virulence gene expression at various time points. Results: Results showed that there was no effect of mycolactone on growth of S. aureus, however, there was a reduction in S. aureus hemolytic activity. S. aureus quorum sensing gene agr, which is responsible for regulation of various virulence factors was downregulated at early timepoints. Conclusions: These results suggest attenuation of S. aureus virulence by mycolactone, while not inhibiting growth. Though more work is in progress, these findings provide insights on the interaction of MU with S. aureus and the role of mycolactone in providing fitness to MU in polymicrobial environments. Furthermore, these data are encouraging toward determining possible treatment outcomes of BU following antibiotic treatment.
Outstanding Abstract Award: Pseudomonas Aeruginosa and Staphylococcus aureus Interactions in Cystic Fibrosis

Primary Author Block:
E. E. Bernardy1, A. T. Chande2, R. A. Petit, III3, I. K. Jordan2, T. D. Read3, J. B. Goldberg1; 1Emory Univ. and Children's Hlth.care of Atlanta, Atlanta, GA, 2Applied Bioinformatics Lab. (ABiL), Georgia Inst. of Technology, Atlanta, GA, 3Emory Univ., Atlanta, GA

Abstract Body:
Pseudomonas aeruginosa and Staphylococcus aureus are the most common bacteria that infect the lungs of people with cystic fibrosis (CF). S. aureus is the primary pathogen during childhood but this prominence switches to P. aeruginosa in adulthood. This change is poorly understood, but it is believed that P. aeruginosa decreases viability of S. aureus. Interestingly, some patients present with chronic coinfection of these pathogens, resulting in decreased lung function and a poorer prognosis. However, what promotes coexistence of S. aureus and P. aeruginosa and the associated health decline is not well understood. To begin to address this gap, we have obtained clinical isolates from the CF Biospecimen Registry (CFBR; a part of the Children’s Healthcare of Atlanta and Emory University Pediatric CF Discovery Core). We have identified three groups among 49 tested CFBR S. aureus isolates based on their interaction with the non-mucoid P. aeruginosa laboratory strain PAO1 and its mucoid derivative. Previously, it has been shown that S. aureus isolates, like the laboratory strain JE2, are killed by non-mucoid PAO1, but not mucoid PAO1 (Group 1). We found that most of our isolates also fall into this group. However, we identified a subset of strains that were killed by both non-mucoid and mucoid PAO1 (Group 2), or were resistant to killing by both strains (Group 3). In order to better understand the genetic factors involved in this interaction, these isolates were fully sequenced, and analysis is currently underway to identify genetic differences between each group. Previous studies have shown that extracellular products secreted by P. aeruginosa kill S. aureus, but there is evidence to suggest that these products may only be active when the bacteria are cocultured. Therefore, we are investigating the extent to which the interaction between P. aeruginosa and S. aureus, in the context of these three groups, is contact dependent. Altogether, our work will provide greater insight into how typically antagonistic bacteria develop a synergistic relationship that can result in polymicrobial infections leading to worsening conditions for people with CF.
Abstract Title:
Conditionally Redundant Antagonism Mediated by Distinct Bacteriocins in Pseudomonas Syringae

Primary Author Block:
K. Hockett1, D. Baltrus2; 1The Pennsylvania State Univ., University Park, PA, 2Univ. of Arizona, Tucson, AZ

Abstract Body:
Plant-associated bacteria exist within complex communities of potentially competing microbes. Bacteria that are regular inhabitants of plant environments, such as Pseudomonas species, encode multiple systems that are known or predicted to antagonize competing microbes. These systems include narrow-spectrum proteinaceous antibacterials, termed bacteriocins. Questions remain regarding the role(s) of multiple antagonistic systems within a single strain. This is particularly true of bacteriocins, where it is common to find 4-6 distinct bacteriocins encoded by a single strain. In this work, we demonstrate that Pseudomonas syringae pv. syringae B728a (Psy) produces multiple bacteriocins that can target another strain of this same species, P. syringae pv. actinidiae (Psa). One bacteriocin is similar to the well described nuclease domain-containing colicins of E. coli, whereas the other bacteriocin is derived from a bacteriophage tail (tailocin). Interestingly, these distinct bacteriocins exhibit conditional redundancy, in that sensitivity to the colicin-like bacteriocin is only manifest after resistance is acquired against the tailocin. Such conditional redundancy resembles theoretical and empirical research demonstrating sequential use of antibiotics is an optimal strategy for delaying or slowing the emergence of resistance. Indeed, we show that resistant colonies arise at a statistically significant higher frequency when Psa is exposed to the tailocin only, rather than both bacteriocins, despite Psa being naturally resistant to the colicin-like bacteriocin. Furthermore, we have shown that the colicin-like bacteriocin can be expressed and purified from a heterologous host and will recapitulate the effect of suppressing the emergence of resistant Psa colonies, whereas a catalytically dead version of this same toxin does not suppress the emergence of tailocin resistant colonies. Mutants that are tailocin resistant, but sensitive to the colicin-like bacteriocin all exhibit a loss of O-antigen synthesis, as assessed by an ether-based LPS extraction followed by SDS-PAGE separation. These results indicate that LPS plays a critical role in mediating bacteriocin sensitivity and resistance. Taking advantage of conditionally redundant targeting between distinct bacteriocins may provide for sustained control of a target pathogen, by slowing the emergence of resistance.
Abstract Title:
Determining the Role of Conserved Operons in Streptomyces Coelicolor In Interspecies Interactions
Primary Author Block:
Y. Ra, B. Bonet, M. Traxler; Univ. of California, Berkeley, Berkeley, CA
Abstract Body:
The study of members of the bacterial genus Streptomyces is largely motivated by their impressive capacity to produce clinically-applicable secondary metabolites, including antibiotics and anti-malarial compounds. However, the factors that regulate secondary metabolism are still not fully understood. Previous studies strongly suggest that interactions with other species are one method to trigger otherwise inactive secondary metabolite biosynthesis pathways (Traxler 2013) and can facilitate novel compound discovery. Previous research describes the presence of several conserved operons, called conservons (cvn), in Streptomyces species, and one of these conservons were found to encode a membrane-bound heterocomplex that resembles G-protein coupled receptors (GPCRs) in eukaryotes (Komatsu 2006). Based on prior RNaseq results, we hypothesized that one or more of these conservons play a key role in how Streptomyces coelicolor responds to an interaction, such as by sensing the presence of another species and creating a response to the interaction downstream by changing gene expression. Thus, we have conducted transcriptomic experiments via RNAseq to determine the function of these conservon proteins. Specifically, we concentrated our efforts on studying cvn-8 in S. coelicolor based on the observation of impaired pigmented secondary metabolite biosynthesis by mutants lacking one or more genes in the cvn8 operon. Preliminary analysis of the transcriptome comparisons show that the mutants had down-regulated expression of genes involved in the biosynthesis of actinorhodin, a pigmented antibiotic, and up-regulated genes involved in production of coelimycin and a cryptic lantibiotic. We are also taking a biochemical approach to search for potential downstream effector proteins in order to fully characterize the function of this conservon and determine how it contributes to regulating secondary metabolism during interspecies interactions.
Abstract Title:
Development of A Microfluidics Platform to Uncover Novel Antibiotics from Actinomycetes

Primary Author Block:
P. Buenbrazo, D. J. McClung, M. F. Traxler; Univ. of California Berkeley, Berkeley, CA

Abstract Body:
Developing new tactics to battle multi-drug resistant bacteria poses a serious challenge for public health. We have missed discovering many natural products from actinomycetes due to our controlled conditions of monocultures, which keeps many of their natural product biosynthesis gene clusters silent (Wiemann & Keller, 2014). We posit that replicating naturally occurring interactions from the soil will stimulate antibiotic production in rare actinomycetes. Actinomycetes produce more metabolites that are unique to individual interactions than common to two or more unique interactions (Traxler, et al., 2013), suggesting that we need to analyze many pairwise interactions to elucidate the true wealth of antibiotic potential of this group of bacteria. I aim to develop a microfluidics device to screen approximately 10,000 pairwise interspecies interactions of rare, soil actinomycetes for synthesis of novel antibiotics. Initial PDMS-based prototypes were made up of four chambers, each separated by a semi-permeable barrier, which physically separates species but allows chemical communication to occur. Each of the inner two chambers houses a different actinomycete species. The outer two chambers house an indicator strain: a naturally multi-drug resistant actinomycete, Amycolatopsis sp. AA4. If the interaction between the inner two actinomycetes stimulates antibiotic production, then it will diffuse into the outer two chambers, potentially inhibiting the growth of the indicator. We are optimizing devices for the most robust barrier that fulfills the requirements of the screen without compromising manufacturing efficiency. From preliminary designs, colored dyes and an unknown antibiotic produced by a streptomycete diffuse well through various barriers. However, simple mazes did not prevent filamentous actinomycetes from growing into the next chamber, demonstrating that the current maze designs are not sufficient to physically separate bacterial species. Our work will set up a high-throughput screening method for discovery of natural products (i.e. antibiotics) from actinomycete interspecies interactions, increasing the efficiency in identifying biologically active small molecules that can be used for future therapeutics.
Abstract Title:
Evolving Bi-Directional Costly Mutualism from Byproduct Consumption

Primary Author Block:
W. Harcombe1, J. Chacón1, L. Chubiz2, C. Marx3; 1Univ. of Minnesota, St. Paul, MN, 2Univ. of Missouri - St Louis, St. Louis, MO, 3Univ. of Idaho, Moscow, ID

Abstract Body:
Mutualisms are common in microbial systems, yet it is unclear how they arise. A two-stage process has been proposed for the evolution of mutualisms that involve exchange of two costly resources1. First, costly provisioning by one species can be selected for if that species gains a benefit from costless byproducts generated by a second species. Selection can then drive the second species to provide costly resources in return. Previously, a synthetic consortium evolved the first stage of this scenario: Salmonella enterica evolved to produce methionine in exchange for costless carbon byproducts generated by an auxotrophic E. coli on lactose plates2. Here we report that further evolution of these partners led to a hyper-cooperative E. coli. In replicate communities there was independent evolution of galactose excretion by E. coli, which was simultaneously quite costly to individual growth and highly beneficial to S. enterica, and thus to growth of the community as a whole. The genomic and metabolic basis of this adaptation aligns with predictions from genome-scale metabolic models. To our knowledge, this is the first experimental example of de novo, bi-directional costly mutualism evolving in a system that initially involved solely uni-directional consumption of byproducts. This validates the plausibility of costly cooperation emerging from initially costless exchange, a scenario widely used to explain the origin of species interactions that are central to life on earth.
Abstract Title:
Global Metabolic Profiling Approach to Characterize Consistency in Polymicrobial Communities

Primary Author Block:
S. Da Silva, N. Lin, Y. Simon-Manso, E. Musteata; NIST, Gaitherburg, MD

Abstract Body:
Pseudomonas aeruginosa and Staphylococcus aureus are pathogens often co-located in human disease states including wound infections and chronic lung infections of cystic fibrosis (CF) patients. Their behavior in co-culture depends on the environment and can lead to robust biofilm formation or antibiotic resistance. Moreover, coinfection typically worsens patient outcomes as compared to monoculture infections. Thus, the co-culture system is an important target for therapeutics. Despite the prevalence of this bacterial system in disease, the biochemical mechanisms involved in the complex interspecies interactions are not fully understood. One challenge associated with studying systems such as this is the lack of robust in vitro models and metrics to standardize measurements and facilitate reproducible experiments. Our objective was to measure intracellular and extracellular metabolomes of a clinically relevant in vitro co-culture model and evaluate the potential of metabolic profiling for reproducibly characterizing interactions in multispecies communities. The two species were grown as mono- or co-cultures for 24 h in artificial sputum medium, which mimics nutrients in CF lung infections and improves clinical relevance versus typical in vitro growth media. We integrated and optimized protocols for microbial culturing and quantification, quenching, and metabolite extraction. Global metabolite profiles were analyzed by liquid chromatography - tandem mass spectrometry (LC-MS/MS).

Principal component analysis indicated that profiles of P. aeruginosa, S. aureus, and the co-culture are statistically differentiable (n = 9) and highly reproducible across all three levels (day, biological and technical replicate). At this point, we could identify few compounds that are common to all three cultures but at different quantities, suggesting up- and down-regulation of pathways in co-cultures such as 2-heptyl-4-hydroxyquinoline N-oxide (HQNO) and N-Butyryl-L-homoserine lactone. In addition, we have found 575 features unique to the co-culture, which are under identification analysis. Overall, our results suggest that LC-MS/MS metabolic analysis is a promising approach to monitor repeatability and reproducibility in polymicrobial communities. Our approach enables comparison and combination of laboratory studies toward an improved understanding of interspecies interactions, their role in disease, and novel therapeutics to target these systems.
**Session Number:** 439  
**Session Type:** Poster  
**Session Title:** MEE09 - Polymicrobial Interactions of Ecological or Evolutionary Significance  
**Session Start Date Time:** 6/10/2018 12:45:00 PM  
**Session End Date Time:** 6/10/2018 2:45:00 PM  
**Session Primary Track:** Microbial Ecology and Evolution  
**Abstract Control Number:** 5495  
**Poster Board Number:** SUNDAY - 986

**Abstract Title:** Understanding the Ecology and Evolution of Polymicrobial Wound Infections  
**Primary Author Block:**  
A. C. da Silva1, T. J. Sloan1, P. Williams1, S. P. Diggle2; 1Univ. of Nottingham, Nottingham, United Kingdom, 2Georgia Inst. of Technology, Atlanta, GA  
**Abstract Body:**  
Chronic wounds (CW) are a common complication of diabetic foot ulcers (DFUs), which are a major burden to health care systems worldwide [1], and can result in lower limb amputation due to the intractability of the infection [2]. DFUs appear because of underlying causes such as peripheral neuropathy, peripheral vascular disease and trauma [3, 4], and often become colonized with polymicrobial infections of Staphylococcus aureus (Sa) and Pseudomonas aeruginosa (Pa) [5]. Polymicrobial infections have been suggested to be more virulent, antibiotic resistant and result in worse patient outcomes [5, 6], but the reasons why remain unclear. In a long-term infection environment like DFUs, there is a high probability of the infecting bacteria evolving considerable phenotypic and genetic diversity, as has previously been shown in chronic lung infections of cystic fibrosis patients [7]. However, it is not known whether this is also the case in CW from DFUs, and whether diversity and/or strain interactions impacts on virulence or antibiotic resistance. To study this, we isolated bacterial populations taken from the bone and soft tissue of four patients and five CW from in vivo DFUs. Initially we determined whether phenotypic diversity is present in Pa populations through the analysis of phenotypes traditionally associated with pathogenicity in chronic wounds. Furthermore, we sampled Sa isolates from two of the CW to analyze interactions between strains of both species that co-evolved in the same or different ulcers. We found that there is phenotypic variation in Pa isolates taken from different patients, but little variation within the same chronic wound, and that antibiotic resistance increases during the course of the infection. We also observed that Sa and Pa strains isolated from the same wound can often co-exist when grown together in synthetic wound fluid. In contrast, when Pa and Sa strains were isolated from different wounds, Pa often outcompeted Sa, possibly because of differences in quinolone levels that we observed between different Pa wound isolates. Our findings are an important step in understanding how ecology influences the evolution of different species within wound infections and how this contributes to antibiotic resistant polymicrobial infections.
Abstract Title: Bacterial Social Interactions Modify Aggregation in Campylobacter jejuni
Primary Author Block: K. Simunovic, I. Mandič Mulec, S. Smole Možina; Biotechnical Faculty, Univ. of Ljubljana, Ljubljana, Slovenia

Abstract Body:
Background: Bacteria are social creatures that through communication coordinate specific tasks such as group movement over surfaces and biofilm formation. They are also able to discriminate kin from non-kin. The mechanisms and benefits of kin discrimination are not yet fully understood, as only a handful of microorganisms have been studied regarding this phenomenon. Methods: In this study we tested for kin discrimination between isolates of Campylobacter jejuni using a swarming assay on 0.5% agar. In addition we applied a co-aggregation assay, where we tested the ability of strains to form aggregates with self or with different strains. We predicted that swarms of phylogenetically related strains (same MLST) will merge, while less closely related (non-kin, different MLST) will form a visible boundary at the contact point of two swarms. We tested strains with ST-21, ST-45, ST-353 and ST-354 MLST profiles and additionally ten strains with variable MLST profiles for boundary formation. We also studied the interaction of 17 C. jejuni knock out mutants (ΔcmeB, ΔcmeR, ΔkpsM, Δomp50 and others) with the parental strain. Results: Results show that C. jejuni strains always merge with self. Merging is also dominant between strains that share MLST as at least 70% of the strains within a MLST group merged with each other, but some also formed boundaries. Strains differing in MLST profiles showed less consistent pattern and we found both merging and boundary formation. Among 17 tested mutants only the ΔcmeF mutant lacking a functional CmeDEF efflux pump, formed a boundary with the parental strain. In selected combinations of strains (both merging and boundary forming) we also applied a co-aggregation assay and found that aggregation with self is more efficient than co-aggregation between strains forming a boundary line. Conclusions: We conclude that the absence of the CmeDEF efflux pump contributes to boundary formation, merging of swarms dominates within MLST type and strains prefer to aggregate with self. However, to our surprise, swarms of different MLST profiles still merge. This suggests that MLST typing alone is not sufficient to predict boundary formation and the potential for kin discrimination between C. jejuni strains. The work also suggests a complex interplay of social traits that contribute to their aggregation.
Abstract Title:
Progression of the Rhizospheric Bacterial Count of Coriandrum Sativum Grown in An Arid Region Like Solapur

Primary Author Block:
V. Mane; D.B.F. Dayanand Coll. of Arts and Sci., Solapur, India

Abstract Body:
Background: The Rhizosphere of any plant is a dynamic environment. An enormous number of microbial activities are continuously going on in this particular region as a result of diversity of microorganisms. It is a micro-biome. It is an active region of soil, often rich in organic matter and other nutrients. The microorganisms play an important role in the improvement of soil fertility and recycling of nutrients. The microbial diversity depends upon the type of plant, soil environment and other physical factors. However, the type and the number of microorganisms change with the time and also it is dependent upon the developmental stage of the plant. Each plant shows a peculiar rhizosphere microflora. Each and every microorganism of this region is carrying out some specific activity. The present study is taken to determine the variation and progression of heterotrophic bacteria in the rhizosphere of Coriandrum sativum. It is a preliminary study done by undergraduate students with the limited resources and hence is restricted to the rhizosphere bacterial count which can grow on nutrient agar. The study not only reflected bacterial diversity but clearly indicated the progression in bacterial count throughout the developmental stages of plant. Thus, this clearly indicates microbial ecological succession. Materials and Methods: The rhizosphere bacterial count was taken from day 8 to 15 in triplicates on Nutrient agar plates. The time period selected for the count was from the seed germination up to the harvest. Results and Discussion: The sample codes are arbitrary. Table no.1: Bacterial count Table no.3: Rhizosphere bacterial count on Nutrient Agar plates Colony count/gm of soil sample=mean count (per 100 mg) X dilution factor (103) X 10<
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The interesting part of this experiment is that bacterial diversity is observed only in early stages of growth. Conclusion: The results reflected not only the diversity in bacterial population but also an increase in the bacterial count i.e. progression with the progress of development of plant growth. The limitations for performance restricted us to count heterotrophic bacteria. But surely we have succeeded in finding the progression in the rhizosphere bacterial count of Coriander.
Abstract Title:
Analysis of Tick Associated Bacterial Diversity Using Nanopore Real-Time Sequencing

Primary Author Block:
S. Huether, D. Brown, P. Lepp; MInot State Univ., Minot, ND

Abstract Body:
Background: Beyond a few individual bacterial species known to be associated with human and animal diseases little is known about the microbiota associated with hard-bodied tick species. What little is known has come primarily from culture based identification methods. Ixodes scapularis, also known as the black-legged or deer tick, is the vector primarily responsible for the transmission of the bacterium Borrelia burgdorferi, the etiological agent of Lyme disease. Dermacentor varibilis, also known as the wood tick or American dog tick, harbors the bacterial pathogens responsible for Rocky Mountain spotted fever and tularemia. Methods: We used PCR-amplified ribosomal RNA genes (rDNA) to identify bacterial species associated with these two hard-bodied tick species. We have analyzed over 80,000 bacterial 16S rDNA sequences from both tick species using a high through-put, real-time, Nanopore sequencing system. Results: We have analyzed the bacterial species richness and evenness among and between these two tick species. Bacterial populations are dominated primarily by small number of closely related bacterial species from the genera Francisella believed to be endosymbionts of ticks. Our methods have not revealed the presence of Borrelia burgdorferi in any of the individual ticks examined to date. Conclusions: The two hard bodied ticks examined exhibited low species richness dominated by a single bacterial species from the genus Francisella.
Abstract Title:
Comparative Genomics of the Nanoarchaeota-Crenarchaeota Symbiosis

Primary Author Block:
A-L. Reysenbach1, Y. Liu1, M. Podar2, E. St John1, M. Stott3; 1Portland State Univ., Portland, OR, 2Portland State Univ., Oak Ridge, TN, 3Univ. of Canterbury, Christchurch, New Zealand

Abstract Body:
The symbiotic Nanoarchaeota have few described species in culture and little additional representation in genomic datasets but represent key taxa for studying archaeal evolution and adaptation. We have expanded the known diversity of Nanoarchaeota through both culture-independent and culture-dependent approaches from different marine and terrestrial geothermal environments, and some patterns are beginning to emerge. All new isolates have different hosts belonging to the Crenarchaeota, and share features such as small reduced genomes and limited biosynthetic capabilities. The terrestrial Nanoarchaeota from New Zealand and Yellowstone National Park, USA, share several unique features such as a greater suite of genes involved in carbohydrate metabolism and archaeal flagellar genes that are expressed. Unlike the marine Nanoarchaeum equitans, the terrestrial Nanoarchaeota do not have split tRNAs that require trans-assembly. While the host of N. equitans, Ignicoccus hospitalis, is a chemolithoautotroph, the two terrestrial nanoarchaeotal hosts thus far studied in culture, have heterotrophic hosts, although there is some evidence from a single cell metagenome that terrestrial Nanoarchaeota may also form symbioses with autotrophs. While no nanoarchaeotes have been isolated from deep-sea hydrothermal vents, we have a draft nanoarchaeotal genome that suggests that the presence of flagella may not be limited to the terrestrial Nanoarchaeota. Given the large evolutionary distances between the different Nanoarchaeota and that they have very different crenarchaeotal hosts, it is likely that the radiation of these lineages were ancient events.
Abstract Title:
Nocardioides Pakistanensis Sp. Nov., Isolated from A Hotwater Spring of Tatta Pani in Pakistan

Primary Author Block:
A. Butt, I. Ahmed; Natl. Agriculture Res. Ctr., Islamabad, Pakistan

Abstract Body:
A Gram-staining positive, non-spore forming, non-pigmented and non-motile bacterium, designated as NCCP-1340T, was isolated from a hot water spring, Tatta Pani, Pakistan. Cells of strain NCCP-1340T were observed to be aerobic, rod shaped, catalase and urease positive but H2S production and oxidase negative. Growth was observed at pH 6.0-8.0 (optimum pH 7.0) and at 20-40 °C (optimum 37 °C). The strain could tolerate 0-8 % NaCl (optimum 2 %, w/v). Phylogenetic analysis based on 16S rRNA gene sequence comparisons revealed that strain NCCP-1340T belongs to the genus Nocardioides and is closely related to Nocardioides iromotensis JCM 17985T (96.8 %), Nocardioides daedukensis KCTC 19601T (96.6 %), Nocardioides jensenii KCTC 9134T (96.1 %) and Nocardioides daejeonensis KCTC 19772T (96.1 %). The DNA-DNA relatedness values of strain NCCP-1340T with N. iromotensis JCM 17985T, N. daedukensis KCTC 19601T and N. jensenii KCTC 9134T were found to be less than 53 %. The DNA G+C content of strain NCCP-1340T was determined to be 71.8 mol %. The affiliation of strain NCCP-1340T to the genus Nocardioides was further supported by chemotaxonomic data which showed the presence of MK-8(H4) as major menaquinone system; iso-C16:0, C17:0, C16:0 10-methyl, iso-C15:0 and C 15:0 as major cellular fatty acids; and diphosphatidyglycerol, phosphatidylethanolamine, phosphatidylglycerol and unidentified glycolipids and polar lipids in the polar lipids profile. The cell wall peptidoglycan contained LL-diaminopimelic acid as the diagnostic amino acid. On the basis of physiological and biochemical characteristics and the phylogenetic analyses, strain NCCP-1340T can be distinguished from the closely related taxa and thus represents a novel species of the genus Nocardioides, for which the name Nocardioides pakistanensis sp. nov. is proposed with the type strain NCCP-1340T (= DSM 29942T = JCM 30630T). Keywords: Nocardioides pakistanensis sp. nov., Hot water spring, Tatta Pani Pakistan.
Microbial Communities At Varying Depths of Siberian Permafrost

Primary Author Block:
A. Almatari1, D. Williams1, E. Spirina2, S. Pfiffner1, E. Rivkina2, K. G. Lloyd1, T. Vishnivetskaya1; 1Univ. of Tennessee Knoxville, Knoxville, TN, 2Inst. of Physicochemical and Biological Problems in Soil Sci., Pushchino, Russian Federation

Abstract Body:
This research seeks to explore the differences in microbial communities with respects to geological age and depth. Samples were collected from Northern Siberia at the Alazeya River in boreholes from the surface to a maximum depth of 24.6 meters. We hypothesize that sediments whose DNA was isolated from shallower depths may show more diversity than deeper sediments due to the age of the sediments and time microbial cells stay frozen. Cores were collected with a slow rotary drill using quality control procedures. Collected cores were subsampled with sterile knife into sterile Whirl-Pak bags and were transported to the University of Tennessee in a frozen state. DNA was isolated from these samples using Fast Spin DNA Kit for Soil®. The 16S rRNA gene was amplified using 27F and 1492R with a further nested amplification using 515F and 806R primers. All samples were cleaned and concentrated using DNA Clean & Concentrator® Kit (Zymo Research). DNA concentration was measured ranging from 1.2-83 ng/µL. After confirming DNA presence without contamination using Agilent Automatic Gel Electrophoresis, correct DNA concentration for MiSeq was determined using qPCR. Sequencing was completed using Illumina MiSeq and comparative analyses were achieved by using the CLC Genomics Workbench. The Greengenes 16S rRNA gene database was used to cluster sequences into OTUs at 95% sequence similarity threshold. Data were normalized through CLC. We see that the samples in bore hole AL1 at depths 1.75-1.8 m and 2.95-3.0 m have a greater alpha diversity than samples from depths of 22.9 m and 24.5-24.6 m. Actinobacteria predominated in the shallow depths of 1-3 m at 47-60 % compared to the 1-6% seen in the deeper sediments (23-25 m). Likewise Firmicutes were more prominent in the deeper sediments at 46-87% compared to the 5-35% shown in the shallower depths. From shallow permafrost sediments to deeper we see changes in bacterial community from aerobic non-spore-forming high GC Gram-positive Actinobacteria to anaerobic spore-forming low GC Gram-positive Firmicutes. The deepest permafrost sample was different and we see a lot of Betaproteobacteria, Burkholderia, which are Gram-negative aerobic bacteria that often found in decaying organic matter. Microbial diversity data from the permafrost strata will add to our understanding of polar microbial ecology.
Abstract Title:
Characterisation of Microbial Populations in Highly Radioactive Storage Facilities in Sellafield, Uk

Primary Author Block:
S. Ruiz Lopez1, N. Cole2, J. Lloyd1, L. Foster1; 1Univ. of Manchester, Manchester, United Kingdom, 2Sellafield Ltd, Warrington, United Kingdom

Abstract Body:
The use of nuclear energy has been of great importance to the United Kingdom, with Sellafield being the largest nuclear site used for both power production and more recently reprocessing activities. This project, via collaboration between the Geomicrobiology Group at the University of Manchester and Sellafield Limited, aims to investigate the biogeochemical conditions of a Fuel Handling Pond (FHP) in Sellafield. The main pre-reprocessing storage pond at the Sellafield site is the Fuel Handling Plant (FHP), a concrete walled indoor pond filled with demineralised water, responsible for receiving, storing and mechanically processing spent nuclear fuel (SNF) from Magnox and Advanced Gas-cooled Reactor (AGR) stations from across the UK. Samples were taken from the FHP at different spatial locations and depths, encompassing subponds and a feeding head tank. The aim of this study is to characterize microbial communities of the storage pond at the Fuel Handling Plant (FHP) to create a microbial database consisting of population density and diversity of microorganisms present to better understand the biological complexity of the pond. The findings are discussed in relation to microbial survival to extreme environments and microbial adaptation to operational changes observed during the six months of analysis, and how microorganisms may impact on the management of the pond. The presence of microorganisms in water samples was studied by using molecular techniques e.g. Illumina high throughput 16S rRNA gene sequencing and the microbial density was determined by real-time quantitative PCR (Q-PCR). Organisms identified by Illumina sequencing were predominantly Proteobacteria, mainly Alpha and Beta in the head tank and main pond sample sites. Greater phylogenetic diversity was observed in the Head Tank samples that feed FHP. The presence of Hydrogenophaga sp. solely in the FHP main ponds suggests the metabolism of hydrogen in the ponds which could be generated by radiolysis of water. Future plans involve further genetic characterisation of the identified communities which will be correlated with the complex biogeochemistry of the pond system. These studies will lead to a better understanding of the factors supporting colonization of the pond, the potential impact of the microbial community on fuel handling operations and the biochemical mechanisms of adaptation to this extreme radioactive environment.
Abstract:

Anti-Proliferative and Anti-Oxidant Activities of Prodigiosin Extracted from Radio-Resistant Streptomyces Sp. Strain Wma-Lm31
Primary Author Block:
W. Sajjad, F. A. Nazir, I. Naz, S. Bano, F. Hasan, A. A. Shah; Quaid-i-Azam Univ., Islamabad, Pakistan

Abstract Body:

Background: Microorganisms have been considered as one of the major source of novel pharmaceutical products since decades. The purpose of current study was to evaluate anti-proliferative and anti-oxidant potential of prodigiosin from radio-resistant Streptomyces sp.

Methods: A radio-resistant bacterium, labelled as strain WMA-LM31, was isolated from desert soil. The strain was characterized via 16S rRNA gene sequencing and also exposed to different doses of ultraviolet (UV) radiation to find its survivability in comparison to E. coli (ATCC 10536), a UV sensitive strain. Strain WMA-LM31 produces a red color pigment in tryptone glucose yeast (TGY) supplemented with casein and 5% NaCl. The colored pigment was extracted using water:methanol and purified by high-performance liquid chromatography on a C18 analytical column. The purified colored compound was then subjected to Liquid chromatography-tandem mass spectrometry. The active fraction was assayed for antioxidant, iron chelation and anti-proliferative activities using standard assay conditions against HeLa and HepG2 cell lines.

Results: 16S rRNA gene sequencing showed that the bacterium clusters to genus Streptomyces with maximum similarity to Streptomyces coelicolor. It was found to be resistant to UV dosage up to 2×10³ J/m² and the pigment extracted showed a lambda max at 532 nm. LCMS/MS analysis of the compound showed molar mass of 324 [m/z]+ matched the formula C20H25N3O, and was identified as prodigiosin. The purified prodigiosin showed antioxidant activity with strong scavenging ability against superoxides up to 62.51% at 10 µg/ml concentration. The percent chelation capacity of purified prodigiosin for iron (Fe²⁺) was 34.739% ± 0.53. Furthermore, prodigiosin showed high inhibitory action against oxidative damages to bovine serum albumin and mice liver lipids in comparison to standard ascorbic acid. IC50 values of HepG2 and HeLa cell lines were 12.66 and 14.83 µg/ml of prodigiosin concentration, respectively, that demonstrates induction of apoptosis in cancer cell lines.

Conclusions: It is concluded, that prodigiosin pigment extracted from Streptomyces WMA-LM31 has strong anticancer, apoptotic and antioxidant properties. Prodigiosin from radio-resistant isolate can be used as a potential source in topical creams as sunscreen.
Abstract Title:
The Paradox of Glycerol: Cellular Protectant and Cellular Stressor

Primary Author Block:
J. E. Hallsworth; Inst. for Global Food Security, Queen's Univ. Belfast, Belfast, United Kingdom

Abstract Body:
Glycerol is a chemically simple and yet biologically complex substance which is produced by many microbes (especially yeasts and fungi) as a compatible solute. It can reduce intracellular water activity and thereby regulates cell turgor, and can also protect macromolecular systems against various types of stress. However, at high concentrations glycerol can itself become a stressor. A series of studies were carried out, using fungal xerophiles as model systems, to (i) unravel the various stress mechanisms by which glycerol can inhibit fungi [1,2], and determine whether glycerol can also (ii) enhance biotic activity of fungi [1,3,4] and (iii) enable fungal germination and hyphal growth beyond the established 0.605 water-activity limit for life [5]. A culture-based approach was used, based on rational design of solute mixtures within culture media to enable the function of the cellular system and its biomacromolecules at biophysical extremes; media were designed over a wide range of water activities. At high concentrations, glycerol reduces water activity to beyond the known limit for xerophile growth and metabolism and, at molar concentrations [1,3-5], also acts as a chaotropic stressor [1,2,6,7]; this polyol does, however, enhance the rate of fungal germination - and reduce the water activity minimum for germination and growth - of extreme fungal xerophiles to ≤ 0.585 water activity [4,5]. Germination of Aspergillus penicillioides spores, at 0.585 water activity (with theoretical limit, derived by extrapolation of data of 0.565) represents a new water-activity limit for the microbial biosphere [2,5,8]. The findings were considered in context of key questions relating to biological control of plant pathogens [9], microbes in the built environment [6], and the astrobiology field.
Numerical Taxonomy and Phylogenetic Analyses of the Microbial Community of Epsomic Basque Lake, BC

Primary Author Block:
J. D. Crisler1, F. Chen2, B. C. Clark3, M. A. Schneegurt1; 1Wichita State Univ., Wichita, KS, 2Jet Propulsion Lab., Pasadena, CA, 3Space Sci. Inst., Boulder, CO

Abstract Body:
Athalassohaline waters that are rich in divalent ions are good analogues for the chemical environments of Mars and the icy worlds. Sulfate salts, along with chlorides, are important in Mars regolith with Ca, Fe, Mg, and Na counterions. Certain lakes in the Pacific Northwest are saturated with MgSO4 as epsomite. Bacterial isolates from epsomic lakes provide insight into the risk of forward contamination of Mars and other celestial bodies by terrestrial microbes on spacecraft. Here we report on the microbial community of Basque Lake, BC, a group of playas that is saturated in MgSO4. More than 60 bacterial isolates were obtained from Basque Lake soils by enrichment culture and repetitive streak-plating using media containing 10% NaCl or 2 M (~50%) MgSO4. Most of the isolates (~75%) were Gram-positive, motile, and produced endospores. Isolates related to Marinococcus halophilus and Virgibacillus marismortui dominated the collection. Halomonas and Salinivibrio were Gram-negative genera found at Basque Lake. Less diversity was captured in the microbial collection from Basque Lake than from closely related epsomic Hot Lake, WA. Nearly all of the Basque Lake isolates grew at 2 M MgSO4, with 65% growing at 60%. Several isolates could grow in saturated (67%) MgSO4 (aw = 0.90) and even at the eutectic temperature (~4 °C) and concentration (43%). Eutectic solutions formed by deliquescing hygroscopic salts may be present on Mars. All of the isolates grew at 10% NaCl with 70% growing at 20% salinity (~3.5 M NaCl; aw = 0.82). Halotolerance and epsotolerance were positively correlated among the isolates, but not for every isolate. Basque Lake isolates grew better at basic pH than acid pH, with 80% growing at pH 9 and 30% growing at pH 10. Only 20% of the isolates grew at pH 5. Numerical taxonomy was used to create trees of relatedness based on 45 phenetic characteristics. These trees show a strong correspondence to phylogenetic trees constructed from 16S rRNA gene sequences. Microbes capable of growth under the extreme chemical conditions on Mars are a particular concern for forward planetary protection should they contaminate a spacecraft. Diverse salinotolerant microbes are capable of growth in high MgSO4 concentrations. Epsotolerant bacteria like those from Basque Lake also are found in spacecraft assembly facilities and in oligosaline common soils.
Session Number: 440  
Session Type: Poster  

Session Start Date Time: 6/10/2018 12:45:00 PM  
Session End Date Time: 6/10/2018 2:45:00 PM  
Session Primary Track: Microbial Ecology and Evolution  
Abstract Control Number: 6413  
Poster Board Number: SUNDAY - 991  

Abstract Title:  
Insights Into the Phylogenetic and Metabolic Diversity of the Prokaryotic Community Dwelling in Hypersaline Soils  

Primary Author Block:  
B. Vera-Gargallo, A. Ventosa; Univ. of Sevilla, Sevilla, Spain  

Abstract Body:  
Hypersaline environments, characterized by a high salt concentration, mainly comprise aquatic and terrestrial habitats. While only a limited number of studies on the microbiota and ecology of saline soils have been carried out, hypersaline lakes and salterns have been thoroughly studied, resulting in a biased knowledge about halophiles and hypersaline habitats. The scarce information about saline soil biodiversity and functions hamper our ability of making predictions of the changes in microbial communities with soil salinization, an increasingly important problem for society and the environment. In an effort to improve our understanding of microbial communities in saline environments, and particularly in soils, here we assess the phylogenetic diversity and metabolic potential of the prokaryotic microbial community dwelling in hypersaline soils (23.98 mS/cm and 54.5 mS/cm) from the Odiel Saltmarshes area (SW Spain) by means of shotgun metagenomics. The comparison of the results with the data available from extensively studied aquatic habitats shows that the considered soils harbor a more diverse microbial community which comprise at least 29 different phyla. While the majority of groups and main inhabitants of these saline soils include well-recognized halophiles belonging to Euryarchaeota, Balneolaeota, Bacteroidetes, Rhodothermaeota and ‘Nanohaloarchaeota’, our results suggest that some taxa may be highly adapted to aquatic habitats (such as Haloquadratum) and that soil structure may provide shelter to organisms with different tolerances to salinity. Furthermore, we report the first genomes reconstructed from saline soil metagenomes, affiliated to Balneolaeota and Bacteroidetes, and assess their phylogenetic relationships, osmoadaptation strategies and abundance in hypersaline environments around the world. Metabolic potential seems to relate to soil habitat properties and structure. Categories such as fermentation, monosaccharides and polysaccharides, membrane transport, iron acquisition, motility and chemotaxis are in higher proportion in soil databases as metabolic versatility may be more advantageous in these highly dynamic, heterogeneous and nutrient poor habitats that soils represent. Also, genes classified into the category of pathogenicity, virulence and secondary metabolites were increased in soil databases.
Abstract Title:
Characterization of Halophiles Producing Stable Hydrolases from the Cabo Rojo Salterns

Primary Author Block:
Y. Molina, J. Rosado-Albino, A. Ghrayeb, E. Fasoli, L. Casillas-Martinez; Univ. of Puerto Rico-Humacao, Humacao, PR

Abstract Body:
The Cabo Rojo salterns consists of two main ecosystems, salt ponds and microbial mats filled with halophiles in need of characterization. In this study, we have tested the capacities of more than 150 isolates from the salterns for their capacities to produce stable hydrolases particularly extracellular esterase lipases, proteases and epoxide hydrolyses. These novel enzymes are very needed as green biotechnological alternatives for biocatalysis. Usually hydrolases are needed for the synthesis of chiral building blocks with high chemo-regio- and stereoselectivity and require to be really stable under different industrial conditions. Of all the microorganisms screened (n=150), 45 isolates exhibited esterase activity, 12 were positive for lipases, 47 for proteases, and 28 for epoxide hydrolyses. Several isolates showed multiple enzymatic activities. The positive hits were initially assayed for enzymatic stability at increasing concentrations of acetonitrile, dioxane, THF, acetone and DMSO, (15%, 30%, 30% and 50% (v/v)). Three esterases, one lipase, two proteases, and two epoxide hydrolyses showed high activity in these organic solvents when compared to the other isolates. These eight isolates were further identified using three methods: (1) biochemical traits analysis using the Analytical Profile Index (API-50C), (2) Fatty acid methyl esters (FAME) lipid comparisons (FAME) and (3) 16SrDNA sequence analysis. The preliminary biochemical comparisons by API indicated that most isolates (n=7) belong to the Bacillus genus. Further FAME analysis indicated three of the isolates have closest relatives to Bacillus sp. with 100% identity to B. subtilis, while the other three remained unknown. The phylogenetic 16SrDNA comparisons indicated a 97% consensus sequence to an Exiguobacterium sp with and six other isolates with 97-99% to B. subtilis. In conclusion, tropical salterns are an excellent ecosystem to search for halophiles producing stable enzymes for the newly growing Green-Chemistry industries.
Session Title: MEE11 - Unusual Microbes and Extreme Environments
Session Start Date Time: 6/10/2018 12:45:00 PM
Session End Date Time: 6/10/2018 2:45:00 PM
Session Primary Track: Microbial Ecology and Evolution
Abstract Control Number: 7396
Poster Board Number: SUNDAY - 993

Abstract Title:
A Comparative Genomic Analysis of Extremely Halophilic Carbon Monoxide Oxidizers

Primary Author Block:
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Abstract Body:
Background: Mo-dependent CO oxidation has largely been known from the domain Bacteria including at least 6 phyla (Actinobacteria, Bacteroidetes, Chloroflexi, Deinococcus-Thermus, Firmicutes, and Proteobacteria), most of which grow with moderate temperature, pH, and salt requirements. Several additional members of the Crenarchaeota (e.g., Sulfolobus) have also been documented as Mo-dependent CO oxidizers (Mo-COX), while metagenomic analyses have provided evidence that Aigarchaeota and Geoarchaeota harbor CO oxidizers as well. Recently, several approaches have revealed Mo-COX among the extremely halophilic Euryarchaeota. Little is known about this group of CO oxidizers, but phylogenetic analyses of coxL, the large sub-unit gene for CO dehydrogenase, show that they form a clade distinct from that of all other Mo-COX isolates, and that they share a common ancestor with Bacteria Mo-COX. The latter observation supports an origin for Mo-dependent CO oxidation prior to the Great Oxygenation Event (ca. 2.45 Gya). To probe this possibility further, and to better understand the extremely halophilic Mo-COX, we have conducted a comparative genomic analysis 7 distinct isolates representing 6 genera. Methods: Isolates were obtained from hypersaline soils and sediments from Hawai`i, the Bonneville Salt Flats (UT) and the Atacama Desert (Chile). Isolate genomes were sequenced by JGI-DOI (Halorubrum sp. BV1) or Michigan State University (all others); after sequence curation and assembly, all genomes were annotated by IMG/MER. Phylogenetic analyses of specific genes and concatenated genes were conducted with MEGA7 (Kumar et al., 2016) after alignments with MUSCLE (Edgar, 2004). Bias due to substitutions for aspartate, glutamate and valine was assessed by removing codons for these residues. Results and Conclusions: Phylogenetic analyses of 17 extreme halophile genomes (7 obtained in this study along with 10 others whose genomes contain cox genes) provides strong support for a topology in which Bacteria and Halobacteria Mo-COX share a common ancestor. Other archaeal Mo-COX form a clade within the Bacteria that appears to have arisen via horizontal gene transfer subsequent to the evolution of Mo-dependent CO oxidation. This result is also supported by observations of synteny for the cox operon. The composition and arrangement of cox genes is highly conserved within the halobacteria and distinct from other lineages. It most closely resembles that of Actinobacteria, its closest phylogenetic neighbor in cox gene trees.
Abstract Title:
Halophilic Bacterium, Halomonas Pacificamml1909 A Bioactive Potential Source of Isolated Novel Compound

Primary Author Block:
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Abstract Body:
Salt pans a potential source of bioactive metabolites. The larger population of halophilic bacteria in saline habitats may be considered for the possibility of discovering novel bioactive agents. This study aimed to evaluate the antioxidant and cytotoxic potential of halophilic bacteria isolated from Kelambakkam Salt pan. Totally 18 sediment samples were collected from reservoir pond, condensation pond and evaporation ponds of Kelambakkam Saltpan. The antioxidant activity of the halophilic crude extracts was measured by the DPPH scavenging activity using the free radicals assay. Out of 63 extracts, 39 exhibited moderate antioxidant activity, one showed strong and one showed highly strong activity. Further, the antioxidant activity of halophilic crude extracts was assessed by TLC autobiography. Among 63 halophilic bacterial crude extracts, 2, 7 and 41 extracts exhibited cytotoxicity against A549, HepG2 and MCF-7, respectively at 1 mg/ml in MTT assay. Totally 41 crude extracts exhibited antioxidant activity and 42 crude extracts showed cytotoxicity and 30 crude exhibited both antioxidant and cytotoxicity. Based on the antioxidant activity and cytotoxicity, 12 halophilic bacterial crude extracts were shortlisted due to their IC50 values against A549, HepG2 and MCF-7 cell lines. The MML1909 revealed as Halomonas pacifica based on the polyphasic taxonomical study as it was found to be most effective strain. The purified crude extract of H. Pacifica MML1909 using column chromatography resulted in 18 fractions. The selected fraction no. 14 was further purified by column chromatography followed by preparatory TLC. The pure compound was characterized by various spectral analyses such as IR, UV, 1HNMR and 13CNMR, which revealed that the molecular formula as C20H30N4O6 and its respective molecular weight as 422.48 by ESI-MS. The IC50 value of pure compound of H. pacifica MML1909 was determined as 45 µg and 25 µg on A549 and MCF-7 cell lines, respectively. Apoptosis was confirmed by flow cytometry and DNA fragmentation analysis. Not much work has been done globally on the bioactivities of halophilic bacteria in genus and Halomonas spp.in particular. The pure compound appears safe molecule as they did not show significant cytotoxicity on normal human vero cell line. These results highlighted the importance of H. pacifica MML1909 as a bioactive potential compound.
Therefore, this compound would serve as a better chemotherapeutic agent for the cancer treatment for which further elaborate research is warranted and it is worth investigating.
Abstract Title:
Metagenomics Insights Into the Halophilic Diversity of Hypersaline Lake in the Desert of Tharparkar, Pakistan

Primary Author Block:
L. M. Cycil1, K. Memon1, S. DasSarma2, W. Pecher3, F. Hasan1; 1Quaid-i-Azam Univ., Islamabad, Pakistan, 2Univ. of Maryland, Baltimore, MD, 3Univ. of Baltimore, Baltimore, MD

Abstract Body:
In this study, we report for the first time, the diverse halophilic communities of un-impacted Dhuyaeen Lake, which is situated at the far south of Pakistan in the desert of Tharparkar (24°28'0N 69°35'0E). This is an alkaline and hypersaline lake, highly exposed to solar radiations. It originated in 2001 after an earthquake (7.7-magnitude) jolted the entire desert causing liquefaction of sand and salt pans. Over 10,000 m² of underground brine erupted, forming mud volcanoes and lakes. The area is politically sensitive, near the India-Pakistan border and inaccessible for geological surveys. As a result, this pristine extreme environment holds clues about evolutionary processes and ecological patterns but the microflora remains poorly studied. Here we used both metagenomics and culturing techniques to study halophilic diversity. DGGE and Illumina sequencing protocols were performed on both brine and wet sediments collected from the sampling site. Sequences were analyzed using QIIME, Basespace, CLC-Genomics Workbench, and Cytoscape. Cultures were isolated at 16-25% salinity. We used bioinformatics tools to bin the genes responsible for halophilic adaptation surveying 256829 and 102730 sequence reads from brine and sediment respectively. Metagenomic data accounted for Bacteria (31% brine; 79% sediment), Archaea (8% brine; 4% sediment) and unclassified (30 % brine; 45% soil) sequences. Abundant phyla in both the datasets included Proteobacteria (24%), Bacteroidetes (25%), p_OP1 (2.4%) and Euryarchaeota (10%). Alpha diversity showed less diversity in brine then the sediments. Genes producing ectoin, betain, extremozymes, bacteriorhodopsin and gas vesicle proteins were characterized. The isolates displayed huge potential in commercial applications. This report laid a foundation for exploring biogeochemical cycling and microbial function in hypersaline environments and has astrobiological implications.
Abstract Title:
Bacillus Altitudinis, A Deep Southern Ocean Microbe - Lifestyle & Genome Mining
Primary Author Block:
U. Halder, Student, R. Bandopadhyay, Scientist; The Univ. of Burdwan, Burdwan, India
Abstract Body:
Understanding the functional role of microbes in the extreme environment is diverse because of higher existing species. Also, Exploitation of microbial EPS is relatively unexplored and research interest is constantly increasing toward characterization and applications of novel EPS as renewable resources. The species B. altitudinis 41KF2bT was first reported at the height of 41km in the stratosphere. Here, B. altitudinis SORB11 was collected from 3.8km deep Southern Ocean. Genomic DNA was isolated and sequenced on the Illumina MiSeq. Genome annotation was performed using the RAST. Comparative genomics was performed using BRIG blast, PHAST, Mauve, Spine & Agent server. Production & optimization of EPS were performed & characterisation was done by SEM, AFM, GPC, GC-MS, FTIR, 1H, 13C, 2D-NMR, TGA, Rheological analysis. The whole genome is 3.6 kbp long with 41.2% mean GC content. Based on a complete 16S rDNA sequence analysis, it showed 100% similarity to type strain B. altitudinis 41KF2bT. Calculations for ANI & dDDH showed 98.4% and 86% values, respectively. 3,867 coding genes were annotated, with 2 rRNAs & 22 tRNAs. It has a unique capacity to resist UV-B more than 10J & total of 102 genes belong to DNA metabolism including 97 genes encoding stress response. The morphology was visualized under TEM before & after UV stress. EPS biosynthesis genes epsC & epsD is present in the genome. The EPS is water soluble, powder like and produced up to 11.5 g/L. EPS is porous & surface topography is disordered random inter/intra molecules in water apparently different from the rod-like chains. Molecular weight is 1.24×103 Da. Mannose & glucose are present as a monomeric component. Furanoid ring of the sugar units, glycosidic linkage along with the identification of non-carbohydrate parts is also observed. 45% of total mass of EPS was degraded while the temperature increased up to 600°C. Rheological activity is also preferable varying the temperature.
Abstract Title:
Aerobic Respiration on Soluble Iron is Expressed Constitutively by Metallosphaera Sedula

Primary Author Block:
N. T. Pham, O. G. Griswold, R. C. Blake, II; Xavier Univ., New Orleans, LA

Abstract Body:
Very little is known about the electron transfer reactions that occur during aerobic respiration on soluble iron by members of the Euryarchaeota, a phylum of Archaea that contains Metallosphaera sedula. This project was conducted to test the Hypothesis that iron oxidation is expressed constitutively in this thermophilic, archaeal microorganism. Methods: M. sedula was cultured organotrophically on yeast extract at pH 1.6 and mixotrophically on yeast extract and soluble ferrous iron at pH 1.5, both at 65o C. The time courses of ferrous iron oxidation as catalyzed by intact cells of M. sedula were monitored using an integrating cavity absorption meter (ICAM) that permitted the acquisition of accurate absorbance data in suspensions of intact cells that scatter light. Initial velocities of iron oxidation were determined from the linear increases in absorbance at 350 nm due to the generation of oxidized iron. Results: Regardless of the immediate growth history of the organism, M. sedula was observed to readily oxidize soluble iron and the kinetics of aerobic respiration on soluble iron by the intact organism conformed to the Michaelis-Menten formalism. It was thus evident that the ability to respire aerobically on iron was expressed in this organism under both growth conditions, which was consistent with the hypothesis. Electron transfer reactions among colored cytochromes in the intact archaeal cells were subsequently monitored in the ICAM in the presence of much higher concentrations of the intact cells. When whole cells were mixed with soluble iron at pH 1.5, the aerobic iron respiratory chain of M. sedula was dominated by the redox status of two abundant cellular chromophores that had maximum absorbance peaks at 442, 483 and 578 nm in the reduced state. The intracellular chromophores were reduced within the time that it took to mix a suspension of the intact archaea with soluble ferrous iron. The reduced chromophores then returned to their original oxidized states as the organisms catalyzed the complete oxidation of the limiting concentration of ferrous iron by the excess concentration of molecular oxygen. Conclusions: The kinetic behaviors of these reduced chromophores were consistent with the hypothesis that their oxidation represented the rate-limiting step in the overall aerobic respiratory process.
Abstract Title:
Bacterial Growth Tolerances to An Iterative Matrix of Ions Relevant to Mars and the Ocean Worlds

Primary Author Block:
H. Zayed1, M. Joad1, A. F. Al Soudi1, A. Mahdi1, H. Zbeeb1, F. Chen2, B. C. Clark3, M. A. Schneegurt1;
1Wichita State Univ., Wichita, KS, 2Jet Propulsion Lab., Pasadena, CA, 3Space Sci. Inst., Boulder, CO

Abstract Body:
Microbial responses to hypersaline environments have relevance to the salty hyperarid surface of Mars and the concentrated brines likely found in Europa and the ocean worlds. Hygroscopic sulfate and (per)chlorate salts may form deliquescent brines with low eutectic points, providing liquid water at the near-surface of Mars. While microbial tolerances to hyperhaline environments rich in NaCl are well described, growth tolerances to extremely high concentrations of other salts are not. It is clear that a range of specific solute effects, and not solely water activity, determines microbial responses to high solute concentrations. When salts are at high concentrations, each ion will impart individual cellular effects that are difficult to discern. We are measuring the growth tolerances of salinotolerant bacteria using an iterative matrix of ions that includes salts relevant to Mars and the ocean worlds. The matrix includes the anions chloride, nitrate, (per)chlorate, phosphate, and sulfate coupled in every combination with the cations Ca, Fe, Mg, K, and Na. In addition, salts of borate, Cs, and Li have been tested. The subjects of the current study are 18 well-characterized bacterial isolates from Hot Lake, BC and the Great Salt Plains of Oklahoma, environments saturated in MgSO4 and NaCl, respectively. These collection encompasses Halomonas, Marinococcus, Nesterenkonia, and Virgibacillus isolates that grow at ≥10% NaCl (1.7 M) or ≥2 M (50%) MgSO4. Strong growth was commonly observed in a variety of salts at ≥1 M concentrations, including LiCl, MgCl2, MgSO4, KNO3, K2SO4, and NaCl. Growth has been observed in ≥1 M perchlorates and ≥2.5 M chlorates of Mg, K, and Na. Certain ions, such as Cs and borate, seem toxic at concentrations low enough as to not appreciably affect water activity. Statistical analyses have been used to examine the complex set of tolerances, allowing the identification of individual ions that appear most deleterious to bacterial cell proliferation. Measuring the toxicity to bacteria to ions at extremely high concentrations helps to define extraterrestrial habitable regions and informs us about the types of microbes on spacecraft that pose greater risks for forward planetary contamination.
Abstract Title:
Metabolic Mechanisms of Interaction Within A Defined Gut Microbiota

Primary Author Block:
G. Medlock1, M. Carey1, D. McDuffie1, N. Giallourou2, J. Swann2, G. Kolling1, J. Papin1; 1Univ. of Virginia, Charlottesville, VA, 2Imperial Coll., London, United Kingdom

Abstract Body:
Metabolic interactions between bacterial species govern important processes ranging from global biogeochemical cycles to colonization resistance to pathogens. However, identifying interacting species within a microbial community and the associated metabolic mechanism of interaction is a daunting task. Here, we present a co-culture resource allocation model (CRAM) that infers metabolites that may govern growth-modulating interactions between species grown in vitro from untargeted supernatant metabolomics data. To develop and test this method, we performed pairwise growth experiments between each of 6 strains from the altered Schaedler flora (ASF), a synthetic community of 8 bacterial strains used to standardize the microbiota of mice in breeding facilities. While co-culture of pairs of strains generally led to negative growth interactions (i.e. strains grew to lower abundance than in monoculture), it also led to higher total biomass production (i.e. together, strains grew to higher density than either in monoculture). We applied the CRAM to untargeted supernatant metabolomics data from these experiments to compare co-culture metabolic profiles to expected profiles based on monoculture behavior. We found striking increases in efficiency in co-culture, as well as differential behavior in co-culture suggestive of cross-feeding. We used the CRAM results to interrogate a putative cross-feeding interaction in which one strain produces amino acids that another strain consumes through Stickland fermentation. We coupled the CRAM with genome-scale metabolic network reconstructions for each of the ASF species to provide parallel support for putative metabolic interactions, then validated these predictions in vitro. This framework for inferring mechanisms of metabolic interaction between species is generalizable to communities of any size, and can easily be modified to interrogate interactions that modulate phenotypes other than growth.
Neutral and Selective Dynamics in A Synthetic Microbial Community

N. Cira1, M. Pearce2, S. Quake2; 1Rowland Inst. at Harvard, Cambridge, MA, 2Stanford Univ., Stanford, CA

Ecologists debate the relative importance of selective versus neutral processes in understanding biodiversity. This debate is especially pertinent to microbial communities, which play crucial roles in areas such as health, disease, industry, and the environment. Here we created a synthetic microbial community using heritable genetic barcodes and tracked community composition over repeated rounds of subculture with immigration. Consistent with theory, we find a transition exists between neutral and selective regimes, and the crossover point depends on the fraction of immigrants and magnitude of fitness differences. Subjecting a community to a step increase in carrying capacity resulted in a transition from neutral to selective regimes, highlighting that using the correct model is essential for predicting community response to change. Taken together these results emphasize the importance of including selection to obtain realistic models of even simple systems.
Microbial communities are ubiquitously found in nature and maintain the huge diversity. It has been considered that microbial diversity contributes to maintain ecological functions. However, how to coexist multispecies and maintain the microbial diversity are unknown although the competitive exclusion principle demonstrated that only strain exhibiting the highest competitiveness occupies the niche. Cupriavidus sp. P-10 and Comamonas testosteroni R2 were co-cultured with phenol as the sole carbon and energy source in a chemostat to reveal the coexistence mechanism under the substrate-competing condition. Simulation depending on Lotoka-Volterra equations predicted that strain R2 became dominant and strain P-10 was washed out. However, these strains coexisted and strain P-10 became dominant in the chemostat culture. The kinetic parameters for phenol indicated that the phenol concentration exhibiting Vmax of strain P-10 was higher than that of strain R2. It was shown that phenol concentration was spatiotemporally heterogeneous in the chemostat, suggesting that strain P-10 obtained a chance to acquire phenol. Actually, a dominant strain changed from strain P-10 to strain R2 corresponding to the decrease of phenol loading rate. When strain P-10 was dominant, the transcription of the gene encoding phenol hydroxylase (LmPH) was observed in only strain P-10. Interestingly, total cells yield in the coexisting culture was higher than distinct pure culture. These results suggested that spatiotemporal heterogeneity allowed strain P-10 to overcome the competition with strain R2 and that strain R2 could survive using metabolites from strain P-10. Coexistence collapsed when strain R2 dominated, where only strain R2 expressed LmPH and strain P-10 washed out. These results suggested that coexistence was organized when metabolic networks were constructed between microorganisms, which would be one of maintenance mechanism of microbial diversity in the nature.
Many species of bacteria are able to collectively sense and respond to their physical environments. A common solution to the challenge of environmental uncertainty is the evolution of cell-cell communication systems known as ‘quorum sensing’ (QS), where individual cells control gene expression via the secretion and sensing of extracellular signal molecules. Despite detailed understanding of the intra-cellular mechanisms of signal production and signal responses, there remains considerable debate over the functional role(s) of QS, how does QS enhance bacterial fitness - or in short, what is it for? Using an agent-based modeling approach, we investigated how different selective challenges drive the evolution of QS complexity and multi-functionality. Specifically, the in silico simulations enable us to track the evolution of multiple QS component traits underlying signal production, signal response and their interaction (auto-induction) under defined conditions of social and physical heterogeneity. We demonstrated that individual’s production rate and signal threshold co-evolve in a fashion that is sensitive to costs and benefits of cooperation. We found that bacteria can coerce others to produce more signals and become less responsive by increasing their signal threshold to avoid paying for the cost of cooperation while gaining the group level benefits. However, when we continuously decreased the genetic relatedness, we found that the coercion strategy eventually collapsed and a cheat strategy evolved. This phenomenon was observed in the evolutionary scenarios regardless of the presence of QS auto-induction mechanism. However, when we investigated the transition period where the coercion strategy was collapsing, we found that bacteria evolved with auto-induction mechanism generally have both a higher overall cooperation payoff than the ones evolved without auto-induction mechanism. This suggests that the auto-induction architecture provides a robust mechanism to sustain cooperation. This work is important as it sheds light on how simple signal-mediated behavioral rules can shape complex collective behaviors in bacteria.
**Session Number:** 462  
**Session Type:** Poster Talk

**Session Number:** 462  
**Session Type:** Poster Talk

**Session Title:** Why Can't We All Just Get Along?: Characterizing Microbial Interactions and Dynamics

**Session Start Date Time:** 6/10/2018 1:45:00 PM  
**Session End Date Time:** 6/10/2018 2:35:00 PM

**Session Primary Track:** Microbial Ecology and Evolution  
**Abstract Control Number:** 9268  
**Poster Board Number:**

**Abstract Title:**  
Bacteriocin-Mediated Interactions Increase with Spatial Scale in Household Pseudomonas aeruginosa Isolates

**Primary Author Block:**  
A. Mojesky, E. Miller, J. Bara, S. Remold; Univ. of Louisville, Louisville, KY

**Abstract Body:**  
Pseudomonas aeruginosa is a Gram-negative bacterium known to abundantly produce a class of narrow spectrum antimicrobial toxins called bacteriocins. While bacteriocins have traditionally been studied in laboratory settings, an emerging field of research is exploring the particular role of these antibiotics in natural microbial assemblages. Recent studies have shown that bacteriocins are important in the spatial structuring of natural microbial populations. Little inhibition has been observed in coexisting environmental isolates of Pseudomonas fluorescens and in species of the Xenorhabdus genus; yet, as spatial scale increases, bacteriocin-mediated interactions among these isolates will increase as well. However, it is not well understood how spatial isolation between isolates of P. aeruginosa may influence the outcome of bacteriocin-mediated interactions, nor whether isolates living in different habitats display differences in bacteriocin-mediated interactions. We collected isolates of P. aeruginosa from bathroom and kitchen sink drains in 70 households located in Kentucky. A 54 x 54 pairwise-inhibition assay was used to assess bacteriocin-mediated interactions among isolates collected from the same drain, isolates collected from a different drain in the same home, and isolates collected from different homes. We hypothesize that bacteriocin-mediated inhibition will increase in household isolates of P. aeruginosa as spatial scale increases, and that isolates from bathroom sink drains and kitchen sink drains would differ in their patterns of inhibition. The results of this study identified the lowest probability of inhibition to be among isolates collected from the same drain and the highest probability of inhibition to be among isolates collected from different homes. Additionally, bathroom sink drain isolates were found have a significantly higher probability of inhibition than kitchen sink drain isolates. These results are consistent with previous findings in other systems and indicate that bacteriocins play an important role in shaping patterns diversity in household isolates of P. aeruginosa.
Session Number: 462
Session Type: Poster Talk

Session Title: Why Can't We All Just Get Along?: Characterizing Microbial Interactions and Dynamics
Session Start Date Time: 6/10/2018 1:45:00 PM
Session End Date Time: 6/10/2018 2:35:00 PM
Session Primary Track: Microbial Ecology and Evolution
Abstract Control Number: 9476
Poster Board Number:

Abstract Title:
Moderator
Primary Author Block:
Sam Brown; Georgia Inst. of Technology, Atlanta, GA

Abstract Body:
Abstract Title:
Reconstruction and of Health-Impact Pathways in the Human Gut Microbiome

Primary Author Block:
D. A. Ravcheev, L. Moussu, I. Thiele; Univ. of Luxembourg, Esch-sur-Alzette, Luxembourg

Abstract Body:

Background: The human gut microbiome (HGM) have been shown to play a crucial role in human physiology, nutrition, health, and disease. Thus, to date, more than 50 human diseases have been demonstrated to be associated with HGM alterations and more than a dozen HGM-produced metabolites are known to be associated with human health state. Here we present results of a computational comparative-genomic analysis of pathways for biosynthesis or catabolism of such health-impact compounds in the HGM genomes.

Methods: We applied genomic analysis to 632 HGM genomes microorganisms found in the human gut belonging to the phyla of Actinobacteria, Bacteroidetes, Euryarchaeota, Firmicutes, Fusobacteria, Proteobacteria, Synergistetes, Tenericutes, and Verrucomicrobia. For the annotation of gene functions, the PubSEED platform (http://pubseed.theseed.org) was used. The gene function annotation was done using available literature data, protein sequence similarity, protein domain structure, and genome-context based approaches, including gene chromosomal clustering and phyletic patterns.

Results: We analyzed six types metabolic pathways associated with production, catabolism, or transformation of health impact compounds, as follows, (1) biosynthesis of vitamin K, (2) biosynthesis of butyrate, (3) biosynthesis of propionate, (4) utilization of ethanolamine, (5) utilization of propanediol, and (6) biotransformations of bile acids. First, for each of health-impact compound, more than one pathway was found in the HGM genomes. Second, distribution of these health-impact pathways in the HGM genomes is a taxonomy-specific, but a distribution of no one pathway is limited by a single microbial taxon. Third, distribution of the analyzed pathways points to an intensive exchange of health-impact compounds or their precursors in the HGM. Finally, comparison of a taxonomic composition of healthy and diseased HGMs demonstrated the different distribution of the analyzed pathways in healthy and diseased microbiomes. For example, genes for bile acid biotransformation were significantly underrepresented in Crohn’s Disease microbiomes, in comparison with healthy HGMs.

Conclusions: We demonstrate that pathways for metabolism of six different health-impact compounds are broadly distributed in HGM organisms. Data on the distribution of these pathways can improve an understanding of a role of the HGM and provide a future prediction of the HGM genes associated with the host health state.
Abstract Title:
Radiation Oncology: A New Way to Study Clin. Microbial Genomic Streamlining in Vivo?
Primary Author Block:
A. Greninger; Univ. of Washington, Seattle, WA
Abstract Body:
Background: Microbial genome evolution is known to be extraordinarily rapid with promiscuous gene acquisition/loss and swift generation of single nucleotide variants. Bioinformatic analyses of microbial genomes require discussion of both core- and pan-genomes, but understanding of whether a given gene is truly required or adaptive is limited in such analysis. Gene essentiality screens using randomly integrating transposon mutagenesis and/or deep mutational scans have been used to experimentally assess the genomic requirements for replication, but they are limited to cell culture and animal models. There is currently no simple method of experimentally studying large-scale microbial genomic evolution in the human host. Methods: We describe a proof-of-concept study on the use of radiation oncology to understand in vivo genomic streamlining. We prospectively sequenced genomes from microbial strains associated with recent hematopoietic cell transplantation (HCT). Results: We describe metagenomic sequencing of a human coronavirus 229E from a patient with AML and persistent upper respiratory symptoms, who underwent HCT. Sequencing revealed a 548-nucleotide deletion, which comprised the near entirety of the ORF4 gene. No minor allele variants were detected to suggest a mixed infection. As part of her pre-HCT conditioning regimen, the patient received myeloablative treatment with cyclophosphamide and 12 Gy total body irradiation. Iterative sequencing and RT-PCR confirmation of 4 respiratory samples over the 4-week peritransplant period revealed that the pre-conditioning strain contained an intact ORF4 gene, while the deletion strain appeared after conditioning and persisted over a 2.5-week period. This sequence represents one of the largest genomic deletions detected in a human RNA virus and is the first description of large-scale viral mutation associated with myeloablation. Previous in vitro studies have hinted at the dispensability of ORF4 in human alphacoronaviruses, but no function has been elucidated for this mysterious gene. Conclusions: Radiation oncology treatments provide an intriguing opportunity for the study of microbial gene essentiality and genomic streamlining in humans. We hypothesize that radiation oncology may prove more fruitful in understanding prokaryotic genomic evolution where gene dispensability is greater than in RNA viruses. We will discuss opportunities for understanding genomic streamlining in real-time in the human host in the setting of different radiation oncology treatments.
Abstract Title:
Comparative Genomics of the Emerging Pathogen Streptococcus Pseudopneumoniae

Primary Author Block:
G. Garriss, P. Nannapaneni, B. Henriques-Normark; Karolinska Inst., Stockholm, Sweden

Abstract Body:
Background: Streptococcus pseudopneumoniae (Sppn) is a gram positive bacterium closely related to the major human pathogen Streptococcus pneumoniae (Spn). While initially considered as a commensal, there is increasing evidence that Sppn is a pathogen (1, 2); however the lack of a specific test for identifying Sppn severely hampers the understanding of its pathogenic potential. Current typing methods discriminate Spn from commensal α-hemolytic streptococci based on optochin susceptibility, bile solubility and capsular serotyping. Atypical phenotypes, lack of a pneumococcal capsule and variations in optochin susceptibility testing conditions leads to common misidentification of Sppn isolates as non-typable (NT) Spn or other species such as S. mitis (1, 3). Our objectives were to gain understanding of the genetic characteristics of Sppn clinical isolates and to identify species-specific markers allowing their precise identification.

Methods: Whole-genome sequencing of 25 presumed Sppn strains from lower respiratory tract infections and core genome phylogenetic analysis including publicly available Sppn, Spn, NT Spn and S. mitis genomes were used to identify the Sppn clade. Collectively these genomes encompass isolates from carriage and invasive disease from children and adults. The defined Sppn clade was further investigated in silico to identify known and potential new virulence genes, acquired antimicrobial resistances (AMR), mobile genetic elements (MGEs) and unique genes. Genetic loci found exclusively in Sppn strains were used for the development of a PCR-based assay that allowed the precise identification of Sppn isolates from other α-hemolytic streptococci. Conclusions: Our study reveals the large number of pneumococcal virulence genes that are part of core genome of Sppn as well as the composite scenario of MGEs and their association with AMR genes. Our novel typing assay provides a simple tool for better understanding the epidemiology and clinical significance of Sppn.
Abstract Title:
Phylogenomic Analysis of Fusobacterium Necrophorum Based on Whole Genome Sequencing and its Assoc. with Disease and Host

Primary Author Block:
A. Jensen; Aarhus University, Aarhus, Denmark

Abstract Body:
Objective: Fusobacterium necrophorum (FN) is the main cause of Lemierre’s syndrome and FN has also been associated with tonsillitis and peritonsillar abscesses, otitis media and colorectal cancer. FN may also be found in asymptomatic carriers. Virtually nothing is known about this bacterium at the molecular and genetic level. The aim of this study was by whole genome sequencing of a large volume of strains isolated from patients with different diseases and hosts to determine the phylogenetic diversity of FN and to identify whether certain phylogenetic lineages of FN may be associated with specific diseases.

Material and Methods: In total, genomes of 85 strains were analyzed. Of these, 70 were from our own strain collection while the genomes of 15 strains were extracted from Genbank. Phylogenomic analysis were based on single nucleotide polymorphism (SNP) analysis using the software program parsnp while pairwise average nucleotide identity (ANI) was calculated in the JSpecies program.

Results:
Phylogenomic analysis based on SNPs of the whole genome sequences revealed that the FN strains grouped into three distinctive clades, corresponding to the two subspecies of FN. The third clade consisted of two penicillin-resistant human isolates of FN. ANI calculations showed that the interclades identity were around 95% supporting separation of FN into subspecies. Strains of F. necrophorum subsp. funduliforme clustered into two distinct clusters. Within both cluster a clonal relationship between most of the strains were observed. Most interestingly, no separation of the strains based on the host or disease could be found. Conclusions: No lineages of FN were associated with specific diseases in humans and invasive strains were phylogenetically similar to strains from local infections and strains isolated from healthy carriers. Also, human isolates of FN were similar to animal strains. Our results indicate that virulence and invasiveness of FN is not associated with a specific phylogenetic lineage. Therefore, FN infections might involve additional factors to cause infections (host specific and other external factors (e.g. viruses)). Preliminary data on the intracluster virulence potential and its relationship to disease will be presented at the congress.
Session Number: 502
Session Type: Rapid Fire

Abstract Title:
Genes Significantly Associated with Human Virulence Revealed Through Comparative Genomics of Vibrio Vulnificus Strains

Primary Author Block:
K. J. Jesser1, R. Nowell2, B. A. Froelich1, R. T. Noble1; 1UNC Chapel Hill Inst. of Marine Sci., Morehead City, NC, 2Imperial Coll. London, Berks, United Kingdom

Abstract Body:
Background: Vibrio vulnificus (Vv), a native and ubiquitous member of bacterial assemblages in marine waters and shellfish, is of interest from a human health perspective because of its potential to infect human hosts. Despite concerns over the increasing number of Vv cases worldwide, Vv infections are relatively rare due to the fact that only a small subset of strains appear to be disease-causing. Methods: To identify genomic indicators of virulence in Vv strains, we have utilized high-quality genomic sequencing data and a statistical method that accounts for phylogenetic relatedness to investigate significant associations between gene presence and isolation source (i.e. clinical or environmental) across 27 Vv genomes isolated in the United States between 2006 and 2012 from blood and oyster matrices. Results and Conclusions: This statistical approach is an improvement over other comparative methods, which are often based only on gene presence or absence and do not consider strain relatedness, and has allowed us to identify genomic regions that are significantly associated with either human hosts or the environment. We have evidence for a common set of genes associated with human disease, which includes previously identified indicators of virulence as well as a suite of new genotypic markers linked to disease causing strains.
**Abstract Title:**
Outstanding Abstract Award: Two Faces of Janus: Group A Streptococcal (Gas) Upper Respiratory Tract Colonization and Invasive Infection: Molecular Diversity and Transcriptomics

**Primary Author Block:**
T. Abraham, S. Sistla; Jawaharlal Inst. of Postgraduate Med. Ed. and Res. (JIPMER), Puducherry, India

**Abstract Body:**
Background: GAS is a strict human pathogen causing a broad spectrum of diseases. The throat and skin epithelia form the primary ecological niches of GAS as well as the sites for tissue intrusion in case of invasive disease. Pathogenic bacteria employ diverse mechanisms to fine-tune the expression of virulence factors and metabolic processes in response to growth conditions encountered in different milieu and stages of infection. Understanding the evolution of niche specialization can reveal new insights about the critical factors essential for the disease development. The present study was undertaken to determine the molecular diversity of colonizing and invasive GAS isolates and the basis of such transition with transcriptome analysis of select isolates.

**Methods:** The study comprised of 107 GAS isolates, (59 from invasive diseases, 48 from asymptomatic throat colonizers). To determine molecular diversity, the isolates were characterized by emm genotyping and MLST. Whole transcriptome analysis was performed on select isolates using Illumina Hiseq 2500 platform, and the gene expressions were estimated using String_Tie program.

**Results:** A total of 41 emm types were identified from clinical isolates with emm82, emm183, emm44, emm66 and emm63 in decreasing order. Six of the emm typable community isolates belonged to emm66, emm80, emm77 and emm75.1. MLST analysis of the randomly selected 36 isolates demonstrated a considerable genetic diversity, with twenty different STs and some new STs (969, 970, 971, 972, 973). More than 400 transcripts were highly expressed, mainly belonging to functional categories involved in the adaptive metabolic shift (ska, RelA, CodY, arcCBA, Sdh, ldh, etc) and virulence gene transcription (Isp, hasABC, hyl, smeZ, saga, crg, scpA, etc). About 49% of the expressed genes are hypothetical ORFs of unknown function. Genes with significant expression changes (DEGs) were identified by FDR<0.10 and log2FC ≥1 and considering the community isolate as wild type. Thirteen transcripts were up-regulated (clpL, int3, spel, speH, hylP2) and one was down-regulated in the blood isolate. In the tissue isolate, twenty transcripts were up-regulated (salA, scpA, mga, emm1, lys, hylP2) and sixteen were down-regulated (srt, ddh, sat, hsdS, Spy_1075). Conclusions: Our study provides insights into the changes associated with the plasticity of GAS transcriptome. Increased understanding of how GAS strains from various habitats respond during ex vivo culture could be helpful in developing novel preventive or therapeutic strategies.
Abstract Title:
Genomic Characterization of Urethritis-Associated Neisseria Meningitidis Shows that A Wide Range of N. Meningitidis Strains Can Cause Urethritis

Primary Author Block:
K. C. Ma1, M. Unemo2, S. Jeverica3, H. Takahashi4, M. Ohnishi4, Y. H. Grad1; 1Harvard TH Chan Sch. of Publ. Hlth., Boston, MA, 2WHO Collaborating Ctr. (CC) for Gonorrhoea and Other Sexually Transmitted Infections, Örebro Univ., Örebro, Sweden, 3Univ. of Ljubljana, Ljubljana, Slovenia, 4Natl. Inst. of Infectious Diseases, Tokyo, Japan

Abstract Body:
Background: N. meningitidis, typically a resident of the oropharynx and the causative agent of meningococcal meningitis and meningococcemia, is capable of invading and colonizing the urogenital tract. This can result in urethritis, akin to its sister species N. gonorrhoeae, the etiologic agent of gonorrhea. Recently, meningococcal strains associated with outbreaks of urethritis were reported to share genetic characteristics with gonococcus, raising the question of the extent to which these strains contain features that promote adaptation to the genitourinary niche, making them “gonococcus-like” and distinguishing them from other N. meningitidis. Methods: A total of 39 urethritis-associated N. meningitidis, representing multiple serogroups and independently collected over a decade and 3 continents, underwent genome sequencing and analysis. The genomes were compared with serogroup-matched N. meningitidis strains isolated from carriage and invasive disease and N. gonorrhoeae strains isolated from men with urethritis. Results: Intact nitrite reductase (AniA), disrupted factor-H binding protein (fHbp), and the lack of capsule are features previously speculated to promote urogenital colonization. However, we found that a considerable number (n=11) of meningococcal urethritis isolates harbor mutations in AniA predicted to result in truncated peptides and a minority (n=7) of these isolates contained alleles associated with frameshifted fHbp. We noted substantial diversity in the capsule biosynthetic locus, including intact, disrupted, and absent capsules, indicating urogenital colonization is possible across a range of capsular phenotypes. In an ST-11 lineage of N. meningitidis previously associated with outbreaks of nongonococcal urethritis, we observed novel signatures of horizontal gene transfer that may have contributed to the relative success of this clade. Conclusions: The meningococcal urethritis strains in this study overall do not share the allelic patterns of AniA, fHbp, or the capsule locus previously reported for urethritis-associated N. meningitidis; however, particular lineages may have undergone further adaptation to the urogenital environment via recombination. Genomic analysis therefore might provide insight into the biological basis for both sporadic and sustained cases of urethritis caused by N. meningitidis.
Session Number: 502
Session Type: Rapid Fire
Session Title: Comparative Genomics of Biomedically Important Bacteria
Session Start Date Time: 6/10/2018 4:30:00 PM
Session End Date Time: 6/10/2018 5:15:00 PM
Session Primary Track: Microbial Ecology and Evolution
Abstract Control Number: 9337
Poster Board Number:

Abstract Title:
Outstanding Abstract Award: Application of A 17th Century Mycobacterium Tuberculosis Genome to Mycobacterial Dating and Phylogeography

Primary Author Block:
S. J. Sabin1, A. Herbig1, Å. J. Vågene1, T. Ahlström2, G. Bozovic3, E. A. Nelson1, C. Arcini4, K. I. Bos1;

Abstract Body:
Ancient pathogen genomics has opened a new avenue for directly studying the temporal and spatial history of certain infectious diseases. Tuberculosis occupies an intense focus of modern medical and microbiological research, but questions persist regarding its origin and earliest association with its human host. Based on the phylogeography of modern Mycobacterium tuberculosis complex (MTBC) genomes, one hypothesis posits that the pathogen followed human Pleistocene migrations out of Africa, yielding an extrapolated date for the most recent common ancestor (MRCA) of ~70,000 years before present. However, the two studies that have incorporated ancient genomes to calibrate the molecular clock have generated much younger MRCA dates of less than 6,000 years. This discrepancy can only be addressed by continued sampling of diverse, high-quality, ancient tuberculosis genomes that provide numerous calibration points to account for potential rate heterogeneity between different MTBC lineages and over time. Using DNA extraction and next-generation library construction methods designed specifically to accommodate ancient and degraded genetic material, we were able to identify DNA belonging to Mycobacterium tuberculosis in a calcified lung nodule from the mummified remains of Bishop Peder Winstrup of Lund (b. 1605 - d. 1679). With a custom-designed in-solution capture method we were able to reconstruct a 141-fold coverage genome of Mycobacterium tuberculosis from a library for which only 0.045% of the metagenomic DNA had been identified as belonging to members of the MTBC. Phylogenetic analysis revealed this sample to be part of the recently defined L4.10/PGG3 sublineage of the Mycobacterium tuberculosis complex. This high-quality, high-coverage 17th century Mycobacterium tuberculosis genome provides an opportunity to apply temporally diverse data to a phylogeographic analysis of the L4.10/PGG3 sublineage, and adds a reliable calibration point for dating the MTBC via Bayesian methods. This ancient genome provides better resolution to the genetic history of this critical pathogen.
Session Number: 502
Session Type: Rapid Fire
Session Title: Comparative Genomics of Biomedically Important Bacteria
Session Start Date Time: 6/10/2018 4:30:00 PM
Session End Date Time: 6/10/2018 5:15:00 PM
Session Primary Track: Microbial Ecology and Evolution
Abstract Control Number: 9574
Poster Board Number:

Abstract Title:
Moderator
Primary Author Block:
Geneviève Garriss; Karolinska Inst.t, Solna, Sweden
Abstract Body: