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CELLULAR AND MOLECULAR
1:00 -3:00 PM
Room TC 214

BIOINFORMATICS SYMPOSIUM:

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February 23, 2017

1:00-3:00 PM

Room TC 214

INBRE Symposium: Metagenomics to Functional Microbiome
Section Symposia: Molecular and Cellular Biology Division
Organizer: Dr. Shahid Karim, Biological Sciences, The University of Southern Mississippi (Shahid.Karim@usm.edu)

Traditionally, microbes have been studied as cultures in the laboratory. However, the vast majority of organism-associated microbial species have never been isolated in the laboratory, presumably because their growth is dependent upon factors or conditions which have not been replicated in the laboratory. Advances in the field of DNA sequencing technologies have opened a new avenue of research, called metagenomics, allowing comprehensive examination of microbial communities without the need for cultivation. Other advanced 'omics' technologies like transcriptomics, proteomics and metabolomics, which measure the biological properties of whole microbial communities, are being used to gain insights into how the microbiome and host organism interact to support health or trigger disease. An emphasis on the approaches used in the field of metagenomics and functional microbiome would serve as an excellent addition to Mississippi Academy of Sciences Annual meeting.

The Mississippi Academy of Sciences annual meeting brings together life and natural sciences from the entire state of Mississippi and Gulf-South region of the United States to connect, brainstorm, and collaborate. The proposed symposium will provide a platform to discuss molecular approaches used in the field of microbiome. Although, the focus of our proposed symposium will be on the "biomedical world", many of the molecular approaches can be applied to a much larger audience of the MAS community. This forum will provide an opportunity to researchers to discuss and cross-cultivate ideas with experts. We expect that the proposed symposium might attract more molecular microbiologists at the annual meeting, as well as expand the current research forum for an increased exchange of ideas across research disciplines within microbiology and computational biology. The symposium will consist of 20 minutes talks with an additional 5 minutes for Q & A session; the full symposium lasting approximately 120 minutes.

CHALLENGES AND ADVANCES IN THE METAGENOMIC AND MICROBIOME CHARACTERIZATION OF BACTERIA IN CLINICAL AND ARTHROPOD VECTOR SAMPLES

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Many bacterial agents are found in human and animal clinical samples as well as in the arthropod vectors which transmit a wide variety of the bacterial pathogens that cause disease in humans and vertebrate hosts. Microbiome characterization exploits deep sequencing of portions of the *16S* rRNA genes of eubacteria. The sequences of bacteria found in each sample are detected by PCR amplification of various *16S* rDNA regions using highly conserved primer sites

in that gene. By using different barcodes to tag the amplicons, numerous samples can be pooled and sequenced together to allow cost efficient use of the power of next generation sequencing platforms. The sequences are then matched to databases of 16S rRNA genes to identify and bin the specific operational taxonomic units (OTUs) that are present in the samples. Although conceptually straightforward, there are significant challenges inherent in both the amplification and subsequent bioinformatic analysis: (1) The number of sequences may not be proportional to the bacterial load in the sample for different OTUs because of varying efficiency in amplification of different targets or varied efficiency in extracting different taxa. (2) Specific bacterial symbionts or pathogens may comprise >95% of the DNA in the sample so the sensitivity of detection of other agents may be subpar because of selective amplification of the dominant agent. (3) Samples may contain other molecules (e. g., mitochondrial rDNA) which amplify to varying degrees with some of the conserved *16S* rDNA primer sites and give background amplicons which render sample amplification inefficient. (4) Different conserved regions of *16S* rDNA may provide greater or lesser separation of specific OTUs and in general, the identification is at the genus level for many agents because of the intrinsic conservation of *16S* rDNA that allows the method to work at all. Several methods have been employed to overcome these difficulties. Improved quantitation of specific agent loads of interest may be obtained by qPCR or digital droplet PCR with agent and species-specific target assays once the community composition is known. Blocking PCR assays have been developed to suppress the amplification of overdominant agents. While many spurious human and animal background sequences can be filtered out using genome sequences for those hosts, for many vector arthropods those sequences are not yet available. An alternative approach is to DNase treat host DNA or preenrich bacteria from samples before nucleic acid extraction of the bacteria. Methods of reducing host rDNA concentrations have also been developed but these are expensive and limited in host species.

Metagenomic characterization of bacteria in target samples is effected by construction of libraries of different size from the DNA present in the sample. The basic limitation for the analysis is the cost of very deep sequencing and the amount of repetitive DNA in the sample which can make de novo bioinformatics assembly very challenging. Although the sequencing costs continue to drop, most of the attention for improving the metagenomic analysis falls in three areas: (1) improved library construction to achieve much longer reads or more readily assembled sequence sets. (2) Selective amplification of targets of interest. We have used this approach to improve arthropod mitochondrial genome and high load vector symbiont genome assemblies. (3) Selective target enrichment of bacteria can be effected by using antibody capture methods or nucleic acid capture baits, either completely synthetic tiled arrays or regions amplified from near bacterial relatives by long range PCR. A combination of these approaches promises to extend the power of metagenomics sequencing to complete genomic characterization of heretofore elusive bacterial agents of interest.

MICROBIAL COMMUNITIES ASSOCIATED WITH THE BIOTRANSFORMATION POTENTIAL OF INSENSITIVE EXPLOSIVES IN SURFACE SOILS

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New explosive formulations that are less sensitive to external stimuli are being incorporated into current munitions. However, very little is known about whether these new insensitive explosives could pose ecological or human risks, especially since many DoD lands are located in critical ecosystems. The objective of this project was to determine if molecular ecology approaches could be used to assess microbial community diversity and function as an early indicator of disturbance in ecosystems and the microbial populations associated with biotransformation. We evaluated the effects and biotransformation potential of the explosives 2,4-dinitroanisole (DNAN), 3-nitro-1,2,4-triazole-5-one (NTO), and the new IMX-104 formulation on soil microbial communities. High throughput sequencing was used to determine changes in community diversity and to infer which phylotypes were correlated with biotransformation of the explosives. Aerobic and anaerobic biotransformation of the explosives was observed in soil microcosms with and without supplemental carbon and nitrogen. With IMX-104, significant hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) degradation did not occur until DNAN and 50% of the NTO had been degraded. Principal Coordinates Analysis (PCoA) of the weighted UniFrac distances showed that the presence or absence of carbon explained 47.33% of the variation in microbial community diversity, indicating that carbon rather than IMX104 had the greatest effect on community diversity. Four families, Sphingomonadaceae, Rhizobiaceae, Comamonadaceae, and Bradyrhizobiaceae, exhibited a statistically significant increase in relative abundance in IMX-104 plus nitrogen supplemented microcosms compared to microcosms without IMX-104. Metagenomic analysis of soil communities is providing valuable information on the types of microorganisms