Next-Generation Metagenomic Sequencing To Explore Environmental Fungal Diversity

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Aims and Description: The diversity of microorganisms on earth remains poorly understood. An estimated 1.5 million species of Fungi comprise a diverse group of organisms that have vital functions as decomposers, symbionts, and pathogens in ecosystems (Hawksworth 2001). However, only about 5% of the estimated number of fungal species have been discovered and described. Unculturable fungi inhabiting rhizosphere, phyllosphere, and other less studied niches are thought to represent a large fraction of the unknown diversity. Therefore, culture-independent technologies are necessary to assess the true biodiversity of fungi and other microorganisms in nature (O'Brien et al. 2005). Sequencing DNA directly from the environment, a technique commonly referred to as metagenomics is an appropriate tool for exploring microbial communities, especially “the uncultured majority”. The goal of the proposal is to use next-generation metagenomic sequencing to study the diversity of microbial communities in the following understudied environments:

1. Fungi in deep-sea hydrothermal ecosystem: Microbial ecology studies have revealed remarkable prokaryotic diversity in hydrothermal vents in the past two decades. However, fungi were unknown in this extreme environment until only a few years ago. The fact that many discovered fungi from hydrothermal vents are siderophore producers indicates that they may play a functional role in seafloor alteration and biomineralization processes (Connell et al. 2009). In my lab, two fungal strains (Penicillium sp. and Cladosporium sp.) have been isolated from hydrothermal vent samples provided by Dr. Costantino Vetriani (Institute of Marine and Coastal Sciences, Rutgers). We postulate that the majority of fungi in the extreme marine environments have not been revealed due to unfavorable laboratory culture conditions. Universal fungal specific primers (ITS1F and ITS2) will be used to amplify the total genomic DNA directly extracted from the sediment samples. The mixed amplicons will be sequenced with the GAIIx and analyzed afterwards.

2. Endophytic fungi in dogwood (Cornaceae) phyllosphere: This will be the first attempt to explore the fungal communities associated with Cornaceae using culture-independent methods. My lab has collected a number of dogwood samples from Europe, Asia and N. America. One or a few samples from each continent will be included in this free run because space is limited. The results will help to discover new species, and to understand plant-fungal coevolution and the factors that shape their distributions.

3. Bacteria and fungi in switchgrass rhizosphere: Switchgrass (Panicum virgatum L.), a warm season C4 perennial grass, is a designated bioenergy crop. Nitrogen management is essential for bioenergy systems because nitrogen is an important cost energetically, economically and ecologically as well. Switchgrass yield response to nitrogen fertilization is variable (personal communication, Dr. Stacy Bonos). It was hypothesized that soil microbes, especially nitrogen-fixing bacteria contribute to the difference. N-fixing bacteria were discovered in sugarcane and miscanthus but not in switchgrass yet. This study will characterize the soil microbial communities of different switchgrass ecotypes in order to test this hypothesis. The knowledge will be useful to increase nitrogen utilization rates. Samples will be provided by Dr. Bonos, who has established a switchgrass breeding program in New Jersey.
Justification and Future Outlook: Recent discoveries on the vast microbial biodiversity with metagenomic pyrosequencing reflect that fact that we lack fundamental knowledge on microbial communities. Next-generation sequencing generates unbiased and quantifiable profiles of mixed samples, which is ideal for biodiversity survey of environmental samples. Next-generation sequencing has enormous potential to further our understanding of microbial biodiversity, the ecological roles of fungi and their geographical distribution. This study will be among the first attempts towards applying next-generation sequencing for high-throughput ecological surveys of fungi, which have the potential to revolutionarily accelerate such ecological studies. In addition, data from these experiments will significantly strengthen proposals to be submitted to NSF, DOE, USDA and other federal funding agents.