DEVELOPMENT OF ANALYTICAL METHODS TO INVESTIGATE ANTAGONISTIC INTERACTIONS BETWEEN BACTERIA AND FUNGI

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Project Goals: Interactions between bacteria and fungi are important determinants of ecosystem function, yet little is known about these interactions or how they operate. This is a critical knowledge gap as these interactions are important in addressing multiple DOE priorities including developing renewable energy sources, understanding the possible effects of Earth system change, and understanding how these interactions may help overcome energy and environmental challenges. Here we outline a range of research questions that we are beginning to address through a new SFA in order to better understand the diversity and function of these bacterial:fungal interactions. Using a combination of bioinformatics-based data mining of existing fungal genome sequencing data and single cell isolation and cultivation techniques, we are beginning to understand the diversity of bacteria that form associations with fungi, and how these associations affect both fungal and bacterial growth.

Abstract

Fungi are cosmopolitan microorganisms with complex genetic make-up and metabolism.[1, 2] Furthermore, this group of microorganisms possesses important roles in ecology, agriculture, forestry and human health. In soil, fungi are one of the most abundant groups of microorganisms and are known to interact with different domains of life, including bacteria. Bacterial:fungal interactions in soils can be positive or negative (synergistic or antagonistic). In the present study, we developed several tools to investigate the potential antagonistic interactions of fungi with bacteria. Fungi have unique responses to stressful conditions, under antagonistic conditions between bacteria and fungi, we hypothesized that there will be certain physiological fungal responses, such as apoptotic-like cell death and production of volatile organic compounds (VOC). We developed methods to determine reactive oxygen species (ROS) and VOC produced by fungi that can trigger apoptotic-like cell death due to antagonistic interactions between bacteria and fungi. Methods to detect two different types of ROS were developed. The ROS investigated were hydrogen peroxide (H_2O_2) and super oxide radical anion ($O_2^{-\cdot}$). In addition to ROS production, fungi are also known to produce VOC. These VOC can represent a significant portion of the fungal metabolome and have been demonstrated to provide information in real-time about metabolic changes that occur in the fungal cell under normal or stressful conditions.[3] We have developed and optimized VOC analysis using solid phase micro-extraction (SPME) coupled to gas chromatography and mass spectrometry (GC/MS). To further investigate the effects of VOC and ROS on fungal survival, we have also developed methods to determine morphological changes associated with apoptosis. Fungi are known to go through early apoptosis and late apoptosis. During early apoptosis there is chromatin condensation. In this study, we used the Hoechst 33258 dye that labels nuclear material, followed by microscopic observation to determine early apoptosis.

Late apoptosis was also investigated by observing hyphal death. For late apoptosis, we used the Evan Blue method, which allowed us to determine plasma integrity of fungal hyphae under normal and stressful conditions. These methods will be essential for the project to gain a better understanding of the antagonistic relationships between bacteria and fungi in soil ecosystems.

References

[1] L. de Aguirre, S.F. Hurst, J.S. Choi, J.H. Shin, H.P. Hinrikson, C.J. Morrison, Rapid Differentiation of Aspergillus Species from Other Medically Important Opportunistic Molds and Yeasts by PCR-Enzyme Immunoassay, Journal of Clinical Microbiology, 42 (2004) 3495-3504.
[2] R.E. McKinney, Environmental pollution control microbiology: a fifty-year perspective, CRC Press, 2004.
[3] L.V. Roze, A. Chanda, M. Laivenieks, R.M. Beaudry, K.A. Artymovich, A.V. Koptina, D.W. Awad, D. Valeeva, A.D. Jones, J.E. Linz, Volatile profiling reveals intracellular metabolic changes in Aspergillus parasiticus: veA regulates branched chain amino acid and ethanol metabolism, BMC Biochemistry, 11 (2010) 33.

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