CULTURING METHANOGENIC ARCHAEA IN CONTROLLED STRESS MICROENVIRONMENTS

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Introduction: Natural environments such geothermal hot springs, soil, tissue and groundwater are heterogeneous at small scales[1,2,3], resulting in a diverse and spatially distributed array of chemical and physical stressors, or microenvironments. Understanding microbial response to environmental stressors at the microscale can provide insights into the rapidity and nature of microbial evolution on Earth and elsewhere. In this study, a specially constructed microfluidic device[2], termed microfluidic gradient chamber (MGC), was fabricated to simulate heterogeneous microenvironments. The MGC was fabricated in 4-inch silicon wafers using standard photolithography methods. Solute transport and microbial growth in the MGC was monitored and characterized by epi-fluorescence microscopy. By varying flow rates across the boundary flow channels, advective or diffusive transport processes controlled the generation of solute concentration gradients, i.e., stressors, across a 1200-well array. A strictly anaerobic methanogenic archaeal species (*Methanosarcina acetivorans*) was successfully grown in the MGC by introducing high salt media along the two boundary channels. *M. acetivorans* produced a large amount of methane gas, which ultimately displaced the liquid media in the 1200-well array, resulting in isolated pockets of microbial growth that were ultimately depleted of nutrients. The results of this study detail procedures for fabrication, characterization of chemical concentration gradients as a function of flow rate, and experimental methods to successfully grow strict anaerobic microorganisms in the MGC. This work forms the basis for future efforts that will focus on exploring the microbial response to stress-promoting concentration gradients.

References: [1] Fouke, B.W. (2011) *Sedimentology*, 58, 170-219 [2] Zhang, Q. et al. (2011) *Science*, 333, 1764-1767 [3] Lin, X (2012) *Appl Environ Microbiol,* 78, 759-757