Temporal and Spatial Organization within a Syntrophic Bacterial-Archaeal Biofilm

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The elucidation of how populations interact in a given community and how the community responds to stress and perturbations can help infer the interplay between stress pathways and gene networks that help optimize bacterial biochemistry. A goal of VIMSS is to determine the molecular determinants that underlie microbial community function and stability. A syntrophic co-culture of the sulfate-reducing bacterium, *Desulfovibrio vulgaris* Hildenborough, and the methanogenic archaeon, *Methanococcus maripaludis*, was selected as a basal community that can directly and indirectly interact as a biofilm. Planktonic growth conditions, in which cells exist as 'non-adhered cells', rarely represent a true state of growth for the majority of microorganisms under *in situ* conditions, and adherent growth is most likely a universal feature. The roles of biofilms have become increasingly more evident in processes from microbial pathogenesis to waste water to metal corrosion; however, relatively little work has been done on anaerobic biofilms, particularly regarding the structure and behavior of non-pathogenic organisms under environmentally relevant conditions. Microbial communities associated with surfaces may incur protection from stresses such as nutrient-limitation, pH, salts, and heavy metals. In addition, proximity and localization within surface-adhered communities may impact functionality in terms of electron- and hydrogen-metabolism. It was hypothesized that hydrogen transfer would dictate co-culture biofilm formation in the absence of sulfate as terminal electron acceptor for *D. vulgaris* and without addition of hydrogen as electron donor for the methanogen. *M. maripaludis* did not form significant biofilms on a glass surface in batch mono-culture experiments, but *D. vulgaris* did. However, *M. maripaludis* did form a pellicle-like structure in batch, static cultures. A biofilm reactor was developed to co-culture *D. vulgaris* and *M. maripaludis* during syntrophic growth, and spatial and temporal organization was characterized using qPCR, epifluorescent microscopy, field emission electron microscopy, methane production and protein and carbohydrate analysis. During early development, the biofilm initiated as a monolayer of *D. vulgaris* cells, and the mainly *D. vulgaris* biofilm contained extracellular filaments that have been previously described. Soon after the development of the D. vulgaris biofilm, *M. maripaludis* cells were observed, and the number of planktonic phase cells declined as the number of biofilm cells increased for both populations. Over time, the methanogenic biofilm stabilized, and the ratio of *D. vulgaris* to *M. maripaludis* cells was approximately 2.5 and this is a similar ratio observed for cultures entirely

populated by planktonic cells. However, at later time points, the planktonic populations had a ratio of approximately 0.2, and this ratio was significantly lower compared to biofilm. Both populations had 1 to 2 log more cells in the biofilm than the planktonic phase. As the methanogenic biofilm developed, extracellular structures continued to be observed. The results suggested that *D. vulgaris* initiated and established a biofilm that then recruited *M. maripaludis*, and the biofilm grew and changed over time as the numbers of both populations increased.