ENIGMA: Cooperation Impacts Structure in a Syntrophic Biofilm of *Methanococcus maripaludis* and *Desulfovibrio vulgaris*

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Project Goal: Elucidate the relationship between structure and function in a methanogenic biofilm that consists of a sulfate-reducing bacterium and methanogenic archaeon. The functions of interest are carbon-compound oxidation and methanogenesis between syntrophic populations. The structure refers to population organization within a self-assembled, biofilm community.

Transfer of reduced carbon and electrons between microbial community members is of interest in anoxic systems, and methanogenesis represents a crucial trophic level that can include sulfate-reducing bacteria and methanogenic archaea. The current work uses a dual-culture approach to examine the structure of a syntrophic biofilm formed by the sulfate-reducing bacterium *Desulfovibrio vulgaris* Hildenborough and the methanogenic archaeon *Methanococcus maripaludis.* Biofilm was grown in a continuously stirred reactor where cells could attach to a silica surface or remain suspended. Under the tested conditions, *D. vulgaris* formed monoculture biofilm, but *M. maripaludis* did not. However, *M. maripaludis* did form pellicles in static, batch cultures while *D. vulgaris* did not form a pellicle. Under syntrophic conditions, a methanogenic biofilm formed and reached steady-state in approximately 7 days based upon protein levels and methane mass flux. Biofilm establishment was dependent upon initial colonization by *D. vulgaris* that was followed by recruitment of *M. maripaludis* into the biofilm matrix. The initial *Desulfovibrio*:*Methanococcus* biofilm ratio was approximately 375:1 but steady-state biofilm reached a ratio of 4:1. Steady-state biofilm was fixed for Fluorescence *in situ* Hybridization (FISH) and confocal laser scanning microscopy (CLSM). FISH revealed a framework of *D. vulgaris* with both single cells and large micro-colonies of *M. maripaludis* interspersed throughout the biofilm. 3D-FISH and CLSM of hydrated intact biofilm confirmed steady-state biofilm irregularity, with ridge, valley and spire macro-architecture. Key structural signatures were observed that confirmed the cooperative nature of the community using a newly developed model. Colorimetric assays indicated cell-associated carbohydrate was composed of .035 µg hexose/µg protein, .017 µg pentose/µg protein and .011 µg uronic acid/µg protein, similar to *D. vulgaris* mono-culture biofilm and approximately 5 times less than *M. maripaludis* pellicles. Filaments presumed to be protein have been observed in dual-culture biofilm matrix with electron and atomic force microscopy, and matrix was sensitive to proteinase K treatment during preliminary work with Catalyzed Reporter Deposition FISH. Syntrophic biofilm 3-D structure appears to be initialized by *D. vulgaris* that provides an advantageous environment for *M.*

maripaludis to establish micro-colonies throughout the *D. vulgaris* scaffold. The coculture biofilm growth mode resulted in a 10-fold higher methane production per *M. maripaludis* biomass than the planktonic only growth mode, and these results indicated that the structure of cooperative interactions between a bacterium and archaeon positively impacted function.

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