Characterization of the *Methanosaeta concilii* Sheath, a Remarkably Resilient Protein Assembly

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Project Goals: Understanding the proteinaceous sheath of *Methanosaeta concili* is critical for understanding how the methanogen interacts with its environment. Given the difficulty of solubilizing the assembly, these efforts will also provide insight into the analysis of such intractable protein subunits.

The sheath of certain methanogens makes up the outermost component of cells. Sheaths assemble into long structures that encapsulate multiple cells in a filamentous segment. The components that compose this structure are critical for two reasons. First, this filamentous layer must regulate entry of critical molecules, acetate specifically, into the cells, as well as the exit of carbon dioxide and methane. There are also mechanisms expected to be present that facilitate interspecies electron transport, a unique capability evidenced in many methanogens, including *Methanosaeta concilii*. The sheath also protects the cells of the methanogens, which can often exist in particularly harsh conditions. Elucidating the components of these structures would provide great insight into their physiology and regulation.

The chemical properties of some methanogen sheaths have been studied, and more recently, the protein identity of the *Methanospirillum hungatei* sheath was discovered. Surprisingly however, no homolog to the *M. hungatei* sheath protein was found in the genome of *M. concilii*, keeping its identity unknown. Further, *M. concilii* sheaths have demonstrated significantly different chemical properties from *M. hungatei* sheaths, and the former appears to be a much more stable assembly. Previous reports have also indicated that the sheaths of these archaea bear significantly more glycan than other identified sheaths. This investigation provides unique challenges and opportunities for analysis of this proteinaceous layer.

We have recently looked to characterize this sheath by exploring protocols to solubilize it and to identify proteins that associate with it. Cells were grown and sheaths isolated in the Gunsalus laboratory. Primarily SDS-PAGE and liquid chromatography coupled with tandem mass spectrometry have been employed in analyses. We have identified a number of protein factors that associate with the sheath and have found conditions to solubilize it, although the sheath protein's identity remains elusive.

By characterizing the sheath of *M. concilii*, we should be able to elucidate the environmental/cellular interface of this organism. This study lays groundwork for understanding the mechanisms by which these archaea comprise extremely durable structures and how they survive and thrive in atypical biological environments.

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