

Identification of S-layer Proteins in the Methanosarcinaceae

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Project Goals: One major goal of this collaborative project addresses the identity, structure and function of archaea envelopes that provide cell protection from environmental challenges. Our model organisms include key methanogenic species involved in anaerobic carbon cycling and methane production. An understanding of their ability to adapt and thrive in changing habitats impacts fundamental processes of microbial biomass transformations, CO₂ sequestration, and energy generation by anaerobic microorganisms.

The cell envelopes of many archaeal species¹ have a proteinaceous surface or lattice termed the surface-layer (S-layer). It is typically composed of only one or two abundant, often post-translationally modified proteins that self-assemble to form a highly organized surface-exposed array. Currently, very little is known about the properties of such surface arrays in any archaean. Surprisingly, over a hundred proteins were annotated to be S-layer or surface associated components in the *Methanosarcina mazei*, *Methanosarcina acetivorans*, and *Methanosarcina barkeri* genomes, reflecting limitations of current bioinformatics predictions^{2,3}. To experimentally address what proteins are present, we devised an *in vivo* biotinylation technique to affinity tag all surface-exposed proteins that overcame challenges in working with these fragile microorganisms. The *Methanosarcina* species were adapted to growth under N₂ fixing conditions to minimize the level of free amines that would interfere with the NHS-label acylation chemistry used⁴. A 3-phase separation procedure was then employed to isolate the intact labeled cells from any lysed-cell derived proteins. The Streptavidin affinity enrichment was followed by stringent wash to remove non-specifically bound proteins, and LC-MS-MS methods were employed to identify the labeled surface proteins. The major surface layer protein was identified in all three species to belong to a small highly conserved group of hypothetical proteins. They were shown to be present in multiple glycosylated forms by using SDS-PAGE coupled with glycoprotein-specific staining, and by interaction with the lectin, Concanavalin A. This family of related S-layer proteins/genes identified in all the sequenced *Methanosarcina* genomes exhibited similar features including a signal P sequence, tandem DUF1608 domains, and a C-terminal hydrophobic transmembrane helix. To address S-layer structure and function, crystallographic studies were performed whereby the *M. acetivorans* S-layer protein DUF1608 domain structure was determined at 2.3 Å. This structure provides a model for S-layer protein assembly onto the cell surface to form a lattice. Finally, several pore types were revealed that would allow for movement of small molecules to the cytoplasmic membrane. In conclusion, these studies reveal a conserved protein signature within the *Methanomicrobia* having distinct protein features and implied architecture that is absent in other archaea.

References

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