An *O*-Glycosylated Archaeal Flagellin

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Project Goals: To elucidate the biological pathways of microbes relevant to microbial biofuel production and to global carbon cycling. These studies employ proteomics and mass spectrometry to characterize protein post-translational modifications.

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Methanospirillum hungatei, an archaeon isolated from sewage sludge, plays an important role in waste treatment and bioenergy by degrading organic wastes to methane. In association with syntrophic organisms, it facilitates chemical conversions that are essential to recycling carbon throughout the environment. Understanding these syntrophic consortia requires not only knowledge of organisms' genetic potentials, but also how the organisms' gene products vary in response to environmental changes (quantitatively and via post-translational modification). Little is known about the modifications borne by proteins within environmental microbes. Glycosylation is particularly important, impacting cell-cell adhesion and recognition, as well as cell stability. We examine flagella from *M. hungatei* and characterize its constituent protein.

In high phosphate (45 mM) medium, *M. hungatei* grows in non-motile, linear chains (up to 9 to 12 cells long) surrounded by a protein sheath within which protein plugs separate individual cells. In low phosphate medium (3-30 mM), however, the organism presents mostly as sheath-enveloped single cells or as short chains made motile by two polar tufts of flagella transecting the multilayered terminal cell plug. High resolution cryo-electron microscopy (cryoEM) structures of flagella sheared from *M. hungatei* JF1 (ATCC# 27890) suggest that the single gene product assembling into this appendage, FlaB3 (Mhun_3140), bears several post-translational modifications.

Enzymatic digestion (trypsin, chymotrypsin, elastase, pronase, and/or pepsin) of flagellar protein and tandem mass spectrometry identified 6 *O*-linked glycosylation sites, each trailing a 508 Da disaccharide, comprising 190 (dimethyl hexose) and 318 Da units (Thr-acetamido-deoxy-hexuronic acid), with the latter sugar linked to peptide. *M. hungatei* presents the first archaeal flagellum found bearing *O*-glycosylation.

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