Detecting Cysteine Modifications in methanogen *Methanosarcina mazei* Gö1

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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.

Archaea in genus *Methanosarcina* are distributed broadly from marine to fresh water environments. They produce methane from a wide range of substrates including acetate, methylamines, and methanol and account for a large percentage of global methane emission. In methanogenesis, several important steps rely on thiol intermediates; ***e.g.***, methyl transfer from tetrahydrosarcinopterin (H4SPT) to coenzyme M (mercaptoethanesulfonate), methane release by oxidation of coenzyme M and coenzyme B to form a heterodimer, and recycling of coenzymes M and B after reduction by heterodisulfide reductase. The importance of thiols to methanogenesis encouraged us to explore cysteine modifications in *Methanosarcina mazei.*

Tryptic peptides were generated with and without reduction/alkylation from cell lysates of *Methanosarcina* cultivated on methanol and on other carbon substrates. Peptides were analyzed by LC-MS/MS to identify proteins and to inventory post-translational modifications. Among the most abundant modifications observed was cysteinylation (Cys+119), identified on over 40 of proteins. Protein cysteinylation was observed not only from cultures maintaining reducing conditions with Na_2S/cysteine addition, but also from those supplementing with Na_2S only. Other modifications detected included Cys+30 (trisulfide in multi-cysteine peptides), Cys+140, Cys+151, and Cys+152. Modified cysteines appeared in active sites of some metabolic enzymes. The significance of these modifications is being explored.

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