DISCOVERY OF TETRAHYMANOL PRODUCTION IN AN AEROBIC METHANOTROPH REVEALS A NOVEL PROTEIN REQUIRED FOR BACTERIAL TETRAHYMANOL BIOSYNTHESIS.

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Gammacerane is a pentacyclic isoprenoid lipid preserved in ancient sediments that is utilized as a biomarker for eukaryotic ciliates and an indicator for water column stratification. Tetrahymanol is recognized as the biological precursor to gammacerane and was first discovered in the ciliated protozoan *Tetrahymena pyriformis*. Subsequent studies have revealed tetrahymanol production in a variety of eukaryotes, including freshwater and marine ciliates, an anaerobic free-living protists and an anaerobic rumen fungus. However, tetrahymanol biosynthesis is not limited to eukaryotes. Two α-proteobacteria, *Rhodopseudomonas* and *Bradyrhizobium*, have been shown to produce tetrahymanol and little is known about the biosynthesis and physiological function of this lipid in these bacterial species. Further, it is unclear how widespread tetrahymanol biosynthesis is in the bacterial domain and what implications the discovery of tetrahymanol production in other bacteria would have for the interpretation of gammacerane signatures in the rock record.

In this study, we demonstrate the production of tetrahymanol by a third bacterial species, *Methylomicrobium alcaliphilum* 20Z, an alkaliphlic halotolerant aerobic methanotroph originally isolated from a soda lake in Siberia. Utilizing comparative genomics and gene deletion analysis, we identify a protein of unknown function that is required for the production of tetrahymanol. Phylogenetic analysis revealed that every sequenced *Rhodopseudomonas* and *Bradyrhizobium* genome contains a copy of this putative tetrahymanol synthase in agreement with the occurrence of tetrahymanol in these species. In addition, this protein is found in the genomes of previously unknown tetrahymanol producers including other alphaproteobacteria, *Methylomicrobium* species, and *Desulfovibrio* species. The tetrhaymnaol synthase is not present in tetrahymanol producing eukaryotes indicating that bacteria and eukaryotes have evolved distinct biochemical mechanisms for producing the same lipid molecule. We have begun studies to determine the biochemical mechanism of the tetrahymanol synthase and hypothesize that it catalyzes the conversion of the hopanoid diploptene to tetrahymanol through a ring expansion. In addition, we are characterizing an *M. alcaliphilum* tetrahymanol synthase mutant for any growth phenotypes associated with the loss of tetrahymanol biosynthesis. Through these studies we will gain a better understanding of tetrahymanol biosynthesis and physiology in the bacterial domain which would lead to a better interpretation of gammacerane signatures in the rock record.