

**ASSESSING THE ROLE OF STEROL METHYLTRANSFERASE HOMOLOGUES  
IN THE METHYLHOPANOID SYNTHESIS PATHWAY OF  
*METHYLOBACTERIUM EXTORQUENS* AM-1**

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Hopanoids are polycyclic triterpenoids that are abundant in the geological record and common in some bacteria. Among the classes of hopanoids, methylhopanoids have been of particular utility to organic geochemists. In this study we test the hypothesis that the gene responsible for hopanoid A-ring methylation is a bacterial homologue of eukaryotic S-adenosyl-L-methionine (SAM)-dependent sterol methyltransferase.

Several cyanobacteria and proteobacteria produce hopanoids methylated at the C-2 position (Summons *et al.*, 1999). In ancient sediments, the detection of 2 $\alpha$ -methylhomohopanes (derived from 2 $\beta$ -methyl-bacteriohopanepolyols) in high amounts relative to desmethylhopanes is usually attributed to ancient cyanobacterial productivity. These compounds have been detected in rocks 2.7 billion years old; this has been interpreted as the earliest evidence for the evolution of oxygenic photosynthesis (Brocks *et al.*, 1999). Similarly, 3 $\beta$ -methylhopanoids are produced by aerobic type I methanotrophs such as *Methylococcus* (Summons & Jahnke, 1992). The detection of their hydrocarbon derivatives in ancient rocks is usually interpreted as a proxy for aerobic methane cycling and used to infer paleoenvironmental conditions (e.g. Brocks *et al.*, 2005).

Despite the importance of these molecules to organic geochemists, very little is known regarding either the genetic basis for hopanoid methylation or the physiological function of hopanoids. Identification of the gene or genes responsible for hopanoid methylation would provide a basis with which to rapidly examine the potential distribution of methylhopanoids in microbes (via screening of genomic databases). Comparative sequence analysis of these genes may shed light on the evolution of this ancient pathway. Understanding the physiological role of these molecules would be a boon to investigations that make paleoenvironmental interpretations upon their detection.

The methyl group on the hopanoid A-ring is known to be derived from L-methionine, likely via SAM (Zundel & Rohmer, 1985). SAM is a methyl donor for a variety of methyltransferase enzymes, including the sterol methyltransferases (SMTs) that operate on the sterol side chain in some eukaryotes. Examination of genomic databases revealed the

presence of SMT homologues in the genomes of several methylhopanoid-synthesizing bacteria that lack sterols, including the cyanobacteria *Synechococcus* and *Nostoc*, the methanotroph *Methylococcus*, and the methylotroph *Methylobacterium*, among others. The translated amino acid sequences for these genes contain a highly conserved region that corresponds to the SAM-binding domain in SMT, but lack the sterol-binding region that is unique to SMT. Due to i) the presence of these genes in a wide variety of methylhopanoid synthesizing organisms, ii) the fact that these genes are homologous to SMT, which operates on triterpenoids, and iii) the likelihood that these genes are SAM-dependent, it has been hypothesized that these genes encode enzymes operating to methylate hopanoid A-rings (Summons *et al.*, 2006).

The  $\alpha$ -proteobacterium *Methylobacterium extorquens* AM1 is a genetically tractable methylhopanoid producer in which we are conducting experiments to test this hypothesis. When grown on succinate, *Methylobacterium* produces C<sub>30</sub>-methylhopanols. Its genome contains a SMT homologue with 35% identity to SMT1 of *Arabidopsis* and a conserved SAM-binding region. We are creating several mutant strains of *Methylobacterium*, each of which lacks a gene we suspect may be involved in hopanoid synthesis (such as squalene-hopene cyclase) or methylation. Examination of the hopanoid content of the mutant strain lacking the SMT homologue will test our hypothesis that this gene participates in A-ring methylation. Physiological characteristics of this and other mutant strains will be compared to wild-type *Methylobacterium*, in an attempt to gain insight into the physiological function of hopanoids and methylhopanoids.

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