Goldschmidt 2026 Abstract
https://doi.org/10.46427/gold2026.36628

Geologic and Molecular Controls on 3-Methylhopanoid Production and Application as Proxies for Bacterial Methanotrophy

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Bacterial hopanoid lipids are widely present in the geologic record and act as important biomarkers for reconstructing bacterial metabolisms and tracing the Earth's biogeochemical history. 3-Methylhopanes are the diagenetic products of 3-methylhopanoids, characterized by methylation at the C-3 position. They are historically used as indicators of bacterial aerobic methanotrophy across the geologic record[1-3]. However, several uncertainties confound their use as proxies for reconstructing methanotrophy: (1) the abundance and distribution of 3-methylhopanes through geologic time are poorly constrained, (2) the environmental factors controlling 3-methylhopanoid production are not well established, and (3) it is unclear what physiological role(s) these compounds play in the cell. To address these uncertainties, we use *Methylococcus capsulatus*, a well-studied obligate methanotroph and known 3-methylhopanoid producer[1], as a model organism, combining genetic approaches, continuous culture (i.e., chemostat) experiments, and lipid analyses of modern and ancient samples to constrain environmental controls on 3-methylhopanoid production, their physiological function, and their application as proxies for methanotrophy.

We extracted and analyzed lipid biomarker assemblages from over 700 well-preserved marine sedimentary rocks spanning more than 25 Phanerozoic localities. All samples have escaped significant thermal alteration, as independently constrained from Rock-Eval pyrolysis. Rock extracts were analyzed by Metastable Reaction Monitoring-Gas Chromatography-Mass Spectrometry (MRM-GC-MS) to build a comprehensive database of Phanerozoic hopane biomarker distributions. Using chemostat experiments with *M. capsulatus* wild type and mutant strains, we examine how CH_4/O_2 ratios and nutrients regulate 3-methylhopanoid synthesis in the cell.

Our biomarker database reveals anomalously elevated 3-methylhopane abundances in early Phanerozoic marine shelf environments, an order of magnitude above typical Phanerozoic baseline values, suggesting enhanced methane cycling during this time interval. Chemostat experiments further constrain the environmental controls on 3-methylhopanoid production and their relationship to secular trends observed in the geologic record. Together, these results establish a robust molecular and geologic framework for interpreting 3-methylhopanoids in modern and ancient environments, with broad implications for reconstructing carbon cycling and microbial ecology throughout the Phanerozoic.

References

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