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Archaeal Lipid Hydrogen Isotope Signatures of the Metabolically Flexible *Archaeoglobus fulgidus* During Autotrophy and Heterotrophy

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The hydrogen isotope compositions (δ^2H) in lipid biomarkers track metabolic processes and can remain stable over geologically relevant time scales, with hydrogen isotope exchange rates ranging from 10^4 to 10^8 years [1]. The lipid δ^2H in photosynthesizing eukaryotes (e.g., leaf wax δ^2H) has therefore been widely used for paleoclimate reconstructions [2]. Microbial lipid δ^2H may reveal additional information about geobiological processes, as microbes live in a broad range of habitats and have wider metabolic capabilities. While bacterial lipid δ^2H has been extensively studied and shown to be quantitatively related to central metabolism [3], archaeal lipid δ^2H remains largely understudied. In this study, we conducted experiments with pure cultures of an anaerobic, hyperthermophilic, and sulfate-reducing archaeon, *Archaeoglobus fulgidus*. This strain was grown on different substrates to test the effects of carbon metabolism (autotrophy on CO_2 *vs*. heterotrophy on lactate) and redox potentials (lactate *vs*. H_2 as electron donors) on their lipid δ^2H. All experiments were conducted with three different δ^2H values of growth medium to apportion the relative contributions of H from distinct sources—protons in water, hydride carrier molecules, and organic substrates—to the final lipid products. Preliminary δ^2H data from these experiments indicate that more lipid-bound hydrogens derive from water during autotrophy compared to heterotrophy. In light of the experimental results, we discuss how this approach can include other metabolisms to enable a generalized framework for interpreting archaeal lipid δ^2H. The empirical framework can be validated by analyzing the δ^2H of relevant environmental samples, allowing us to assess the potential application of the H isotope composition of archaeal lipids as paleoenvironmental proxies.

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