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Temperature effects on the stable carbon isotopic fractionation of bacteria using different CO_2 fixation pathways

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Biological processes typically preferentially incorporate the lighter isotope into the biomass than the heavier one. In example, the isotopic fractionation of stable carbon isotopes (^{12}C versus ^{13}C) during CO_2 fixation can provide important insights into carbon cycling in natural environments. The most widely distributed CO_2 fixation mechanism – the Calvin-Benson-Bassham (CBB) cycle – displays a high stable carbon isotopic fractionation and results in the production of biomolecules depleted in ^{13}C relative to the inorganic carbon source. In studies of past as well as modern environments this effect has been used to distinguish between biotic and abiotic processes. However, some microorganisms use CO_2 fixation pathways that result in very low isotopic fractionations, such as the reverse tricarboxylic acid cycle (TCA) or the 3-hydroxypropionate pathway. Both of these processes lead to biomolecules (e.g., fatty acids) that have very similar stable carbon isotopic compositions ($\delta^{13}C$) as the educt inorganic carbon.

At hydrothermal vents, where *in situ* microbial production is an important source of organic matter for higher trophic levels, it is of great interest to understand the mechanisms of carbon cycling by the vent-associated microorganisms. Recent studies[1,2] propose different contributions of CBB and reverse TCA mediated CO_2 fixation in hydrothermal systems, where microorganisms using the CBB and reverse TCA cycle co-exist. Although vent communities have been shown to be comparable in composition at different sites, vents are very dynamic systems where microorganisms have to readily adapt to fluctuations in geochemical gradients and temperatures. Since temperature effects on $^{13}C/^{12}C$ fractionation during synthesis of biomolecules are not yet fully understood this study investigates the influence of temperature on stable carbon isotopic fractionations via the CBB and reverse TCA cycle. The chosen model organisms were *Sulfurimonas denitrificans* and *Thiobacillus denitrificans* that use the reverse TCA cycle and CBB for carbon fixation, respectively. Both strains are mesophilic and grow optimally at respective 22˚C and 30˚C, with a temperature range of 4 to 37˚C. Our results show that at optimal growth temperatures both organisms display isotopic fractionations that are expected from their respective CO_2 fixation pathways: Biomass and fatty acids of *S. denitrificans* have $\delta^{13}C$ values around -5 to 0‰ that are very close to the dissolved inorganic pool (DIC, 1.4‰), while those of *T. denitirificans* are 15 to 25‰ lighter than DIC. However, at elevated temperatures (≥33˚C) fatty acids of both strains become very ^{13}C-depleted with values around -30‰Ḟatty acids of *T. denitrificans* reach similarly light $\delta^{13}C$ values of -31‰ when grown at temperatures ≤ 14˚C, while *S. denitrificans* fatty acids remain ^{13}C-enriched with values around 0‰Ṫhese results demonstrate that the CO_2-fixation pathway does not strictly dictate isotopic fractionation factors, but that also temperature seems to play an important role. Notably, the highest fractionations concurred with lowest cell densities, potentially suggesting that not only temperature but also microbial fitness might play an important factor in the observed changes in isotopic fractionations among the investigated strains.

(1) Olins et al., 2013 Geobiology, 11, 279-293.

(2) Reeves et al., 2014 Environmental Microbiology, 16, 3515-3532.