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δ13C compositions of bacteriohopanetrol isomers reveal bacterial processes involved in the carbon cycle

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Bacteria play key roles in the carbon cycle. In many sediments and peatlands, methanotrophic bacteria consume a portion of released methane, reducing the emissions of this potent greenhouse gas. In marine oxygen minimum zones (OMZ) and other anoxic settings, anaerobic ammonium oxidizing (anammox) bacteria remove bioavailable nitrogen while performing chemoautotrophic carbon fixation. Methanotrophic and anammox bacteria synthesize a wide number of complex bacteriohopanepolyols (BHPs), comprising notably several stereoisomers of bacteriohopanetetrols (BHT), which are used as biomarker lipids. While BHT-17 β (H), 21 β (H), 22*R*, 32*R*, 34*S* (BHT-34*S*) is ubiquitous in the environment, its 34*R* stereoisomer (BHT-17 β (H), 21 β (H), 22*R*, 32*R*, 33*R*, 34*R*; BHT-34*R*) has only five known producers: the freshwater anammox genera 'Candidatus Brocadia', the aerobic acidic peatland methanotroph *Methylocella palustris*, the nitrogen-fixing aerobic bacteria *Frankia* spp., and the aerobic acetic acid-producing bacteria *Acetobacter pasteurianus* and *Komagataeibacter xylinus*. BHT-*x*—another BHT isomer of unknown stereochemistry—has only one known producer, the marine anammox bacteria 'Candidatus Scalindua' (Schwartz-Narbonne et al., 2020). The occurrence and extent of these

different carbon cycle processes can be assessed by measuring the concentrations of these BHT stereoisomers and changes in their δ^{13} C values (Hemingway et al., 2018; Lengger et al., 2019). However, the ¹³C fractionation associated with the different carbon assimilation pathways of these bacteria has been minimally assessed, resulting in poorly constrained ranges in δ^{13} C values and difficulty in interpreting isotope results.

We used a gas chromatography-combustion-isotope ratio mass spectrometry (GC-C-IRMS) method to measure the δ^{13} C of BHT-34*S*, BHT34*R*, and BHT-*x* of cultured bacteria ('*Ca*. Scalindua', '*Ca*. Brocadia', *Methylocella tundrae*, *Frankia* spp., and *Komagataeibacter xylinus*). These δ^{13} C values were combined with bulk isotopic measurements of the bacterial biomass and δ^{13} C analyses of the bacterial growth substrates to establish carbon isotopic fractionation from substrate to biomass to BHT lipid. We demonstrated that bacteria using different metabolic pathways produced distinct fractionation factors between substrate and BHTs, which potentially allows for distinguishing BHT-34*R* produced by '*Ca*. Brocadia' and methanotrophs from other freshwater producers (e.g. in peatlands). Measurement of BHT-specific fractionation factors allowed us to better constrain the contribution of anammox bacteria to fixed carbon in OMZ. This work expands the application of BHT isomers to isotopically identify carbon cycle processes.

References

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