BACTERIOHOPANEPOLYOL ANALYSIS BY GAS CHROMATOGRAPHY-TRIPLE QUADRUPOLE MASS SPECTROMETRY FOR ANAMMOX BIOMARKER DETECTION

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Introduction

Bacteriohopanepolyols, or BHPs, are bacterial membrane lipids with promising and diverse biomarker applications. In particular, a rare stereoisomer of the ubiquitous bacteriohopanetetrol (BHT) is of interest, as the anaerobic ammonium oxidation (anammox) bacterium genus ‘\textit{Candidatus Scalindua}’ is its only known producer in marine systems \cite{1}. Analysis of “BHT isomer” could present a valuable approach to investigate marine anoxia and its relationship to the nitrogen cycle \cite{1}. This is of particular interest as anthropogenic inputs are causing expansions and fluctuations of modern marine oxygen minimum zones \cite{2}. As BHPs can be preserved in sediments for at least ~55 Ma \cite{3}, BHT isomer has potential as a marker of marine anoxia and nitrogen cycling during paleo-anoxic events. Further, measuring the full suite of BHPs alongside BHT isomer may allow us to interpret additional environmental signals, as BHPs can indicate stress conditions, and serve as biomarkers for other bacterial communities \cite{4}. This understanding of past nitrogen cycle responses to marine anoxia will be valuable to predict future change.

However, the high molecular weight and high polarity of BHPs makes their analysis by gas chromatography (GC) difficult, thus necessitating chemical degradation procedures, which result in loss of functional group information. This is problematic for measurements of BHT isomer, as the stereochemical differences to BHT are found on the side-chain which is removed during the cleavage step \cite{5}. Developments have been made in GC measurements of intact BHPs to measure some commonly occurring BHPs, like bacteriohopanetetrol, aminotetrol, and anhydroBHT, and the GC-FID quantification was found to be more accurate than LC-MS in the absence of authentic standards \cite{6}. Here, we significantly expand the applicability of GC for BHP analysis to include a suite of BHPs previously undetected using GC methods. We then apply this method to the analysis of complex environmental matrices: marine sediments, and peats, using novel SPE preparatory procedures for environmental BHP analysis. GC-MS methodology was evaluated by analysing biomass with known BHP contents (e.g. \cite{7}). Using one set of chromatographic conditions, we were able to achieve baseline separation of BHT and BHT isomer; another set of conditions was required for analysis of aminoBHPs. To enable detection of BHPs in complex environmental samples, triple quadrupole GC-MS-MS selected reaction monitoring (SRM) transitions were optimized for a wide range of BHPs. Modern peat and marine samples containing BHT isomer have been analysed using this method. The next step of this work will employ this high-specificity method to investigate the role of anammox and other bacteria in widespread paleo-anoxic events such as the PETM, allowing a better understanding of the paleo-nitrogen cycle.
References


