

**BACTERIOHOPANEPOLYOLS IN HOLOCENE SEDIMENTS OF ACE LAKE
(ANTARCTICA) AND THEIR POSSIBLE BIOLOGICAL SOURCES AS REVEALED
BY 16S rDNA STRATIGRAPHY**

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Anoxic Holocene and Pleistocene sediment record provide an archive of preserved labile biomolecules such as nucleic acids (Coolen et al., 2004) and most likely also for intact BHPs. For example, post-glacial Ace Lake (Vestfold Hills, Antarctica) developed from a melt-water filled freshwater lake (>9400 years BP; Unit III sediments) to a stratified fjord with sulfidic bottom waters (starting 9400 yr BP; Unit II sediments) and the present-day isolated saline lake with still sulfidic, but sulfate-depleted and methane-saturated bottom waters (~final 3000 years; Unit I sediments). Previous studies have shown that methanogens and Type I methanotrophic bacteria thrive respectively in the sulfate-depleted and suboxic waters of the lake and their fossil biomarkers were found only in the Unit I sediments.

The excellent preservation conditions and Holocene changes in microbial communities linked to environmental changes made Ace Lake an excellent setting to search for biomarkers such as bacteriohopanepolyols (BHPs) and to identify their possible biological precursors for the first time using both stable isotopic fingerprinting of periodic acid-cleaved BHPs and preserved ribosomal RNA gene (rDNA) sequences, the latter being species-specific phylogenetic markers.

The hopanoids including BHPs are a group of pentacyclic triterpenoids which are biosynthesised by many prokaryotes and occur in a wide range of environments (e.g. Talbot *et al.*, 2003) and are known to be the most abundant natural product on earth.

A wide range of structural variation is observed in the BHP side chain with many structures differing in terms of the number, position and nature of the functional groups. Rapid degradation of intact BHPs or incorporation in organic matter is known to occur but even if preservation conditions are less optimal, information regarding the bacterial source can in certain cases be accessed via characteristic ¹³C isotope signatures of individual hopanoid components and/or the preservation of some structural details. Therefore, (intact) BHPs serve as markers to describe past microbial populations and processes at a relatively high resolution.

Structural identification of the intact BHPs found in Ace Lake provides strong evidence for the occurrence of aerobic methane oxidation mediated by both Type I and possibly Type II methanotrophs as well during the freshwater lake phase. The compounds observed in the Unit I, sediments although usually considered to be indicative of Type II organisms, in this case seem likely to have been produced by a Type I organism producing a lipid signature similar to that of the Louisiana Slope mussel Type I methanotrophic endosymbiont (Jahnke et al., 1995). The methanotrophic origin could be further confirmed by compound specific isotope ratio analysis of the terminal alcohol products produced during periodic acid cleavage as lipids produced by methane oxidizing bacteria usually have a highly depleted isotopic signature. The presence of Type I methanotrophs related to mussel endosymbiotic Type I methanotrophs in Unit I was confirmed by 16S rDNA based phylogenetic analysis of Type I 16S rDNA sequences and those sequences were not recovered from Unit II and III sediments. Sequences with up to 95% sequence similarity to Type II methanotrophic species of the genera *Methylosinus* and *Methylocystis* were found in both Unit I and III and were below the detection limit in Unit II.

The only component present, which is considered to be directly indicative of cyanobacteria, is 2-methyl bacteriohopanetetrol (Summons *et al.*, 1999). This component is present in all of the unit III samples but is absent from all of the other samples. 16S rDNA stratigraphy revealed that its most likely source was a *Synechococcus* related to species of this group found in modern freshwater environments. A succession towards to the salt requiring *Synechococcus* str. Ace occurred during the marine incursion and this is still the only described pelagic cyanobacterium in Ace Lake today and is not a likely source of 2-methyl BHT.

Our results showed that a both DNA and intact BHPs of bacteria which thrived in the suboxic zone survived degradation in the Holocene Ace Lake sediment record and phylogenetic analysis of fossil 16S rDNA sequences verified and further refined possible biological sources of the intact BHPs.

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