



Chemical traces of bacteria in ancient rocks can tell us something about how oil formed, and can even provide clues about past environments.

Sampling sediment and water from Priest Pot. Inset: Under the microscope - bacteria from the bottom waters of Priest Pot. Only a few can be identified - the groups of cells like egg boxes are *Thiopodia*, the large curved filament is *Chloronema* and the clusters of small dark rod shaped cells are *Chlorobium*. We are using DNA and lipid analyses to characterise them.

Bacteria live virtually everywhere on and below the surface of our planet, from high in the atmosphere to deep within sediments and sedimentary rocks beneath the oceans. They comprise the most diverse and abundant forms of life on Earth, and probably have done so for nearly all of geological time.

Evidence for bacteria in very ancient sediments comes from microscopic fossils over 3.5 billion years old. Such fossilised bacteria are rarely found, but a group of organic molecules (called hopanes) from bacteria occur in all rock strata, including rocks as old as 1,800 million years. These distinctive 'chemical fossils' come from complex chemicals found in the fatty membrane that surrounds bacterial cells, and provide evidence of bacterial activity on the ancient Earth.

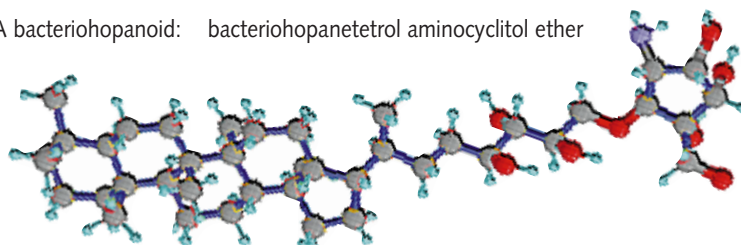
Hopanes have a very practical importance to the petroleum industry. Their composition varies between rock samples and the oils derived from those

rocks, probably because different bacteria lived in the different environmental conditions when the rocks were deposited. This means that hopane 'fingerprints' can distinguish between different oils, and tell geologists something of how those oils and their source rocks were formed – information of great importance in oil exploration.

However, in order to unlock more information from ancient hopanes we need to know about their parent molecules (bacteriohopanoids) in living bacteria, and how these molecules are converted to the geologically-stable

hopanes. In order to find out more, we studied bacteria in a small freshwater lake in the English Lake District – Priest Pot. We discovered that the composition of these bacteriohopanoids varies from one sediment layer to another, probably in response to changing populations of bacteria in the lake. It therefore looks as if the origin of hopanes in the geological record is more complicated than previously thought. This complexity may actually provide more valuable information about the nature of ancient environments and the types of bacteria which lived there.

A bacteriohopanoid: bacteriohopanetetrol aminocyclitol ether



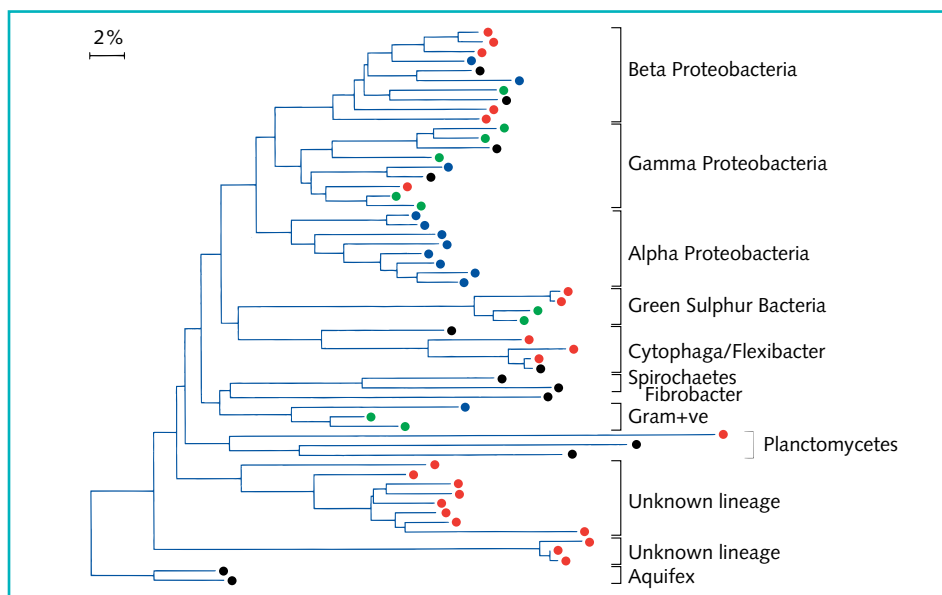
Chemical fossils of ancient bacteria: the science in detail

Hopanoids, a group of organic molecules derived from bacteria, are ubiquitous in ancient sedimentary rocks, and must preserve valuable information relating to ancient bacteria and the environments in which they lived. However, to use these 'chemical fossils' more effectively, we need a greater understanding of their sources and the various transformations which hopanoids undergo in modern sediments to form geologically stable products.

This project has combined novel chemical analyses to study the transformation of biohopanoids to geohopanoids with state-of-the-art molecular biological analyses to characterise the bacterial populations that are the source of the biohopanoids.

The hopanoids found in bacteria have several different chemical groups in their side-chain and are not amenable to conventional organic geochemical analysis. We used a method that cleaves their functionalised side-chain before analysis by gas chromatography-mass spectrometry. We found that although bacteriohopanetetrol (widely regarded as the major precursor of geohopanoids) was abundant in the lake sediments, it was not the predominant biohopanoid. More complex biohopanoids, that can be measured only indirectly by this analysis, were much more abundant.

The composition of the hopanoids also varied with sediment depth. One explanation for this is that changes in the bacterial populations have led to changes in the hopanoids delivered to the sediment from the water column. This offers the possibility that environmental changes which influence the bacterial populations in the water column may be recorded in sedimentary hopanoid composition – a signal which may be preserved (albeit after diagenetic overprints) in the geological record. However, this link would be confounded if hopanoids are also bio-synthesised in the sediment under anoxic



Phylogenetic tree derived from 16S ribosomal RNA sequences from the dominant bacteria present in Priest Pot and cultured bacteria known to produce hopanoids. The dominant bacterial populations in Priest Pot sediments are not closely related to known hopanoid producing bacteria.

- bacteria known to produce hopanoids
- bacteria known not to produce hopanoids
- bacteria from Priest Pot sediments
- cultured bacteria whose hopanoid content is unknown

conditions. Complementary studies characterising the bacterial populations present in the sediments have therefore been done.

Methods

Traditional population assays typically characterise less than 0.1% of the bacterial population present. We therefore adopted molecular biological techniques based on sequence analysis of bacterial nucleic acids. These were extracted from the same sediment samples used for hopanoid analyses, in order to characterise the dominant bacterial populations present.

We found that microbial taxa related to known hopanoid-producing strains were not dominant in the sediments. Interestingly, many rRNA sequences not corresponding to known bacterial taxa were found in Priest Pot sediments.

Future work

We are continuing to determine the bacterial populations and hopanoid composition both in the water column and in additional sediment horizons. These analyses will further test our hypothesis that the hopanoids reflect bacterial inputs from the lake water column, and are sensitive to changes in environmental conditions. The results should comprise a major step forward in the confident application of hopanoids as biomarkers of bacterial inputs to ancient environments.

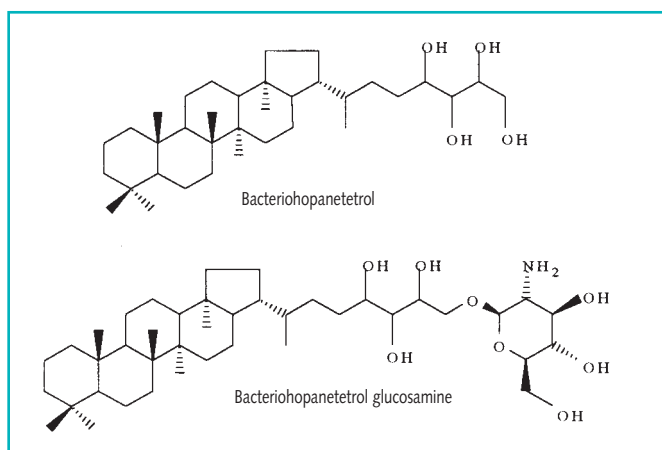
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Chemical structure of two biohopanoids. Bacteriohopanetetrol glucosamine is one of the more complex biohopanoids, with a composite group attached to the end of the side chain. It is this and other 'composite' hopanoids that constitute the bulk of biohopanoids in a small freshwater lake (Priest Pot).