ANCIENT BIOMOLECULES INITIATIVE

Molecular Signatures from the Past

Ancient life in salt mines?

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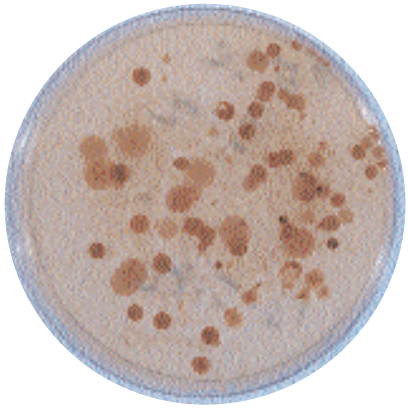
Recent evidence of fossil bacteria in a Martian meteorite has raised the possibility that Mars once harboured life, and maybe still does. We have been trying to detect pockets of ancient bacterial life on Earth which may have survived for hundreds of millions of years in a dormant state.

The red salt crust on the surface of Lake Magadi, Kenya (and other salt lakes around the world) is coloured by salt-loving microbes called halobacteria.

Below: Colonies of halobacteria grown from salt mine sample (230 million years old) in a special salty growth medium.

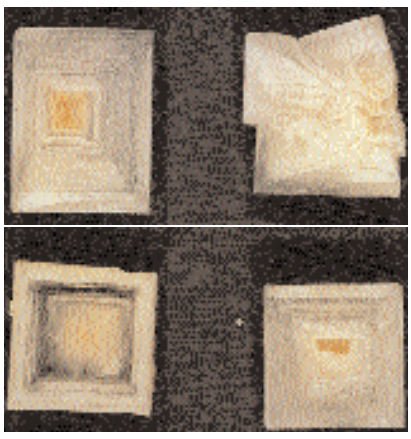


When life first appeared on Earth it faced harsh conditions - probably high temperature, extremes of acidity or alkalinity, high salinity and no oxygen. Similar conditions still occur on Earth, and these hostile environments are populated by bacteria called 'extremophiles' (because their environments are considered to be extreme by us!) Extremophiles provide clues about how life might have established itself on this planet or elsewhere and how it might survive.

Our search for ancient bacteria has concentrated on one kind of extreme environment: salt. Microbes called halobacteria live in salt lakes around the world. We already know that these bacteria can become trapped inside the salt crystals where they survive for many years. Salt mines contain the remains of ancient salt lakes (there are two in Britain over 200 million years old). Could the ancient inhabitants of the original lake still be trapped in a dormant state in rock salt?

We have occasionally grown halobacteria from within ancient salt crystals, but have yet to prove that these are ancient bacteria, not modern contaminants. We are also developing methods to find traces of halobacterial DNA in ancient rock salt, taking care to avoid contamination by present-day DNA.

It is too soon to say whether ancient bacteria still survive in rock salt. Revived micro-organisms or ancient DNA could answer key questions about the rate of evolutionary change. Our experience with extremophiles in earthly environments (like salt mines) will surely influence the search to detect life on Mars or elsewhere.



Salt crystals grown in the laboratory can trap red-pigmented halobacteria.

Pieces of ancient salt (230 million years old).

The Ancient Biomolecules Initiative is a five-year programme to understand the fate of biological molecules in archaeological and fossil materials, and to explore the applications of this new knowledge.
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Natural Environment Research Council

Ancient life in salt mines: the science in detail

Accounts stretching back nearly a century suggest that micro-organisms, usually bacteria, may survive in environments of ancient origins, uncontaminated by present-day activities. For the past decade, research in our laboratory has centred on isolating viable halobacteria from ancient salt deposits (20-260 million years old). Our hypothesis is that these organisms are themselves ancient, having survived for millions of years as relict populations from the days when the sites were salt lakes. We know that halobacteria become trapped inside brine fluid inclusions within halite crystals. Indeed, ancient halite often contains fluid inclusions that have captured material that might have a microbial origin.

We would expect a microbe revived from dormancy to be more primitive at the molecular level than its modern descendants. We therefore analysed and compared the sequence of particular genes of salt mine bacteria with modern salt lake bacteria. Members of one group of halobacteria (the haloarculas) unusually possess two or more dissimilar genes that are involved in protein synthesis (16S rRNA genes). If the gene multiplicity is due to duplication in the distant past, then fewer differences would be expected between a haloarcula revived from dormancy after 200 million years compared to an isolate that has had the time to mutate and evolve. If the duplication is due to gene transfer from another organism, then a revived haloarcula would possess greater differences between the genes than those of a contemporary surface isolate where crossover mechanisms would reduce differences between gene sets with time.

Our results to date, comparing 60 genes from 20 haloarculas, show no clear pattern of gene difference related to geological age.

Number of base changes between different 16s rRNA genes of haloarculas.

Isolate	Number of genes	Base differences between genes A/B	B/C	A/C	Age of site where isolated
Ha marismortui	2 (A, B)	52			Present day salt lake
Ha sinaiiensis	2 (A, B)	26			Present day salt lake
T 3.2	3 (A, B, C)	52	53	7	Halite deposit 65 million years
T 208.9	2 (A, B)	51			Halite deposit 65 million years
WB4 E.4	2 (A, B)	53			Halite deposit 240 million years
CFN ES	3 (A, B, C)	28	31	2	Halite deposit 240 million years
Br 6	3 (A, B, C)	56	59	4	Halite deposit 240 million years

The table shows that both surface (present day) and salt mine isolates (ancient ?) have similar differences between genes. However, detailed sequence analysis might still indicate statistically significant differences between subterranean and surface types.

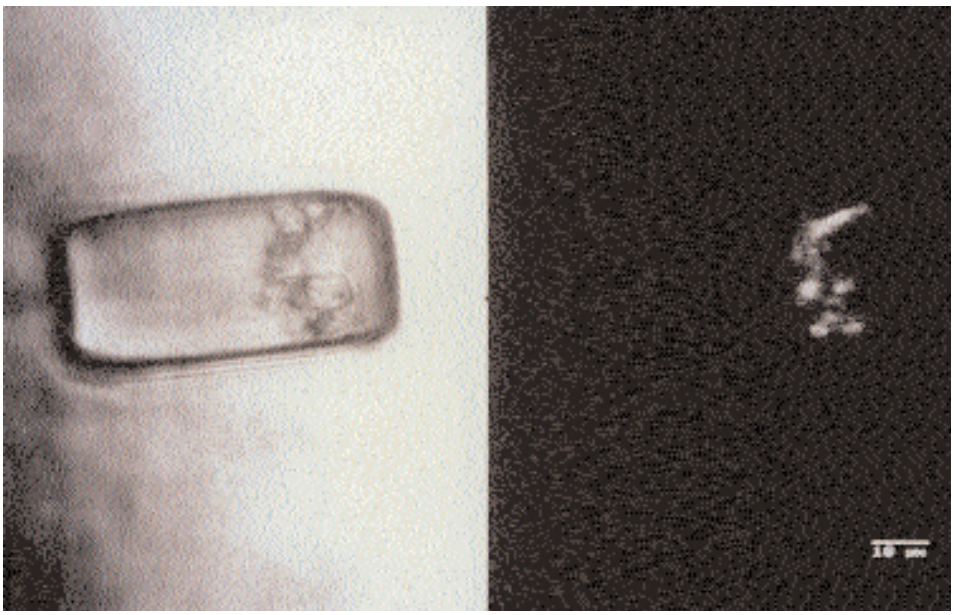
Methods

We have extracted DNA from haloarculas that have been isolated from salt mines in England (235-260 Ma), Thailand (65 Ma) and Poland (20 Ma) and from present day salt lakes. By digesting the DNA with enzymes and separating the fragments by electrophoresis on an agarose gel, fragments containing different 16S rRNA genes can be identified, extracted and the genes amplified by the polymerase chain mechanism (PCR) for subsequent sequencing and analysis.

In a complementary approach, in order to detect very small amounts of halobacterial DNA in ancient salt crystals, we are using PCR as a sensitive technique for detecting very small amounts of 16S rRNA genes. Stringent precautions are necessary to avoid contamination, including surface sterilisation and cleaning with alcohol. So far, we have concentrated on optimising the DNA detection system - we can now detect DNA from less than 50 halobacterial cells. The next step is to apply it to ancient crystals.

Future Work

We hope to improve our sampling procedure by drilling into pockets of liquid in ancient halite using specially adapted lasers, so that we can date and extract the brine contents and use our optimised methods to look for halobacteria and halobacterial DNA in these very small volumes of ancient brine.



Visible light (left hand) and UV light (right hand) micrograph of ancient salt crystals shows the fluid-filled pockets. The fluorescence shown in the right hand picture probably indicates organic material in the fluid.

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