Comparative analysis of 16S r-RNA genes from Piora Dolomite reveals a new group of endolithic Archaea

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Introduction

Certain endolithic conditions in rocks allow microorganisms to develop into thriving communities. In this study, we extracted DNA from a biolayer inside a dolomite rock in order to define which organisms are part of the ecosystem.

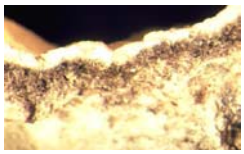
These results are part of a broader study of the endolithic community in the Piora Dolomite. Previous results already showed the presence of Bacteria and amoeba and now we add a new group of Archaea and also mosses to this list. The Archaea form a group, branching into the non-thermophilic marine Crenarchaeotae.

Material

The Piora Dolomite ($CaMg(CO_3)_2$ is a gypsum ($CaSO_4.2H2O$) containing dolomite with a sucrosic texture. Outcrops near Lago Cadagno (Fig. 1) display a green to brown biolayer which penetrates the rock from 1 to 5 millimetres (Fig. 2). The outer layer of the dolomite looks like a product of recrystallization, however this still has to be confirmed.

Fig. 1 (Right): The Piora Dolomite, outcropping near Lago Cadagno.

Fig. 2 (Below): The brownish biolayer in the dolomite rock has a thickness of 2 mm.



DNA Extraction & PCR

Subsamples (~0.1 g) were taken from the biolayer by scratching the surface.

16S r-RNA genes were extracted using the method as in Sigler & Zeyer (2002) with a buffer containing 50 mM NaCl, 50 mM EDTA, 50 mM Tris and 5% SDS at pH 8.0 and phenol-chloroform-isoamyl-alcohol.

Polymerase Chain Reactions were executed with different primer pairs:

- Arch008s + 1512Ur (Eukarya)
- Arch89F + Arch915R (Archea)
- Bact-008-b-S + bact1524as (Bacteria)
- uni 519s + S-Univ-1393-A (Universal)

The procedure was only continued with products from the PCR with eukarya and archeal strains, since previous studies have yielded results about Bacteria.

The PCR products were cloned into *E. coli* for seperation of the strains and 'blue-white selection'.

RFLP Analysis

Restriction Fragment Length Polymorphism with HinfI and HaeIII digestion enzymes, was used to analyse the different DNA strains and to make a selection for sequencing.

Archeal (Fig. 2) and Eukaryote (Fig. 3) strains were selected for sequencing (marked 'X').

Endolithic Archaea

The Archeal sequences form a group, branching into the non-thermophilic marine Crenarchaeotae. A number of soil isolate clones (unpublished) fall within this group (Fig. 4).

The closest identified relative is *Cenarchaeum symbiosum* (Preston et al., 1996), a psychrophilic crenarchaeon inhabiting the tissues of a temperate water sponge. This symbiotic archaeon grows best at 10°C and other close relatives are non-thermophilic crenarchaeotes that inhabit diverse marine and terrestrial environments (Preston et al., 1996).

The discovery of this group shows again that Archeae inhabit not only extreme environments, moreover they also thrive in more 'friendly' environments, in such a way that they form a subsurface endolithic ecosystem.

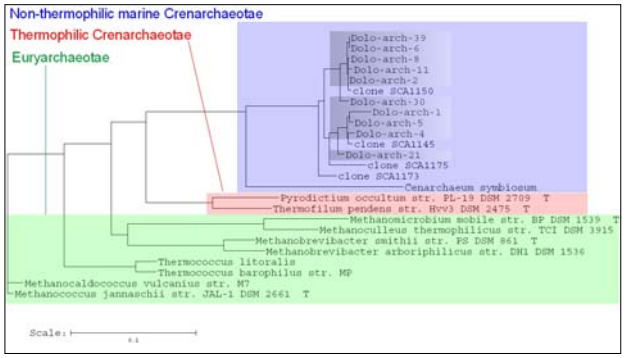


Fig. 4 : Phylogenetic tree for detected endolithic archea (shaded): the closest relative of the discovered group, branching into the non-thermophilic marine Crenarchaeotae, is *C. symbiosum*. The evolutionary distance is more than 10%. Distance within the group are in the order of a few per cent.

Bryophyta

The endolithic biolayer contains mosses, closely related (Fig. 6) to the Pottiaceae and Dicranaceae, however exact identification was not possible. This result was confirmed by the detection of moss rhizoids (Fig. 5).

In this environment, Pottiaceae are more likely to thrive than Dicranaceae (pers. comm. E. Urmi). The closest related and named moss species is *Leptobryum pyriforme*, better known as *thread moss*. Most likeley, the rhizoids of moss growing on the rock, penetrated the dolomite, and were rendered detectable for the used techniques.

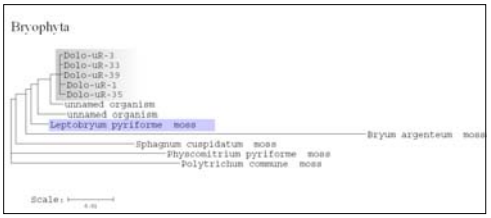


Fig. 6 : Phylogenetic tree for detected moss species (shaded). The closest known relative is *L. pyriforme* (Pottiaceae).

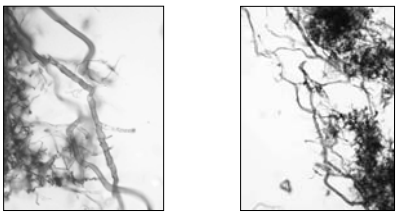


Fig. 5 : 200- (Right) and 400- (Left) fold magnification of moss rhizoids, recognized by the typical compartments. Angular twists in the rhizoids could be caused by growing between the grains of the dolomite rock.

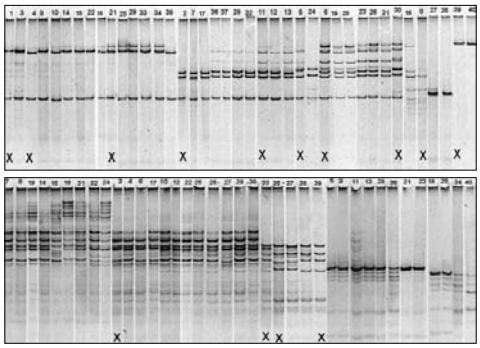


Fig. 3 : RFLP pattern for archeal (top) and eukaryote (bottom) strains.

Conclusion

A group of endolithic Archaea (non-thermophilic Crenarchaeotae) and most likely epilithic moss species with endolithic rhizoids, closely related to *L. pyriforme*, are found in the biolayer in the Piora Dolomite (near Lago Cadagno) by phylogenetic analysis using PCR and sequencing techniques.

Together with earlier found bacteria and amoeba (pers. comm. Th. Horath), they form a fascinating community of rock inhabiting organisms.

These results show that the environment inside the Piora Dolomite rock is life-supporting. The presence of a biolayer suggests a rather high activity and success of survival.

Outlook

Next steps in this study will be to

- define the nutrient sources in this ecosystem: the Piora Dolomite ($CaMg(CO_3)_2$) is a gypsum ($CaSO_4.2H_2O$) which can have important implications for the geobiochemical processes
- define biochemical interactions between the different groups of organisms: does symbiosis occur; do different organisms occupy discrete places in the biochemical pathways;…

References:

C.M. Preston *et al.* (1996). *A psychrophilic crenarchaeon inhabits a marine sponge:* Cenarchaeum symbiosum *gen. nov., sp. nov.* Proc. Natl. Acad. Sci. USA 93(13): 6241-6246.

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