Sequencing of 16S rDNA from endolithic microorganisms in dolomite rocks of the Piora valley

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Introduction

Endolithic is Greek and means living inside stones. The ecological role of endolithic organisms lays within the colonization of places with little or no life; this way, they play a pioneer role in the outspreading of life. There are three distinct forms:

- chasmolithic: living in fissures
- cryptoendolithic: living in pores
- euendolithic: active colonization of the rock by penetration of the stone

Characteristic for endolithic microorganisms is the:

- Variable biodiversity between different locations
- Absence of nutriment limitation in stones rich in calcite and in contact with the atmosphere
- Light conditions of 0,01 – 0,5% sunlight
- C-uptake of 0,15 - 0,5 mg CO_2 / mg chla * h
- Slow growth rate

The succession between the different communities is as follows:

- Hyphes of fungi penetrating the rock
- Cyanobacteria in form of cocci
- Symbiosis between cyanobacteria and fungi
- Spreading of eucaryotic algae
- Colonization by the protonemas of mosses

Possible sample locations are:

- Sandstone
- Calcareous stone
- Gypsum
- Granite

The colonization of the first three kinds of stones in the list is more common because they are soft rocks, not as hard as granite. In mountainous regions organisms are subject of many stress factors as:

- Strong UV-radiation
- Considerable changes in temperature between night and day (max: -20°C to +40°C)
- Water as a limiting factor

Specific adaptations observable in organisms are:

- Scytonemines: pigments functioning as UV-protection (visible as black stripes on rocks)
- Gelatinous envelopes: protection against desiccation

The Microorganisms studied in this report were collected from the Piora region, Ticino, Switzerland, 2000m above sea level, in dolomite rock containing gypsum. Biodiversity was estimated to be high as there are strong selection factors. Genomic DNA was extracted from the stone and the 16S rDNA was amplified with the two bacterial primers S-D-Bact-0008-b-S-20 (5'-AGA GTT TGA TCM TGG CTC AG-3') and S-D-Bact-1524-a-A-18 (5'-AAG GAG GTG ATC CAR CCG-3') The PCR-product was then cloned with the Invitrogen TOPO TA cloning kit. The diversity was determined by RFLP (Restriction fragment length polymorphism). The number of different species present after analysis of the RFLP was 30 out of 40, eight of which have been sequenced. This report focuses on the preparation for and the sequencing of the bacterial 16S rDNA. The aim was to determine the species present by comparison with sequences from Genbank databases.

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Results

We submitted the eight sequences for comparison to both NCBI Blast and GCG, and received the following results.

DOLO-1	*Chlorella saccharophila*	90.0%
	Uncultured eubacterium	84.5%
DOLO-4	*Mycoplana dimorpha*	92.0%
	Bartonella visonii	89.4%
DOLO-5	*Chelatobacter heintzii*	97.5%
	Bartonella visonii	93.8%
DOLO-6	*Xanthomonas vescicatoria*	96.0%
	Xanthomonas vescicatoria	96.8%
DOLO-36	Uncultured eubacterium	98.0%
	Prophyrobacter sp. KK348	96.5%
DOLO-37	*Chlorella saccharophila*	90.0%
	Uncultured eubacterium	84.6%
DOLO-39	*Curtobacterium flaccum*	92.0%
	Clavibacter michiganensis	88.2%
DOLO-40	*Brevundimonas variabilis*	99.0%
	Brevundimonas variabilis	90.4%

Table 1: BLAST (odd rows) and GCG results.
Of the eight sequences sampled, only four (Dolo-5, Dolo-6, Dolo-36 and Dolo-40) show a significant match. In one case (Dolo-1 and Dolo-37) our sequence matches the chloroplastic 16rRNA of a green alga.

We observe a high degree of diversity within the sample, and apparently at least two of the eight sequences (Dolo-4 and Dolo-39) were never identified before.

Another important observation is that *C. saccharophila* appears twice in the result. The concern is, due to our clone selection based on RFLP (Fig.1), that we have not been carefully enough to select the different clones. Looking close to the RFLP pattern, we have to admit that Dolo-01 and Dolo-37 are good candidates for representing the same strain. In fact sequences 1 and 37 differ in only 3 bases out of the 1.5kb of the whole sequence.

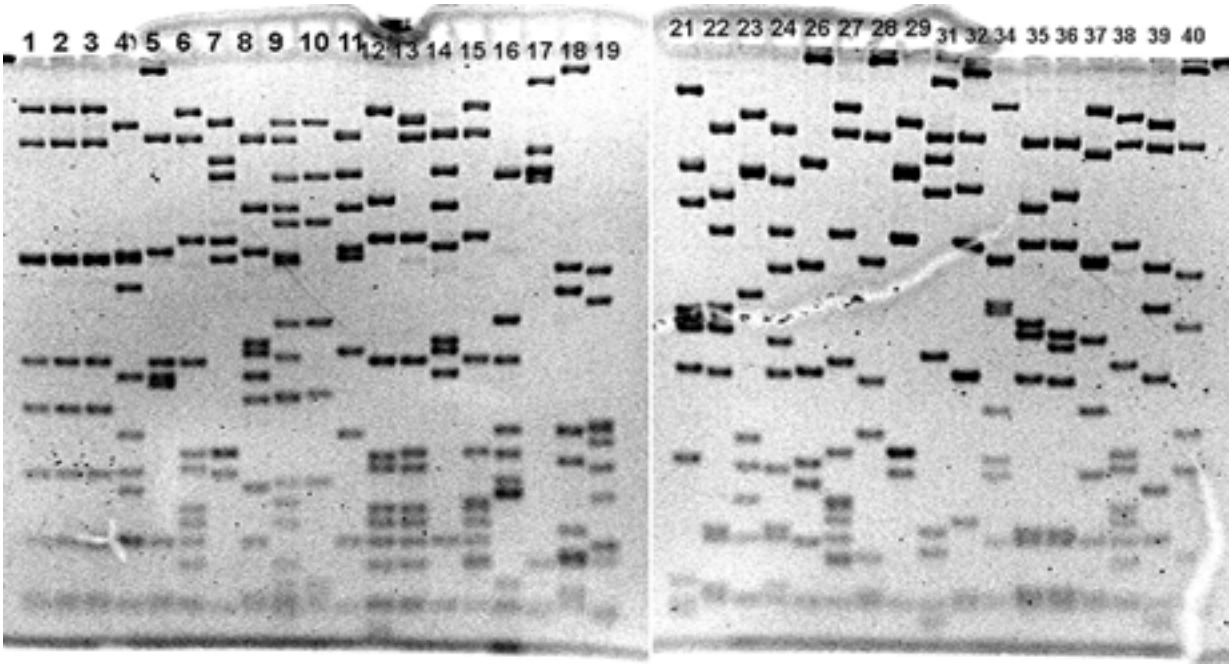

Figure 1: RFLP of the 36 Dolo-clones cut by *Hinf* I and *Hae* III (Plasmids 20, 25, 30, and 33 did not contain any insert)

With this insight we run a similarity matrix (Ribosomal Database Project II, Michigan State University) on our dataset, and we can reject the hypothesis that sequences 4, 5, 36, and 40 were representing the same organism (on the other hand, a BLAST of a consensus of these sequences provided compatible results, giving as possible result either a *Rhizobium sp.* or a *Orchobactrum sp.*16S rRNA with 93% respectively 95% matching). The high similarity between Dolo-4 and Dolo-5 is nevertheless interesting. Another evidence supporting the fact that we are confronted with a high degree of diversity and not with some kind of artefact, is that almost all the differences in the sequences are not randomly distributed, but rather clustered in homologous variable regions (not shown).

Even if the results of the similarity matrix match the ones from the individual BLASTs and GCGs for most organisms, we still know little about Dolo-4 and Dolo-39, and as they could be new forms, experiments with traditional microbiological techniques will be necessary.

Similarity Matrix version 1.1
written by Niels Larsen

Date : Sat Jan 19 13:02:28 2002
RDP data : Small Subunit rRNAs
Columns : 2341 alignment positions were used
Matching : Full ambiguity code matching
Template : 1

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
DOLO-04	**1**	.944	.932	.931	.839	.844	.890	.896	.794	.788	.842	.809	.896	.897	.793	.788
Rps.spHGJF	**2** .056		.933	.933	.843	.842	.880	.883	.798	.790	.836	.806	.885	.887	.796	.790
DOLO-05	**3** .068	.067		.975	.830	.837	.879	.894	.791	.792	.845	.812	.876	.879	.789	.792
Z94817	**4** .069	.067	.025		.840	.844	.894	.902	.784	.787	.844	.814	.884	.887	.784	.787
DOLO-06	**5** .161	.157	.170	.160		.973	.825	.833	.797	.799	.809	.798	.815	.820	.796	.799
Xan.oryza2	**6** .156	.158	.163	.156	.027		.833	.840	.800	.798	.810	.795	.821	.826	.800	.798
DOLO-36	**7** .110	.120	.121	.106	.175	.167		.974	.789	.789	.825	.796	.867	.870	.788	.789
AB016518	**8** .104	.117	.106	.098	.167	.160	.026		.789	.792	.823	.795	.874	.876	.788	.792
DOLO-37	**9** .206	.202	.209	.216	.203	.200	.211	.211		.925	.797	.796	.785	.787	.999	.925
Clrl.mir_C	**10** .212	.210	.208	.213	.201	.202	.211	.208	.075		.800	.802	.784	.786	.924	1
DOLO-39	**11** .158	.164	.155	.156	.191	.190	.175	.177	.203	.200		.903	.819	.822	.796	.800
AF060679	**12** .191	.194	.188	.186	.202	.205	.204	.205	.204	.198	.097		.790	.793	.796	.802
DOLO-40	**13** .104	.115	.124	.116	.185	.179	.133	.126	.215	.216	.181	.210		.993	.786	.784
Cau.variab	**14** .103	.113	.121	.113	.180	.174	.130	.124	.213	.214	.178	.207	.007		.787	.786
DOLO-01	**15** .207	.204	.211	.216	.204	.200	.212	.212	.001	.076	.204	.204	.214	.213		.924
Clrl.mir_C	**16** .212	.210	.208	.213	.201	.202	.211	.208	.075	0	.200	.198	.216	.214	.076	

Table 2: Similarity matrix. Rsp.sp: *Rhodopseudomonas sp. strg. H*, former *R. marina*, third match was *Ochrobactrum sp.* (94.1%, not shown); Xan. oryza: *Xanthomonas oryzae* second match *X. vasicola* (97.3%, not shown); Z94817: *Mesorhizobium genosp.*; AB016518: *Poprhyrobacter sp.*; Clrl. mir: *Chlorella mirabilis*, *C. saccharophila* second match (91.2%, not shown); AF060679: Kineococcus-like bacterium AS2953; Cau. variab: *Caulobacter variabilis* (former *Brevundimonas variabilis*)