Title of Experiment	**Endolithic Microbial Communities In Dolomite Rocks**
Supervisor	Thomi Horath, horath@botinst.unizh.ch , ☎ 01 634 82 86, Lab P1-36
Textbook Chapters	Madigan M.T., J.M.Martinko and J. Parker:"Brock -Biology of Microorganisms", 9th Edition,Prentice Hall,1999.ISBN:0-13-085264-3 or ISBN:0-13-081922-0 (hard cover version). Section "Endolithic Algal Communities" in Chapter 17.6 "Algae" Schlegel, Hans Günter und Zaborosch, Christiane:"Allgemeine Mikrobiologie", 7.Auflage,Thieme Verlag 1992.ISBN:3-13-444607-3 Chapter 3.21, "Aerobe, oxygene, phototrophe Bakterien: Cyanobakterien" Sambrook, J., E. F. Fritsch and Th. Maniatis (1989) "Molecular Cloning - A Laboratory Manual", 2 nd ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York. Pages 1.25-1.28, "Small-Scale Preparations of Plasmid DNA"
Objectives and Research Questions	What kind of organisms live in the first 1 to 5mm thick layer of a dolomite rock from Piora (about 1980m above sea level)?
Background	If one could expect life on Mars, it would most likely be present below the surface or inside rocks (endolithic)."Endolithic" is ancient Greek. "ενδον" means: inside, at home, in the town, in the castle. "λιθος" means: stone, rock. The organisms we are interested in are present on earth but not easily seen, because they grow below the surface layer of rocks. This might also be the reason why endolithic organisms have not been investigated intensively so far. This kind of endolithic microorganisms is normally found at depths of 1 to 5mm, but sometimes as deep as several centimeters (D.W.Larson, http://eqb-dqe.cciw.ca/eman/reports/publications/milton/milton5.htm). Endolithic organisms which grow in much deeper layers and those which grow closer to surfaces may also be interesting because of their suspected ability to detoxify or immobilize pollutants such as volatile organic compounds (Hoi-Ying N. Holman, http://infrared.als.lbl.gov/pubs/HYH-SEIRA.pdf) . Phototrophic microorganisms such as cyanobacteria and green algae present in endolithic communities produce sugars and other organic compounds which can be decomposed by heterotrophic prokaryotes and by fungi. Perhaps even phototrophic proteobacteria and chemoautotrophic organisms are able to live inside rocks. Growing endolithic organisms *in vitro* needs patience. Those which grow at all seem to grow very slowly on low nutrient medium. Endolithic cyanobacteria whose growth seems to be controlled by the intensity of light were also described from Mono Lake, a hypersaline and highly alkaline water in California, and heterotrophic extremophiles growing under the high saline and alkaline conditions were isolated from the same communities (H.Sun et al., http://www.astrobiology.com/asc2000/abstract.html?ascid=361)
Selected Literature	P.S. Amy, D.L. Haldeman, D. Ringelberg, D.H. Hall, and C. Russell, "Comparison of Identification Systems for Classification of Bacteria Isolated from Water and Endolithic Habitats within the Deep Subsurface" Applied and Environmental Microbiology (1992) 58: 3367-3373.

	M. Tretiach , A. Geletti, "CO_2 exchange of the endolithic lichen Verrucaria baldensis from karst habitats in northern Italy", Volume 111, Issue 4, pp 515-522, Oecologia (1997) Amann R. and Ludwig W., "Ribosomal RNA-targeted nucleic acid probes for studies in microbial ecology", FEMS Microbiology Reviews 24 (2000) 555-565 Woese, C.R. 1987. "Bacterial Evolution," Microbiol. Rev., vol. 51, pp. 221-271.
WWW-links	http://www.astrobiology.com/asc2000/abstract.html?ascid=361 http://eqb-dqe.cciw.ca/eman/reports/publications/milton/milton5.htm http://infrared.als.lbl.gov/pubs/HYH-SEIRA.pdf
Practical Work	• Plasmid isolation and Sequencing of some 16S rRNA PCR clones from a dolomite DNA extraction. • Cultivation and optical investigation of microbial organisms originating from dolomite material. • Isolation of pure cultures from a growing mixed culture, determination of their 16S rRNA sequences and characterisation of the so far unknown strains, if there are any. • Construction of a phylogenetic tree with the DNA-sequences employing the ARB programm.
Materials and experimental Protocols	All needed material is available in the lab (microscope, phenol, phenol-chloroform, chloroform, -20°C fridge, 1.5 ml tubes, 0.2 ml tubes, Taq Polymerase, dNTP's, etc.) Protocols: DNA-extraction Agarose gel electrophoresis PCR (polymerase chain reaction) DNA restriction TOPO TA cloning Plasmid preparation RFLP (restriction fragment length polymorphism Sequencing with ABI Big Dye Terminators Cultivation of "blue greens"
Goals and Experiences gained	Familiarity with • preparing a liquid or solid growth medium for microorganisms. • sequencing a cloned PCR-fragment and the following computer based analysis of the sequence. • basic molecular techniques
Timing	Most of the work can be done during the 4 course weeks. Progress in growing some slowly growing cyanobacteria and algae can be observed after 2 to 6 months.
Reporting	Oral presentation, written report
Questions to be answered	What kind of endolithic organisms can be detected by sequencing of the 16S/18S rDNA? What kind of endolithic organisms do we expect to find? Can we find clones which have the same DNA-sequences like the strains cultured from the dolomite rock ?

Laboratory Rules and Precautions	We will follow standard working techniques and precautions: No eating, drinking or smoking in the lab. No contaminations of media and reagents. Waste and contaminated material should be sterilized before disposal. Molecular techniques are sensitive methods. To avoid false positive results, please clean the bench before using it, wear clean gloves, use sterile tubes and pipettes, also prepare the negative and positive controls for each reaction. Some of the used chemicals are dangerous to your health even in low concentrations: phenol, chloroform, ethidium bromide. Always wear gloves when handling them. UV radiation can cause damage to eyes and skin, use protective glasses.