Title of experiment	**Molecular analysis of endolithic microbial communities in dolomite rocks**
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Textbook reading	Chapter "Endolithic Algal Communities" in Madigan, M.T. J.M. Martinko and J. Parker (2000) *Brock, Biolog of Microorganisms.* 9-th edition. Prentice Hall, ISBN 0-13-085264-3, page 738 Pages 1.25 - 1.28: “Small-scale preparations of plasmid DNA “ Pages 1.85 - 1.87: “Identification of bacterial colonies that contain recombinant plasmids” Pages 6.3 - 6.20: “Agarose gel electrophoresis” in 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.
Research questions and objectives	Molecular characterization of microorganisms present in Dolomite rocks. How many different microorganisms can be found in this extreme enviroment?
Background	The reason that our view of bacterial diversity is small and underestimated lies in the fact that the organisms are small and that culturing them is often limited by the lack of knowledge about their groeth needs. One approach to study bacterial diversity is exploring different habitats, especially from extreme environments, e.g. high-mountain lakes or stones which may contain unique microorganisms. Colonizers of such extreme environments are pioneer organisms and well adapted to tough conditions like cold, hot, dry, or low-nutrient concentrations. Association with a surface in a structure known as “biofilm” is one of the preferred microbial life strategies for efficient nutrient scavenging at low nutrient concentrations. In this formation many kinds of bacteria, archaea and fungi live together in an optimally adaptated way which maximizes the organisms access to nutrients. Accurate study of the community structure of biofilms have not been possible with conventional methods, i.e. based on the cultivation of the biofilm-forming microorganisms in the laboratory and characterizing them by classical taxonomy, since the community diversities within the biofilms may be reduced through selective culturing conditions. The applications of molecular approaches e.g. PCR-based methods is becoming a robust tool to assess microbial diversity without the need for cultivation.
Selected literature	A series of articles in “Antarctic microbiology”, edited by E.Imre Friedmann, 1993, Wiley-Liss, Inc.New York, ISBN 0-471-50776-8 Dang, H. and C.R. Lovell. 2000. Bacterial primary colonization and early succession on surfaces in marine waters as determined by amplified rRNA gene restriction analysis and sequence analysis of 16S-rRNA genes. Appl. Env. Micobiol. 66: 467-475.

	Hugenholtz, P. and N.R. Pace. 1996. Identifying microbial diversity in the natural environment: a molecular phylogenetic approach. Trends Biotechnol. 14: 190-197. Watnick, P. and R. Kolter. 2000. Biofilm, city of microbes. J. Bact. 182: 2675-2679 Woese, C.R. 1987. Bacterial evolution. Microbiol. Rev. 51: 221-271
www.Links	http://www.arb-home.de http://www.ncbi.nlm.nih.gov/BLAST/
Practical work	Genomic DNA extraction from dolomitic rock samples. PCR amplification of the 16S/18S-rRNA genes from the extracted DNA. Cloning of PCR products and selection of the clones for sequencing by restriction fragment length polymorphism (RFLP).
Materials and experimental protocols	*DNA extraction*: waterbath, centrifuge, hood, phenol-chloroform *PCR*: PCR reagents, PCR machine *gel electrophoresis* and *gel documentation* *RFLP*: restriction enzymes and buffer, incubator *Cloning of PCR-products*: cloning kit (Invitrogen), LB medium, antibiotics, IPTG, X-gal
Goals and experience gained	Familiarity with bacterial cultivation and purification (aseptic techniques) Basic knowledge of some molecular techniques in microbiology Data processing and interpretation of the results
Timing	Cultivation, phenotypic characterization, and molecular analyses can be performed during the duration of the course if everything works as planned.
Reporting	Oral presentation, written report
Laboratory rules and precautions	Standard microbiological working techniques and precautions are required. No eating or drinking in the laboratory. Avoid microbial contaminations. All the waste and contaminated material should be sterilized before disposal. Molecular techniques are sensitive methods, e.g. PCR. To avoid the false positive results, be aware during preparations: clean the place before using it, wear the really clean gloves, use DNAase-free tubes and pipettes, prepare also the negative and positive controls. Some of the chemicals that will be used can be dangerous for your health: phenol (very toxic), chloroform (toxic), ethidium bromide stains (carcinogenic). Please always wear the gloves when handling these substances. UV radiation can cause damage to eyes and skin, use protective glasses and wear a coat with long sleeves.