Title of Experiment	**Molecular analysis of biofilm-forming bacteria from cold, oligotrophic habitats**
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Textbook Chapters	Madigan, M.T. J.M. Martinko and J. Parker (2000) *Brock, Biolog of Organisms*. 9th edition. Prentice Hall, ISBN 0-13-085264-3. Contents in chapters 5,6,7,9,10,12,16 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, - pages 1.85-1.87: Identification of bacterial colonies that contain recombinant plasmids, - pages 6.3-6.20: Agarose gel electrophoresis Ehrlich, H.A (Ed.) (1992) PCR Technology: Principles and applications for DNA amplification. Oxford University Press, Inc. Pages 1-38. Part One: Basic methodology
Objectives and Research Questions	Characterization of biofilm-forming bacteria present in and well adapted to cold, oligotrophic habitat conditions. How many different microorganisms living in biofilm can be obtained from this extreme enviroment? Are they cultivable organisms (if so, what are their phenotyphic characters?) or are they viable-but not (yet)-cultivable organisms which we can clone?
Background	Searching for and studying microorganisms from harsh environments might lead to the discovery of special physiological and ecological traits which are characteristic for life under extreme conditions. We have chosen cold, nutrient-poor, high mountain lakes (the Jöri lakes) as study sites where we are searching for psychrophilic, oligotrophic bacteria. Microorganisms living under these oligotrophic conditions have adapted benthic biofilm formation as their prefered life strategy. Biofilms are habitats which contain rather dense populations interacting with each other and which exchange nutrients and macromolecular cell components. In our work we focus on bacteria-dominated biofilms, in which we expect to find a large microbial diversity consisting of many different species. Accurate analyses of the community structure in these biofilms requires conventional methods, i.e. based on the cultivation of the biofilm-forming microorganisms, but also molecular techniques which allow us to trace the presence of organisms which cannot be cultured easily. One of the fallacy of the cultivation approach is its selectivity which excludes a large number of organisms that do not respond to the enrichment methods applied. The applications of molecular approaches e.g. PCR-based and recombinant DNA methods on the other hand, are now becoming robust and powerful tools to assess bacterial diversity without the need for cultivation (culture-independent studies), but they do not give us a lot of information about the physiological abilities of the organisms.

Selected Literature	• Costerton, J.W., Z.Lewandowski, D.E.Caldwell, D.R. Korber, and H.M. Lapin-Scott. 1995. Microbial biofilms. Ann. Rev. Microbiol. 49: 711-745. • 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	**Biofilms:** www.erc.montana.edu/bf-basic-99/default.htm www.gasp.med.harvard.edu/biofilms/jbmini/movie.html www.personal.psu.edu/faculty/j/e/jel5/biofilms www.Buckman.com/eng.biofim3.htm www.asmusa.org/edusrc/biofilms/index.html **Molecular biology**: www.protocol-online.net/molbio/index www.isic.ucla.edu/is3/tutorials
Practical Work	• Bacterial cultivation and isolation from environmental samples using standard microbiological techniques. • Purification of genomic DNA, either from the biofilms (total community DNA from the environmental samples) or from pure cultures (isolates). • PCR amplification of the 16S-rRNA genes from total community DNA and from pure cultures. • Cloning of PCR products from total community DNA and selection of the clones. • Plasmid isolation and verification of some clones. • Restriction Fragment-Length Polymorphisms (PCR-RFLP) analysis of the 16S-rRNA genes of the clones and isolates
Materials and experimental Protocols	Cultivation and purification of the biofilm-forming bacteria: preparing the medium, aseptic methods, incubation, phenotyphic characterization. Basic molecular analysis : preparing the solutions, pipetting, contamination-free working practices. Some important steps and the material and equipment required: *DNA extraction*: waterbath, centrifuge, fume hood *PCR*: PCR reagents, setting of PCR machine, gel electrophoresis and gel documentation apparatus *PCR-RFLP*: restriction enzymes and buffer, incubator, gel electrophoresis apparatus *Cloning of PCR-products*: cloning kit (Invitrogen), LB medium, antibiotics, IPTG, X-gal Sequencing and sequence analyses of r-RNA-genes

Goals and Experiences gained	Familiarity with bacterial cultivation and purification (aseptic techniques) Basic knowledge of some molecular techniques in microbiology Data processing and interpretation of the sequencing results
Timing	Cultivation, phenotypic characterization, and molecular analyses can be performed during the duration of the course.
Reporting	Oral presentation, written report
Laboratory Rules and Precautions	Standard working techniques and precautions are required: no eating, no drinking, smoking and applying cosmetics in the laboratory. Avoid contaminations during work, either with any other bacteria which are not from the samples, and the contaminations from any other biochemical materials which are not to be added to the reaction assay. All the waste and contaminated material needs to be sterilized before disposal. Molecular techniques are sensitive methods, e.g. PCR. To avoid false positive results, please work precisely during the preparations: clean the bench before using it, wear clean gloves, use DNAse-free tubes and pipettes, prepare negative and positive controls. Some of the chemicals that will be used could be dangerous to your health: phenol (very toxic), chloroform (toxic), ethidium bromide stains (carcinogenic). Please always wear gloves when handling them. UV radiation can cause damage to eyes and skin, use protective glasses and a labcoat with long sleeves.