Title of Experiment	**Phenotypic and genotypic characterization of metal corroding biofilms**
Supervisor	Christine Lehmann, Institut für Pflanzenbiologie, Abteilung Mikrobiologie chleh@botinst.unizh.ch ☎ 01 63 48281, Lab P1/24
Textbook Chapters	➢ Bergey's Manual of Determinative Bacteriology ➢ Chapters 6 to 10,12 and 16 in Madigan M.T., J.M. Martinko and J. Parker:"Brock -Biology of Microorganisms", 9th Edition,(BBOM,International Edition), Prentice Hall, 1999. ISBN: 0-13-085264-3. ➢ Chapters 4 (section 17) and 7 in Lengeler J.W., G. Drews, H.G. Schlegel (eds):"Biology of the Prokaryotes ", Thieme Stuttgart,1999.ISBN:3-13-108411-1.
Objectives and Research Questions	Characterization and identification of bacteria growing in biofilm assemblages on metal surfaces based on: ➢ phenotypic features of microorganisms originating from the biofilms ➢ genotypic identification of clones obtained from the biofilm community Familiarity microorganisms involved in metal corroding processes Access to the biofilm literature
Background	The most common 'lifestyle' in the microbial world is probably the living of microbes closely together, forming communities in so called **biofilms,** aggregates and mats. Microorganisms in biofilms live embedded in a slimy exopolymer matrix forming a thin layer attached to all kinds of surfaces. Microbes living in such a community derive a lot of important advantages from such a 'lifestyle'. They are better protected from an adverse environment, nutrient supply and the removal of metabolites might be fast without removing the biomass and genetic exchange is facilitated by the close proximity of cells. 'Biofilm-bacteria' are phenotypically often different from free living bacteria (Stickler, 1999). Understanding surface-associated bacterial communities is important in many fields of microbiology (e.g. medicine, ecology, botany, material engineering etc.) since they have an important economic and health impact. Among engineers, metal corrosion was generally thought to be an abiotic process, but more recently the concept of **MIC** (**microbially influenced corrosion)** integrates the role of microorganisms in the corrosion processes of metal and non-metal surfaces. Worldwide more than 200 x10 ^{9} $ annually are estimated to be lost due to metal corrosion. It is now aknowledged that bacteria are capable to influence the kinetics of corrosion processes through their physiological activities. Nevertheless, there is a great lack of knowledge about the species composition involved, the development of biofilm communities on surfaces and the actual biochemical processes which lead to metal corrosion.
Selected Literature	Papers will be provided by the supervisor: ➢ Amman R.I., Ludwig W., and Schleifer K.H.1995. Phylogenetic identification and *in situ* detection of individual microbial cells without cultivation. *Microbiol. Rev.* 59, 143-169. ➢ Costerton J.W., Lewandowski Z.,Caldwell D.E., Korber D.R. and Lappin-Scott H.M.1995. Microbial biofilms. Ann. Rev. Microbiol. 49 , 711-745. ➢ Pratt L A and Kolter R. 1999. Genetic analyses of bacterial biofilm formation. *Curr. Op. Microbiol.* 2 , 598-603. Stickler D.1999.Biofilms. *Curr. Op .Microbiol.* 2 , 270-275.

www. Links	
Practical Work	Standard microbiological techniques will be used for phenotypic characterization of microbial species grown in biofilms on metal surfaces, such as: ➢ Differential staining (e.g. DAPI staining, gram staining, staining with tetrazolium compounds) and microscopic observation of the microorganisms. ➢ Identification of bacteria growing in biofilms based on substrate utilisation patterns (*Biolog*) Molecular standard protocols will be followed for genotypic characterization: ➢ Analysis of clones, i.e. sequencing (handouts of special protocols and cf. also project 3) ➢ If time allows, analysis of the sequences obtained will be performed (see special lectures and project 3)
Materials and experimental Protocols	➢ Pipettes, sterile pipette tips, sterile Eppendorf tubes, *Biolog* microtiter plates, DAPI stain, gram stain, tetrazolium compounds ➢ DNA extraction and sequencing facilities ➢ Please write your own protocol following the instructions given and the experiences made during the experiments.
Goals and Experiences gained	➢ Aseptic handling and manipulation of microbes ➢ Basic knowledge of some conventional microbiological and molecular tools used in microbial diagnostics of biofilms (fluorescence microscopy, spectrophotometric methods, sequencing steps) ➢ Theoretical background of molecular analysis of biofilms (cf. Lecture)
Timing	The experiments are performed during the four weeks of the course.
Reporting	Writing of a precise protocol (important for the written report), oral presentation and written report (see time schedule)
Questions to be answered	How do you define a biofilm? What is a bacterial ‘clone’?
Laboratory Rules and Precautions	Standard working techniques and precautions for working with microorganisms are required. Please do not eat, drink and smoke in the lab. If handling mutagenic or toxic compounds (e.g. DAPI, polyacrylamide), wear lab coat and protective gloves. When working wiht UV light, wear protective googles. Do not be afraid but estimate the potential hazard caused by inhalation and/or contact with chemicals. Ask instructor about a potential risk if you are in doubt.