Molecular analysis of biofilm-forming bacteria from cold, oligotrophic habitats

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ABSTRACT
In the laboratory sessions of the course, we tried to isolate prokaryotes from Jöri lake XIII, which is a cold, oligotrophic high mountain habitat. We were interested in finding out what kind of microorganisms could be expected to live under the harsh conditions there.
We used molecular and physiological methods to characterize the cultures. In the end we focussed on one isolate and identified a species which had 98% similarity in the base sequence of its 16S-RNA-gene with three other species listed in the data bank: *Rhizobium giardinii, Sinorhizobium sp.* and an uncultured soil bacterium.

INTRODUCTION
During four weeks of the course we were searching for new species. We received 26 already axenic cultures from Jöri lake XIII and we also tried to isolate species on our own from natural and artificial surfaces, like Teflon or PVC, on which biofilms had been grown while they were immersed in the lake. With physiological and molecular methods we tried to find out what species were present in the biofilms. The aim of our work was to answer the following question: How many different microorganisms are living there? What known species are closely related to the microorganisms which we find there? Are the spieces which we can detect specially adapted to the conditions which prevail in the high mountain habitat ?

PROCEDURES & METHODS
For the molecular analysis we used the following procedures:
We isolated the 16S-rDNA in two different ways:
To get the DNA of the 26 already isolated (pure) cultures, we used the CTAB-method to extract the genomic DNA. We then amplified the 16S-rDNA with PCR using universal bacterial primers. The forward primer was S-D-bact-0008-s (5`-AGA GTT TGA TCM TGG CTC AG-3`), the reverse primer was S-D-bact-1524-as (5`-AAG GAG GTG ATC CAR CCG-3`). With the help of PCR-RFLP (Restriction Fragment-Length Polymorphism) we determined the 16S-rDNA profiles obtained with two different restriction enzymes: *Rsa*I and *Hpa*II. After DNA-purification we made a PCR for sequencing.
To get the genomic DNA from the total community, we first suspended small pieces of biofilm in 2ml of distilled water. With the CTAB-method, we extracted the DNA from the suspensions of the whole communities from Teflon (TEF), PVC (PVC I-III), natural surfaces (NS I,II) and from the sediment (5cm deep: S5 and 10cm deep: S10). This is a useful method since not all species can be cultivated. In the following PCR-amplification step, we always used the same bacterial primers as before and in the case of S5 and S10 we also tried universal archaea primers. Forward primer: SD-Arch-0013-s (5`-TTG ATC CYG CCG GAG-3`) and reverse primer: SD-Arch-1517-as (5`-ATC CAG CCG CAG RTT C-3`). We then cloned the DNA into *E. coli* with a TOPO TA cloning kit by transformation (Invitrogen BV, Netherlands). The plasmid vector used was pCR 2.1-TOPO. After plating on an LB-medium containing either Kanamycin or Ampicillin, we could select the clones which contained a plasmid. Since we had also added Xgal to the medium, and only picking the white colonies, we could be sure to select bacteria with recombinant plasmids containing 16S-rDNA-inserts. We now checked, how many different *E. coli* colonies (with different 16S-rDNAs) we had and therefore first

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made a PCR with the whole cell DNA as template. By using M13-primers (M13 forward (-40) primer (5`-GTA AAA CGA CGG CCA G-3`) and M13 reverse primer (5`-CAG GAA ACA GCT ATG AC-3`)) we only amplified the inserted 16S-rDNA and not the one of *E. coli*. With the PCR-product we made a PCR-RFLP and found out which of our colonies contained the same 16S-rDNA insert. After that we extracted the plasmid DNA of the different clones by alkaline lysis. In order to obtain enough material for sequencing we amplified the 16S-rDNA by PCR. The PCR-products were purified before the sequencing-PCR using blue-white microcon-100 sample reservoir (Amicon, Inc., Beverly, MA 01915, USA). After the sequencing-PCR by the Sanger-method we removed unincorporated “Big Dye Terminators” by a Sephadex G50 gel filtration using MultiScreen 96-well filtration plates (Millipore). For sequencing we used the ABI Prism TM 377.

RESULTS

From the 26 already isolated cultures we found 6 to be different. We sequenced the 16S-rDNA of one of them and found by comparison with a database 98% similarity with three species: *Rhizobium giardinii, Sinorhizobium sp*. and an uncultured soil bacterium. For the total communities we only did all the procedures with NS I as we had some difficulties obtaining *E. coli* colonies. However we selected 20 clones from NS I and found that we had 8 different 16S-rDNAs. There wasn’t enough time to sequence them all. With the archaea-primers which we applied to DNA from the samples S5 and S10, no amplified 16S-rDNA products could be found. The reason for this could be that there were not enough archaebacteria to extract DNA or an inhibitor which was present in the sediment extract inhibited the PCR reaction.

DISCUSSION

Unfortunately the four weeks were not enough time to sequence all our obtained 16S-rDNA and to answer all the questions. It also remains to be determined what organism we had found. Is it a new one or is it one of those three? To answer this questions further investigations should be made. For example we should compare our sequence with other databases and make phenotypic characterizations.

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