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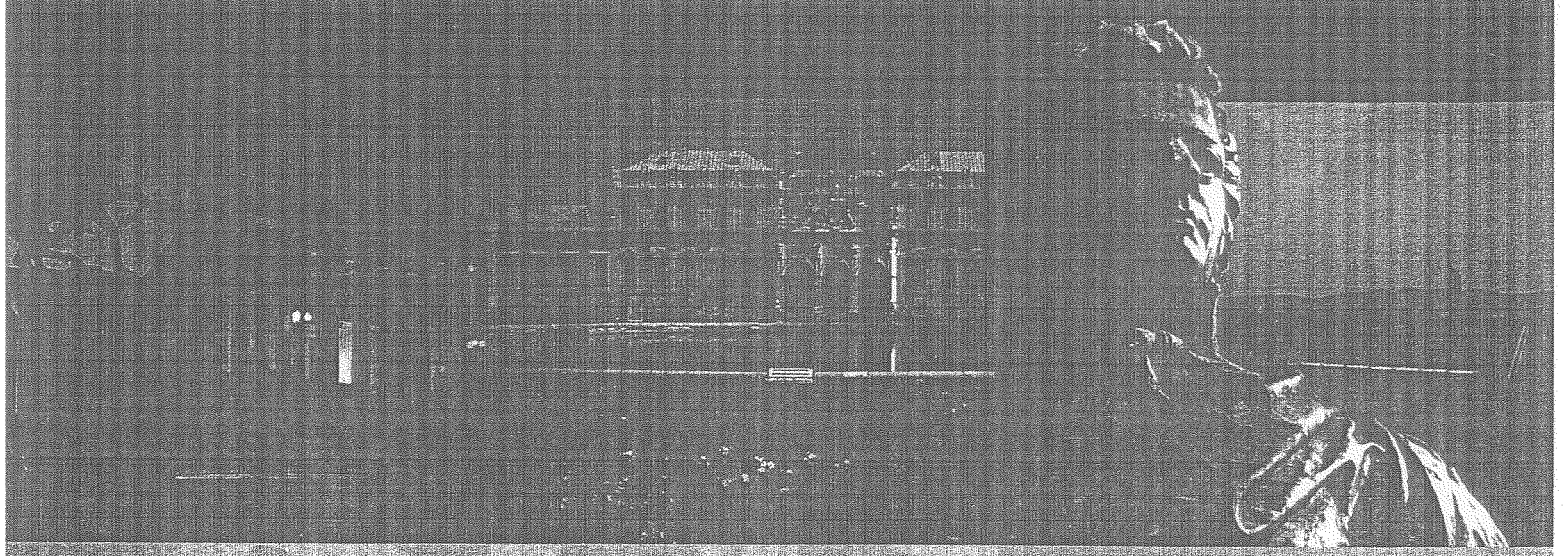

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BOOK OF ABSTRACTS

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E OF PIF-LIKE GENES IN ACTINOMYCETES AND ATORY INFLUENCE ON THE MOBILE GENETIC ELEMENT PSAM2

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The mobile genetic element pSAM2 from *Streptomyces ambofaciens* is capable of conjugal transfer and integrates via a site specific phage-like mechanism into the host chromosome of various Gram-positive bacteria, and therefore, represents a promising tool for genetic manipulation of Actinomycetes. Previous studies have shown that a specific regulation mechanism prevents redundant exchange between two strains harboring pSAM2. One copy of the pSAM2 gene pif (pSAM2 immunity factor) is sufficient to abolish conjugal transfer of pSAM2 into host strains already carrying this element. In several sequenced actinomycete genomes, pif-like genes have been found on pSAM2 related integrated plasmids. The presence of pif-like genes in non sequenced Actinomycetes, which represent a genetic barrier for pSAM2-transfer, was detected by Southern-hybridization with soil isolates, mostly *Streptomyces* sp. The acquisition of detected pif-like genes via pSAM2 related mobile genetic elements during evolution could be demonstrated by fosmid library construction of one representative pif-positive Streptomyces sp. and subsequent sequencing which revealed a highly conserved pif and other pSAM2 genes. Both, pif-positive and negative strains were then used as recipients in conjugation experiments measuring potential differences in pSAM2-transfer rates caused by the presence of pif homologues in these strains. An activity preventing pSAM2 transfer was tested for several identified pif-like genes by expressing them in a highly receptive streptomycete and determining the resulting decrease in pSAM2 transfer efficiency by conjugation. The results indicate that the dispersion of pSAM2 and pif-like genes in many distinct Actinomycetes may limit the application of this mobile element as universal vector system.

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DETECTION AND CHARACTERIZATION OF UNCULTURED AMMONIA-OXIDIZING BACTERIA (AOB) IN A METAGENOMIC LIBRARY FROM LAKE KINNERET, ISRAEL

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Ammonia-oxidizing bacteria (AOB) are an essential element of the nitrogen cycle in aquatic and terrestrial ecosystems. These microorganisms have shown to be remarkably resilient to isolation and culturing. Studies based in molecular markers have demonstrated that in situ diversity of AOB exceeds the diversity of culture collections. However, these molecular techniques generally are limited to the detection of single molecular markers, which in most of the cases do not reveal the metabolic diversity of the organisms in the environment. Metagenomics, the culture-independent genomic analysis of an assemblage of microorganisms, has the potential to answer fundamental questions in microbial ecology. In this study we present results of this approach to study ammonia-oxidizing bacteria (AOB) in Lake Kinneret, Israel. The presence of genomic DNA from AOB in a fosmid library prepared with surface water from Lake Kinneret was tested using the gene of ammonia monooxygenase *amo*A as functional anchor. In the initial screening, 18 out of 145 pools of 96 clones were positive. These products were evaluated by denaturing gradient gel electrophoresis (DGGE) and sub-cloning of the *amo*A PCR products. A surprisingly diverse assemblage of *amo*A sequences was detected in the fosmid library, containing sequences related to seven different clusters of *Nitrosomonas* and *Nitrosospira*. These clones were used for further characterization of large sub-genomic DNA.

USING THE AMOCAB OPERON TO STUDY AMMONIA OXIDIZING BACTERIA (AOB) IN LAKES AND THE BALTIC SEA

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In ammonia-oxidizing bacteria (AOB), the enzyme ammonia monooxygenase (AMO) catalyzes the oxidation of ammonia to hydroxylamine. AMO has three subunits, AMO-C, AMO-A and AMO-B encoded by the genes *amo*C, *amo*A and *amo*B, which form an operon. Up to now, sequence information from environmental samples is available mainly for amoA. New primers for amplification of the complete amoCAB operon were developed and tested in sediment samples from Lake Plußsee, Lake Schöhsee and the Baltic Sea. In order to compare community composition of AOB, denaturing gradient gel electrophoresis (DGGE), differences in the size of IS (intergenic spacer) region between *amo*C and *amo*A and clone libraries of the whole amoCAB operon were applied. DGGE band patterns from each environment were different, while the size of ISs was similar in the two lakes but differed in the Baltic Sea. From the cloned PCR products, 43 clones from Baltic Sea, 7 clones from Plußsee, and 25 clones from Schöhsee were obtained. Most of the sequences of an inner fragment of amoA were related to *Nitrosospira*, one clone from Lake Plußsee was similar to *Nitrosomonas* and few clones were placed between AOB and methane-oxidizing bacteria (MOB). For the first time, the analysis of amoCAB sequences from environmental samples allowed an insight into the differential evolution of these coupled genes.

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STABLE ISOTOPE PROBING INTEGRATED WITH METAGENOMICS: COSMID LIBRARIES CONSTRUCTED FROM ^{13}C-DNA OF ^{13}C-BIPHENYL (PCB)-DEGRADING BACTERIA IN ENVIRONMENTAL SAMPLES

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DNA-SIP is an important approach for identifying nucleic acids of members of microbial communities actively metabolizing specific ^{13}C-labeled substrates. This study aims to explore and recover genes and operons for new biodegradative steps involved in the oxidative detoxification of the polychlorinated biphenyl, from uncultured microbial communities by producing a cosmid library from the SIP-metagenome of biphenyl-utilizing organisms. Microcosms containing sediment slurries from the River Raisin (Monroe, MI, USA) were constructed and provided with ^{13}C-biphenyl, which was utilized by indigenous microorganisms under aerobic conditions within 14 d resulting in the production of ^{13}C-labeled DNA from these organisms. One of potential problems in the use of ^{13}C-DNA for constructing cosmid libraries is the necessity to recover large amounts of high quality and high molecular weight (HMW) DNA. In this study, we recovered HMW DNA directly from sediment and scaled up CsTFA density gradient procedures to separate up to 100 ug of total DNA based on buoyant density. The overall scheme for creating the SIP-cosmid libraries is that ^{13}C labeled-DNA is randomly sheared, end-repaired, size-selected, ligated into cosmid vector, and transfected into *E. coli*. Over 1700 cosmid clones were produced, with each clone containing around 25kb environmental DNA from organisms that derived carbon from biphenyl. The cosmid library constructed ^{13}C-labeled DNA should provide more concentrated and relevant sequences to PCB degradation than direct (or conventional) environmental libraries.