Nocardioform actinomycetes in activated sludge: phylogenetic classification and in situ identification on the basis of 16S rRNS analysis

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The analysis of complex microbiota present in activated sludge is of utmost importance for the understanding and possible control of severe separation problems in sewage treatment, such as sludge bulking or sludge scumming. Previous studies indicated that nocardioform actinomycetes are frequently responsible for these conditions which not only affect the efficiency of sewage treatment but also represent a threat to public health due to spread of pathogens[1]. Among these are ubiquitous mycobacteria or nocardioform actinomycetes such as *Rhodococcus equi*, *Nocardia asteroides* or *Gordona bronchialis*, i.e. organisms that may be part of the microbial flora of the scum[2]. However, isolation and identification of these filamentous, nocardioform actinomycetes is hampered by their fastidious nature. Most species are still uncultivable and their taxonomy is by and large unclear.

To study the ecology of these microorganisms at the molecular level, we carried out a comparative sequence analysis of nocardioform 16S rDNA sequences which derived from a clone library of 16S rRNA gene fragments amplified from bulk sludge DNA[3]. The DNA was extracted from an activated sludge sample of the waste water treatment plant Marienfelde (Berlin). 16S rRNA gene fragments (approx. 500 bp) were selectively amplified by using 16S rDNA amplification primers specific for eubacteria. Amplicons were ligated in pUC 19 and used to transform competent *E. coli* cells. Colony hybridization with oligodeoxynucleotide probe MNP1 (specific for nocardioform actinomycetes and mycobacteria) detected 27 out of 3000 recombinant clones in our 16S rDNA clone library. The sequence data from the MNP1 probe-positive clones were used for comparative sequence analysis and construction of dendrograms. Although all sequences differed from hitherto sequenced species in databases, comparative sequence analysis allowed the phylogenetic classification of the bacteria from which the 16S rDNA fragments derived.

In parallel, part of the respective sludge sample was spread on agar plates with modified Czapek-Dox medium to isolate nocardioform actinomycetes. The characterization of the culture isolates revealed a high discrepancy between the two approaches. Only one 16S rDNA sequence of a culture isolate was represented in the clone library. This indicates that culture conditions select species, which represent only a small fraction of the organisms in the activated sludge and therefore were not represented in the clone library. However, conventional culture methods failed to grow bacterial species most abundant in the clone library.

Based on the nocardioform 16S rDNA sequences from the clone library we synthesized specific, fluorescent oligonucleotide probes. In situ hybridization of activated sludge from the same waste water treatment plant using these specific probes identified the respective organisms in the sludge.

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PCR of DNA from dried blood spots on filter paper coupled to a simple DNA enzyme immunoassay for rapid detection of HIV-1

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For the HIV PCR technique to be implemented as a tool in large scale studies it is essential that the sample preparation and the hybridization procedures are easy to perform and ensure the specificity of the amplified DNA. Therefore, we developed a method for the detection of proviral HIV-1 DNA from blood samples dried on filter paper appropriate for long-term storage. To simplify the analysis of amplified products we adapted the previously described colorimetric microtitre plate hybridization assay[1] to the detection of HIV-1 specific sequences.

Amplification of DNA from blood spot materials. Blood spots were prepared dropwise on filter paper and allowed to dry down at room temperature. The filters