INVESTIGATION OF A METAGENOMIC LIBRARY FOR AEROBIC TOLUENE DEGRADATION GENES

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Introduction. Metagenomic analysis of polluted sites holds great promise in bioremediation. It has the potential to give access to all genes involved in pollutant degradation from cultured and not-yet-cultured bacteria. Therefore, discovering gene sequences from the little characterized or totally unknown microorganisms by this approach may help the design of novel specialized strains and enzymes useful for bioremediation.

Our study was conducted on sediment from a former gasworks site located in Düsseldorf-Flingern, Germany (1). Our aims were: (i) to develop and to apply metagenomic tools for assessment of microbial adaptation to contaminant stress in sediments polluted with benzene, toluene, ethylene and xylene (BTEX) and (ii) to detect aerobic toluene degradation patterns based on genetic screening of the metagenomic library for the toluene 4-monooxygenase gene (*tmoA*) which encodes the alpha-subunit (subfamily 2) of the hydroxylase component of the multicomponent toluene monooxygenase.

Methods. High quality and high molecular weight DNA was extracted from a low cell density in the sediment. The extraction method used direct lysis of bacterial cells within the sediment followed by a phenol-chloroform extraction. The DNA was then purified using a centrifugal concentrator coupled with Q-Sepharose treatment. This protocol resulted in pure DNA of appropriate size range to construct a metagenomic library in the pCC1FOS vector with the CopyControl™ fosmid library system (Epicentre, USA). Macroarrays containing 53,760 fosmid clones with an average insert size of 40 kb representing 537 *E. coli* genome equivalents were produced at Libragen, France. The library was screened by colony blot hybridization for toluene monooxygenase using a digoxigenin-labeled *tmoA* probe. Positive clones detected by hybridization were tested for amplicon production by PCR using primers described by Hendrickx *et al* (2005) (2).

Results. 1/ The developed HMW DNA extraction and purification protocol yielded 1.5 μg DNA/100 g sediment of pure DNA.

2/ DNA cloning produced a 55,296-clones library.

Restriction fragment length polymorphism with *BamHI* showed a high variability of inserts with an average size of **40 kb.** The library corresponds to 537 *E. coli* genome equivalents (Fig.1).
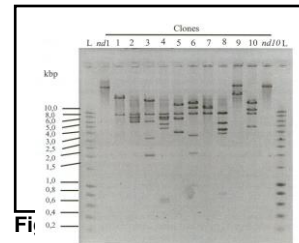

Fig ... h polymorphism with *BamHI* of 10 clones. L. Smart Ladder, nd1 and nd2: undigested clones.

3/ Assuming an equal abundance of genomes within the library, about 40 % of the screened genomes contained a *tmoA* gene.

The 79 clones detected by hybridization and showing a negative amplification of *tmoA* contain mismatches with the TMOA-F/TMOA-R primer set. Together with the 27 clones having negative hybridization and positive amplicon production may contain a *tmoA*-like gene that is divergent from previously described sequences (Table 1).

Table 1. Genetic screening of macroarrays targeting *tmoA*.

Clone numbers	Hybridization	PCR *tmoA* Amplicon production
50	+	+
79	+	-
27	-	+

Conclusions. The combination of macroarray screening by hybridization and PCR appeared to be a powerful tool to capture all *tmoA*-like genes in the studied polluted site that might be missed by primers designed on the basis of cultured toluene degraders. Sequencing of these clones is expected to considerably enrich the catalogue of known genes involved in toluene degradation.

References.
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