Supplementary Material

Phylogenetic and functional diversity within toluene-degrading, sulphate-reducing consortia enriched from a contaminated aquifer

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Running title: Diversity within toluene-degrading consortia
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Material and Methods - Single strand conformation polymorphism (SSCP) analysis of bacterial 16S rRNA genes

The SSCP fingerprinting method was used to follow changes in the phylogenetic composition of the cultures during enrichment. Samples were taken after 11, 21 and 24 months for Zz5-7 and Zz53-56 and 6, 8 and 10 months of enrichment for Zz9-00 (sampling time points A, B and C, respectively). Analysis was based on the highly variable V4-V5 region of the 16S rRNA gene which was PCR-amplified with the primers UniBac515f (5’-GTGCCAGCAGCCGCG-3’) and 5’-phosphorylated UniBac927r-Ph (5’-Ph-CCCGTCAATTYMTTTGAGTT-3’). The following PCR programme in a PTC-200 Thermal Cycler (MJ Research, MA) was used: 4 min initial denaturation at 94°C, 30 cycles of amplification with 20 s at 94°C, 30 s at 54°C and 1 min at 72°C, and 10 min of final extension at 72°C. Fifty microlitres of PCR reaction contained: 18 µL of nuclease-free water, 25 µL of Taq PCR master mix (Qiagen, Hilden, Germany), 1.5 mM MgCl₂, 5 pmol of each primer and 2 µL of template DNA. After purification of PCR products with the E.Z.N.A. Cycle Pure Kit (peqlab GmbH, Erlangen, Germany) according to the manufacturers’ instructions, 15 µL of purified product were digested for two hours at 37°C with 6 units Lambda exonuclease (New England Biolabs). After addition of 1 volume 2 x SSCP sample buffer (95% formamide, 10 mM NaOH, 0.25% bromophenol blue, 0.25% xylene cyanol FF) the samples were denatured for 2 min at 95°C, immediately chilled on ice for 5 min and stored at -20°C until electrophoresis.

SSCP electrophoresis was run on an 0.6% MDE gel (Biozym, Germany) in a TGGE Maxi chamber (Biometra, Germany) in 1 x TBE buffer [1] at 400 V and 26°C for 17 h. The gel was silver stained according to Bassam et al. [2] with the modification that 3% NaOH was used as developing bath. After drying, the gel was scanned and the image was analysed using the Phoretix 1D software (Nonlinear Dynamics, UK). A hierarchical cluster analysis of the SSCP pattern was performed applying the Jaccard similarity index and the UPGMA algorithm. All
statistical evaluation was done with the statistical software R version 2.10.0 [3]. To visualise the dissimilarities in community composition between the samples, nMDS plots were calculated based on the Jaccard similarity index.

**Results - Community shifts during enrichment**

To follow variations in community composition during enrichment of the consortia, SSCP community fingerprinting targeting the V4-V5 region of the 16S rRNA genes was performed from the cultures at three different sampling times (Fig. S1). NMDS analysis of the SSCP patterns revealed that the communities of the enrichment cultures Zz5-7 and Zz53-56 were more similar to each other than to that of Zz9-00 (Fig. S2). Although some community shifts during enrichment were detected for all consortia, the SSCP profiles of each consortium at the three sampling times remained similar to each other. Irrespective of sampling time, however, the consortia were clearly distinct from each other.

**Fig. S1.** Image of the SSCP gel from the enrichment cultures Zz5-7, Zz53-56 and Zz9-00. A, B, C refers to the sampling times as indicated in Materials and Methods.
Fig. S2. NMDS analysis of the SSCP patterns applying the Jaccard similarity index. The enrichment cultures Zz5-7 and Zz53-56 are clearly separated from Zz9-00. A, B, C refers to the sampling times as indicated in Materials and Methods.

References

