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Anaerobic degradation of benzene by marine sulfate-reducing bacteria

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Benzene, the archetypal aromatic hydrocarbon is a common constituent of crude oil and oil-refined products. As such, it can enter the biosphere through natural oil seeps or as a consequence of exploitation of fossil fuel reservoirs. Benzene is chemically very stable, due to the stabilizing aromatic electron system and to the lack of functional groups. Although the anaerobic degradation of benzene has been reported under denitrifying, sulfate-reducing and methanogenic conditions, the microorganisms involved and the initial biochemical steps of degradation remain insufficiently understood. Using marine sediment from a Mediterranean lagoon a sulfate-reducing enrichment culture with benzene as the sole organic substrate was obtained. Application of 16S rRNA gene-based methods showed that the enrichment was dominated (more than 85% of total cells) by a distinct phylotype affiliated with a clade of Deltaproteobacteria that include degraders of other aromatic hydrocarbons, such as naphthalene, ethylbenzene and m-xylene. Using benzoate as a soluble substrate in agar dilution series, several pure cultures closely related to Desulfotignum spp. and Desulfosarcina spp. were isolated. None of these strains was able to utilize benzene as a substrate and hybridizations with specific oligonucleotide probes showed that they accounted for as much as 6% of the total cells. Incubations with 13C-labeled benzene followed by Halogen in situ Hybridization – Secondary Ion Mass Spectroscopy (HISH-SIMS) analysis showed that cells of the dominant phylotype were highly enriched in 13C, while the accompanying bacteria had little or no 13C incorporation. These results demonstrate that the dominant phylotype was indeed the apparent benzene degrader. Dense-cell suspensions of the enrichment culture did not show metabolic activity toward added phenol or toluene, suggesting that benzene degradation did not proceed through anaerobic hydroxylation or methylation. Instead, benzoate was identified in analyses of metabolites with benzene-grown cultures, suggesting an activation of benzene via carboxylation.