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Effect of oxygen limitation on the enrichment of bacteria degrading either benzene or toluene and the identification of *Malikia spinosa* (Comamonadaceae) as prominent aerobic benzene-, toluene-, and ethylbenzene-degrading bacterium: enrichment, isolation and whole-genome analysis

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Abstract:

The primary aims of this present study were to evaluate the effect of oxygen limitation on the bacterial community structure of enrichment cultures degrading either benzene or toluene and to clarify the role of *Malikia*-related bacteria in the aerobic degradation of BTEX compounds. Accordingly, parallel aerobic and microaerobic enrichment cultures were set up and the bacterial communities were investigated through cultivation and 16S rDNA Illumina amplicon sequencing. In the aerobic benzene-degrading enrichment cultures, the overwhelming dominance of *Malikia spinosa* was observed and it was abundant in the aerobic toluene-degrading enrichment cultures as well. Successful isolation of a *Malikia spinosa* strain shed light on the fact that this bacterium harbours a catechol 2,3-dioxygenase (C230) gene encoding a subfamily I.2.C-type extradiol dioxygenase and it is able to degrade benzene, toluene and ethylbenzene under clear aerobic conditions. While quick degradation of the aromatic substrates was observable in the case of the aerobic enrichments, no significant benzene degradation, and the slow degradation of toluene was observed in the microaerobic enrichments. Despite harbouring a subfamily I.2.C-type C230 gene, *Malikia spinosa* was not found in the microaerobic enrichments; instead, members of the *Pseudomonas veronii / extremaustralis* lineage dominated these communities. Whole-genome analysis of *M. spinosa* strain AB6 revealed that the C230 gene was part of a phenol-degrading gene cluster, which was acquired by the strain through a horizontal gene transfer event. Results of the presens study revealed that bacteria, which encode subfamily I.2.C-type extradiol dioxygenase enzyme, will not be automatically able to degrade monoaromatic hydrocarbons under microaerobic conditions.

Keywords: *Malikia spinosa*, BTEX degradation, bioremediation, petroleum hydrocarbons, groundwater