



Evaluating the aerobic xylene-degrading potential of the intrinsic microbial community of a legacy BTEX-contaminated aquifer by enrichment culturing coupled with multi-omics analysis: uncovering the role of *Hydrogenophaga* strains in xylene degradation

Sinchan Banerjee¹ · Anna Bedics¹ · Péter Harkai² · Balázs Kriszt² · Nagaraju Alpula^{1,3} · András Táncsics¹

Received: 23 June 2021 / Accepted: 20 December 2021 / Published online: 6 January 2022
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Abstract

To develop effective bioremediation strategies, it is always important to explore autochthonous microbial community diversity using substrate-specific enrichment. The primary objective of this present study was to reveal the diversity of aerobic xylene-degrading bacteria at a legacy BTEX-contaminated site where xylene is the predominant contaminant, as well as to identify potential indigenous strains that could effectively degrade xylenes, in order to better understand the underlying facts about xylene degradation using a multi-omics approach. Henceforward, parallel aerobic microcosms were set up using different xylene isomers as the sole carbon source to investigate evolved bacterial communities using both culture-dependent and independent methods. Research outcome showed that the autochthonous community of this legacy BTEX-contaminated site has the capability to remove all of the xylene isomers from the environment aerobically employing different bacterial groups for different xylene isomers. Interestingly, polyphasic analysis of the enrichments disclose that the community composition of the *o*-xylene-degrading enrichment community was utterly distinct from that of the *m*- and *p*-xylene-degrading enrichments. Although in each of the enrichments *Pseudomonas* and *Acidovorax* were the dominant genera, in the case of *o*-xylene-degrading enrichment *Rhodococcus* was the main player. Among the isolates, two *Hydrogenophaga* strains, belonging to the same genomic species, were obtained from *p*-xylene-degrading enrichment, substantially able to degrade aromatic hydrocarbons including xylene isomers aerobically. Comparative whole-genome analysis of the strains revealed different genomic adaptations to aromatic hydrocarbon degradation, providing an explanation on their different xylene isomer-degrading abilities.

Keywords Xylene · *Hydrogenophaga* · Groundwater · Aromatic hydrocarbons · Biodegradation · Catechol 2,3-dioxygenase

Responsible Editor: Robert Duran

✉ András Táncsics
tancsics.andras@uni-mate.hu

¹ Department of Molecular Ecology, Institute of Aquaculture and Environmental Safety, Hungarian University of Agriculture and Life Sciences, Gödöllő, Hungary

² Department of Environmental Safety, Institute of Aquaculture and Environmental Safety, Hungarian University of Agriculture and Life Sciences, Gödöllő, Hungary

³ Department of Biotechnology, Microbial Biotechnology Research Unit, Kakatiya University, Warangal, India

Introduction

Xylene is one of the volatile organic compounds composed of a central benzene ring with two methyl groups attached as substituents, arranged in three various positions to create three distinct isomers: *m*-xylene, *o*-xylene, and *p*-xylene (Marshall and Rodgers 2008; Kandyala et al. 2010; Yan and Zhou 2011). It is a toxic monoaromatic hydrocarbon and is highly mobile in the environment, either in the gaseous, liquid, or solid phase (Mazzeo et al. 2013). Among BTEX compounds, xylene is most commonly used as a solvent in industrial coatings and the petrochemical industry (Boon-saner et al. 2011). Only in the USA, the amount of xylenes (mixed isomers) released in 2012 from all factories was 7,482,435 kg (including disposal and release on-site and

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