New Genes for Monitoring of In Situ Remediation of Aromatic Hydrocarbons: Outdoor Mesocosm Study

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Aromatic and polyaromatic hydrocarbons are listed as priority soil and groundwater pollutants. Microorganisms have evolved specialized pathways to use the aromatic compounds as their carbon and energy source. The aerobic degradation is usually initiated by activation of the aromatic ring through oxygenation reactions and formation of a few dihydroxylated central intermediates - substrates for extradiol ring-cleavage dioxygenases, which are key enzymes in the degradation of aromatic compounds. Our research is focused on the metagenomic organization and diversity of the extradiol dioxygenase genes and *meta*-cleavage pathways, active in highly contaminated with mono- and polyaromatic hydrocarbons environments. So far, only bacteria, fully equipped with genes for complete degradation of the pollutant of interest were isolated. Moreover, the laboratory cultivation techniques cannot reproduce the natural environmental conditions. It can be anticipated that significant fraction of so far unknown bacteria may possess novel genes or pathways.

Functional metagenomic approach was used to identify new genes involved in the metabolism of aromatic hydrocarbons in soil contaminated with jet fuel. High-throughput sequencing revealed a different from the so far known archetypal organization. The new genes belong to the uncultivable soil bacterial population and count for over 55% of the extradiol ring-cleavage dioxygenases detected in the particular soil. These key catabolic genes were used for monitoring of the biodegradation capacity of the microbial communities and for evaluation of the intensity and efficiency of the remediation treatment in situ.

The only reliable experimental system for exploring the genes distinctive for the biodegradation potential of the soil microbial population is the outdoor mesocosm cultivation using soil from real contaminated sites. Jet fuel was introduced to increase the TPH concentration over 20 000 mg kg^{-1} dry soil. A remedial response of the microbial community was detected by the increase of both bacterial and catabolic gene abundance. It corresponded to a faster reduction of the PAH compounds when compared to the control mesocosms. Polymerase chain reaction and high-throughput sequencing were applied for detection of new extradiol dioxygenase genes and their transcripts during the cultivation, and for identifying concomitant taxonomic changes in the soil population.

The outdoor mesocosm study and verification of the novel dioxygenase genes activity in situ was carried out to support our hypothesis that the high contamination is a driving factor for evolution of the biodegradation potential of the autochthonous microbial communities.