

Appetite for danger - genetic potential for PCP degradation at historically polluted groundwater sites

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Pentachlorophenol (PCP) is a priority pollutant of exclusively anthropogenic origin. Formerly used commonly in timber preservatives, PCP has persisted at polluted groundwater sites decades after its use was banned, typically as the last detectable contaminant component. Notorious for its toxicity and poor biodegradability, little is known about the genetic potential and pathways for PCP degradation in the environment. The only fully characterized mineralization pathway is initiated by the enzyme coded by chromosomal *pcpB* gene, previously detected in PCP degrading Sphingomonadaceae bacteria isolated at two continents. However, there is no information about the abundance or diversity of any PCP degradation related gene at contaminated sites in situ.

Our aim was to assess whether *pcpB* and/or sphingomonads seem to play a role in in situ degradation of PCP, by studying whether *pcpB* i) is detectable at chlorophenol-polluted groundwater sediments, ii) responds to PCP concentration changes, and iii) shows correlation with the abundance of sphingomonads or a specific sphingomonad genus. Novel protocols for quantification and profiling of *pcpB*, with primers covering full known diversity, were developed and tested at two sites in Finland with well-documented long-term chlorophenol contamination history: Kärkölä and Pursiala. High throughput sequencing complemented characterization of the total bacterial community and *pcpB* gene pool.

The relative abundance of *pcpB* in bacterial community was associated with spatial variability in groundwater PCP concentration in Pursiala, and with temporal differences in groundwater PCP concentration in Kärkölä. T-RFLP fingerprinting results indicated and Ion Torrent PGM and Sanger sequencing confirmed the presence of a single phylotype of *pcpB* at both geographically distant, historically contaminated sites, matching the one detected previously in Canadian bioreactor clones and Kärkölä bioreactor isolates. Sphingomonad abundance generally correlated positively with *pcpB* abundance. Sphingomonad and *pcpB* ranges in the same sample were comparable regardless of differences in sphingomonad community composition between different groundwater wells.

These first cultivation-independent results of *pcpB* abundance and diversity at contaminated sites indicate that *pcpB* confers competitive advantage in environments contaminated with the priority pollutant PCP and may be related to its degradation in situ; its relative abundance amongst bacteria reflected PCP concentration, and it seemed to be widely shared among various sphingomonad genera. Interestingly, decades under contamination pressure have led to no diversification of *pcpB* gene at these sites, suggesting constrained distribution and evolution of genetic potential for PCP degradation. It remains open whether the present form of the gene or pathway is the single most efficient one in these environments, or whether inoculation with diverse degrader strains isolated elsewhere could further enhance PCP bioremediation.