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Quantification of phenoxyalkanoic acid degraders and investigation of PCR inhibiting effects in aquifer sediment by real-time PCR

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Detection of microbial genes using PCR is challenging in oligotrophic environments like aquifer sediment due to low amounts of target DNA and the presence of compounds inhibitory to PCR. The aim of the study was to detect and quantify phenoxyalkanoic acid degraders in MCPP polluted aquifer sediments, in which MCPP degradation has been shown, and microcosms by real-time PCR, to elucidate which are the mineralizing microorganisms and to investigate the presence of PCR inhibiting compounds.

DNA was extracted from 50 sediment samples using bead beating combined with freeze-thawing. The selected target genes were *tfdA*, *tfdB* and *tfdC* involved in 2,4-D degradation, and *sdpA* and *rdpA* involved in MCPP degradation, testing multiple primers. 50 microcosm enrichments for MCPP degraders from the sediment were set up to achieve higher copy numbers of target genes. To elucidate which are the mineralizing microorganisms in microcosms 16S rDNA from DGGE gels was sequenced. In order to investigate the presence of inhibiting compounds, sediment extracts spiked with DNA-extracts from a culture containing the *gfp* gene at two different concentrations were used in PCR with *gfp* specific primers. Also, the absorbance at 400 nm was measured spectrophotometrically in the DNA extracts as indicator for presence of humic acids.

tfdA was most efficiently detected using a newly designed primer set and *tfdA* copy numbers up to 10^3/g in three samples was observed. Sequencing of sediment PCR products revealed 100% homology to *Ralstonia eutropha* JMP134 *tfdA*. *tfdC* was detected in the same three sediment samples, whereas *tfdB, sdpA* and *rdpA* were below detection limit in all samples. The concentration of *tfdB* and *tfdC* increased in 3 microcosms, while *tfdA* was not detected. 16S rDNA extracted form microcosms sediment showed highest similarity to *Arthrobacter*, *Acinetobacter* and *Micrococcus* genera. However, due to co-extracted impurities the PCR signal was significantly inhibited (t-test, P=0.95) up to 100% in 15 of 50 samples at low *gfp* concentration (10^3 *gfp* copies/PCR) and in 4 of 50 samples at high *gfp* concentration (10^6 *gfp* copies/PCR). High absorbance at 400 nm (humic acids) was observed in 1 sample, which also was the only sample showing a complete inhibition of PCR.

Screening of different primers and target genes allowed the detection and quantification of *tfdA* and *tfdC* in aquifer sediment by real-time PCR, which shows that it is possible to quantify specific phenoxy acid degraders even under these difficult subsurface conditions. Absence of *tfdA*, an increase of *tfdB,* and *tfdC* in only 3 microcosms, and presence of genera not typical in phenoxy acid degradation, suggests that MCPP degraders in the aquifer sediment may differ from known phenoxy acid degraders and thus may not contain known MCPP degradation genes. The investigation of inhibiting compounds in the sediment revealed that when the amount of artificially spiked target genes (*gfp*) in a sample was low, the presence of inhibiting compounds had a larger impact on PCR than when the concentration of target genes was high. One sample contained high concentration of substances with absorption spectra like humic acids and in this one sample total PCR inhibition was found. However, in all samples but this one, inhibition was not severe enough to prevent detection by PCR, which shows potential for the use of real-time PCR in this environment using suitable primers. However, inhibition may contribute significantly to the uncertainty of real-time PCR results.