Resumē

Resumē

Fossil fuels are currently the primary industrial energy source on Earth. They are principally composed of complex hydrocarbons in either long-chain or cyclic conformation. Industrial use of petroleum, diesel, oil, tar and other coal-derived products inevitably leads to pollution of the environment. The most serious pollution is caused by polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs) that are not easily removed from soil after a spill. Long-chain and cyclic conformation makes fossil fuel hydrocarbons difficult to break down. However, certain free-living soil microorganisms have adapted to utilising these PAHs/PCBs as a source of energy. In many cases, their efficacy is greatly enhanced by the presence of plants. By inhabiting the rhizosphere, microbes form a mutualistic relationship with the plant, receiving nutrients from it and in return providing a less polluted environment in which the plant can grow. The purpose of this study was to elucidate some of the microbial population diversity in PAH/PCB-polluted soils in South Africa through the use of denaturing gradient gel electrophoresis (DGGE).

In an initial study, DGGE was employed to separate soil communities in polluted and unpolluted soils into a genetic fingerprint, the main bands of which were sequenced and subjected to a BLAST analysis through a database for possible identification of species present. Phylogenetic and distance studies indicated that unpolluted soils have a far greater species diversity. It thus was evident that PAH/PCB pollution of

soil leads to a decrease in microbial diversity by selecting for microorganisms with the ability to activate metabolic pathways allowing them to utilise the pollutants as an alternative source of carbon.

Population diversity of pro- and eukaryotes found within polluted and non-polluted soils was compared. DGGE was employed to determine the genetic fingerprint of each population. Following this, dendogram analyses based on Shannon indices were done to determine PAH breakdown potential of prokaryotic vs. eukaryotic communities. A higher diversity and better adaptation potential were evident within prokaryotic than eukaryotic communities in pollution-stressed environments, indicating that the prokaryotic component of these samples had the greatest PAH-metabolism potential.

To determine the capacity for PAH/PCB metabolism by the organisms within the soil samples being studied, the presence of *xylE* and *ndoB* genes, responsible for toluene/xylene and naphthalene biodegradation, respectively, was determined. DGGE was performed to analyse genetic diversity between these two genes, based on community fingerprints. Polluted soil communities tended to have comparable community diversity within their functional genes, depending on their physical situation, plant species proximity and soil conditions. In general, soil contained indigenous microbes with a high natural potential for biodegradation of PAHs/PCBs.

A portion of the 16S gene of eight bacterial isolates representing the most dominant culturable taxa in the polluted soils was sequenced and analysed for identification purposes. These identifications were conducted in conjunction with the use of the

catabolic gene probes *xylE* and *ndoB* to establish the hydrocarbon degrading capacity of the isolates. *Pseudomonas*, from the rhizosphere of *Cyperus esculentus*, was the most common PAH-degrading genus found in this study. Considering the well-established rhizosphere competence and PAH-degrading capacity of *Pseudomonas*, this genus seems to be the best suited for bioaugmentation purposes in South Africa.

The presence of the *nifH* gene, the general marker gene of nitrogen-fixing bacteria in communities from unpolluted and polluted soils, was determined. It was hypothesised that bioremediation could be enhanced by nitrogen addition to polluted environments. Nested-PCR of the *nifH* gene was conducted on a diagnostic basis and was followed by DGGE of the product to determine the functional gene diversity within pollution-dwelling, nitrogen-fixing bacterial communities. Nitrogen-fixing microorganisms were present in all the soils sampled but, in only 80% of the pure cultures isolated from polluted and unpolluted soils and rhizospheres. Although different rhizospheres and pollutants were examined, it was found that of the polluted soils studied, most *nifH* gene diversity of polluted soils existed within machinery oil polluted, wood chip mulched, non-rhizosphere soil. Thus, it would appear that the more polluted the soil the higher the free microbe nitrogen fixation diversity possibly due to environmental stress.