Chapter 6

Conclusion

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The purpose of this study was to evaluate the DGGE technique for determining microbial diversity in PAH/PCB-polluted soils. The technique proved to be appropriate and gave a good indication of different diversities, i.e. pro- and eukaryotes, across different gene groups viz. 16S, *ndoB*, *xylE* and *nifH*. A great deal of work has been done on determining bacterial soil diversity based on 16S DGGE analysis because 16S rRNA genes are most commonly used to give an overall indication of the bacterial species composition of a sample. A partial sequence of this gene from soil was analysed by means of DGGE by Throbäck *et al.* (2004). However more complex functional genes have also been targeted for determining microbial diversity among specific communities. Milcic-Terzic *et al.* (2000) successfully isolated diesel, toluene and naphthalene-degrading microbial consortia from diesel-polluted soils by screening for the presence of *xylE* and *ndoB* from petroleum and diesel-polluted soils using PCR with gene-specific primers. Similarly, the general marker gene in nitrogen-fixing bacteria has been targeted for community analysis by Rosado *et al*. (1998). They successfully used *Paenibacillus azotofixans nifH* species-specific primers in DGGE analyses of soil samples. In addition to this, Zehr and McReynolds (1989), Simonet *et al*. (1991), Widmer *et al*. (1999) and Yeager *et al.* (2005) successfully designed four new sets of degenerate primers for universal targeting of the *nifH* gene in microorganisms. All of these PCR-DGGE applications proved to yield valuable microbial diversity information.

This study revealed a great deal of information concerning microbial diversity, with respect to species and functional genes, in different soil environments. Representatives of South African soils were found to have a higher general microbial rhizosphere diversity than found in non-rhizosphere soils. This finding supports previous studies in the tropics, where it was established that plant roots play an important role in rhizoremediation of PAH/PCB-polluted soils by creating a haven for microorganisms involved in the process (Merkl *et al.* 2005). It has also been globally shown that the mere presence and diversity of these microbes directly improve the bioaugmentation of PAHs/PCBs in polluted soil (Glick 2003). DGGE was ideal for the analyses conducted in this study, as it is capable of screening multiple samples and genes, which can then be analysed simultaneously yielding many different types of information about the environments studied. Smalla *et al.* (1998), Marshall *et al*. (2003), Zuccaro *et al*. (2003) and Foucher *et al.* (2004) made use of several probes designed for PCR-DGGE in the analysis of different microbial rhizosphere communities, and found that it revealed far more community data than more conventional methods such as for example BIOLOG. In accordance with international literature (Milcic-Terzic *et al.* 2000), the *xylE* and *ndoB* genes identified diesel, toluene and naphthalene-degrading microbial consortia from PAH/PCB polluted soils in South Africa. These genes were targeted in order to assess the bioremediation potential of microbial consortia in petrol, diesel and crude oil-polluted soils (Greer *et al*. 1993). Finally, nitrogen-fixing capacity and species diversity in South African PAH/PCB polluted soil was successfully determined by targeting the *nifH* gene in DGGE analysis as supported by Rosado *et al*. (1998).

This thesis constitutes the first study of its kind in South Africa and has provided the basis for further more in-depth environmental microbial diversity studies. Now that the effectiveness of DGGE as a technique for assessing microbial diversity in the stressed environment of PAH/PCB polluted soils has been established, it can be applied to many more environments. Further such studies should include a higher number of samples across a wider range of environments, pollutants and other factors. South Africa has many unique environments, some of which are extreme, in which microbial diversity has not yet been studied, e.g. man-made vs. natural, urban vs. rural, desert vs. sub-tropical, industrial, mines, various types of underground sites, hot water springs, warm and cold ocean currents. The understanding of microbial community diversity, interaction and response to different environments is paramount to the application of microbes for the good of mankind. Until recently, the wealth of microbial diversity in the environment seemed immeasurable but with the application of techniques such as DGGE, considerably more data can be acquired to better understand microbes and their habitats.