

Molecular techniques for the detection of waterborne pathogens

Cloete T E and Theron J.

Department of Microbiology and Plant Pathology, University of Pretoria
Pretoria, South Africa

Detection of pathogenic organisms provides information as to the safety and public health risks associated with a given water supply. In response, molecular techniques are being and have been developed as means to identify microorganisms in water. The basic underlying principle of DNA fingerprinting is the generation of one-dimensional patterns of macromolecules or fragments from isolates and the reference strains the isolates are considered to be related to. The patterns should neither be too complex to prevent analysis nor too simple to obscure actual genomic dissimilarities. Providing the potential for discrimination, isolates and reference strains that exhibit a high degree of pattern similarity can be considered related. Electrophoretic analysis of whole cell proteins have been used less extensively due to problems associated with the interpretation of the data caused by effects such as the composition of growth media on the protein banding pattern and the superposition of proteins. The different polymorphism-based procedures are generally coupled to a PCR reaction. In the amplified ribosomal DNA restriction analysis (ARDRA) technology, PCR-amplified 16S rRNA genes are digested with restriction endonucleases and the resulting fragments separated electrophoretically. Presence or absence of the restriction site within two strains will cause differences in the length of the DNA restriction fragments and the complexity of the pattern depends upon the number of target sequences and position of restriction sites. Comparison of the generated patterns to those obtained from a database will allow assignment of isolates to species or species clusters in those cases where the banding patterns are highly similar. The separated DNA fragments may also be transferred to filters for hybridization with probes specific for an organism of interest. Two other protocols for generating DNA fingerprints use a single primer to amplify fragments with PCR before examination on agarose gels. PCR amplification of repetitive extragenic palindromic sequences (REP-PCR) takes advantage of repetitive sequences found in the microbial genome. In the randomly amplified polymorphic DNA (RAPD) or arbitrarily primed PCR technology, a short oligonucleotide primer (about 10 nucleotides), usually with random sequence that is not specific for a

particular gene is used as a primer to amplify fragments. These methods yield DNA fingerprints comprised of multiple, differently sized DNA amplification products following separation by gel electrophoresis. One of the most promising technologies for microbial source tracking is, however, amplified fragment length polymorphism (AFLP) analysis. AFLP analysis appears to have the same taxonomic range as other fingerprinting techniques, but this technology combines several advantages of these different techniques, which in most cases results in the highest power of discrimination. This technology is based on the selective amplification of a subset of genomic restriction fragments using PCR. For AFLP, purified genomic DNA is digested with two restriction enzymes, one with an average cutting frequency and a second one with a higher cutting frequency after which oligonucleotide adapters are ligated to the genomic DNA restriction fragments. The sequence of the adapters and the adjacent restriction site serve as oligonucleotide primer binding sites for subsequent amplification of the restriction fragments by PCR. Selective nucleotides extending into the restriction fragments are added to the 3' ends of the adapter-specific PCR primers such that only a subset of the restriction fragments are recognized and amplified. The subset of amplified fragments is then analysed by denaturing polyacrylamide gel electrophoresis to generate the fingerprint. Since relatively small amounts of DNA are digested and detection of AFLP fragments does not depend on hybridization, the AFLP analysis method is more reproducible and robust than other fingerprinting techniques and displays more fragments than other fingerprinting techniques.