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**MOLECULAR SYSTEMATICS AND GENETIC FINGERPRINTING OF  
HYDROCARBON-DEGRADING BACTERIA IN FUEL SAMPLES**

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Microbial spoilage of hydrocarbon fuels is a widespread problem for the oil industry. The inability to grow contaminants and complex taxonomy of many bacterial species often limits the effective identification of the spoilage organisms by routine microbial culture. Molecular techniques provide a rapid and effective means to overcome the latter problems. We developed a strategy to examine the bacteria associated with fuel spoilage that combined cultivation with: (a) genetic fingerprinting to identify strains associated with contamination, and (b) 16S rRNA gene sequence analysis to facilitate their systematic identification. A total of 30 samples of contaminated fuels from a range of sources were analysed. PCR fingerprinting of single colonies was performed by Random Amplified Polymorphic DNA (RAPD) analysis enabling a collection of genetically unique contaminating strains to be assembled. This avoided unnecessary repetitive analysis of the same strain within a given sample and from an industrial perspective, the genetic fingerprinting also formed a means to track single strains and to identify the source of contamination. 16S rRNA gene sequencing of 65 cultivable strains identified the following distribution of contaminating bacteria: 83% were Gram negative including 15 *Pseudomonas sp.*, 1 *Serratia sp.*, 3 *Marinobacter sp.*, 8 *Burkholderia cepacia* complex *sp.*, 1 *Shewanella sp.* and 1 *Pantoea sp.*, and 17% were Gram positive including *Bacillus sp.*, and *Staphylococcus sp.* In summary, a combined approach of cultivation and molecular analysis of bacterial fuel contamination revealed a diversity of causative species. The techniques show possibilities for tracing problematic sources of contamination and for development of rapid identification tests and novel strategies to prevent biofouling.