Genetic diversity among petroleum hydrocarbon degrading bacteria isolated from crude oil and oily sludge contaminated sites

Contamination of soil by crude oil in India is a major concern considering the huge network of oil pipelines that transport crude oil to and from various refineries. Crude oil spills occur due to leakage from joints of the crude oil pipelines, accidents of oil tankers during transportation of crude oil, anthropogenic and pilferage activities. Oily sludge (hazardous hydrocarbon waste generated by petroleum refineries) contamination is also a major environmental concern since many of its constituents are highly toxic, carcinogenic and are poorly biodegradable in nature. Petroleum hydrocarbons in nature are degraded by diverse group of microorganisms, which are capable of utilizing hydrocarbons as sole source of carbon and energy. Documentation of bacterial diversity of a hydrocarbon-contaminated site is essential because it helps in isolation and identification of novel bacterial strains having capability to degrade wide range of the recalcitrant compounds of crude oil and oily sludge.

The present investigation encompassed an approach where the bacterial diversity of 7 geoclimatically different crude oil and oily sludge contaminated sites (five oil refineries and two oil exploration sites) was documented. Age of hydrocarbon contamination at these sites also varied. Diversity was evaluated by cultivation based and gene cloning approaches. A total of 159 cultivable hydrocarbon degrading bacterial strains were isolated by enrichment cultures techniques. Biochemical characterization of the bacterial strains was studied based on Biolog microbial identification (substrate utilization profiles). Of the total bacterial strains isolated, both Gram negative and Gram positive bacteria were obtained but Gram negative bacterial strains were dominant. Identification of bacterial strains was performed by sequencing the genes encoding 16S rRNA. Bacterial strains were predominantly affiliated to g subclass of proteobacteria. Genus Pseudomonas and Acinetobacter were the most frequently occurring genus at all the sites. Of the total Pseudomonas sp. isolated from different sites, P. citronellolis were the most dominant strains at all seven contaminated sites. Bacterial strains showed total petroleum hydrocarbon degradation in a range from 20 % -95 %. Genetic diversity was evaluated in the bacterial strains by whole genome analysis with repetitive DNA sequences (ERIC, REP and BOX) based DNA fingerprinting and PCR based ribotyping. Bacteria from different sites showed heterogeneous genomic profiles elucidating a distinct genetic diversity among them. Presence of unique amplicons distinguished bacterial strains isolated from specific geoclimatic locations. Bacteria isolated from diverse sampling sites were showing similarities in their rep-PCR profiles(suggesting that strains were regionally endemic). rep-PCR fingerprints were not species specific since a species isolated from different sites was also showing variation in the genomic DNA fingerprints. This suggested the existence of intraspecies diversity. The ribotype profiles discriminated the strains based on the 16S-23S-rDNA internally transcribed spacer polymorphism.

Intraspecies genetic diversity was studied in the 29 strains of Pseudomonas citronellolis isolated from different hydrocarbon contaminated sites. Intraspecies diversity was evaluated by rep-PCR DNA fingerprinting, fluorescent amplified fragment length polymorphism, amplified 16S ribosomal DNA restriction analysis, PCR based and restriction fragment length polymorphism of 16S-23S rRNA internally transcribed spacer. The rep-PCR fingerprinting delineated the 29 P. citronellolis strains into 12 genotypic groups distributed among 7 distinguishable ribotype patterns. The rep-PCR genotypes were specific to isolation site of strains. Genotypic distinctions of 29 different P. citronellolis strains isolated from different sampling sites reflected the difference in location of sampling sites with respect to geo-climatic regions of India. The fact that soil at these sites was contaminated with different types of crude oil and oily sludge may also be a contributing factor. Strains of P. citronellolis isolated from Digboi refinery were most genotypically diverse which could be because soil at Digboi refinery was contaminated with crude oil and oily sludge for nearly 100 years, allowing for genotypic diversification. Ribotypes of P. citronellolis strains showed multiple amplicons that strongly indicated the polymorphism of rRNA spacer region. ARDRA clustered 29 strains of P. citronellolis into XIV genotypic groups. ARDRA showed the differential distribution of the strains in the genotypic groups, which was not related to the isolation site of the strain. ARDRA genotypic clusters had heterogeneous distribution of the P. citronellolis strain. Distinct ARDRA patterns of 29 P. citronellolis strains could be due to increased mutation under stress, facilitating adaptation of strains to stressful environments. This could also be due to the different types of crude oil
and oily sludge contamination and varying age of contamination at the sites. FAFLP segregated 29 *P. citronellolis* strains into 8 genotypic clusters, which were specific to isolation site of strains. The 16S rRNA gene cloning approach detected presence of different bacterial species and non-culturable bacterial population at the crude oil and oily sludge contaminated site, which were not obtained by the cultivation based studies. Of the 50 16S rDNA clones randomly analyzed by sequencing, 31 unique sequences were identified. 9 of the 16S rDNA sequences of the clones gave the closest sequence match to uncultured bacteria which were mostly belonging to proteobacteria. 6 of the 16S rDNA clone sequences were giving homology match with the yet un-identified bacteria.