16S rDNA phylogenetic and RAPD-PCR analysis of petroleum polycyclic aromatic hydrocarbons degrading bacteria enriched from oil-polluted soils

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Abstract:

Abstract Petroleum polycyclic aromatic hydrocarbons (PAHs) are health risks to human, as they can be toxic, mutagenic and carcinogenic. As the Kingdom of Saudi Arabia is one of the major petroleum-producing countries, they inevitably suffer from worsening this environmental problem. Therefore, removal of these compounds from the environment is a necessity for ensuring human health. In this study, 54 bacterial isolates were obtained by enrichment techniques from oil-contaminated soil samples. Out of them, seven gram-negative bacterial strains, KKU-J1, KKU-J2, KKU-J4, KKU-J7, KKU-J9, KKU-J14 and KKU-J17, exhibiting ability for petroleum PAHs degradation were selected. The isolates showing the highest growth during screening as demonstrated by the increase in their optical densities (OD600) and a concentration-dependent growth in all examined PAH compounds that grew in it, with strains KKU-J2, KKU-J7 and KKU-J17 were the best. The highest optimum growth rate of 0.333±0.0, 0.364±0.0160, 0.333±0.0, 0.364±0.016 and 0.357±0.004(OD600)0.357±0.004(OD600) was recorded for the strains KKU-J2, KKU-J7 and KKU-J17, respectively, when the level of phenanthrene was 100 mg/l. On the other hand, strain KKU-J2 was found to be the best one (0.413±0.00.413±0.0) when the level of naphthalene was 100 mg/l. Molecular identification of the selected isolates was detected based on 16S rRNA gene amplification and partial sequence determination. Alignment results and the comparison of 16S rRNA gene sequences of the isolates to the 16S rRNA gene sequences available in GenBank database, as well as the phylogenetic analysis, confirmed the accurate position of the isolates as Sphingomonas paucimobilis KKU-J1, Pseudomonas alcaligenes KKU-J2, Micrococcus antarcticus KKU-J4, Arthrobacter oxydans KKU-J7, Stenotrophomonas rhizophila KKU-J9, Kocuria rhizophila KKU-J14 and Shinella zoogloeoides KKU-J17. RAPD–PCR fingerprinting was carried out for the seven isolates, and the DNA patterns revealed that there is no correlation between the RAPD profile and geographic origin sites where these isolates were collected from. This study indicates that the contaminated soil samples contain a diverse population of PAH-degrading bacteria, and the use of soil-associated microorganisms could be recommended for PAHs bioremediation in the environment.

Keywords:

Enrichment culture Isolation 16S rRNA gene sequencing Phylogenetic analysis Petroleum PAHs-degrading bacteria Oil-polluted soils

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