ABSTRACT

The increased generation of petroleum hydrocarbon waste has been stated as one of the most critical environmental problems. Though microbial bioremediation has been widely used for waste treatment but their application in petroleum hydrocarbon waste treatment is limited since they have toxic effects on the microbial cells.

In the present research work, soil samples from the petroleum contaminated sites were screened for petroleum hydrocarbon degrading microbes. Among the thirty two petroleum degrading bacteria, PS11 strain was selected for further work on the basis of highest zone of crude oil utilization. Phenotypic characterization and phylogenetic analysis confirmed strain PS11 to be *Geobacillus stearothermophilus*. The bacterial strain was able to grow in presence of petroleum hydrocarbons as evidenced by increase in cellular dry biomass after 6 h of incubation. However, PS11 cells exhibited a delayed growth pattern inthe presence of crude oil in the growth medium in comparison to those growing in absence of crude oil. The strain was also capable to grow in presence of wide range of other hydrophobic solvents with log *P*-values between1-4,whereasalcohols having very low log *P*-value had inhibitory effect on growth. Transmission electron micrograph of PS11 cells grown in the presence of 10% (v/v) crude oil for 24 h showed convoluted cell membrane and accumulation of crude oil in the cytoplasm, indicating the adaptation of the bacterial strain to the petrol. The mechanism of solvent tolerance of PS11 was ascertained by gas chromatography analysis of metabolic transformation products of crude oil and other solvents as well as membrane phospholipids. Results showed that PS11 cells growing in presence of crude oil (10 % v/v) for 15 days could degrade both alkanes and aromatic compounds. It degraded aromatic compounds more readily than alkanes. The membrane phospholipids composition of *G. stearothermophilus* PS11 was also altered in the presence of crude oil. Decrease in phosphatidylethanolamine (PE,11%) and phosphatidylglycerol (PG,14%) paralleled increase in cardiolipin (DPG, 15%) and diphosphatidylglycerol (PGL,128%) in presence of crude oil was noted. Significant changes were also observed in the membrane fatty acid. Similarly, an increase (22.4%) of the iso-fatty acids was noted in cell membranes adapted to 10% (v/v) crude oil with

concomitant decrease of the anteiso-fatty acids (17.6%) and straight-chain saturated fatty acids (17.8%). Hence, the solvent adaptation property of *G. stearothermophilus*PS11 seems to be related to both restoration of membrane fluidity and metabolic transformation of hydrophobic solvent to less toxic products.

Furthermore, the role of plasmid DNA in petroleum hydrocarbon degradation property of PS11 was determined. For this plasmid DNA was cured from the bacterial cell by treating with acridine orange. Plasmid cured culture of PS11 were unable to grow in presence of crude oil or any of the solvents indicating the involvment of plasmid encoded gene(s) in petroleum hydrocarbon degradation. The cured plasmid was subsequently transformed into competent*Escherichia coli*JM109 cells. The resultant transformants gained the ability to grow in presence of catechol, a common intermediate of aromatic hydrocarbon meta-degradation pathway. The role of plasmid in petroleum hydrocarbon degradation was further confirmed by PCR amplification of one of the gene of aromatic hydrocarbon metabolic pathway i.e. catechol 2, 3 dioxygenase, using primers designed from the sequence conserved region and plasmid DNA as template, which yielded aPCR product ofabout 900 bp.Homology analysis of the deduced amino acid sequence with the known catechol 2, 3 dioxygenase genes from other bacteria by BLAST programrevealed highest similarity score with *Geobacillus stearothermophilus* DSMZ6285 strain.

*Geobacillusstearothermophilus*PS11 was further employed for enzyme catalyzed biodiesel production. For this, an extracellular, organic solvent tolerant, alkaline lipase from isolated PS11 cells was produced. Optimization of production conditions by OVAT approach enhanced the lipase production by 2.46 folds. The 27KDa lipase was purified by 8.04 fold with 22.6% yield. Optimum pH for lipase was 10. It showed 100% stability in the pH range 8 to 11 for 2h. The enzyme showed maximum activity at 50°C and retained 50% activity at 70°C for 2h. Lipase was stable in presence of wide range of solvents including BTEX, methanol etc. Gas chromatography result confirmed the catalytic activity of lipase in biodiesel production by enzymatic trans-esterification of vegetable oils in presence of methanol.

Thus, *Geobacillusstearothermophilus*PS11 could serve as a potential tool for biodegradation of petroleum hydrocarbons and biodiesel production.