RESEARCH PAPER

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Plasmid incidence and utilization of kerosene by hydrocarbonoclastic fluorescent pseudomonads isolated from local soil

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Environmental concerns have led to the need of sustainable use of natural resources. It is important to use natural bacterial population for environmental cleaner techniques. One of those strategies is biodegradation of natural or man made xenobiotic compounds. The present investigation is on isolation of hydrocarbon utilizing pseudomonads from local red soil. The isolates were identified to species level by performing biochemical and physiological tests as *P. aeruginosa*, *P. aureofaciens*, *P. putida* and *P. fluorescens*. The isolates were screened for the utilization of petroleum hydrocarbon (kerosene) and the conditions (temp and pH) optimum for its utilization. The isolates were screened for their ability to utilize petroleum hydrocarbon (kerosene) as their sole source of carbon and energy. Biodegradation results revealed that, the highest growth was showed by *P. putida* followed by *P. fluorescens*, *P. aureofaciens* and *P. aeruginosa*. The results evidenced that, all the four isolates harbored two low molecular weight plasmids one with 3Kb and the other with 10kb to 12Kb.

Key words : Hydrocarbonoclastic bacteria, Fluorescent pseudomonads, Kerosene utilization, Plasmids.

INTRODUCTION

Environmental pollution is a cause of major concern affecting ecosystems globally. Fuels are significant pollutants of soils and ground water because of leaks of under ground storage tanks and defectiveness of transfer lines (Council on Environmental Quality, 1981). As industrialization expands, petroleum hydrocarbons became a greater potential source of contaminants in the water and soil environments (Margesin and Shinner, 2001). Bioremediation is the newest method of oil spill cleanup and far more effective than any of the mechanical methods used (Desai and Banat, 1997). Microbes are the main degraders of petroleum hydrocarbons in contaminated ecosystems. The most prevalent bacteria that degrade the hydrocarbons belong to the genus *Pseudomonas* (Atlas and Cerniglia, 1995) and it is classified as the most common hydrocarbonoclastic microorganisms in the list of Bossert and Bartha (1984). Interest in pseudomonads has increased because of their possible use in detoxifying chemical wastes through a wide range of enzymatic metabolic activities (Raijimarkers *et al.*, 1995). Ojo (2006) reported the use of native bacterial consortium with petroleum hydrocarbon utilizing capabilities in Southwest Nigeria. Bioremediation of long chain hydrocarbons such as kerosene, diesel and waste oil was reported by Livingston *et al.* (1976). A large kerosene spill in New Jersey was cleaned up by a combination of physical and biological techniques (Dibble and Bartha, 1979). Kerosene utilizing microorganisms belonging to the genus *Mycobacterium* and *Pseudomonas* were reported by Haas *et al.* (1941).The effective utilization of petroleum hydrocarbons *viz*., diesel, kerosene and petroleum waste by *P. aeruginosa* was reported earlier by Modi and Patel (1968).

Plasmids that have been found to harbor genes encoding the transformation of environmental pollutants are known as catabolic plasmids. The plasmid mediated bacterial utilization of various carbon compounds which could be found in the complex mixture of crude oil was also reported earlier (Chakraborthy, 1976). Presence of catabolic plasmids in *P. putida* was reported by Park *et al.* (2003). The present study was carried out to investigate the presence of plasmids in four isolates of hydrocarbonoclastic fluorescent pseudomonads and their biodegradation ability of kerosene oil.

MATERIALS AND METHODS

Microorganisms:

Fluorescent pseudomonads were isolated from soil collected at Acharya Nagarjuna Univesity Campus, Guntur district, Andhra Pradesh, India. For the isolation, fluorescent pseudomonad selective King's B medium (King *et al.*, 1954) with the composition of (g/L^{-1}): Protease peptone – 20.0, Purified Glycerol-15.0, K_2HPO_4-2.5, $MgSO_4.7H_2O$-6.0, Agar agar-20.0 and pH-7.2 was used. A total of fifteen isolates were isolated and among them four abundant isolates were identified to species