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Isolation and Characterisation of Bacteria with Multiple Traits: Hydrocarbon Degradation, Antibiotic-Resistance and Metal Tolerance.

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I. INTRODUCTION

Increasing soil population all over the world has instigated global concerns as enormous quantities of toxic chemicals and heavy metals like cadmium, lead, mercury, petrochemicals, insecticides, polycyclic aromatic hydrocarbons (PAHs) and Chlorophenols are finding their way into the environment, effecting the land and soil, causing soil pollution and thus posing a threat and menace to health and well-being of people and ecosystem. The ubiquitous dissemination, low bioavailability, high perseverance of contamination like poly-hydrocarbon and metals in soil have the potentially destructive effects of human death, envisages to study the biodegradation of PAHs (polycyclic aromatic hydrocarbons) and PACs (polycyclic aromatic compounds). The diversity of micro-organisms that diminish the PAHs/PACs can be utilised in the advancement of bioremediation techniques. The role of metal-tolerant, (PAH) degrading bacteria helps in the biodegradation of organic compounds at miscellaneous polluted sites. The isolation of (PAHs)-degrading bacteria from contaminated soil samples collected from garages and petrol pumps and NCR region was carried out in the present study. Also, the bacterial samples were tested for the tolerance towards 4 heavy metals-arsenic (AS), lead (PB), cadmium (cd), and mercury (Hg) .morphological studies and biochemical tests were conducted to find the genera of the bacterial samples. The study indicated the hydrocarbons were degraded by the isolates P1, P2, P4, P5, P5*, G1, G3. These isolates were also found to be tolerant at a high concentration of metals (arsenic, cadmium, mercury, and lead) as minimum inhibitory concentration (MIC) was also calculated. Antibiotics susceptibility of the isolates was tested against various antibiotics. Thus the study suggests that the isolates identified as pseudomonas aeruginosa, Acinetobacter Baumanii and Klebsiella pneumonia are not only PAH-degrading but metal tolerant and antibiotics-resistant too and are of immense potential for bioremediation of contaminated soils

II. MATERIALS AND METHODS

Collection of samples

The major materials and the standard methods employed in the present study are as follows: Soil sample ,Test tube ,Distilled water ,Pipette ,Petri plate, L-rod, Permananent glass marker, Wire loop, Bunsen burner, Nutrient agar, Bushnell Hass agar, Other routine microbiological equipment .

soil samples were collected from an automobile workshop in Valanchery, malappuram district. The samples were collected in sterilized polythene bags, from a depth not exceeding 6 inches. Polythene bag is well labelled with the name of the site of collection and date before being processed.

Isolation of petrol degrading bacteria

Isolation of bacteria from oil-contaminated soil samples were followed in Erlenmeyer flask containing Bushnell Hass (BH) broth medium. These 50 ml Erlenmeyer flask containing 20 ml BH medium were sterilized by autoclaving at 15 lbs pressure for 20 min at 121°C. 1 g of soil with 1% petrol and a set of the flask containing 1% petrol in BH medium without soil sample were also run as substrate control. All flasks were then incubated at 37°C, 150 rpm for 3 days on orbital rotatory shaker and observed for development of turbidity in the medium. 1 ml aliquot of broth medium from all flasks was serially diluted up to 10 -5 and vortexed for a few minutes. Then, 0.1 ml of these dilutions (10-3, 10-4, 10-5) were spread aseptically on freshly prepared BH agar plates and were incubated at 37°C for 48 hours. The microbial colonies that appeared with characteristics of bacterial morphology were isolated and purified on the BH agar medium.

Screening of petrol degrading ability of isolated bacterial strains

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The isolated colonies were further checked for their growth on Bushnell Hass media containing 5% of petrol. The colonies were aseptically streaked on plates containing BH media and petrol as the only source of carbon, followed by incubation for 2 days (48 hours) at 37°C. The resultant colonies that showed the best growth on the media were selected and identified by using Gram's staining method and by doing biochemical characterization.

MICROSCOPIC EXAMINATION

To differentiate the Gram positive and gram negative nature of the bacterial isolate and also for observing the microscopic morphology the isolate was subjected for Gram staining. Hanging drop method was used to observe the motility character of the pure isolates.

Biochemical And Physiological Examination

The tests include Indole, Methyl Red, voges-Proskauer and Citrate (IMViC)tests, Carbohydrate fermentation test, Oxidation tests, Urease tests and Catalase test.

Determination of minimum inhibitory concentration (MIC) of heavy metals

The isolated colonies were checked for tolerance towards heavy metal. MICs were determined by the plate dilution method against heavy metals Hg, Cu, Pb, and Zn by constantly augmenting the concentration of the heavy metals on BH media plates, which contain 1% petrol till no colonies grew on the plates. The initial concentration that was used was 2mM and thereby, constantly increasing the concentration every time (2000mM for arsenic and cadmium) and (160mM for mercury and lead) on BH media plates. The minimum concentration restricting microbial growth is contemplated as the MIC.

Antibiotic sensitivity of the bacterial isolates

Isolated petroleum degrading and heavy metal resistant isolate were tested for antibiotic resistance and sensitivity, by Kirby- Bauer's disc diffusion method Bauer. The antibiotics used for the test were Tetracycline, Chloramphenicol, Gentamicin, Ampicillin, Cefotaxime and Nalidixic acid. Once incubated for 18 hours, the micro-organism was categorized against antibiotics as sensitive or resistant, depending on the diameter of the zone of inhibition as provided in the standard antibiotic disc chart.

III. RESULT

Several colonies were isolated from the soil of automobile industry. And also the petrol degradation potential of the isolates were studied and quantified. Most of the colonies were mucoid and small. It was found that the isolate had maximum petrol(hydrocarbon) degrading ability .

TABLE 1: COLONY CHARACHTERISTICS

COLONY	SIZE	PIGMENTATION AND COLOR	FORM	ELEVATION
C1	Pinpoint	Off-white	Mucoid	Raised

TABLE 2 :STAINING

COLONY	GRAM STAINING	MOTILITY	CAPSULE STAINING	ENDOSPORE STAINING
C1	Gram positive cocci	Non motille	Non capsulated	Non sporing

The heavy metal tolerance of the isolate was also studied and determined for heavy metals. Antibiotic sensitivity of the isolate was studied by using cefotaxim, gentamycin, ampicillin, nalidixic acid, chloramphenicol and tetracycline. The organism was sensitive to cefotaxim, tetracycline, and chloramphenicol. This shows that these strains had multiple antibiotic resistance. The isolate was identified by biochemical methods.The cleaning or breakdown of petrol or hydrocarbon in the soil or environment by microorganism is called microbial degradation. Biochemical results are given here.

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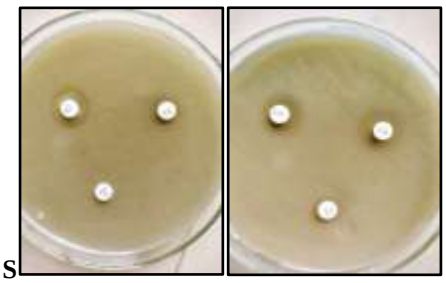
TABLE 3 : BIOCHEMICAL TESTS

COLONY	IMVIC TEST				UREASE TEST	HYDROGEN SULPHIDE TEST	TRIPLE SUGAR TEST	OXIDASE TEST	CATALASE TEST
	INDOLE	METHYL RED	VOGES PROSKAUER	CITRATE UTILIZATION					
C1	–ve	+ve	–ve	+ve	–ve	–ve	–ve	–ve	–ve

TABLE 4 : CARBOHTDRATE FERMENTATION

COLONY	GLUCOSE	FRUCTOSE	LACTOSE	SUCROSE
C1	+ve	+ve	+ve	+ve

SCREENING OF PETROL DEGRADING BACTERIA : STREAK PLATED ON BH AGAR PLATE



S Antibiotic resistance test

IV. DISCUSSION

In the current study, samples of soil were collected from petrol pumps and garages of Valanchery. From these soil samples, an isolate was recovered, which had the hydrocarbon-degrading ability. The screening was done on Bushnell Hass media containing 1% of petrol. The petrol degradation potential of the isolate was studied. The heavy metal tolerance of isolates was also studied and determined for heavy metals lead, copper, Zinc and mercury. Antibiotic sensitivity of the isolates was studied using antibiotics Ampicillin, Tetracycline, Gentamicin, Chloramphenicol, Cefotaxime and Nalidixic acid. This shows that these strains had multiple antibiotic resistance traits The cleaning/breakdown of petrol or hydrocarbon in the soil or environment by bacteria, yeast, and fungi is referred to as microbial degradation (Broomjimans et al., 2009).

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Researchers like (Jan et al., 2003; Atlas, 1992; Yakimor et al., 2007) have shown that mixed population of bacteria with battery of enzymes are capable of degrading hydrocarbon present as contaminant in soil, freshwater, and marine environments through oxygenases which are substrate-specific and act on the hydrocarbon initially. Biodegradation of hydrocarbons can be done either directly by the bacteria by attaching itself to the substrate or through the biosurfactants production (an indirect mechanism) as reported by (Mittal and Singh, 2009). (Das and Mukherjee, 2005, modification of target site, and development of metabolic pathways by bacteria (Kim et al., 2006). A study by Oyetibo et al. (2010) reported heavy metal resistant and antibiotic-resistant among bacterial isolates to gentamycin,rifampicin, and o9loaxcin. The resistance of the organisms to the antibiotics con9irms the correlation between resistance metal ions and antibiotics (Oboh et al., 2006).

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