INTERNATIONAL JOURNAL OF RESEARCHES IN BIOSCIENCES, AGRICULTURE AND TECHNOLOGY
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www.ijrbat.in

ISOLATION, CHARACTERIZATION AND VALIDATION OF LIPID DEGRADATION BY THE BACTERIA FROM WASTE ENGINE OIL CONTAMINATED SOIL

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ABSTRACT:

The bacterial strain were isolated to study the diversity of bacteria from waste engine oil contaminated soil sample which can be useful for the bioremediation of waste engine oil contaminated area. The soil samples were collected from four different places and diluted up to four dilutions and grown on nutrient media. The bacterial isolates were identified by morphological and physical observation, growth on differential media and characterized by performing different biochemical tests. The isolates were analyzed on Tributyrin agar medium.

Key words – Bacterial strain, engine oil, nutrient media and Tributyrin agar medium

INTRODUCTION

At present pollution is considered as a major problem of the world. It could be either organic or inorganic in nature. Quantitative comparison shows that, hydrocarbons are of major concern as an organic pollutant which may be in various forms. The most problem creating environmental organic pollutant is the petroleum derivatives, mostly hydrocarbons.. The most common petroleum derivatives include alkanes, other aliphatic, as well as aromatic compounds and other minor constituents [1, 2].

One of the source responsible for polluting the soil with hydrocarbons is used engine oil. It consists of Petroleum ether or Benzene ,Gasoline, Naphtha, Mineral spirits, Kerosene, Fuel oil, Lubricating oil, Paraffin wax, Asphalt or Tar. Used motor oil typically has much higher concentrations of polycyclic aromatic hydrocarbons (PAHs) than unused motor oil. Naphthalene, a constituent of used engine oil has harmful effects on liver, kidneys, heart, lungs, and nervous system. This used engine oil also contaminates soil, sediments and ground water which can lead to long term hazardous effect due to presence of PAHs.[3]. Impact associated with workshop i.e. seepage of used engine oil includes loss of fertility, water holding capacity, permeability and binding capacity of the soil. [4]

Bioremediation has become an alternative way to degrade oil pollutants from the polluted sites, where the addition of specific microorganism likes bacteria, cyanobacteria, algae, fungi, and protozoa which can improve biodegradation efficiency of polluted soil. [5] These microorganisms can degrade a wide range of target constituents present in oil sludge. [6]Other microorganisms such as fungi are also capable of degrading the hydrocarbons in engine oil to a certain extent. However, they take longer period of time to grow compared to their bacterial counterparts. [7]

This study was conducted to identify and characterize the bacterial isolates which are capable of degrading petroleum product.

MATERIALS AND METHODS

Soil Sample:-

Samples were collected from the three different garage 0r mechanical workshops located in Khapri Naka (Wardha road), Nagpur.viz: Sunil garage, Pratap car washing garage and Papu truck garage

The locations had no grasses growing on them and soil samples were collected at three different locations at each mechanical workshop. The soils were characterized by hardened surfaces and blackish and brownish in colour.

The samples were collected from four different places of Nagpur and serial diluted up to 4 dilutions and by spread plate method on nutrient agar medium .The bacterial isolates so obtained were characterized on the basis of their appearance and physical character and by subculture on nutrient rich media at 37˚C, followed by biochemical tests

Identification of bacterial isolates

Gram staining:-

The different isolates obtained were subjected to Gram staining and the reactions were recorded.

Growth on Differential and selective media:-

The different bacterial isolates were grown on different selective media such as Mannitol salt agar, EMB agar and MacConkey agar for identification

Biochemical tests:-

Biochemical tests were performed by using mother culture of bacterial isolate which were isolated from waste engine oil and diesel rich soil and following biochemical test were performed Carbohydrate Test (Lactose And Dextrose), Citrate Test, Indole Test, Triple Sugar Iron Agar Test, Methyle Red Test, VP Test, Hydrogen Sulphide Test, Urease Test, Gelatin Test, Litmus Test,

Tributyrin egg yolk agar test:-

Tributyrin agar base and egg yolk was prepared and isolated bacterial cultures were streaked on

the medium and incubated for 24 hr at 37˚C in incubator and observed for lipid degradation

RESULTS AND DISCUSSION

The isolates that gave tributyrin test positive were B,C,D,E,G,H ,I. . Tributyrin test is the test for lipid degradation and all the isolates obtained from the engine oil contaminated soil samples gave the tributyrin test positive with egg yolk as the tributyrin rich source. These organisms can be used for degrading oil spills that pose environmental hazards .Based on the biochemical tests for characterization ***Proteus vulgaris*** , ***Pseudomonas aeruginosa*** , ***Staphylococcus aureus*** , ***Micrococcus luteus*** and ***bacillus cereus*** were found to be the organisms involved in lipid degradation

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Table no. 1 showing gram staining and differential media results

SAMPLE	AGAR SLANT CULTURAL CHARACTERISTICS	GRAM STAINING	DIFFERENTIAL MEDIA			
			MacConkey	mannitol salt agar	EMB	Blood agar
A	thick smooth shiny white growth	+ cocci	baby pink	Growth, slight change in colour	white purple	growth
B	thin moist shiny golden growth	violet rod – ve	Pink	Growth, complete colour change	white purple	Growth
C	thick abduntant smooth yellow slightly spreading growth	_ rod	Purple	no growth, no colour change	ink blue	growth
D	thick waxy shiny yellow raised growth	pink	No growth	no growth, no colour change	ink blue	growth
E	shiny orange yellow growth waxy thick	violet cocci +ve	Colourful	Growth, complete no colour change	No growth	growth
F	thick smooth shiny white growth viscous	violet cocci	no growth	no growth, no colour change	Ink blue	growth
G	thick white growth somewhat opaque	pink cocci +ve	no growth	Growth, slight colour change	no growth	Growth
H	thick shiny white growth abduntant opaque growth	+ cocci	violet pink	Growth, complete change		
I10-3	thin orange yellowish growth shiny smooth	_ cocci	no growth	Growth, no colour change	White	Growth
J10-4	thick abduntant white somewhat transparent growth	cocci +ve	Whitish Transparent	Growth, partial colour change	dark red	Growth

Table no. 2 showing different biochemical tests result

Sample	Carbohydrate test DEXTROSEE	Carbohydrate test LACTOSE	Starch hydrolysis	Citrate test	Indole test	Triple sugar iron test	Methyl red test	VP test	Hydrogen sulphide test	Urease test	Gelatin test	Litmus test	Tributyrin test	
													Oil	Egg
A	+	+	+	+ BLUE	+	–	–	+	+	+ Yellow	+	Acid	+	–
B	+	–	+	+ BLUE	–	+ Acid +gas	+	–	+	– No change	–	Alkaline	–	+
C	+	+	+	+ BLUE	–	+ Gas +acid	–	+	–	+ Yellow	–	Acid	–	+
D	+	–	+	– GREEN	–	– no growth	+	+	+	– No change	–	Alkaline	–	+
E	+	–	+	– GREEN	+	–	–	–	+	– No change	–	Acid	–	+
F	+	–	+	+ BLUE	–	+ Acid	+	–	–	+ Slight change	–	–	+	+
G	-	-	+	– GREEN	–	– Bacteria grown	–	–	+	+ Orange	+	Acid+alkaline reduction	–	–
H	-	+		+ BLUE	+	+ Acid	+	+	–	+ Yellow	–	Acid+alkaline reduction	–	+
I	–	–	+	– GREEN	–	– Bacteria grown	–	–	–	– No change	–	Acid+alkaline reduction	+	+
J	+	+	+	+ BLUE	–	+ Gas+ acid	–	+	+	+ Yellow	+	Acid+alkaline reduction	–	–

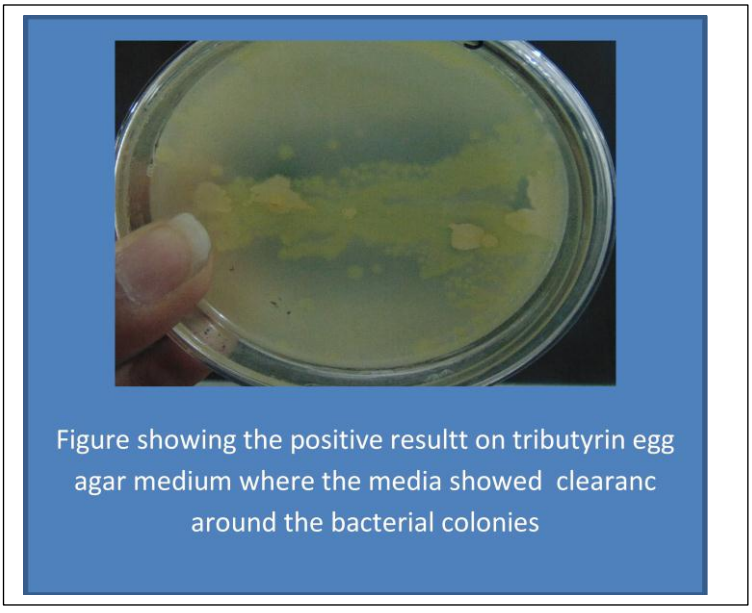

Figure showing the positive resultt on tributyrin egg agar medium where the media showed clearanc around the bacterial colonies