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Research Article

Isolation and Characterization of Lipolytic Bacteria from Oil Contaminated Soil from Petrol Bunk at Southeast Bangalore.

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A B S T R A C T

The isolation of oil contaminated sites and gravimetric analysis of degradation in which, two bacterial formed maximum clearing zones on minerals salt medium. An increase in cell number indicating that the bacterial isolation was responsible for the oil degradation .the collected oil contaminated sites at kormongala, Indra nagar, MTTC culture , micrococcus spp., Bacillus spp., pseudomonas spp., which are able to utilize the oil in soil as carbon sources, were added to oil contaminated soil sample , the growth profiles were determined by monitoring the optical density, dry weight and Ph of the culture utilizing lubricating oil as sole sources of carbon, Bushnell Haas media supplemented with petrol, kerosene and diesel as sole carbon sources was used for isolation of bacteria capable of degrading these petroleum fractionates. From three soil sample and two water sample, a total of nine bacterial strains were isolated capable of degrading petrol, kerosene and diesel with varying tolerance capacities, the isolates were identify by using standard biochemical test and morphological studies and it was determined that these strains belong to six bacterial genera .the present study suggest that the isolated bacterial species could be employed for bioremediation in environment polluted with petroleum and its products, indigenously from the soil and water contaminated with crude oil in the vicinity of oil drilling well were found to be most efficient crude oil utilize as turbidity observed by spectrophotometrically. In the various study of lipolytic bacteria concluded that the taken of oil contaminated soil from from petroleum bunk and to identify their biochemical characterization by using various sources. How it's helpful for characterize by using of lipolytic bacteria.

Keywords – Isolate lipolytic bacteria; oil contaminated soil, physiological parameters, gravimetric analysis.

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INTRODUCTION

In the past years a large number of ecosystem have been changed by the growing influence of human activity, many people have become aware of the need to protect ecosystem as well as to evaluate the damage caused by contamination[1,3,5]. Most of the petroleum goes in the ecosystem via leak of coastal oil refiners this fact increased the interest of scientists to investigate the oil distribution and its fate in the environment especially the marine environment [14,16]. Past analysis of reported oil spills indicated that most of the oil comes from tankers, barges and other vessels as well from land pipeline spills [2, 6].

Shipping accidents have a serious impacts on the surrounding environment, the consequence include serious, widespread and long term damage to marine ecosystem, terrestrial life, human health and natural resources, it is very important to characterize oil spills as already known sources, this can help environment to predict the behavior of oil and estimate the long term impact on the environment, it is necessary to select an appropriate cleanup methods [2, 3, 16].

Conventional remediation methods include physical removal of contaminated material; these methods also use

chemicals, especially shoreline cleaner which are often organic solvents with or without surfactants [4, 12].

There is an increased interest in promoting environmental methods in the process of cleaning oil polluted sites, these methods are less expensive and do not introduces additional chemical to environment, compared to physiochemical methods, bioremediation offers a very feasible alternative for an oil spill response , this technique is considered an effectives technology for treatment of oil polluted one reason is that the majority of the molecules in the crude oil and refined produced are biodegradable[4,5,14].

Microbial degradation of petroleum hydrocarbon is one of the major practices in natural decontamination process [17,18]. Presence of petroleum hydrocarbon has been reported to influence the biodiversity, distribution and pollution of microorganism in an environment, prolonged exposure and high oil concentration may cause the development of liver or kidney disease, possible damage to the bone marrow and an increased risk of cancer [5,8,9].

The indigenous bacteria which degrade hydrocarbon are isolated from oil contaminated petrol bunk sites and screened for their hydrocarbon degradation efficiency, they were further characterized by morphological, cultural and biochemical techniques. Microorganism have enzyme systems enables them to degrade and utilize diesel oil as a sources of carbon energy [6, 13]. The main aim to isolate the lipolytic bacteria from various sites of Bangalore and to identify the biochemical characteristic by using of some biochemical test, so that to know the impact of lipolytic Bacteria from oil contaminated soil of petroleum bunk[7, 16]

MATERIALS AND METHODS

Isolation of lipolytic bacteria:

Soil sample

Soil was collected from different areas Hrbr Layout, Kalyannagar, Hebbal and Kammanhali.

Soil sample from each sites of petrol pump was taken and transferred into sterile polythene bags to minimize moisture losses during transportation and samples was subjected to bacteriological analysis.

Isolation of Bacteria

- Soil sample was serial diluted up to 10^5 dilution factors.
- A dilution factor 105 was spread plated on BHM media agar.
- Incubated for 24 hour at 37^0 c.

Identification of lipolytic bacteria

Gram staining and motility test was done and observed under microscope.

Biochemical characterization of isolates

Indole test

Peptone broth was prepared and sterilized at 121^0c for 15 min and inoculated with test organism, incubated the medium at 37^oc for 24 hours. Added 1 ml of kovac reagent to tubes including control

Methyl Red test

Prepared MR-VP broth in two flasks, inoculate the broth with the test organism and incubated for 24 hours at 37^0c, after 24 hours of incubation transferred 5 ml of broth into two test tubes. To the each broth culture added 5 drops MR indicator the tubes and shake them.

Voges proskauer test

Prepared MR-VP broth in two flask, inoculates the broth with the test organism and incubated for 24 hours, prepared barritt reagent of A and B , After 24 hours all agar slant were examined for the presence of growth and coloration of the medium.

Citrate utilization test

Prepared citrate agar slant and inoculated each of the test organism into appropriately labeled tubes by means of a loop, the slant was left uninoculated that serve as control, incubated for 24 hours at 37^0c.

Catalase test

Transferred small quantity of culture from the plates on glass slide , added 1 drop of 3 % H_2O_2 observe bubbles.

Oxidase test

Taken oxidase disc in clean microscopic slide, pasted the culture on the oxidase disc and observed for color changes.

Nitrate test

Prepared nitrate broth and inoculated each of the test organisms into its appropriately labeled tubes means of a loop. The last slant was left uninoculated that serve as control, incubated all culture for 24 hours at 37^oc , after 24 hours add one dropper full of sulfanilic acid and one dropper full of α naphthylamine to each broth.

Starch test

Prepared starch agar and inoculated each of the test organism into its appropriately labeled tubes by means of a loop, the last plates was left un inoculated that serve as control, incubated all culture for 24 hours at 37^0 c. after 24 hours all agar slants were examined for the presence of growth and zone formation on the medium.

Gelatin test

Prepared gelatin slant and inoculated each of the test organism into its appropriately labeled tubes by means of a loop. The slant was left un inoculated that serve as control, incubated all culture at the bacterium optimal growth temperature for up to 1 week and checked every day for gelatin liquefaction. Gelatin normally liquefies at 28^0c and above, so to confirm that liquefaction was due to gelatinase activity.

Physiological parameters

Effect of Ph

Prepared nutrient broth in 5 flasks, which was sterilized and 1 ml of engine oil was added to all the flask except the control flask which was set blank, remaining flask was adjusted to ph 3, 5, 7, 9 and labeled respectively inoculated the test organism and incubate at 37^0c for 72 hours and OD 600 nm was taken at every 24 hours intervals [8].

Effect of temperature

Prepared nutrient broth in 5 flask, which was sterilized and 1 ml of engine oil was added to the entire flask except the control flask which was set blank, remaining flask was kept at temperature RT 30, water bath 60, incubator 37^0c and fridge 4^0c. Flasks were inoculated with test organism and incubate at 37^0c for 72 hours OD 600 nm was taken at every 24 hour intervals [9].

To examine the degradation of oil by gravimetric analysis

Oil Degradation

For examining the degradation of oil, BHM medium supplemented with 5g/l of engine oil was used. About 50 ml medium was dispensed in 250 ml conical flask, the media was inoculated with 0.1 ml of crude oil degrading bacteria and incubated at 28^0c for 7 days on a rotary shakes at 175 rpm.

Extraction of crude oil

For estimation of oil degradation rates by gravimetric analysis 5 ml of n- hexamine was added to above flask, the contents were transferred to a separating funnels and extracted. Extraction was carried out twice to ensure complete recovery of oil, the extract was treated with 0.4 g of anhydrous sodium sulphate to remove the moisture and decanted into a breaker leaving behind sodium sulphate to remove the moisture and decanted into a breaker leaving behind sodium sulphate .the amount of residual oil was measured after extraction of oil from the medium and evaporating it to dryness in warm bath 40^0c , the volume of extracted oil was deducted from the previously weighed breaker[10,11].

RESULTS AND DISCUSSION

Table 1: Biochemical Test

Test	Gramstaining	Motility	Indole	Methyl red	Voges Proaskauer	Citrate	Catalase	Gelatin	Nitrate	Oxidase	Starch	Triple sugar test	Urease
Pseudomonas spp.	+	--	--	--	--	+	+	+	+	+	--	**K/NC**	+
Bacillus spp.	+	--	--	--	+	+	+	+	+	+	+	**K/K**	--

Table 2: Effect of pH

Organism	pH	DAY1	DAY2	DAY3
Pseudomonas spp.	03	0.01	0.02	0.02
	05	0.02	0.03	0.05
	07	0.39	0.42	0.50
	09	0.35	0.40	0.43
	05	0.02	0.04	0.07
	07	0.14	0.20	0.50
	09	0.02	0.04	0.05

Table 3: Effect of Temperature

Organism	Temperature ^{0}C	Day1	Day2	Day3
Pseudomonas Spp.	04	0.02	0.02	0.02
	30	0.26	0.32	0.48
	37	0.36	0.45	0.55
	60	0.01	0.01	0.01
Bacillus Spp.	04	0.02	0.02	0.02
	30	0.04	0.08	0.11
	37	0.15	0.25	0.36
	60	0.01	0.01	0.01

Table 4: Oil recovered

Isolates	Weight of beaker with oil gm.	Weight of beaker gm.	Recovered oil gm.
Pseudomonas spp.	47.55	47.25	0.30
Bacillus spp.	48.55	48.10	0.45

Table 5: Protein Estimation

Sample	Concentration µg/ml	O.D (600nm)
Pseudomonas spp.	660	0.95
Bacillus spp.	620	0.89

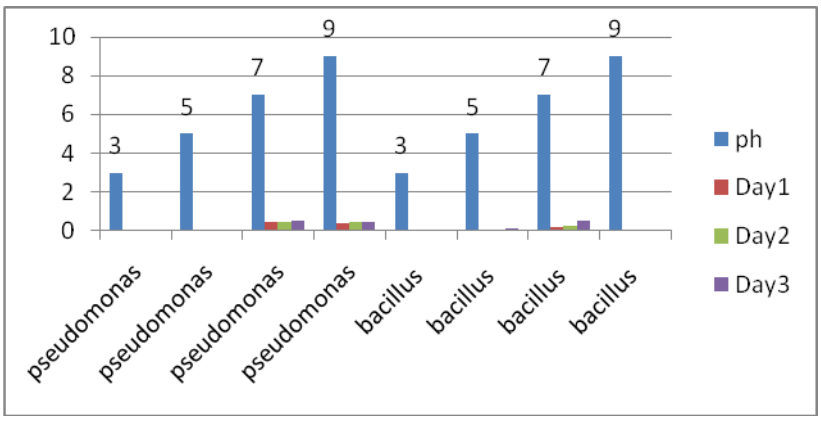

Figure 1: Effect of pH

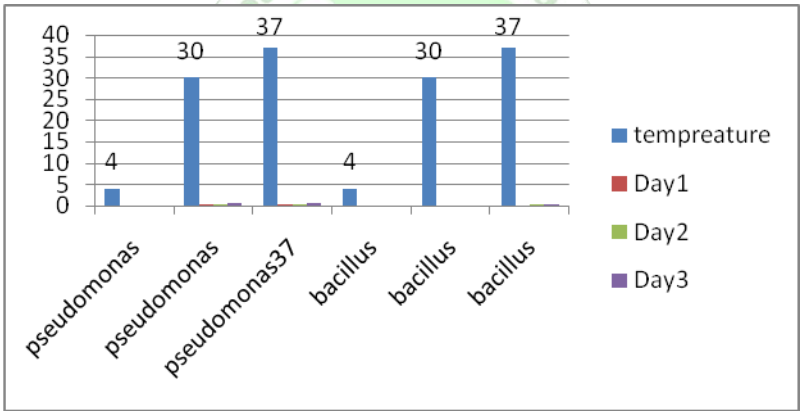

Figure 2: Effect of Temperature

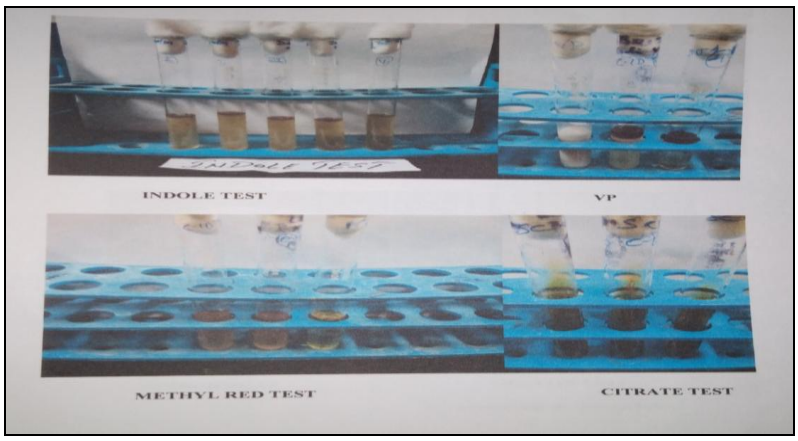

Figure 3: Biochemical Tests

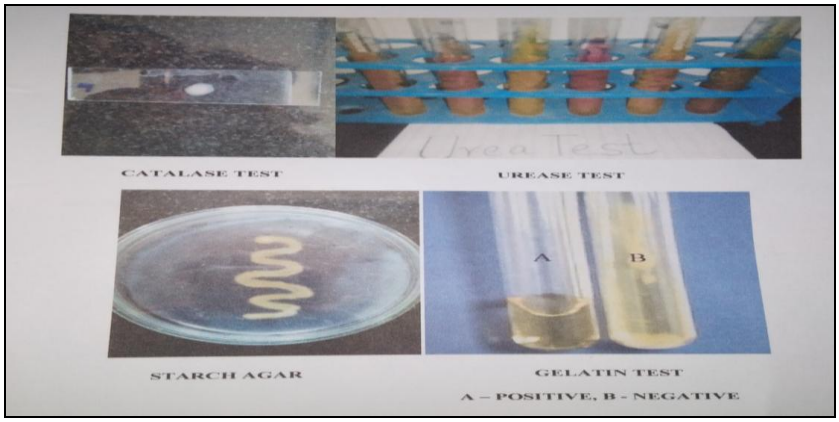

Figure 4: Biochemical Tests

CONCLUSION

The ability of the bacterium to withstand the effects of reduce water activity is great ecological importance. Production of valuable metabolites by microorganism has always been one of the main areas of intense scientific research over the years. The present study was undertaken to isolate lipolytic bacteria from contaminated soil sample and subsequent optimization of culture parameters, which include temperature, ph, incubation period, agitation periods for isolation and characterization of lipolytic that effect of carbon source, nitrogen source metals ions were further assessed to determine in maximum lipolytic bacteria from the contaminated soil which was taken from different areas of Bangalore. Selection bacterial culture was achieved by culture in the different media which was taken. The main purpose of isolate lipolytic bacteria from oil contaminated soil which indicate more ph and temperature resistant that help to grown the such bacteria using of such nutrient choosing such compound which helps to identify the particular bacteria by using of biochemical characterization.

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Author's contribution

Ajay kumar sahu and Rahul Nemani prepared and conceived designed the experiment and draft the manuscript under the guidance of Prangya P. Acharya and Dr. Rupali Sinha and also prepared all media, reagent for bacterial growth and biochemical test. Subhanil Sengupta and Bibekananda Pradhan participate the work and design some idea and coordination.

Conflict of interest - nil

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