RESEARCH PAPER | Biotechnology | Volume : 5 | Issue : 3 | March 2015 | ISSN - 2249-555X

Biochemical Characterization of Oil Degrading Bacteria Of The Great Indian Thar Desert

KEYWORDS: Crude oil, degradation, enrichment technique, whole cell protein

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ABSTRACT *In order to develop environmental technologies for crude oil degradation it is essential to isolate and characterize specific microbial species for evaluation of their efficacy in utilization of hydrocarbons before application to field conditions for bioremediation. 24 bacterial isolates capable of utilizing crude oil as a carbon source were isolated from various contaminated sites using the enrichment technique. On the basis of morphological, cultural and biochemical tests 8 isolates were selected to be the potential crude oil utilizers. Further, the screening of the isolates was done by estimation of whole cell protein and growth in terms of turbidity when crude oil was supplied as the sole source of carbon. Two isolates were chosen to be the most potential utilizers of hydrocarbons as an energy source.*

Introduction

Globalization of petroleum based industries has led to the release of pollutants in the environment by anthropogenic activities. (Castro-Gutierrez et al., 2012). Pollution results in the deterioration of both biotic and abiotic components of the ecosystem as some hydrocarbon components have been considered belonging to families of carcinogenic and neurotoxic organ pollutants.(Santhini K.et al.,2009) It is not legible to ban petroleum based industries and its production as crude oil is the principal source of energy and no reliable alternative energy source has been substituted yet. (Trindade et al., 2005, Al-Saleh and Obuekwe, 2005). Soils contaminated with hydrocarbons have significant higher population of hydrocarbon degrading micro-organisms. Microorganisms have enzyme systems to degrade and utilize Hydrocarbons as a source of carbon and energy. (Ezeji et al., 2005)

Bioremediation aims at using microorganisms for degradation of pollutants and today it's been a choice of scientists because other methods such as surfactant washing and incineration lead to production of more toxic compounds and they are non-economic. (Margesin, 2000; Balba et al., 2002; Urum et al., 2003).

Many bacterial strains can degrade or transform the hydrocarbon components to the non-toxic, non- hazardous, biodegradable and environmentally friendly compounds. This mechanism is known as a biodegradation. The biodegradation is one of the primary ways for eliminating crude oil from polluted sites and the most environmentally friendly method. (Del Arco and De Franca, 2001; Bharathi and Vasudevan, 2001). Bioremediation is accomplished by adding exogenous microbial populations or stimulating indigenous ones for raising the rates of the natural degradation to significantly higher levels (Watanabe, 2001). Many oil-degrading bacteria have been isolated and their degradation potential is investigated. The population of microorganisms found in a polluted environment degrades petroleum differently and at a different rate than micro-organisms in relatively clean environment. (Santhini et al., 2009).

Indigenous and adapted microorganisms are more efficient for biodegradation of oil pollutant. The adapted organism degrades oil pollution normally but rate depends on different factors lke microbial composition, geology of polluted site, type of the pollutant and chemical conditions at the contaminated site (Sepahi A. et al., 2008).

In the current study, we aimed at isolating microorganisms from the oil spilled sites of the desert ecosystem which were further characterized by morphological, cultural and biochemical techniques. Their biodegradation potential was determined with respect to the degree of pollution hazard.

Materials & Methods
Collection of soil samples:

Total 10 soil samples from diesel shed, garages, petrol pumps, railway station fuel points, which are contaminated by petroleum refinery products like diesel, petrol and lubricant oil and near the oil drilling sites were collected in presterilized bags from the depth of 0.5 to 1.0 cm surface and subsurface. All the samples were then carefully transferred to laboratory and stored at 4°C before analysis.

Isolation and screening:
Samples were inoculated into Mineral salt Medium: Bushnell and Haas Medium in 250 ml Erlenmeyer flasks with 2% crude oil as sole source of carbon. The flasks were incubated on rotary shaker at 200 rpm for 7 days at room temperature, there after the cells were transferred to fresh media with crude oil as the sole carbon source. Further the suspensions from each flask were streaked on Nutrient Agar plates to obtain isolated colonies (Santhini, et al., 2009).

Isolation of Crude Oil degrading bacteria from different sampling sites
A total of 24 isolates were isolated from 10 soil samples as per to their ability to utilize hydrocarbon as their energy source

Volume : 5 | Issue : 3 | March 2015 | ISSN - 2249-555X

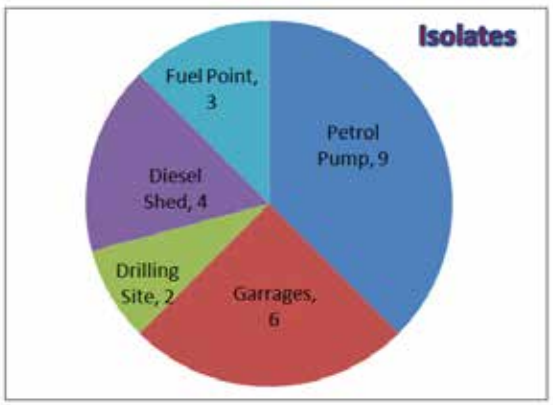


Fig 1

Morphological Characterization of Selected Strains

Out of 24 isolates, 8 isolates were selected and examined for their size, shape, margin, consistency, opacity, elevation, pigmentation, Gram reaction and cell morphology as described in Bergey's Manual of Determinative Bacteriology (Holt, 1994).

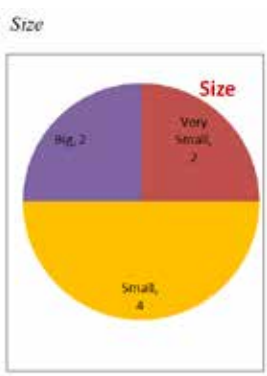


Fig 2

Fig 3

Gram's Reaction

It was carried out by standard Gram's staining procedure.

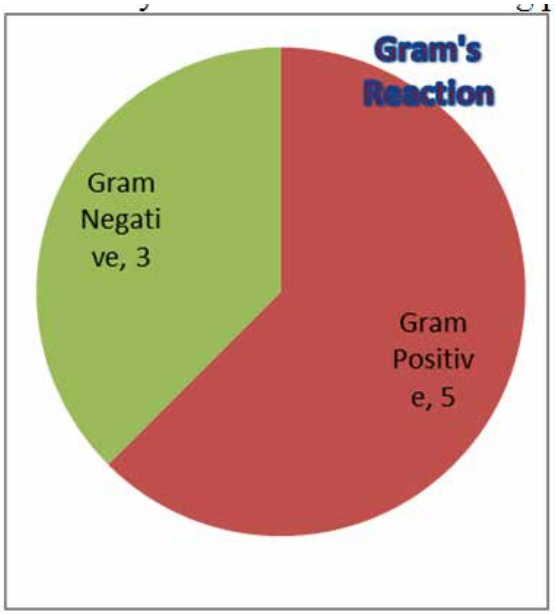
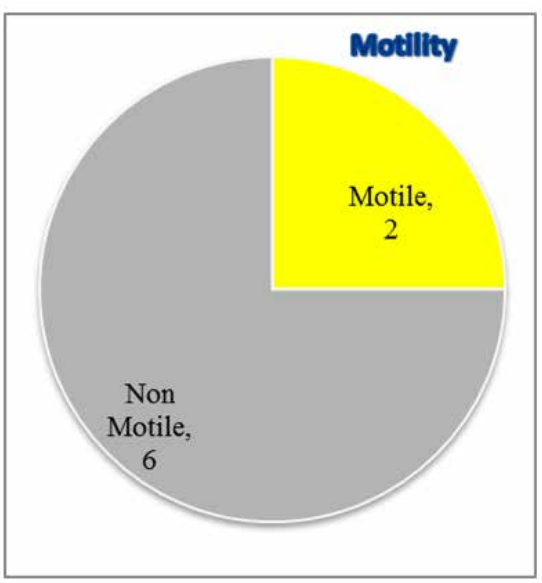


Fig 4

Motility



Cultural Characteristics of Selected Isolates

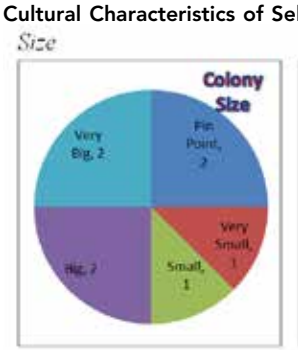


Fig 6

Fig 7

Volume : 5 | Issue : 3 | March 2015 | ISSN - 2249-555X

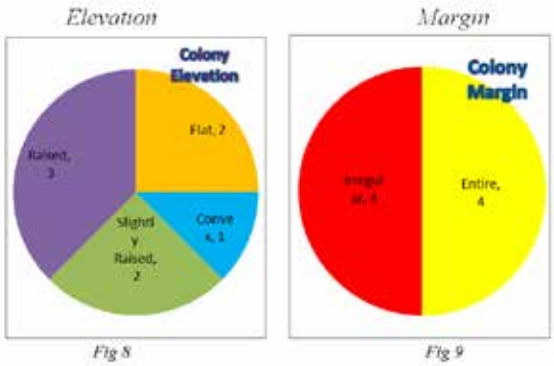


Fig 8

Fig 9

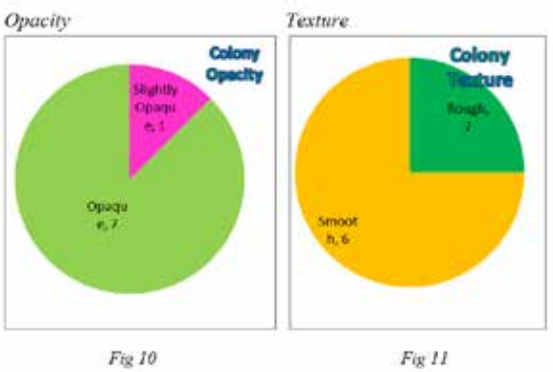


Fig 10

Fig 11

Pigment

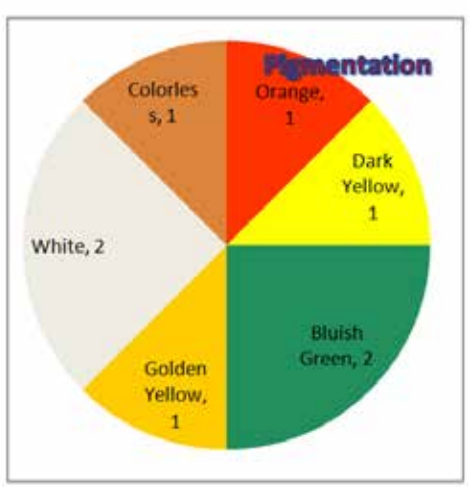


Fig 12

Biochemical Characterization of Selected Isolates
Biochemical characterization aimed at testing utilization of citrate utilization, urease production, H2S production, oxidase production, catalase production, starch hydrolysis, casein hydrolysis, M.R. Test, V.P. Test, lipid hydrolysis and utilization of glucose (K.R. Aneja, Experiments in Microbiology, Plant Pathology and Biotechnology, New Age International Publishers, 2003)

Catalase production:
Catalase degrades hydrogen peroxide (drop of 3% H_2O_2) and releases oxygen which is detected as effervescence. Prompt effervescence indicates catalase production.

Glucose Utilization:
It determines the ability of an organism to ferment/digest glucose which is indicated by the production of acid/gas. Those microorganisms which produce acid in both close and open tubes are fermentative while those producing acid only in the open tubes are called oxidative.

Starch Hydrolysis:
It determines the ability of the microorganisms to produce extracellular amylase and hydrolyze starch using iodine solution as an indicator. Yellow zone around a colony in an otherwise blue medium indicates amylolytic activity.

Caesin Hydrolysis:
This test detects the ability of microorganisms to hydrolyze the major milk protein-Caesin by the production of proteolytic enzyme proteinase (caesinase) that breaks the peptide bond by intervening water in the molecule. Formation of a clear zone in the opaque medium adjacent to the bacterial colony implies to casein hydrolysis.

Hydrogen Sulphide Production:
It detects the ability of microorganisms to degrade sulphur containing amino acids to produce H2S (hydrogen sulphide) , changing the color of the medium to black or brown. SIM (Sulfide indole motility) agar is used for the test.

Oxidase Production:
It indicates the presence of cytochrome oxidase which catalyzes oxidation of reduced cytochrome by oxygen. Colonies giving deep purple blue color with oxidase reagent (1% tetra methyl-p-phenylenediamine hydrochloride) implies to the ability of microbes to oxidise amines.

Lipid Hydrolysis:
It detects the ability of the microorganisms to produce lipolytic enzymes. Presence of clear zone around colonies on tributyrin agar plates indicates hydrolysis of lipids.

IMViC Tests:
Designed to specifically differentiate gram negative bacilli.

Citrate Utilization:
Tests the ability of the microorganisms to utilize citrate present in Simmon's media, as a sole source of carbon. Blue color indicates the positive results. Bromothymol blue is used as an indicator.

Methyl Red Test(MR Test) &Voges-Proskauer (VP)Test :
These tests are used to differentiate two major types of facultatively anaerobic enteric bacteria that produce large amounts of acid and those that produce the neutral product acetoin as end product. MRVP Broth is used as medium.

In MR Test, methyl red indicator in the pH range of 4 will remain red-positive test if yellow then negative test.

In VP Test, crimson-ruby pink color, most intense on sur-

Volume : 5 | Issue : 3 | March 2015 | ISSN - 2249-555X

face indicates positive test while no change indicates negative test.

Urease Production:
It detects the ability of the microorganisms to produce urease enzyme. Phenol Red is used as an pH indicator which turns the inoculated medium purple pink when urease is produced. (indicating alkaline pH due to the conversion of urea into ammonia)

Isolate P1

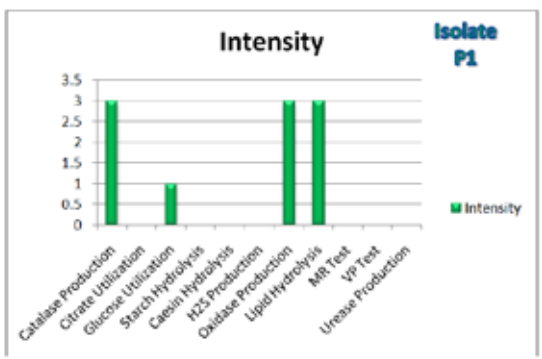


Fig 13

Isolate P2

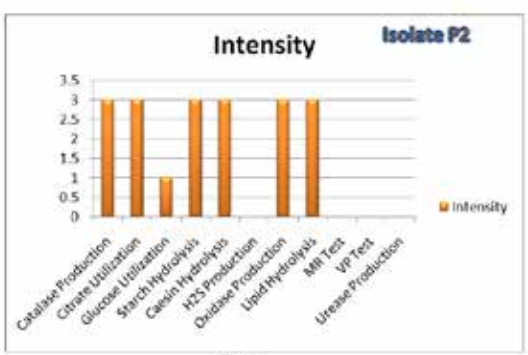


Fig 14

Isolate P3

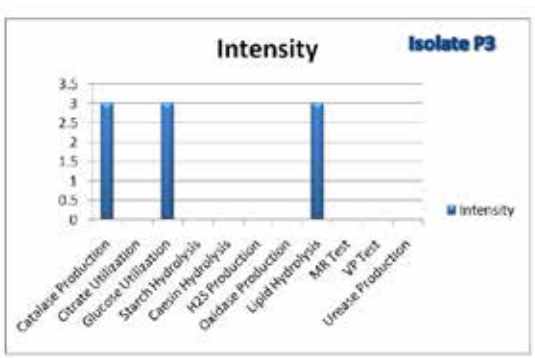


Fig 15

Isolate P4

Isolate P4

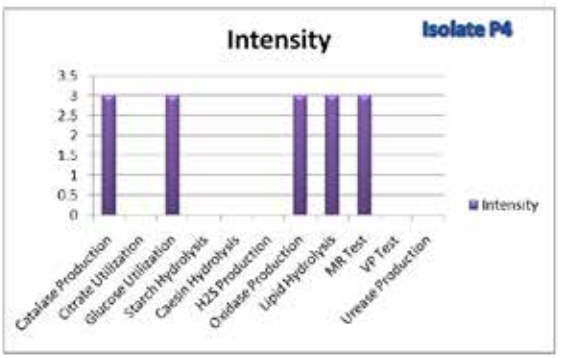


Fig 16

Isolate P5

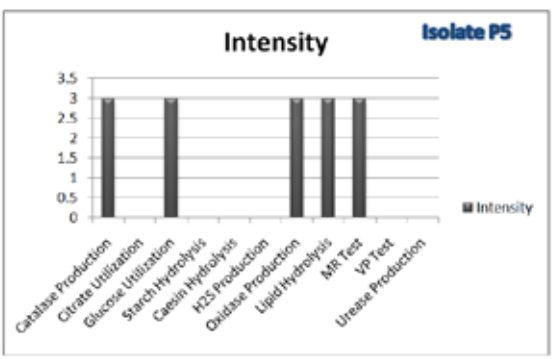


Fig 17

Isolate P6

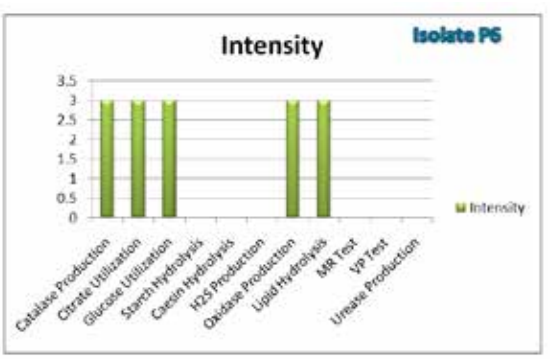


Fig 18

Isolate P7

Volume : 5 | Issue : 3 | March 2015 | ISSN - 2249-555X

Isolate P7

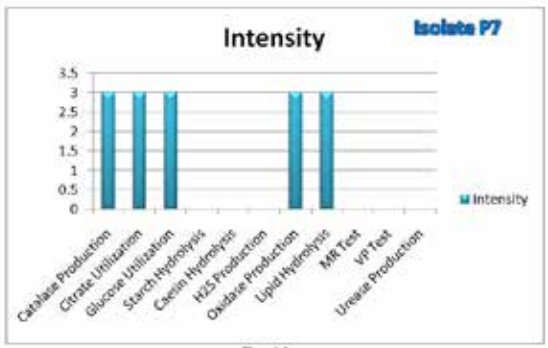


Fig 19

Isolate P8

Isolate P8

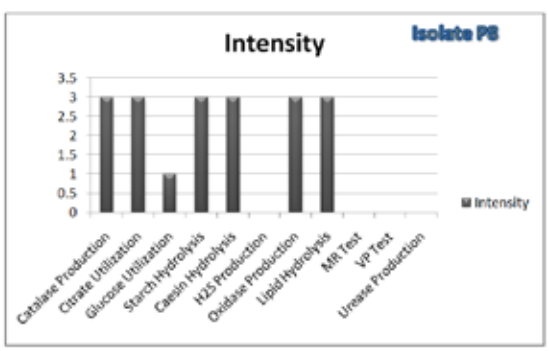


Fig 20

Fig 21

Fig 22

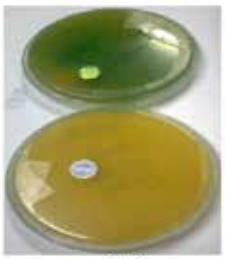


Fig 23

Fig 24

The preliminary identification of all the eight strains was done on the basis of above described morphological, cultural and biochemical characteristics.. Screening for hydrocarbon degradation potential was determined by whole cell protein which directly indicated that increase in whole cell protein implies the ability of bacteria to grow on crude oil as the sole carbon and energy source (Hanson, 1993).

Diversity and crude oil utilization ability of some isolates at the end of 48 hours with crude oil as the sole source of carbon.

The growth of screened bacteria in terms of whole cell protein was determined by hydrolyzing 1.0 ml of cell suspension with 1N NaOH at 100^0 C for 10 min followed by quantitative estimation of protein by Folin Lowry's method. (Hanson, 1993).

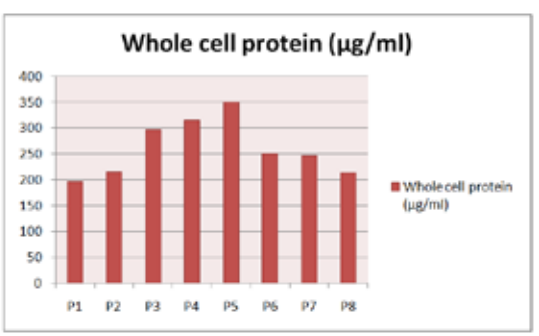


Fig 25

Out of the eight isolates, the isolates P4 and P5 obtained indigenously from the soil in the vicinity of oil drilling site were found to be most efficient crude oil utilizers as observed by the visual turbidity and whole cell protein. So, these two bacterial strains were used for further investigation henceforth.

Results & Discussions

The ability to isolate high numbers of certain oil degrading microorganisms from oil polluted environment in desert ecosystem is commonly taken as evidence that these microorganisms are the active degraders of the environment.

In the present study we isolated 24 strains of bacteria from the oil spilled sites of the Thar Desert. Out of these, 8 strains were selected primarily for the Morphological, Cultural & Biochemical Characterization. Crude oil utilization potential of the strains was determined in terms of visual turbidity and the whole cell protein. Out of 8, two isolates have shown the most efficient biodegradation potential and hence were used for the further investigations.

Volume : 5 | Issue : 3 | March 2015 | ISSN - 2249-555X

Acknowledgement:
Authors are thankful to the Principal and Management of Mahila PG Mahavidyalaya, Jodhpur (Rajasthan) for the Laboratory Facility

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