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Research Article

ISOLATION AND CHARACTERIZATION OF *BACILLUS* SP. AS AN OIL DEGRADING STRAIN FROM CONTAMINATED SOIL

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A B S T R A C T

A study was conducted to isolate and identify the microorganisms for oil degradation abilities from oil contaminated sites. An oil-degrading bacterium isolated from petroleum contaminated soil collected from Manglam Petroleum, Mavli, Udaipur, Rajasthan. Bushnell Haas (BH) broth was used as the enrichment medium with 1% (v/v) oil to isolate oil degrading bacteria from a mixed population of microbes of soil samples. Isolate showed oil degradation abilities, only 2.5 ml oil was recovered out of 25 ml after seven days incubation period and there was a decrease in width of oil layer from 6mm to 2mm after seven days incubation. The isolate was identified as Bacillus sp. by various morphological, biochemical and physiological characterization tests based on Bergey's Manual.

INTRODUCTION

Oil spills have been a major issue across decades. Oil spills are a major menace to the environment because they severely damage the surrounding ecosystems (Head *et al*., 2006). Various oil spills occurs throughout the years in various area of world that causes damage to our surrounding terrestrial and marine ecosystem. engine oil can also be considered as one of the source responsible for polluting the soil with hydrocarbons. Due to their relative persistence and potential for various chronic effects (like carcinogenicity), PAHs (and particularly the alkyl PAHs) can contribute to long term (chronic) hazards of jet fuels in contaminated soils, sediments, and ground waters [Irwin, *et al*.,1997]. Various conventional methods are available to treat oil contaminated site such as use of chemicals or peat moss but are costly and some time ineffective. Biodegradation by naturally occurring microorganisms is the most basic, reliable and cost effective mechanism for removal of environmental pollutants, including crude oil, (Cappello *et al*., 2007). It has been observed that micro-organism found at oil contaminated soil naturally have ability of oil degradation. Various studies reported oil degrading bacteria from oil contaminates sites (M. Hassanshahian *et al* 2012; Moorthi, *et al*., 2008; Emtiazi, *et al*., 2005; Bragg, *et al*., 1994; Singh and Lin 2008; Udeani, *et al*., 2009; Barathi and Vasudevan., 2001; Head and Swannell., 1999 and Ortega *et al*., 2003).

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The present study is based on isolation and characterization of oil degrading bacteria species for biodegradation of oil from oil contaminated site.

MATERIALS AND METHODS

Sample collection

Soil samples were collected from oil contaminated sites of Manglam Petroleum, Mavli and were packed in sterile poly bags and transferred to the laboratories. (Okoh, 2003 and Ojo, 2006).

Isolation of bacteria from soil sample

Bushnell Hass (BH) medium was used for the isolation of crude-oil degrading bacteria. BH media was supplemented with 1% (v/v) crude oil as the sole carbon source. Oil degrading bacteria were isolated from the collected soil samples by adding 1 gm of soil to Erlenmeyer flasks containing 100 ml of medium, and the flasks were incubated for 10 days at 37^{o}C on rotaryy shaker (180 rpm). Then 5ml aliquots were removed and placed in fresh medium. After sub culturing, inoculums from the flask were streaked out on BH agar with oil and phenotypically different colonies on BH agar were purified by quadrant streaking method.

Morphological and Biochemical characterization of isolates

Characterization of isolates was done by studying morphological (Gram staining and shape) and biochemical characteristics (catalase activity, oxidase activity, carbohydrate fermentation, citrate utilization, nitrate reduction). The tests were used to identify the isolates according to Bergey's

Manual of Systematics Bacteriology (Claus and Berkeley, 1986).

Screening of purified cultures for degradation of used oil

Oil degradation studies of purified cultures were performed by using BH broth with oil. The components for preparing 100ml Nutrient broth were dissolved in 75ml distilled water and 25ml of engine oil was added, pH was maintained to 7. Media with oil was autoclaved at 15 psi for 20 minutes. Cooled media was inoculated with 1ml of 24 hour old grown culture of the respective pure cultures. The inoculated flasks were incubated at 120 rpm in a shaking incubator at 37^{o}C for 7 days. Width of oil and media layer in the flask was recorded on zero day and 7th day. And also the oil degradation was quantified by studying the oil recovery after 7th day of incubation.

RESULTS

Isolation of bacteria from soil sample

Ten bacterial strains were isolated from enrichment cultures that were maintained at 30^{o}C for 7 days. Two isolated strains that showed higher growth rates on crude oil were selected among the 10 isolates for further study.

Morphological and biochemical characterization of isolates

Two selected isolates were characterized by detecting their cultural, morphological and biochemical characteristics (Table 1). The investigation results indicated that both the isolates were gram-positive, rod-shaped bacteria and gave positive reaction for catalase activity and citrate utilization. Both the isolates gave negative reaction for fermentation of lactose, indole, Methyle red and oxidase activity. The above results obtained for morphological and biochemical characteristics were further matched with Bergey's Manual of Systematics Bacteriology (Claus and Berkeley, 1986) and isolates were recognised as *Bacillus subtilis*.

Table 1 Colony morphology of oil degrading isolates

S.No.	Isolate	Colony colour	Colony shape
1	PP11	Off White	Irregular and wrinkled

Table 2 Gram staining results of oil degrading isolates

S.No.	Isolate	Gram Staining	Cell Shape
1	PP11	+	Long rods

Table 3 Results of different biochemical tests for oil degrading isolates

S.No.	Isolate	Biochemical tests								
		Lactose	Dextrose	Sucrose	Indole	MR	VP	Citrate	Catalase	Oxidase
1	PP11	-	+	+	–	–	+	+	+	–
2	PP12	-	+	+	–	–	+	+	+	–

Screening of purified cultures for degradation of used oil

Table 4 & 5 below show the quantification of oil degradation by two methods used in this study. 25ml of oil was added in 75ml of BH broth and incubated for 7 days. After 7 days incubation 5 and 6 ml oil recovered from the broth by both the strain. Strain PP11 and PP12 showed 80% and 76% reduction in oil respectively. The oil width also tested before incubation and after incubation. Oil width reduced up to 58% and 50% by both the isolates respectively.

Table 4 Oil degradation studies (width)

Isolate	Width of Oil on Zero Day(mm)	Width of Oil on 7th Day(mm)
PP11	6	2.5
PP12	6	3.0

Table 5 Oil degradation studies (oil recovery)

Isolate	Volume of Oil on Zero Day(ml)	Volume of Oil on 7th Day (Recovery) (ml)
PP11	25	5
PP12	25	6

DISCUSSION

A total of 10 bacterial isolates were recovered on BH agar supplemented with crude oil Out of them 2 isolates were selected on the basis of good growth. Morphological characterization of isolates showed these isolates as Gram-positive Bacillus species. It shows that Gram-positive bacteria have the ability of oil degradation which was also reported in other studies of oil degradation (Ghazali *et al*., 2004, Hassanshahian, M. *et al*. and Khan *et al*., 2011). In this study isolates showed high level of oil degradation up to 80 % and was identified as oil degrading bacteria. Similarly *Bacillus* spp. has been identified as oil degrading bacteria in various studies (Annweiller *et al*., 2000; Ijah and Antai, 2003; Sorkhoh *et al*., 1993; Korda *et al*., 1997; Rahman *et al*., 2002; Sepahi *et al*., 2008). Ijah and Antai, (2003) also reported *Bacillus* spp. as the crude oil utilizing bacteria isolated from highly polluted soil samples.

CONCLUSION

Soil sample were collected from oil contaminated sites. Cultures were purified by quadrant streaking techniques. Purified cultures were characterized by the various morphological and biochemical tests according to bergey's manual. The isolate showing maximum oil degradation abilities was gram positive, citrate and catalase positive and oxidase negative and recognized as *Bacillus* spp. *Bacillus* spp. has ability of hydrocarbon bioremediation. *Bacillus* spp. forms endospores, these endospores are tolerant to high levels of hydrocarbons in soil. Isolates belonging to the *Bacillus* spp. could be effective in clearing oil spills (Ghazali *et al*., 2004 and Khan *et al*., 2011). Preliminary screening of purified culture was also done by recovering oil from the flask and estimating the amount of oil left after degradation.

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