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A Study on Oil Degradation Potential of a *Coccus spp.* Isolated from Oil Contaminated Sites in Lucknow, India.

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Abstract:

Today bioremediation is considered as the most effective and pollution free method for removing crude oil pollution from contaminated sites, as this method makes use of microorganism. The study was designed to evaluate the capability of bacterial strains inhabiting oil contaminated sites to degrade unused engine oil. Four different bacterial species were isolated from oil contaminated sites in Lucknow. One of the isolate (MJP1103) showed maximum oil degradation potential in Minimal salt media supplemented with 1-5 % crude oil, the result showed increase in growth & protein concentration throughout incubation period for culture MJP1103.

Key words: Crude oil, *Coccus*, Degradation, Bioremediation.

Introduction:

Environmental Pollution with petroleum and petroleum products like (engine oil, petrol, diesel etc), has been recognized as one of the most serious problems[1]. Engine oil is a complex mixture of hydrocarbons and other organic compounds, including some organometallic constituents[2]that is used to lubricate the parts of an automobiles engine, in order to keep everything running smoothly[3].

Contamination of soil & water bodies with engine oil is a serious ecological problem, primarily in the mechanic workshops, garages. Oil production activities releases a large amount of hydrocarbon in terrestrial & aquatic environment. The level of soil pollution by petroleum products & oil sludge has reached millions of cubic meters [4] [5].

Prolonged exposure and high oil concentration may cause the development of liver or kidney disesase, possible damage to bone marrow and an increased risk of cancer [6][7].

Today use of microorganisms for removing crude oil pollution from contaminated sites as bioremediation is considered by scientists because other physical & chemical methods such as surfactant washing and incineration lead to production of more toxic compounds [8][9][10][11].

The microbial community has potential to detoxify hazardous organic compounds through transformation, mineralization or [5]. Potential of hydrocarbon degrading bacteria & fungi has been estabilished by many researchers[12]. The biggest advantage of microbial bioremediation is there no side effects in comparison to the other traditional methods.

Keeping in mind the harmful effects of oil pollution and the previous research work being carried out in this field viz. [11] [13][14][15]. The present study was designed to study the oil degradation potential of microbes inhabiting oil contaminated sites for oil degradation potential.

Methods And Materials:

Collection of Soil Sample

As the previous studies reported the presence of oil degrading microbes in soil. Hence, soil sample was collected from specific location within mechanic workshop that had heavy spillage of oil in Lucknow, the soil sample was black in color.

Isolation and Purification of Oil Degrading Culture

Bacterial species were isolated by serial dilution agar plate method. Four different colonies picked from the mixed culture plate on the basis of differences in colony morphology were named as MJP1101, MJP1102, MJP1103 & MJP1104 and purified by quadrant streaking, purity of cultures was cross checked by Gram's staining.

Evaluation of Oil Degradation Potential of Isolated Cultures

The pure culture MJP1101, MJP1102, MJP1103 & MJP1104 were screened for oil degradation on minimal salt media supplemented with1% unused engine oil [11][16]. 100ml of MSM (0.8 g NaCl , 0.8 g KCl , 0.1 g $CaCl_2$, 2.0 g Na_2HPo_4 , 0.2 g $MgSO_4$, 0.1 g $FeSO_4$, 8.0 g Glucose, 2.0 g NH_4Cl pH 7.2) was prepared in a 250 ml conical flasks and was supplemented with 1% unused engine oil and autoclaved. Overnight nutrient broth of all the 4 culture was inoculated into each flask and incubated in shaker at 120 rpm and 37°C. The oil degradation potential was quantified by tracking the increase in turbidity, viable cell count on NA plates and protein concentration[17] throughout incubation period. As the growth of the culture

and increase in concentration of protein in minimal media indicates the ability of the culture to utilize used engine oil as carbon and energy sources, this property was checked in order to know the oil degradation potential of culture.

Staining and Biochemical Characterization of Isolate (Culture MJP1103)

According to Bergey's manual [18] various staining (Gram's staining, Endospore staining) & biochemical tests (Catalase test, Carbohydrate fermentation test) were performed in order to characterize the isolate showing maximum oil degradation potential.

Evalulation of Oil Degradation Potential of Culture MJP1103 at Higher Oil Percentage

Culture MJP1103 was further screened for oil degradation potential on MSM supplemented with 3% & 5% unused engine oil.

The oil degradation potential was quantified by tracking the increase in turbidity throughout incubation period (6 days), viable cell counts on NA plate and concentration of protein throughout [17].

Results:

Isolation of Bacteria from Soil Sample

Microbes from oil contaminated soil were isolated by serial dilution method and four different colonies (MJP1101, MJP1102, MJP1103 & MJP1104) obtained in mixed culture plates were selected based on differences in colony morphology. Mixed cultures were further purified by quadrant streaking to obtain pure culture.

Evaluation of Oil Degradation Potential of Isolated Cultures

The ability of isolated cultures to utilize oil as a source of carbon and energy was confirmed by studying their growth and protein profile. *Table 1* below shows the growth and protein profile of all the four cultures and *Figure 1-2*, show the graphical representation of growth and protein profile of the culture MJP1103. It can be seen from the results that the culture MJP1103 shows maximum growth throughout the incubation period as indicated by OD at 600nm and also the cultures protein concentration during studies indicate its ability to grow in minimal media thus showing its oil degradation potential.

Table 1: Growth and Protein Profile of Isolated Cultures

DAYS	GROWTH PROFILE		PROTEIN PROFILE (BRADFORD'S METHOD)	
	OD at 600nm	GROWTH ON NAM PLATE	OD at 595nm	Concentration of Protein in mg/ml
1	MJP1101-0.08 MJP1102-0.40 MJP1103-0.47 MJP1104-0.01	LAWN	MJP1101-0.01 MJP1102-0.15 MJP1103-0.14 MJP1104-0.02	MJP1101-0.003 MJP1102-0.053 MJP1103-0.049 MJP1104-0.007
2	MJP1101-0.20 MJP1102-0.59 MJP1103-0.58 MJP1104-0.08	LAWN	MJP1101-0.02 MJP1102-0.11 MJP1103-0.12 MJP1104-0.16	MJP1101-0.007 MJP1102-0.038 MJP1103-0.042 MJP1104-0.056
3	MJP1101-0.28 MJP1102-0.92 MJP1103-0.76 MJP1104-0.30	LAWN	MJP1101-0.18 MJP1102-0.03 MJP1103-0.04 MJP1104-0.20	MJP1101-0.063 MJP1102-0.01 MJP1103-0.014 MJP1104-0.070
4	MJP1101-0.45 MJP1102-0.87 MJP1103-0.81 MJP1104-0.44	LAWN	MJP1101-0.14 MJP1102-0.02 MJP1103-0.01 MJP1104-0.15	MJP1101-0.049 MJP1102-0.007 MJP1103-0.003 MJP1104-0.053
5	MJP1101-0.43 MJP1102-1.03 MJP1103-0.95 MJP1104-0.49	LAWN	MJP1101-0.15 MJP1102-0.05 MJP1103-0.08 MJP1104-0.14	MJP1101-0.053 MJP1102-0.017 MJP1103-0.28 MJP1104-0.049
6	MJP1101-0.80 MJP1102-1.06 MJP1103-1.02 MJP1104-0.42	LAWN	MJP1101-0.15 MJP1102-0.08 MJP1103-0.27 MJP1104-0.17	MJP1101-0.053 MJP1102-0.028 MJP1103-0.95 MJP1104-0.056

*Figure 1:*Growth Profile of MJP1103

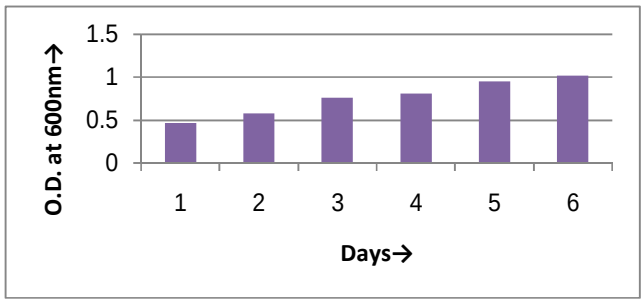
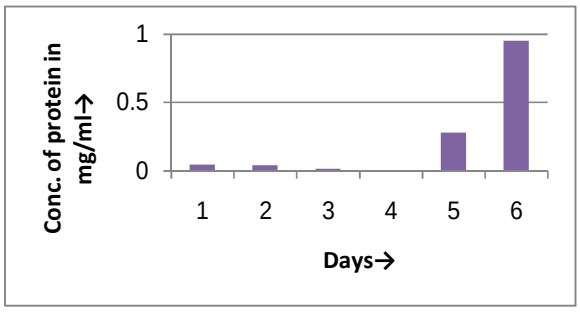


Figure 2: Protein Profile of MJP1103



Staining and Biochemical Characterization of Culture MJP1103

Table 2 below shows the result of various staining and biochemical activities of culture MJP1103 showing maximum oil degradation potential which were carried out as per the Bergey's manual.

Table 2: Staining and Biochemical Characterization of culture MJP1103

TEST	RESULTS
Gram staining	Negative
Cellular morphology	Cocci
Cellular arrangement	Single (mono)
Endospore staining	Positive
Catalase test	Positive
Glucose fermentation test	Positive

Evaluation of Oil Degradation Potential of Culture MJP1103 at Higher Oil Percentage

The culture MJP1103 showing maximum oil degradation potential was further studied for its oil degradation potential at higher oil percentages. Table 3 & Figure 3-6 below show the growth and protein profile of culture throughout incubation period.

Table 3: Growth and Protein Profile of MJP1103 at higher oil percentage

DAYS	GROWTH PROFILE		PROTEIN PROFILE	
	OD at 600nm	GROWTH ON NAM PLATES	OD at 595nm	Concentration of Protein in mg/ml
1	3% -0.03 5% -0.10	LAWN	3% -0.08 5% -0.09	3% -0.02 5% -0.03
2	3% -0.16 5% -0.04	LAWN	3% -0.34 5% -0.30	3% -0.12 5% -0.11
3	3% - 0.06 5% -0.03	LAWN	3% -0.13 5% -0.14	3% -0.046 5% -0.049
4	3% -0.25 5% -0.28	LAWN	3% -0.14 5% -0.13	3% -0.049 5% -0.046
5	3% -0.44 5% -0.36	LAWN	3% -0.21 5% -0.23	3% -0.074 5% -0.08
6	3% -1.10 5% -1.30	LAWN	3% -0.27 5% -0.30	3% -0.095 5% -0.11

Figure 3: **Growth Profile of MJP1103 in 3% oil**

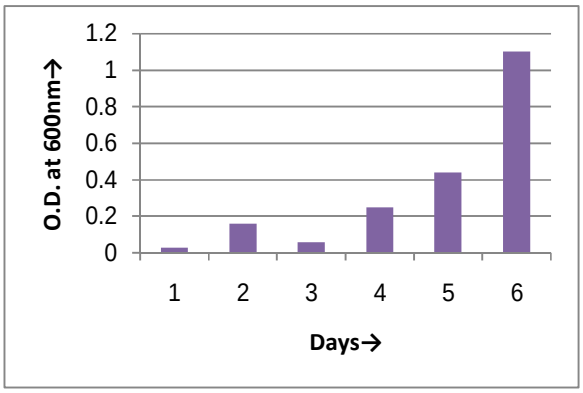


Figure 4: **Growth Profile of MJP1103 in 5% oil**

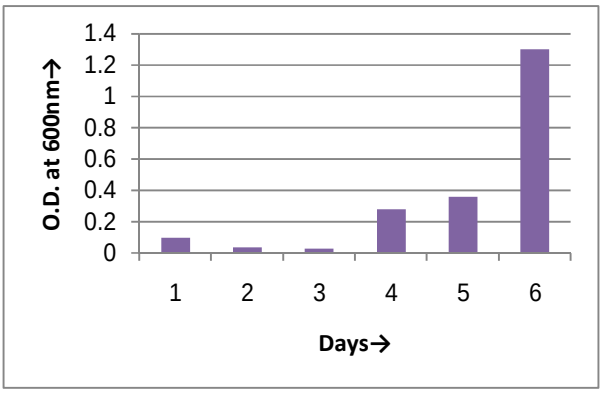


Figure 5: **Protein Profile of MJP1103 in 3% oil**

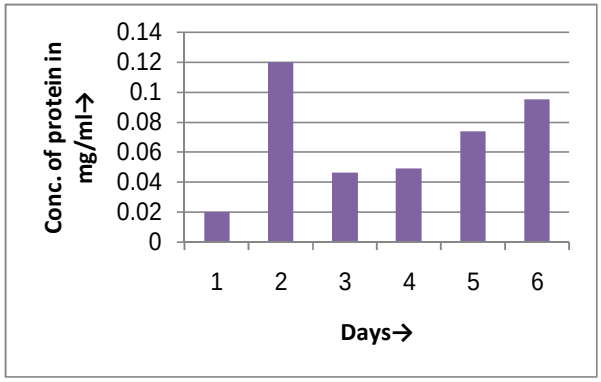
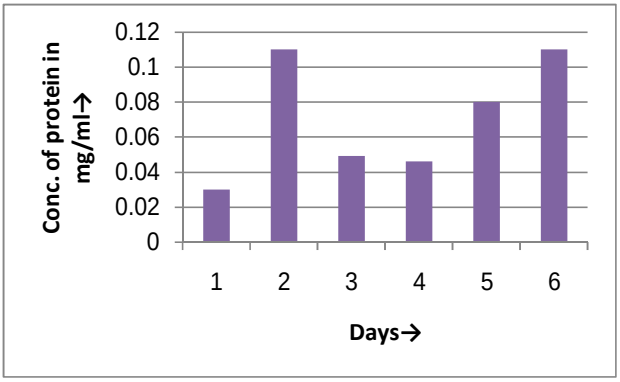


Figure 6: **Protein Profile of MJP1103 in 5 % oil**



Discussion:

In this study the soil sample was collected from oil contaminated site because the capability of native bacterial population to mineralize engine oil hydrocarbons in oil contaminated sites have been confirmed earlier by many scientists[11][19] . Further microorganisms were isolated from oil contaminated soil by serial dilution agar plate techniques as previously done by [1][20] . Purified culture was characterized for various staining and biochemical tests according to Bergey's manual and was found to Gram negative mono *coccus spp*, Endospore former, Catalase positive, Glucose fermentor.

The oil degradation potential was evaluated by studying growth and protein profile of the cultures under study throughout incubation period as increase in number of cell and protein reflects the ability of the cultures to degrade & utilize crude oil as carbon source. This technique has been used in several studies to determine the oil degradation potential of bacteria in engine oil [11] [16] . Oil degrading microbes have the potential to enhance our understanding of roles played by microbes in the natural genesis of long term effect of petroleum product pollution & to enhance new remediation.

Conclusion:

Oil spills are a major threat to the environment as they adversely affect the surrounding ecosystem. Bioremediation is the best way to treat the oil contamination. The microorganisms present at oil contaminated sites have ability to degrade toxic contaminants present in oils into Nontoxic forms.

Till date several works are going on to isolate different types of microbial strain that have ability to degrade oil. In our experiment we isolated a *coccus spp*. Which was having a good potential of engine oil degradation and it can be a novel source for the remediation of oil contaminated sites.

Future prospects of the present study includes the further oil degradation studies at the oil contaminated soil and water bodies and also increment in oil degradation potential by adding various metal ions in the oil degradation media.

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