Isolation, Characterization and Strain Improvement of Oil Degrading Bacteria from Oil Contaminated Soil Sample

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Abstract— Oil is any hydrophobic chemical matter that encompasses viscosity at ambient temperature. Oil spills enter the ocean by the help of shipping travel, drains, dumping and can be very toxic for ocean creature. In this present research article bacteria was isolated from oil contaminated soil , strain improvement was done by physical method applying uv rays, media was optimized for that bacteria.

Keywords: PAHs, Isolation of Oil Degrading Bacteria, PHs

I. INTRODUCTION

The essential elements of life air, water and soil are contaminated constantly due to rapidly increasing population, urbanization and industrialization. The overall annual intake of petroleum hydrocarbon around the world is very high. Petroleum and petroleum hydrocarbons are good source of energy but also causes pollution that affects terrestrial as well as marine ecosystems. Leaks, operational or accidental oil spill generally occur during exploration, production, refining, transport and storage of petroleum and petroleum products. Each polluted sites have their own characteristics related to the typology of the pollutants and their concentration levels, hydro geological characteristics of the environment, biogeochemical soil properties etc[1].The compounds of petroleum are reported as potent mutagens which is responsible for increased incidence of cancer; besides chronic exposure can also affect pulmonary, gastrointestinal, renal and dermatologic systems[2].

Aliphatic and aromatic hydrocarbons are the two main components of petroleum hydrocarbons (PHs). Aliphatic Hydrocarbons are comparatively easily degraded by potent microorganisms. But aromatic hydrocarbons are hard to degrade because of their complex structures[6]. Low molecular weight polycyclic aromatic hydrocarbons (PAHs) are petrogenic sources (produced by incomplete combustion of petroleum) composed of 2-3 aromatic rings and high molecular weight PAHs are composed of about 4-6 aromatic rings, most important pyrogenic sources (production by combustion of petroleum)[7]. Polycyclic aromatic hydrocarbons (PAHs) are toxic to the environment.

II. MATERIALS AND METHOD

The laboratory experiments were conducted in accordance with standard laboratory procedures, at MRD LifeSciences Pvt. Ltd., Lucknow.

A. Collection of Crude Oil Contaminated Soil Samples:

Soil samples were collected from three different oil contaminated sites of automobile workshops near- (1) VibhutiKhand, (2) Amity Tiraha, (3) Patrakarpuram area, Gomati Nagar, Lucknow. The soil samples were collected randomly 3 cm beneath the surface using sterile spatula and to maintain the moisture level the samples were stored in a shady place in room temperature for further use.

B. Isolation of Bacterial Colonies by Direct Culture Technique

Isolation step was performed using the method of serial dilution. 1g of soil sample was inoculated in 10mL of sterile saline water (0.85 NaCl, w/v). After then the samples were serially diluted up to 10^{-10} dilution, 50µL of 10^{-8},10^{-9} and 10^{-10} dilutions were transferred to nutrient agar for spread culture. The plates were inverted at 37ºC for 24 hrs. After incubation, morphology of the colonies were studied. Morphologically 5, 7, 6 distinct colonies were selected and purified on nutrient agar plates to get distinct pure colonies for further experiment from soil sample 1, 2 and 3 respectively. Under the morphological aspects, the shape, margin, pigmentation, elevation, surface, texture, opacity were observed and noted.

C. Screening

The isolated colonies were taken and tested for their ability to produce bio surfactant using analytical method of oil spreading technique. Screening of purified bacterial cultures for oil degradation potential was performed in Minimal Salt Media supplemented with 5% used crude oil. The media contains the following components per litre (pH 7)-KH_2PO_4: 3g/L, Na_2HPO_4: 6g/L, NaCl: 5g/L , NH_4Cl: 2g/L , $MgSO_4.7H_2O$: 0.2g/L, Dextrose: 8g/L, Agar: 20g/L, Crude oil: 5% (v/v).The media was supplemented with limited amount of carbon source. So, after certain period of time, the microbe degraded oil

D. Strain Improvement

1) UV Exposure

One day old bacterial suspensions (45 µL) of CPMH1 were spreaded in 12 nutrient agar plates, 6 each. Control plates were taken for both the bacterial cultures. After spreading, the bacteria inoculated plates were exposed to UV radiation for 1min, 2min, 3, 4, 6, 8 and 10 minutes respectively. Following the UV treatment, the plates were incubated for 37ºC for 24 hrs. After observing the results, two isolated colonies were selected from each UV treated plates (6, 8 and 10 minutes) for further experimental work on CPMH1. The selected UV treated bacterial colonies were then checked for oil degrading potential on minimal salt agar media with supplementation of oil as a sole carbon source. The resultant mutated colonies were then screened for improvement in oil degrading property.

E. Transformation

Genomic DNA was isolated from E.*coli* and plasmid DNA was isolated from CPMH1. Both DNA was cut and ligated to form recombinant DNA then competent cell of E.*coli* was prepared. Recombinant DNA was transformed in E.*coli*.

F. Blue White Screening

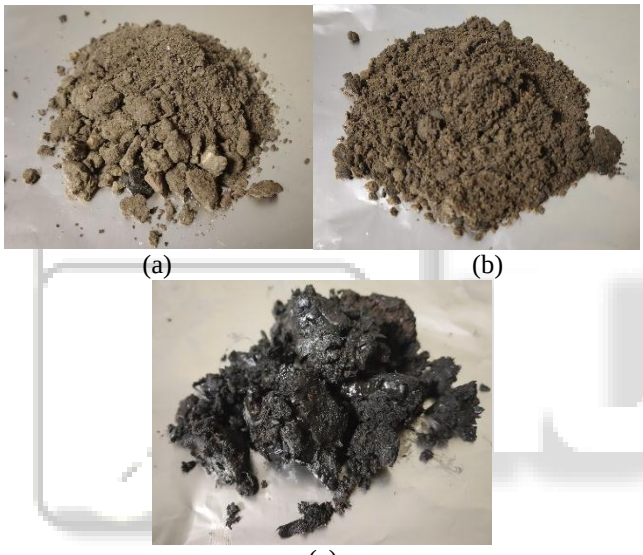
Required amount of NAM was prepared, autoclaved and poured in Petri plates. After solidification, the following were added-X Gal: 20µL, IPTG: 40µL Amphicillin: 5µL, Transformed cell: 20µL.The petriplates were incubated at 37ºC for 24 hrs for observation of the results.

2.7: Characterization of bio surfactant producing Bactria:

Different biochemical test i.e. gram staining, methyl red, urease test etc was performed.

III. RESULTS

A. Collection of Oil Contaminated Soil Sample



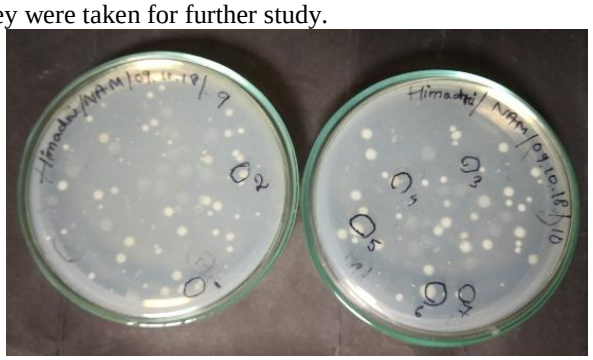
(a) (b)

(c)

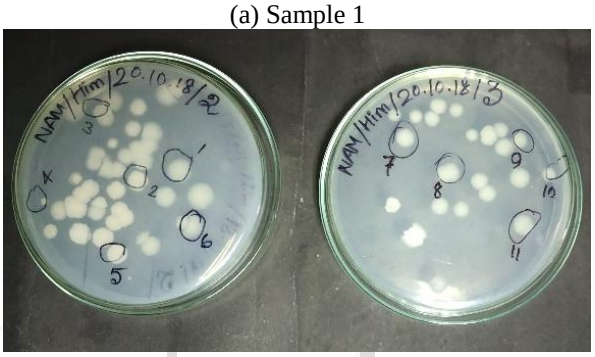
Fig. 1: Soil sample collected from three different oil contaminated area- (a) Sample 1, (b)Sample 2, (c) Sample 3.

B. Isolation of Oil Degrading Bacteria

Colonies 7, 11 and 8 were isolated from soil sample1, sample2 and sample3 respectively; by serial dilution and spread plate method. The selected bacterial isolates were streaked on nutrient agar plates to obtain pure culture and they were taken for further study.



(a) Sample 1



(b) sample 2 & 3

Fig. 2: Isolated bacterial colonies on agar plates. The morphological characteristics of the selected colonies were observed and noted down for further experiment.

Sample no	Bacterial Isolates	Shape	Margin	Pigmentation	Elevation	Surface	Texture	Opacity
Sample 1	CPMH01	Circular	Entire	Non-Pigmented	Flat	Smooth	Soft	Opaque
	CPM02	Irregular	Entire	Non-pigmented	Flat	Smooth	Soft	Opaque
	CPMH03	Filamentous	Entire	Non pigmented	Flat	Smooth	Soft	Opaque
	CPMH04	Spindle	Discrete	Non pigmented	Raised	Rough	Hard	Translucent
	CPMH05	Circular	Lobate	Yellow	Umbonate	Rough	Hard	Opaque
Sample 2	CPMH06	Circular	Entire	Non Pigmented	Convex	Smooth	Hard	Opaque
	CPMH07	Irregular	Curled	Orange	Flat	Rough	Hard	Opaque
	CPMH08	Filamentous	Descret	Non pigmented	Umbonate	Smooth	Gummy	Translucent
	CPMH09	Rhizodes	Lobate	Yellow	Flat	Rough	Hard	Opaque
	CPMH10	Rhizodes	Descret	Non pigmented	Convex	Smooth	Soft	Opaque
	CPMH11	Circular	Entire	Non pigmented	Plumbonate	Rough	Hard	Opaque
	CPMH12	Circular	Entire	Non pigmented	Convex	Smooth	Hard	Translucent
Sample3	CPM13	Irregular	descret	Non-pigmented	convex	Smooth	Soft	Opaque
	CPMH14	Filamentous	descret	Non pigmented	Flat	Smooth	Soft	Opaque
	CPMH15	Spindle	entire	Non pigmented	Raised	Rough	Hard	Translucent
	CPMH16	Circular	entire	Yellow	Umbonate	Rough	Hard	Opaque
	CPMH17	Circular	convex	Non	Convex	Smooth	Hard	Opaque

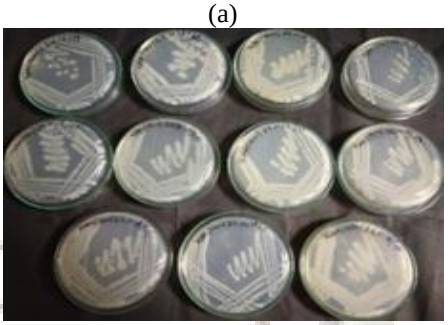
				Pigmented				
	CPM18	Irregular	descret	Non-pigmented	Flat	Smooth	Soft	Opaque

Table 1: Morphological characteristics of the potent bacterial isolates

C. Purification

Purification of culture was done by Quadrant streaking plate method. The selected colonies were streaked in nutrient agar plates with the help of sterilized inoculation loop.

(a)



(b)

Fig. 3: Purification of obtained mixed cultures of (a) sample1, (b) sample2 &3

D. Screening

Every colony was screened and it was found CPMH01 gave higher zone of hydrolysis.

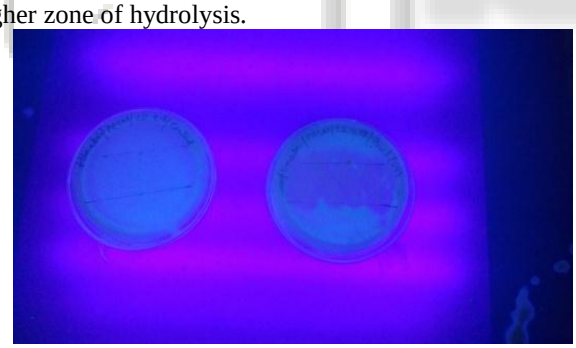


Fig. 4: screening for oil degradation by CPMH01

Bacterial isolates	Screening result
CPMH01	+++
CPMH02	++
CPMH03	+
CPMH04	+
CPMH05	+
CPMH06	_
CPMH07	_
CPMH08	_
CPMH09	_
CPMH10	_
CPMH11	_
CPMH12	_
Cpmnh13	_
CPMH14	++
CPMH15	+
CPMH16	+
CPMH17	++
CPMH18	+

Table 2: screening for oil degradation

E. Strain improvement of oil degrading bacterial isolate CPMH01

1) UV exposure- bacterial isolate CPMH01 was treated with UV for different time interval.

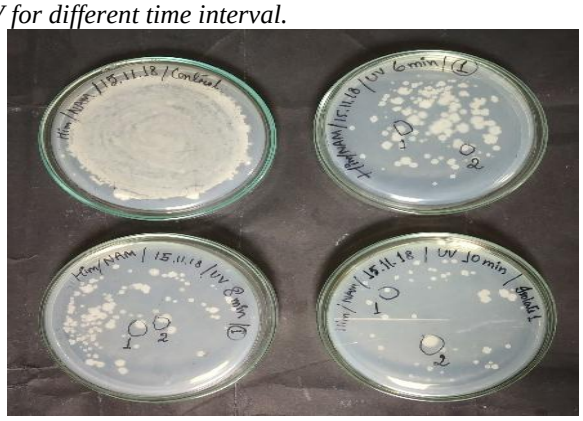


Fig. 5: UV exposure to working bacterial isolates

F. Reassessment the activity of oil degradation of UV treated strain-

Reassessment was done to detect potential of mutated microbes to hydrolysed oil.

UV treatment was given to bacterial strain for different time period and result was seen.

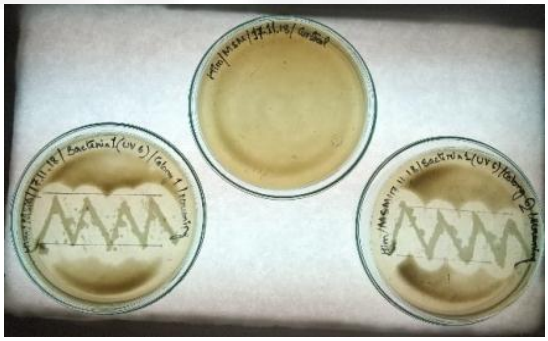


Fig. 6: screening for oil degradation by bacterial mutated strain CPMH01 for 8 min.

All the mutated strain of CPMH01 has potential to degrade the oil but uv mutated strain for 8 min have greater potential to hydrolyse the oil.

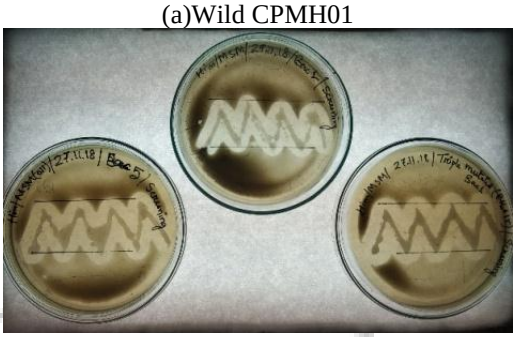
CPMH01 (bacterial isolate)	Zone of hydrolysis
UV treated for 6 min.	30mm
UV treated for 7 min.	20mm
UV treated for 8 min.	31mm
UV treated for 10 min.	28 mm

Table 3: zone of hydrolysis by uv mutated bacterial isolates.

G. Comparative assessment of oil degrading potential between wild strain and mutated strain

Both UV mutated and wild strain of CPMH01 was streaked on screening media for oil degradation and it was noticed, UV mutated strain of CPMH01 has greater potential to eat the oil.

(a)Wild CPMH01



(b) mutated CPMH01

Fig. 7: comparative assessment of oil degrading potential between wild and uv treated strain.

Bacteria	Zone of hydrolysis
wild	31 mm
Mutated	38mm

Table 4: comparative assessment for oil degrading potential, mutated bacteria has more potential.

H. Genomic DNA isolation from E. Coli

Genomic DNA was isolated from E. *coli* by using manual protocol.

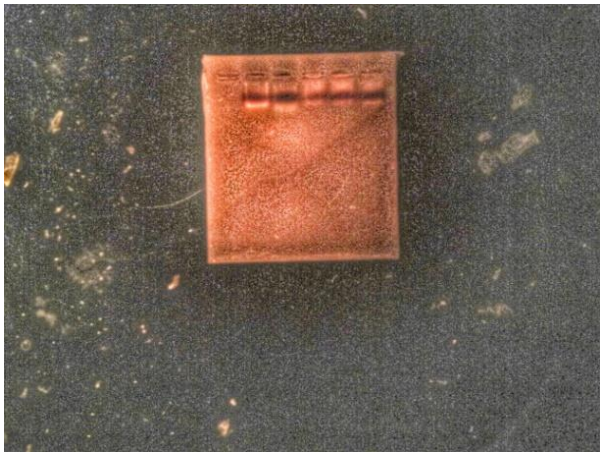


Fig. 8: Agarose gel (0.7 %) electrophoresis of genomic DNA isolated from E. coli.

I. Plasmid DNA isolation

Plasmid DNA was isolated from CPMH01 mutated bacteria and result was by agarose gel electrophoresis.

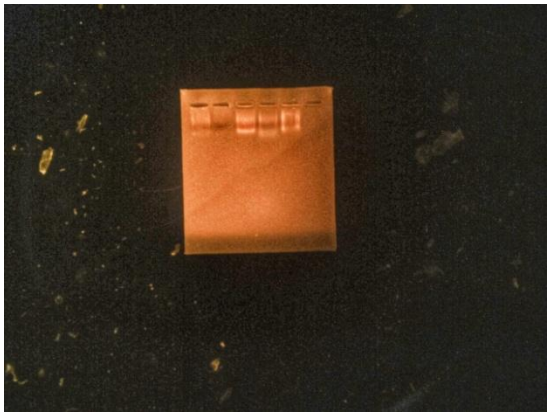


Fig. 9: Agarose gel (1%) electrophoresis of plasmid DNA isolated from CPMH01

J. Blue White Screening:

Transformation was checked by blue white screening and result was seen.

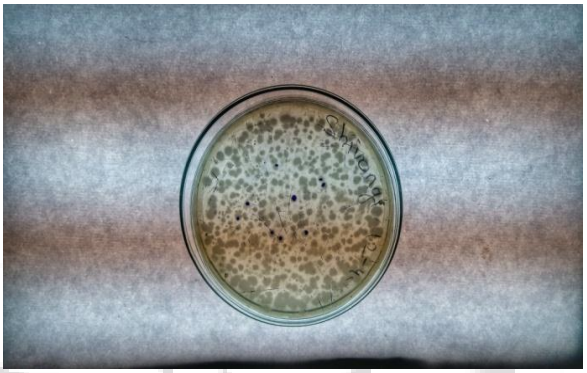


Fig. 10: Blue white screening to detect transformation.

K. Characterization of bio surfactant producing bacteria CPMH01

Name of the test	Results
Indole test	Negative
MR test	Negative
VP test	Negative
Urease activity	Positive
Glucose fermentation test	Positive
Lipid hydrolysis	Positive
Starch hydrolysis	Negative
Catalase activity	Positive
Gram stain	Round shaped, gram positive

Table 5: Different biochemical test were done to detect bacteria.

L. Media optimization

Minimal media was selected a primary media as MM

s.n	Factors	Modified media	STANDARD MEDIA
		minimal media	K_2HPo_4 3g/ l
			Na_2HPo_4 6g/l
			Nacl 5g/l
			NH_4cl_2 3g/l
			$Mgso_4$ 0.1g/l
			Dextrose 8g/l
			Oil 1%
1	oil	Mm1(blank)	1%
		Mm2	0.5%

		Mm3	1.5%
		Mm4	2%
2	dextrose	Mm5(blank)	6 g/l
		Mm6	7g/l
		Mm7	8g/l
		Mm8	9g/l
3	Nacl	Mm9(blank)	5g/l
		Mm10	6g/l
		Mm11	7g/l
		Mm12	8g/l
4	$Mgso_4$	Mm13(blank)	0.2g/l
		Mm14	0.3g/l
		Mm15	0.4g/l
5	P^H	Mm16(blank)	7
		Mm17	6
		Mm18	7
		Mm19	9
		Mm20	11

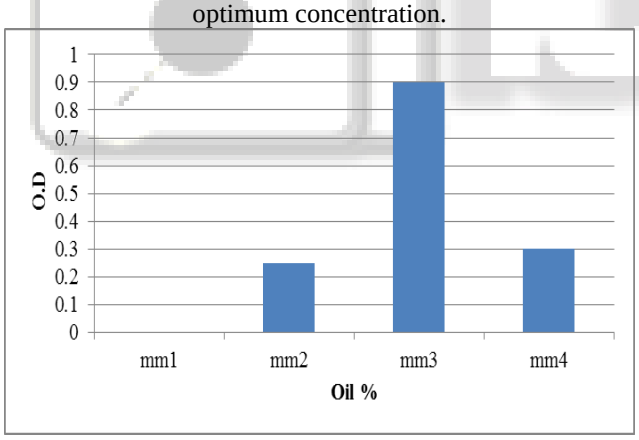
Table 6: media was optimized by using one time one factor method.

M. Modified media for Oil Degradation

1) Oil Concentration Optimization

OIL %	OD at 620 nm
MM1	0.0
MM2	0.25
MM3	0.9
MM4	0.8

Table 7: different concentration of oils was used to find optimum concentration.

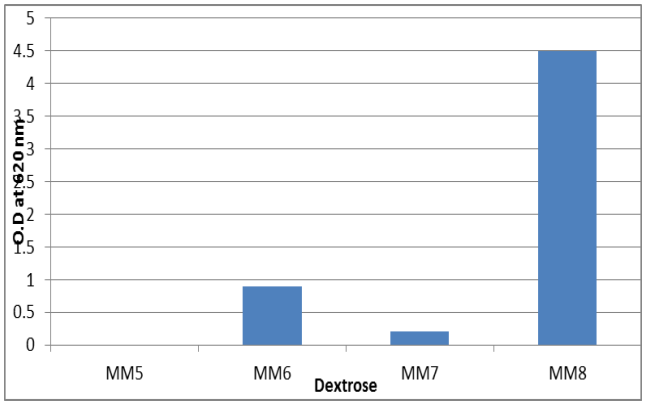


Graph 1: best growth of bacteria was notice in mm3.

2) Concentration of Dextrose Optimization

dextrose	O.D at 620
MM5	0.00
MM6	0.9
MM7	0.2
MM8	0.1

Table 8: different concentration of dextrose was optimized to find best growth of bacteria.

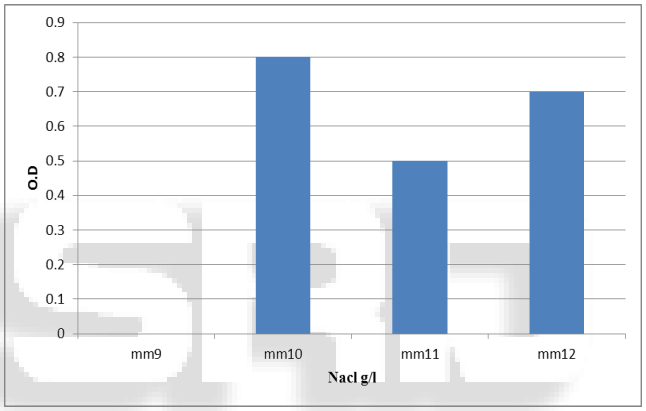


Graph 2: maximum growth of bacteria was shown by mm8.

3) Salt concentration optimization

Mm9	0.00
Mm10	0.8
Mm11	0.5
Mm12	0.7

Table 9: different concentration was used to find optimum concentration of salt.

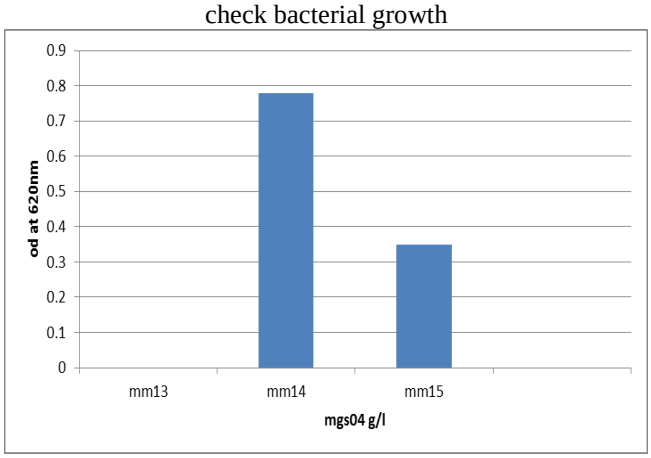


Graph 3: best concentration of Nacl was shown by mm10.

4) $Mgso_4$ optimization

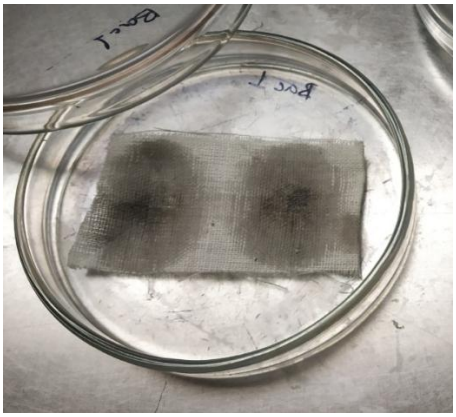
$Mgso_4$	OD at 620
Mm13	0.00
Mm14	0.78
Mm15	0.3

Table 10: different concentration of mgso4 was used to check bacterial growth

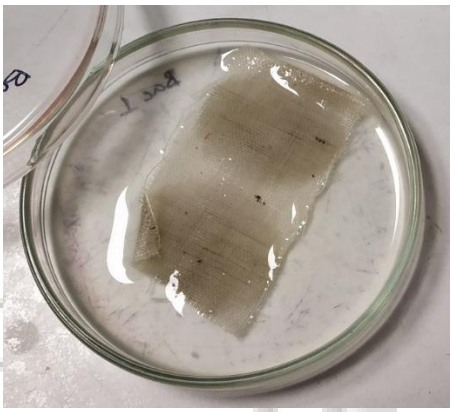


Graph 4: optimum concentration of mgso4 was found in mm14.

IV. APPLICATION



Control oil treated cloth



Oil treated cloth with s. Aureus

Fig. 4.1: oil dipped clothes were incubated with S. aureus; it was found bacteria degraded oils

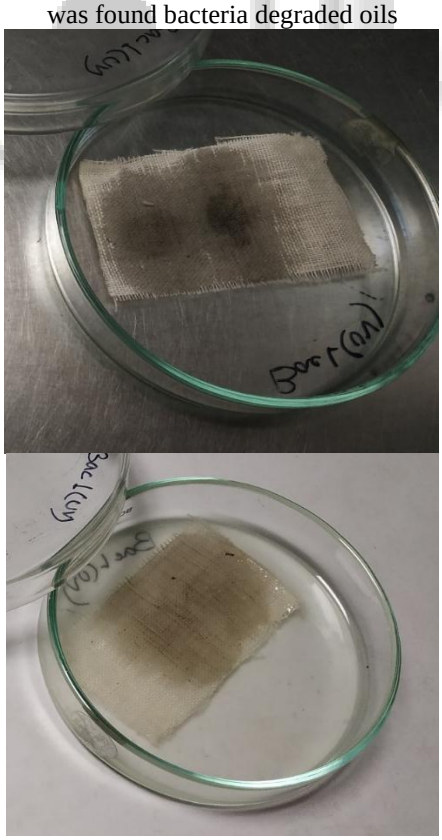


Fig. 4.2: transformed bacteria was incubated with oil dipped clothes, oil concentration was reduced.

V. CONCLUSION

Oil waste generated during the processing, transportation, refining of petroleum, which are spilled out or disposed on the soil and water surface are serious environmental treats. Many studies articles have documented the potentials of microorganisms to degrade oil both in the laboratory and field trials. In this study, we have isolated and investigated oil degrading bacteria from oil contaminated soil; identified as Staphylococcus aureus by observing the biochemical tests. The potency of oil degradation of the wild strain was improved or enhanced by UV mutation (subjecting to sequential UV radiation) and Transformation with E. coli genome. The findings of the experiment reinforce to investigate the enhancement of oil degrading capacity of potent wild bacterial strains; highlighting the need for more scientific research to study biodegradation in this environment

REFERENCES

[1] Galdames A., Mendoza A., Orueta M., Garcia I.S.S., Sanchez M. Virto I. and Vilas J.L. Development of new remediation for contaminated soils based on the application of zero-valent iron nanoparticles and bioremediation with compost. Resource-Efficient Technologies 2017; 3:166-176.

[2] Alvim G. M. and Pontes P. P. Aeration and sawdust application effects as structural material in the bioremediation of clayey acid soils contaminated with diesel oil. International Soil and Water Conservation Research 2018; 6: 253-260.

[3] Syrmanova K.K., Kovaleva A.Y., Kaldybekova Z.B., Botabayev N.Y., Botashev Y.T. and Beloborodov B.Y. Chemistry and Recycling Technology of Used Motor oil. Oriental Journal of Chemistry 2017; 33(6):3195-3199.

[4] Abdulsalam S., Adefila S.S., Bugaje I.M. and Ibrahim S. Bioremediation of Soil Contaminated With Used Motor Oil in a Closed System. Journal of Bioremediation & Biodegradation2012; 3:12.

[5] Santhakumar M., Sivalingam S., Dharmapal D. and Sadras S.R. Characterization of dioxygenases and biosurfactants produced by crude oil degrading soil bacteria. Brazilian Journal of Microbiology 2017; 48:637-647.

[6] Al-Hawash A.B., Dragh M.A., Li S., Alhujaily A., Abbood H.A, Zhang X. and Ma F. Principles of microbial degradation of petroleum hydrocarbons in the environment. Egyptian Journal of Aquatic Research 2018; 44:71–76.

[7] Vieira G.A.L., Magrini M.J., Bonugli-Santos R.C., Rodrigues M.V.N and Sette L.D. Polycyclic aromatic hydrocarbons degradation by marine-derived basidiomycetes: optimization of the degradation process. Brazilian Journal of Microbiology 2018.

[8] Hamzah A., Sabturani N. &Radiman S. Screening and Optimization of Biosurfactant Production by the Hydrocarbon-degrading Bacteria. SainsMalaysiana 2013; 42(5): 615–623.

[9] Sharma D., Ansari M.J., Al-Ghamdi A., Adgaba N., Ali Khan K., Pruthi V. & Al-Waili N. Biosurfactant production by Pseudomonas aeruginosa DSVP20

isolated from petroleum hydrocarbon-contaminated soil and its physicochemical characterization. Environ SciPollut Res 2015.

[10] Borah D. and Yadav R.N.S. Bioremediation of petroleum based contaminants with biosurfactants produced by a newly isolated petroleum oil degrading bacterial strain. Egyptian Journal of Petroleum 2017; 26:181-188.

[11] Dixit H., Lowry M., Mohsin U., Moond M., Kumar S., Chokriwal A. and Gupta S. Screening and Identification of Diesel Oil Degrading Bacterial Isolates from Petroleum Contaminated soil of Barmer. Journal of Pharmaceutical, Chemical and Biological Sciences 2018; 6(1):34-40.

[12] Keskin N.O.S., Han D., Ozkan A.D., Angun P., Umu O.C.O. and Tekinay T. Production and structural characterization of biosurfactant produced by newly isolated Staphylococcus xylosus STF1 from petroleum contaminated soil. Journal of Petroleum Science and Engineering 2015; 133:689–694.

[13] Patel N.K. and Pethapara H. Isolation and screening of hydrocarbon degrading bacteria from soil near Kadi (Gujarat) region. International Journal of Research in BioSciences 2013; 2(4):10-16.

[14] Joshi P.A. and Shekhawat D.B. Screening and isolation of biosurfactant producing bacteria from petroleum contaminated soil. European Journal of Experimental Biology 2014; 4(4):164-169.

[15] Leal A.J., Rodrigues E.M., Leal P.L., Julio A.D.L., Fernandes R.D.C.R., Borges A.C. and Totola M.R. Changes in the microbial community during bioremediatrion of gasoline- contaminated soil. Brazilian Journal of Microbiology 2017; 48:342-351.

[16] Adams F.V., Niyomugabo A. and Sylvester O.P. Bioremediation of Crude Oil Contaminated Soil Using Agricultural Wastes. Procedia Manufacturing 2017; 7:459-464.

[17] Assadi M.M. and Tabatabaee M.S. Biosurfactants and their Use in Upgrading Petroleum Vacuum DistillationResidue: A Review. Int. J. Environ. Res. 2010;4(4):549-572.

[18] Farag S., Soliman N.A. and Abdel-Fattah Y.R. Statistical optimization of crude oil bio-degradation by a local marine bacterium isolate Pseudomonas sp. sp48. Journal of Genetic Engineering and Biotechnology 2018.

[19] Kumari N., Vashishtha A., Saini P. and Menghani E. Isolation, Identification and Characterization of Oil Degrading Bacteria Isolated from the Contaminated Sites of Barmer, Rajasthan. International Journal of Biotechnology and Bioengineering Research 2013; 4(5):429-436.

[20] Jayanthi R. and Hemashenpagam N. Isolation and identification of petroleum hydrocarbon degrading bacteria from oil contaminated soil samples. International Journal of Novel Trends In Pharmaceutical Sciences 2015; 5(3):102-106.

[21] Anitha J., Jeyanthi V. and Ganesh P. Production and characterization of biosurfactant by Bacillus and its applicability in enhanced oil recovery. International Journal of Advanced Research in Biological Sciences 2015; 2(5):7–16.

[22] Mukherjee A., Chettri B., Langpoklakpam J.S., Basak P., Prasad A., Mukherjee A.K., Bhattacharyya M., Singh A.K &Chattopadhyay D. Bioinformatic Approaches Including Predictive Metagenomic Profiling Reveal Characteristics of Bacterial Response to Petroleum Hydrocarbon Contamination in Diverse Environments. Scientific Reports 2017; 7:1108.

[23] Zaidan U.H., Rahman M.B.A, Salleh A.B., Basri M. and Othman S.S. Physicochemical Characterization of Lactose-Based Ester as Potential Pharmaceutical Biosurfactant. International Journal of Pharmaceutical Sciences Research 2017; 4: 120.

[24] Mahalingam P. U. and Nithya S. Isolation, characterization and identification of bacterial biosurfactant. European Journal of Experimental Biology 2014, 4(6):59-64.

[25] Marzan L.W., Hossain M., Mina S.A., Akter Y. and Chowdhury A.M.M.A. Isolation and biochemical characterization of heavy-metal resistant bacteria from tannery effluent in Chitttagong city, Bangladesh: Bioremediation viewpoint. Egyptian Journal of Aquatic Research 2017; 43: 65-74.

[26] Kalita D. and Joshi S.R. Study on bioremediation of Lead by exopolysaccharide producing metallophilic bacterium isolated from extreme habitat. Biotechnology Reports 2017; 16:48-57.

[27] Kafilzadeh F., Ebrahimnezhad M. and Tahery Y. Isolation and Identification of Endosulfan-Degrading Bacteria and Evaluation of Their Bioremediation in Kor River, Iran. Osong Public Healtrh Res Perspect 2015; 6(1): 39-46.

[28] Hesnawi R.M. and Mogadami F.S. Bioremediation of Libyan Crude Oil-Contaminated SoilunderMesophilic and Thermophilic Conditions. APCBEE Procedia 2013; 5: 82-87.

[29] Dey U., Chatterjee S., Mondal N.K. Isolation and Characterization of arsenic-resistant bacteria and possible application in bioremediation. Biotechnology Reports 2016; 10:1-7.

[30] Abd-Elnaby H.M., Abou-Elela G.M., Ghozlan H.A., Hussein H. and Sabry S.A. Characterization and bioremediation potential of marine Psychrobacter species. Egyptian Journal of Aquatic Research 2016; 42:193-203.

