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ENHANCING PETROLEUM HYDROCARBON DEGRADATIVE PROPERTIES OF *PSEUDOMONAS* SPECIES BY INDUCED MUTATION

[1]Mandalaywala Hetal P., [2]Ratna Trivedi
Environmental Sciences Department, Shree Ramkrishna Institute of Computer Education and Applied Sciences, M.T.B. college campus, Athwalines, Surat-395001, Gujarat, (INDIA)

ABSTRACT

Petroleum hydrocarbons are one of the major environmental pollutant owing to its well-spread use. Being harmful to environment and living forms, it is essential to treat and safely dispose it. Petroleum hydrocarbons are recalcitrant in nature and thus are not easily degraded in nature. Nevertheless, hydrocarbon degrading property of Pseudomonas species is well documented. In this study,Pseudomonas species isolated from a hydrocarbon based contaminated site were isolated and exposed to UV radiation for a specific time period to induce mutation. These mutated strain were then checked for their degradation capacity as compared to the wild type. The mutated strains showed enhanced degradation rate. The mutated and wild strain were then identified by 16sRNA sequencing. This study can contribute in the development of new strategies in bioremediation techniques of petroleum hydrocarbons.

Keywords: Induced mutation, enhancing degradative property, petroleum hydrocarbon degradation, *Pseudomonas*species.

1. INTRODUCTION

Petroleum products are major source of energy for industrial and domestic life. Due its increasing use and over exploitation, hydrocarbon based pollution has become a matter of concern. Pollution may arise due to accidental spillage, leakage, transportation and so on. The amount of natural crude oil seepage was estimated to be 600,000 metric tons per year with a range of uncertainty of 200,000 metric tons per year. [Nilanjana D*et al*, 2011] Hydrocarbons create extensive pollution. Hydrocarbons are known to be harmful for living beings and environment.

Thus the treatment and safe disposal is inevitable. There are many physical, chemical and biological treatment methods to breakdown hydrocarbon based pollutants and rendering it harmless.

Bioremediation is one such treatment method. Bioremediation is cost effective and non-invasive method. Different variations in the process has been studied so far in this field to make the process more effective, like bioremediation using specific indigenous microorganisms, use of consortium, and so on. Moreover, it is also known thatmutation can have impact on the fitness of an organism. [Erick Denamur and Ivan Matic, 2006]

In this study the hydrocarbon utilizing bacteria isolated from a contaminated source were manually mutated using UV radiation to check its effect on the hydrocarbon degrading property. The isolates were then identified as*Pseudomonas* species by 16s RNA sequencing method.

2. METHODOLGY

Six strains of Pseudomonas species were isolated from petroleum based polluted site by screening and isolation methods. [RehmanNaziya*et al*,2014] These strains were checked for their hydrocarbon degrading capabilities.

2.1 Hydrocarbon degradation: Strains were inoculated in Tributyrin broth (TBA) with 0.5 % Petrol as a substrate. After 120 hours, OD was measured so as to determine the increase in growth. Increase in growth implies strains are able to consume petrol as substrate and multiply.

2.2 Induced mutation: The strains were streaked on to Tributyrin Agar in a petri plate and was exposed to UV radiation for definite time, to induce mutation. [Naveen Kumar, 2010]

These mutated strains were then checked for their petroleum hydrocarbon degrading activity by similar method as in wild type. That is by inoculating in TBA broth with 0.5% petrol and OD was checked after 120 hours.

2.3 Emulsification Index: Both wild type and mutated strains were tested for its emulsification index. 2ml of48 hours old culture were suspended in TBA broth in a test tube, which was topped by equal amount of oil. Then, the mixture was vortexed at high speed for 1 min and allowed to stand for 24 hours. The emulsion index (E24) is the height of the emulsion layer (cm) divided by total height (cm), multiplied by 100). [V. Saravananet al, 2012]

2.4 Phylogenetic Study: The isolates were identified by 16 sRNA sequencing at Saffron's Gene lab, and further submitted to NCBI. Accession numbers obtained are mentioned in table 2 & 3.

The sequences of wild strain and mutated strains were then compared using various phylogenetic tools to determine the similarity between the corresponding wild strain with mutated strain.

3.RESULT AND DISCUSSION

In both the cases of wild type and mutant type, OD was check by a photoelectric colorimeter at 610 nm after 120 hours of incubation in incubator shaker at 30° C. The values were measured and recorded. The figure 1 shows the graph of the OD of both wild type and mutant type.

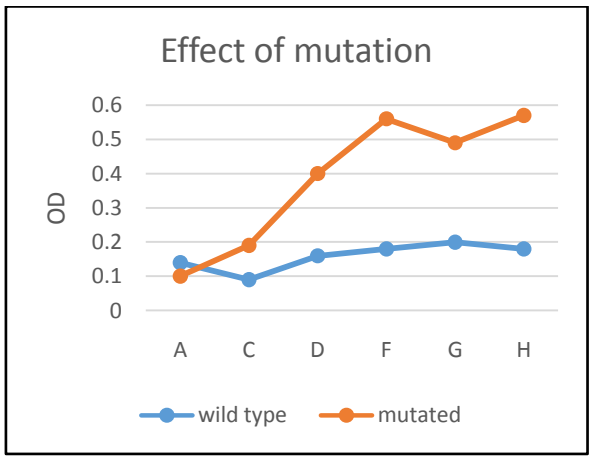


Figure 1. Comparison of mutated and wild type strains

The graph clearly shows that, the mutated strains were able to consume the petroleum hydrocarbons and multiply at faster rate as compared to the wild type.

Table 1. Result of Emulsification index

	Emulsification Index (%)
Wild type :	
A	33.3
C	50.0
D	40.0
F	50.0
G	46.6
H	50.0
Mutant strain:	
A	48.1
C	48.1
D	36.1
F	48.1
G	42.1
H	60.0

The emulsification Index also shows that the emulsification Index of mutant strains are higher than that of wild strains. The strains were identified by 16s RNA sequencing method as follows:

Table 2. Wild type strains identified:

Isolate	Isolate name	Accession number
A	*Pseudomonas aeruginosa* strain RRLP1	KU314415
C	*Pseudomonas aeruginosa* RRLP1	KU314416
D	*Pseudomonas aeruginosa* Strain AS-1	KU314417
F	*Pseudomonas aeruginosa* Strain JQ-41	KU314418
G	*Pseudomonas aeruginosa* Strain SI5(1)3	KU314419
H	*Pseudomonas spp.* Strain 14-1	KU314420

Out of the mutant type strains, the strains showing highest OD i.e., with most enhanced petroleum hydrocarbon degradative activities viz., mutant F and mutant H were identified by 16s RNA sequencing method.

Table 3. Mutant strains identified:

Isolate	Isolate name	Accession number
F	*Pseudomonas aeruginosa* strain SNP0614	KX225388
H	*Pseudomonas aeruginosa* strain SNP0614	KX225389

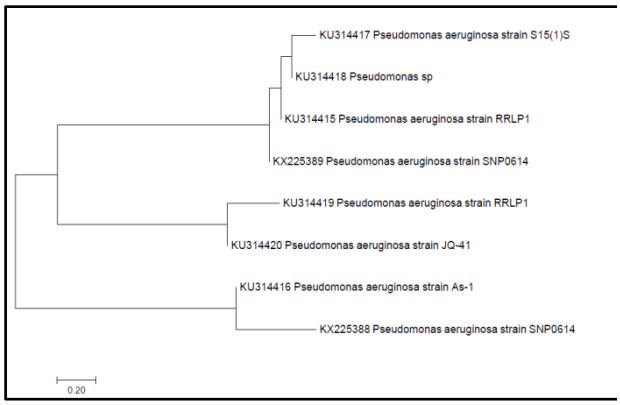
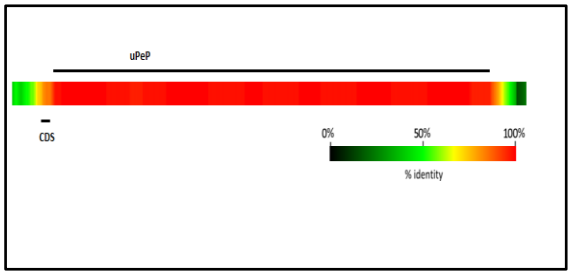


Figure 2. Phylogram of isolates

The sequences were also studied for comparative genomics by the use of a software VISTA.

Figure 3. Comparative genomics by software VISTA.



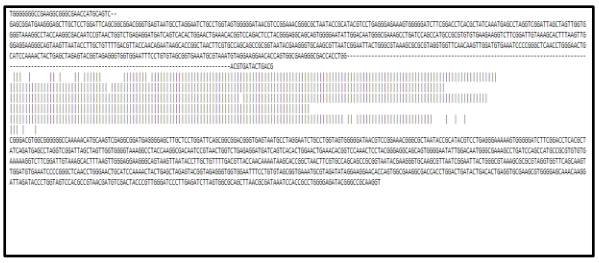
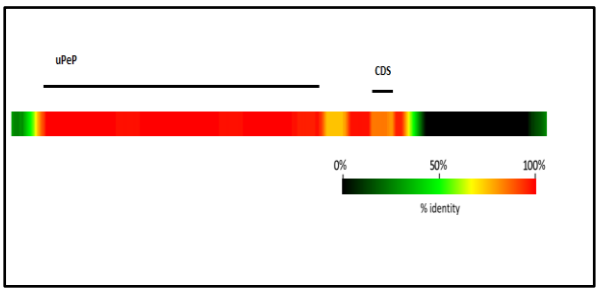


Figure 3. (a) & (b) Heatmap diagram and sequence alignment of wild type (F) KU314418 & its mutant strain KX225388



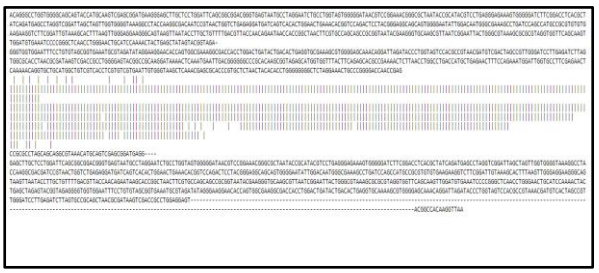


Figure 4. (a) & (b) Heat map diagram and sequence alignment of wild type (H) *KU314420 & its mutant strain KX225389*

It can be seen that the resemblance is quit high in both the cases, which is shown by uPeP. There are a few CDS positions as well. This shows the remarkable similarity between the mutant and corresponding wild strain. This was further confirmed by their protein sequence analysis.

The nucleotide sequences of isolates (Wild type and mutated) converted to protein sequences using ExPASy Translate tool and

then was compared to the standard Rhamnolipid producing rhlAB gene protein sequence by using Emboss matcher Protein alignment tool. The similarities are as shown in the table below.

Table 4. Comparison of sequences of standard Rhamnolipid with sequences of isolates

Isolate	A	C	D	F	G	H	F Mutated	H Mutated
Similarity with Rhamnolipid	45.2 %	61.5 %	77.8 %	45.2 %	45.2 %	45.2 %	45.2 %	45.2 %

4.CONCLUSION

From this study it can be concluded that induced mutation enhanced the degradative properties of *Pseudomonas* species.

The comparative genomics study showed that the nucleotide sequence of mutant and wild strains of the isolates were quite similar. But when the sequences were compared on protein level, the difference among the mutant and wild type strains were observed.

This study can provide guidance for strategic steps for bioremediation of petroleum hydrocarbons by *Pseudomonas* species. Further research in this regard is advised with different strains and different substrates.

5. REFERENCES

1. Chibuike, G. U., Obiora, S. C (2014). "Bioremediation of hydrocarbon-polluted soils for improved crop performance." International Journal of Environmental Sciences 4(No.5).
2. Chinenye C. Chijioke-Osuji, P. N. I.-N. a. E. J. D. B. (2014). "Biodegradation of Crude Oil Polluted Soil by Co-Composting with Agricultural Wastes and Inorganic Fertilizer." Journal of Natural Sciences Research 4.
3. Erick Denamur and Ivan Matic (2006). “Evolution of mutation rates in bacteria” Molecular Microbiology. 60(4), 820–827. doi:10.1111/j.1365-2958.2006.05150.x
4. FarinazleenMohamadGhazali, Raja Noor Zaliha Abdul Rahman, Abu BakarSalleh, MahiranBasri.,(2004)" Biodegradation of hydrocarbons in soil by microbial consortium. " InternationalBiodeterioration& Biodegradation. Volume 54
5. Ilyina A., C. S. M. I., Villarreal Sanchez J. A.*, Ramirez Esquivel G., and C. R. J. (2003). "Isolation Of Soil Bacteria for Bioremediation of Hydrocarbon

Contamination." ВЕСТН. МОСК. УН-ТА.

6. Naveenkumar.S, M. N., Ganesan. S ,Manivannan.S.P , Velsamy. G (2010). "Isolation, Screening and In Vitro Mutational Assessment of Indigenous Soil Bacteria for Enhanced Capability in Petroleum Degradation." International Journal of Environmental Sciences 1.
7. Nilanjana Das and Preethy Chandran (2010). "Microbial Degradation of Petroleum Hydrocarbon Contaminants: An Overview." SAGE-Hindawi Access to Research Biotechnology Research International 2011.
8. Oliveira, I. N. S.-G. a. V. M. d. (2013). "Microbial Hydrocarbon Degradation: Efforts to Understand Biodegradation in Petroleum Reservoirs." http://dx.doi.org/10.5772/55920.
9. RehmanNaziya N. M. Aziz., S. M. B., Dixit P.P. and Deshmukh A.M. (2014). "Screening of Biosurfactant Producing Microorganisms from Oil Contaminated Soils of Osmanabad Region, Maharashtra, India." International Science Journal 1(1)
10. V. Saravanan and S. Vijayakumar (2012). "Isolation and screening of biosurfactant producing microorganisms from oil contaminated soil". *J. Acad. Indus. Res.* Vol. 1(5). 264-268