INTERNATIONAL JOURNAL OF PLANT, ANIMAL AND ENVIRONMENTAL SCIENCES

Volume-1, Issue-2 :2011 *www.ijpaes.com* *ISSN 2231-4490*

ISOLATION AND MOLECULAR CHARACTERIZATION OF HYDROCARBON DEGRADING BACTERIA FROM TANNERY EFFLUENT

Vivek kumar chaudhary[1] and Debajit Borah[2*]

[1]Dept. of Biotechnology, Beehive College of Advance Studies, Dehradun, India, Pin-248197
[2*]Dept. of Biotechnology, Center for Studies in Biotechnology and Bio informatics, Dibrugarh University, Assam, India, Pin-786004

ABSTRACT: The study on two hydrocarbon degrading bacteria *Bacillus spp.* and *Corynebacterium spp*. isolated from tannery effluent indicated that *Bacillus spp.* has a greater potential to degrade hydrocarbon when grown in a medium containing used engine oil. Degradation potential of *Bacillus spp.* was tried to increase by inducing mutation by UV radiation and the mutants were identified by performing RAPD.
Keywords: Tannery effluent *Corynebacterium spp., Bacillus spp.,* mutation, molecular characterization.

INTRODUCTION

An oil spill is the release of a liquid petroleum hydrocarbon into the environment due to human activity, and is a form of pollution. The term often refers to marine oil spills, where oil is released into the ocean or coastal waters. The oil may be a variety of materials, including crude oil, refined petroleum products (such as gasoline or diesel fuel) or by-products, oily refuse or oil mixed in waste (Christopher, 2004). Interest in the microbial biodegradation of pollutants has intensified in recent years as humanity strives to find sustainable ways to clean up contaminated environments. These bioremediation and biotransformation methods endeavor to harness the astonishing, naturally occurring, ability of microbial xenobiotic metabolism to degrade. The ability to isolate high numbers of certain oil-degrading microorganisms from an environment is commonly taken as evidence that those organisms are the active degraders of that environment (Okerentugba and Ezeronye, 2003). Bioremediation is a process that uses microorganisms, fungi, green plants or their enzymes to return the natural environment altered by contaminants to its original condition (Chaudhary A. *et al.* 2009). Although, hydrocarbon degraders may be expected to be readily isolated from a petroleum-polluted environment, the same degree of expectation may be anticipated for microorganisms isolated from a total unrelated environment.

The aim of the present study was to isolate hydrocarbon degrading bacteria from tannery effluent samples collected from a few numbers of tannery industries located in Agra (U.P), India and to do their mutational and molecular characterization with the help of Randomly Amplified Polymorphic DNA (RAPD) technique associated with restriction digestion.

METHODS AND MATERIALS

Modified method of Mandri and Lin (2007) was used to isolate hydrocarbon degrading bacteria from the tannery effluent samples. Tannery samples were diluted up to 10^{-3} fold and added 1% v/v to 100 ml of MEM broth supplemented with 1% v/v used engine oil taken in a 250 ml Erlenmeyer flask and maintained at 150 rpm for 72 hrs at 36°C in a rotary shaker incubator (Lab-line No. 3940). Now 1 ml of each broth were transferred to MEM agar plates containing 1% v/v used engine oil and incubated at 32°C for 3-5 days. After incubation the bacterial colonies were sub cultured and identified by various staining techniques and biochemical characteristics prescribed by Bergey's Manual of Systematic Bacteriology (Vos D. P. *et. al,* 2009). The hydrocarbon degradation potential of the isolates were characterized by observing O.D. at 600 nm of the isolates on LB broth containing different concentrations of used engine oil maintained at 36°C for 48 hrs (Borah D., 2011).

To induce mutation on the bacterial isolates by UV radiation to increase its potential to degrade used engine oil:

The isolated *Bacillus spp.* (H, U1, U2) were exposed to UV radiation for 5 min, 10 min, 15 min respectively and the growth was characterized by inoculating in LB broth containing different concentrations of used engine oil and O.D. was taken at 600 nm after 48 hrs of incubation at 36°C. Mutants were identified by doing RAPD (randomly amplified polymorphic DNA) associated with restriction digestion using a random primer and the scoring results were run in software "Statistica" for the construction of linkage map of the samples.

RESULTS & DISCUSSION

Hydrocarbon degrading microorganisms were isolated by using the methods of T. Mandri and J. Lin (2007). The bacterial colonies were isolated from tannery effluent samples and were identified as *Bacillus spp*. and *Corynebacterium spp*. on the basis of various staining techniques and biochemical tests prescribed by Bergey's Manual of Systematic Bacteriology (2nd edition). Hydrocarbon degradation potential of both the isolates were determined taking the absorbance at 600 nm after 72 hrs of incubation of the isolates in MEM broth containing 1% v/v used engine oil as a carbon source. The O.D. of *Corynebacterium spp*. and *Bacillus spp*. were found to be 0.420 and 0.733 respectively (Table 1) at 600 nm.

Table 1: OD at 600 nm of isolated bacterial samples:

Sample (inoculated in 10 ml MEM with 1% v/v used engine oil)	OD at 600nm (42 hrs of incubation at 36°C)	OD at 600nm (72 hrs of incubation at 36°C)
Corynebacterium spp.	0.366	0.420
Bacillus spp.	0.451	0.733

The degradation potential of *Bacillus spp.* was characterized by inoculating the sample in LB broth containing different concentration of used engine oil and the O.D. was taken at 600 nm after 72 hrs of incubation at 36°C (Table 2). An increase in O.D. with the increase in concentration of used engine oil in the media represents an increase in the bacterial cell population as they are utilizing the engine oil as their sole carbon source. *Bacillus spp*. was found to show the maximum hydrocarbon degradation potential and the degradation potential was tried to increase by inducing mutation by exposing to UV radiation for different time durations (Table 3). DNA samples of all the samples were isolated (Fig 1) and digested by restriction enzyme *EcoRI* (Fig 2).

Table 2: Variation in the O.D. of *Bacillus spp*. with respect to the change in concentration of used engine oil in the medium:

Volume of MEM broth (in ml) inoculated with Bacillus spp.	Volume of used engine oil	Incubated for 48 hrs at 36°C	Incubated for 72 hrs at 36°C
10	10 µl	0.663	1.456
10	20 µl	0.798	1.498
10	30 µl	0.863	1.674
10	40 µl	0.910	1.959
10	50 µl	1.082	2.097

Table 3: Variation in O.D. at 600 nm of UV treated *Bacillus spp*.

Sample code	Time of UV exposure	Medium used	24 hours	48 hours
H	5 min	LB broth with 100 µl used engine oil	1.268	1.575
U1	10 min	LB broth with 100 µl used engine oil	0.959	1.444
U2	15 min	LB broth with 100 µl used engine oil	1.796	1.954

Where,
S= non UV treated *Bacillus spp*.,
H= 5 min UV treated *Bacillus spp.*,
U1= 10 min UV treated *Bacillus spp.* and
U2= 15 min UV treated *Bacillus spp.*

Hierarchical Clustering (Joining) Result:
Number of variables: 4
Number of cases: 10
Joining of variables
Missing data were case wise deleted
Amalgamation (joining) rule: Unweighted pair-group average
Distance metric is: Euclidean distances (non-standardized)

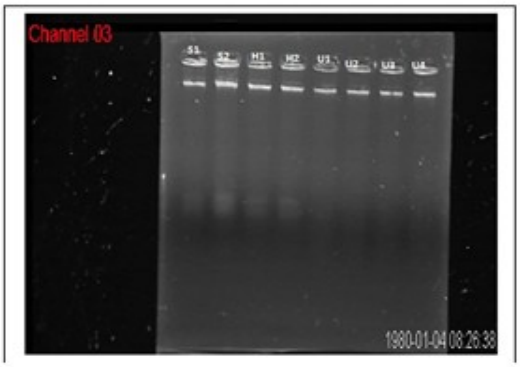

Fig 1: DNA bands of the samples:

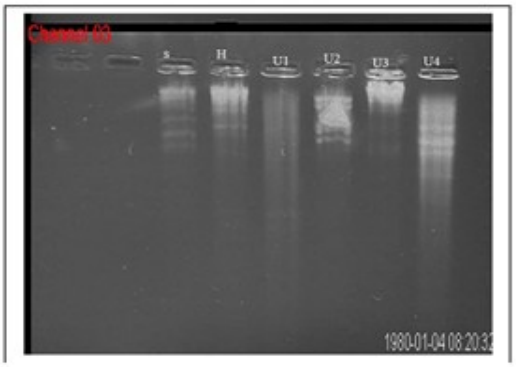

Fig 2: Restriction digestion of the samples:

The mutants were identified by performing RAPD (Fig 3) associated with restriction digestion using a random primer and scoring was done with all the samples by comparing the DNA bands with the UV untreated *Bacillus spp.* sample (S) and scoring results (Table 4) were run in a software "Statistica" to construct a linkage map (Fig 4). The linkage distance determined were tabulated (Table 5).

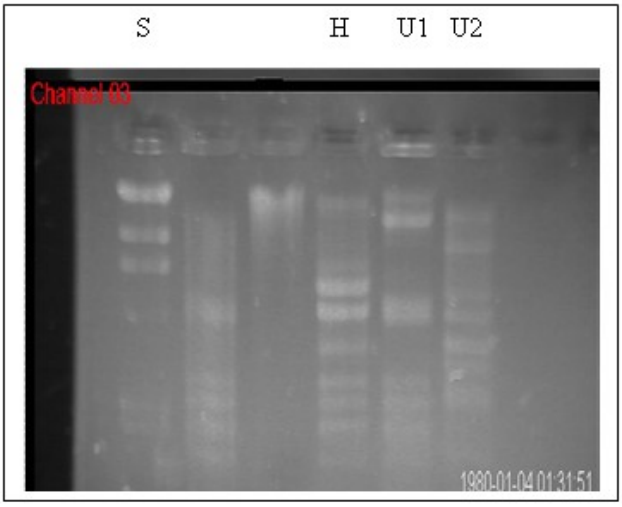

Fig 3: DNA fingerprinting of Bacillus spp. samples after RAPD of sample S, H, U1, U2 (S= non UV treated, H= 5 min UV treated, U1= 10 min UV treated and U2= 15 min UV treated).

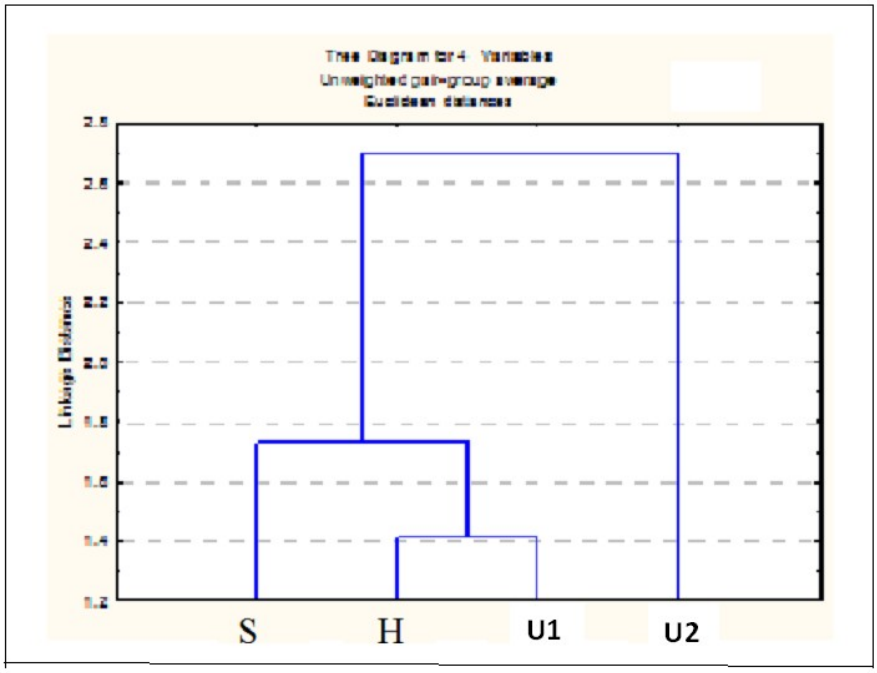

Fig 4: Linkage map showing linkage distances among the samples

Table 4: Scoring results of the samples after RAPD

NO. OF CASES \ SAMPLES	S	H	U1	U2
1	1	0	0	0
2	0	1	1	0
3	1	0	0	1
4	1	0	0	0
5	0	1	0	0
6	0	1	1	1
7	0	1	0	1
8	0	1	1	1
9	0	1	1	1
10	1	1	1	1

Table 5: Table showing the linkage distances among the samples

Linkage distance among the samples	Linkage units
S and H	1.8
S and U1	1.8
S and U2	2.7
H and U1	1.4
H and U2	2.7
U1 and U2	2.7

CONCLUSION

Our present study shows *Bacillus spp.* having maximum oil degradation potential. It has some advantages over the *Pseudomonas spp.,* as it is less pathogenic in comparison to the *Pseudomonas spp*. (Borah D., 2011), and can be handled in normal lab conditions and also the mutation was said to be successful to increase its degradation potential.

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