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Growth Potential Assessment of Actinomycetes Isolated from Petroleum Contaminated Soil

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Abstract

Uncontrolled release of hydrocarbon compounds that are carcinogenic, mutagenic and are potent immunotoxicants into soil and ground water poses a serious threat to human and animal health. Due to their extensive use, they cause serious environmental pollution which has drawn attention towards the research. In the present study total 134 indigenous actinomycetes isolates were obtained from different Petrol contaminated sites (N=40). Fifty one actinomycetes strains were able to grow on 5% crude oil containingineral salt Medium showingaximum growth at temperature 30°C and pH 7.5. They were identified on basis of cultural, morphological and biochemical characteristics as *Streptomyces* sp.1, *Streptomyces* sp.3, *Streptomyces* sp.2, *Rhodococcus* sp., *Nocardia* sp.2 and *Nocardia* sp.1. Isolates were tested for their growth potential on Mineral Salt Broth/Agar supplemented with hydrocarbons *viz.* Crude oil, Anthracene, Coronene, Napthacene, Acenapthene at concentrations 5%, 10% and 15% incubated for 5 days, 10 days and 15 days. All the isolates utilized the hydrocarbons as sole carbon and energy sources in an unequal rate thus suggesting genetic disimilarities in respect of oil degradation capabilities. The study clearly demonstrates that Gram-positive actinomycetes showed good growth potential on hydrocarbon as substrate and support its effective use in hydrocarbon degradation.

Keywords: Actinomycetes; Crude oil; Anthracene; Coronene; Napthacene; Acenapthene

Introduction

The extensive use of petroleum products lead to severe contamination of the environment, becoming a great threat to the natural habitat [1]. One of the most urgent problems of the modern world is environment purification from different toxic residues. Oil hydrocarbons are the principal pollutants of the environment. Today great attention is paid for the working out of ecologically safe biological technology for rehabilitation and biodegradation of soil contaminated with crude oil [2].

As a result of the increase in automobiles, the number of gasoline/diesel station and automobile service station is ever increasing. In gasoline, diesel station and service stations, oil is spilled during transfer and servicing operations. During accidental spills, action has to be taken to remove or remediate or recover the contaminant immediately, whereas in the gasoline and diesel stations the spills due to leakage may be small but continuous and prolonged. Because of its persistence, the chance for groundwater contamination is greater [3].

Hydrocarbon degradation is a highly oxidative process in which molecular oxygen is necessary. Therefore, for maximal degradation both aeration and agitation are required. Agitation of medium maintains homogenous chemical and physical conditions, disperses the dissolved oxygen into smaller bubbles thereby increasing the interfacial area. As the agitation speed increases, oxygen transfer rate increases resulting in higher degradation [4]. The actinomycetes are important oil degraders and the strains of *Streptomyces rochei, Streptomyces plicatus, Streptomyces diastaticus, Nocardia, Frankia* and *Rhodococcus fascians* have commonly been isolated from oil wells and soils. Actinomycetes possess many properties that make them good candidate for application in bioremediation of soil contaminated with organic pollutants. They play an important role in the recycling of organic carbon and are able to degrade complex polymers. Some reports indicated that *Streptomyces* flora could play a very important role in degradation of hydrocarbons. Many strains have the ability to solubilise lignin and degrade lignin-related compounds by producing cellulose and hemicellulose degrading enzymes and extracellular peroxidases. In some contaminated sites, actinomycetes represent the dominant group among the degraders [5]. Actinomycetes are gram positive filamentous bacteria and, are good choice as biosurfactant producer because of their abundance in soil and their major roles in recycling of material in nature. Moreover, they have been found to produce many kind of metabolites including antibiotics, pigments, enzyme, biosurfactants [6].

Hydrocarbon biodegradation in soil can be limited by many factors, for example microorganism type, nutrients, pH, temperature, moisture, oxygen, soil properties and contaminant presence [7].

They produce extracellular enzymes that degrade a wide range of complex organic compounds and spores that are resistant to desiccation. In addition, the frequently occurring filamentous growth favours the colonization of soil particles [8]. In the case of the actinomycetes, the surfactant activity is due to the production of extracellular biosurfactants, specially glycolipids, like trehalose lipids produced by *Rhodococcus* species [9-11] or the lipopeptide produced by *Arthrobacter* sp. strain MIS38 [12], cellular biosurfactants such as mycolic acids that give adherence of the microbial cells to hydrophobic phases in two-phase systems [13]. Many actinomycetes can degrade different pollutants, including several pesticides. For example, members of the genus *Arthrobacter* degrade 4-Chlorophenol [14], Atrazine [15] and Monocrotophos [16] and *Streptomyces* sp. degrades Alachlor

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[17]. The nocardioform actinomycetes (*e.g. Rhodococcus*, *Gordonia*, and *Mycobacterium*) are known hydrocarbon degraders [18-20] and degrade PAH in soil. In some contaminated sites they also represent the dominant group among the degraders [18,21].

The purpose of this study was to examine the hydrocarbon degradation capabilities of actinomycetes in contaminated soil and also to determine the capabilities of the recovered actinomycetes to grow on petroleum and their derivatives.

Materials and Methods

Screening of samples

The soil samples (100 g each) were collected from fuel oil pumps at different location of North India like, Mathura Oil Refinery, Lucknow Railway carriage wagon sites and different petrol pumps of Lucknow for the isolation of oil degrading microorganisms. The samples were collected in pre-sterilised glass bottles and transported to the laboratory for analysis. Enumeration and isolation of heterotrophic bacteria and actinomycetes was carried out through serial dilution agar plating technique using Casein Glycerin (Starch) agar, and Mineral salt medium (HIMEDIA) respectively [5].

Isolation of actinomycetes from hydrocarbon contaminated soil

Isolation of actinomycetes was carried out through tenfold serial dilution agar plate technique using Casein Glycerin (starch) Agar medium (CGA) at pH 8.0. 1g of air dried soil sample was mixed with 9 ml sterilized distilled water. The mixture was shaken vigorously for 15 minutes and allowed to settle for 5 min. 1 ml aliquots of soil suspension (diluted to 10^{-1}) was transferred to 9 ml sterilized distilled water and subsequently diluted upto 10^{-4}. Aliquots from 10^{-4} dilution was pour plated using CGA medium in duplicates. Uninoculated plate served as control. Plates were incubated at 28 ± 2°C for 7 days. Morphologically different colonies were selected and streaked over Mineral Salt Agar medium supplemented with 5% for screening and incubated at 28 ± 2°C for 7 days. Growth was examined regularly. Isolates were maintained on CSA slant subcultured regularly at 15 days interval and incubated at 28 ± 2°C for 5 to7 days and then stored at 4°C [5].

S. No.	Sample sites	Sample size N=40	Average cfu/g (10^5)
1	Mathura Oil refinery	10	**4.3**
2	HP Petrol Pump, Lucknow	10	2.7
3	Railway carriage wagon, Lucknow	10	1.7
4	Bharat Petrol Pump, Lucknow	10	1.1

Table 1: Enumeration of actinomycetes isolated from hydrocarbon contaminated soil.

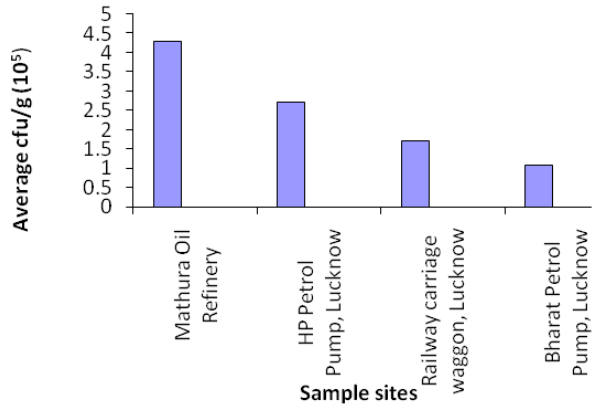


Figure 1: Distribution of population of actinomycetes in hydrocarbon contaminated soil samples.

Identification of actinomycetes isolated from hydrocarbon contaminated soil

The identification was done by on the basis of morphological and biochemical characteristics as per Bergeys Manual of Systemic Bacteriology [2].

Effect of temperature and pH on growth

The effect of temperature and pH on the growth of isolates was studied using Mineral salt medium supplemented with (5%) crude oil inoculated with the isolates and exposed to different temperatures (10°C, 20°C, 30°C, 40°C and 50°C) and pH (5.5, 6.5, 7.5, 8.5 and 9.5). Uninoculated tubes served as control. At 5-days intervals, total viable count (log cfu/ml) was observed [22,23].

Growth potential of actinomycetes isolates on model hydrocarbons

Biodegradation capability of the actinomycetes was determined by the method given by Mills et al. [24]. Selected petroleum derivatives as representative hydrocarbon were assessed to check the growth of isolated actinomycetes on individual hydrocarbons supplemented medium. For these overnight cultures (1%) of actinomycetes were transferred to tubes, containing 5 ml Mineral Salt medium with 5%, 10%, and 15% model hydrocarbons (Crude oil, Anthracene, Coronene, Napthacene, Acenapthene). All the tubes were diluted upto 10^{-5} dilution and plated on mineral salt agar medium. An uninoculated plate was used as control. The plates were incubated at 28˚C for 15 days. Growth was measured in terms of cfu/ml.

Results and Discussion

Enumeration of actinomycetes [Average cfu/g (10^5)] from hydrocarbon contaminated soil

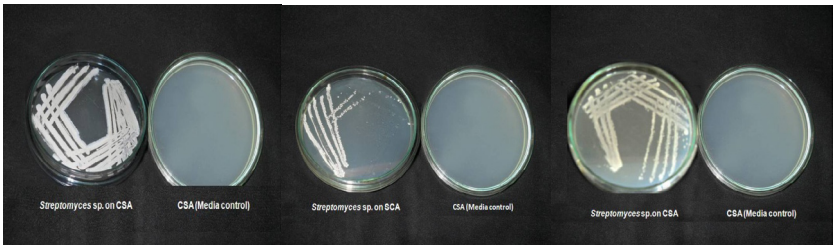
Highest population of actinomycetes was found in the sample collected from Mathura oil refinery sites, Mathura (4.3×10^5 cfu/g) followed by HP Petrol pump sites, Lucknow (2.7×10^5 cfu/g), Railway carriage wagon sites, Lucknow, (1.7×10^5 cfu/g) and least populated site was found in samples collected from Bharat Petrol pump site, Lucknow (1.1×10^5 cfu/g) (Table 1 and Figure 1).

Isolation and identification of actinomycetes from hydrocarbon contaminated soil

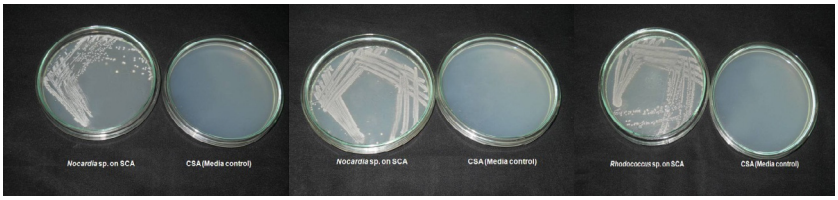
40 samples from hydrocarbon contaminated soil of 4 different sites were used for isolation of different Hydrocarbon degrading actinomycetes. In the present study six species of actinomycetes were identified as *S. sp1, S. sp2, S. sp3, N.sp1, N. sp2 and R. sp* 1 (Figure 2).

Distribution of actinomycetes isolated from different sites potent for growth at 5% concentration of hydrocarbon contaminated soil (Petrol)

Among the 40 samples screened for growth of actinomycetes in Mineral Salt agar supplemented with 5% crude oil, 51 isolates showed positive result. Six different isolates were identified that showed positive result namely *Streptomyces* sp.1, *Streptomyces* sp.3, *Streptomyces* sp.2, *Rhodococcus* sp., *Nocardia* sp.1 and *Nocardia* sp.2. Out of ten samples of Railway fuel oil installation sites, Allahabad, 20 actinomycetes were



Streptomyces sp 1 **Streptomyces sp 2** **Streptomyces sp 3**



Nocardia sp 1 **Nocardia sp 2** **Rhodococcus sp 1**

Figure 2: Bacterial species isolated from oil contaminated sites.

Sample site	Sample size n=40	No.of actinomycetes isolated	No. of isolate positive for growth at 5% supplement	Incidence of isolates capable for hydrocarbon degradation					
				Streptomyces sp.1	*Streptomyces* sp.3	*Streptomyces* sp.2	*Nocardia* sp.2	*Nocardia* sp.1	*Rhodococcus* sp.
Mathura Oil refinery	10	**59**	**20**	**7(35%)**	6(30%)	1(5%)	4(20%)	2(10%)	0(0%)
HP Petrol Pump, Lucknow	10	24	12	3(25%)	2(16.6%)	1(8.3%)	**4(33.3%)**	1(8.3%)	1(8.3%)
Railway carriage wagon, Lucknow	10	40	15	**7(46.6%)**	4(2.66%)	1(6.6%)	2(13.3%)	0(0%)	1(6.6%)
Bharat Petrol Pump, Lucknow	10	11	4	**3(75%)**	1(25%)	0(0%)	0(0%)	0(0%)	0(0%)

Table 2: Distribution of isolates from hydrocarbon contaminated soil.

screened as potent actinomycetes for growth in Mineral Salt agar medium supplemented with 5% crude oil (hydrocarbon). Highest incidence of Streptomyces sp.1 were found from the samples of Bharat Petrol Pump site (46.6%), Mathura oil refinery site (35 %), Mathura and Railway carriage wagon site, Lucknow (75 %) while at HP Petrol Pump, Lucknow *Nocardia* sp.2 (33.3%). had highest incidence. In a similar study conducted by Milic et al. [22]; Jayabarath et al. [25]; Sharma and Pant [4] the results are comparable showing high occurrence of *Streptomyces*, *Nocardia*, and *Rhodococcus* which were measures of incidence and growth of actinomycetes; They represent the dominant group among the degraders (Table 2 and Figure 3).

Optimization of physical growth parameter of isolates

Effect of temperature on growth of actinomycetes at 5% hydrocarbon (Crude oil): Actinomycets isolated from different hydrocarbon contaminated soil showed minimum growth at high temperature and low temperatures. All isolates namely *Streptomyces* sp.1, *Streptomyces* sp.3, *Streptomyces* sp.2, Nocardia sp.2, *Nocardia* sp.1, and *Rhodococcus* sp. showed highest growth at temperature 30°C (6.49 log cfu/ml, 5.52 log cfu/ml, 5.52 log cfu/ml, 6.548 log cfu/ml, 6.56 log cfu/ml and 6.56 log cfu/ml) statistical analysis of data reveled significant differences due to isolates and temperature (Table 3 and Figure 4).

Effect of pH on growth of actinomycetes at 5% hydrocarbon (Crude oil): The optimal pH that supported growth of isolates from hydrocarbon contaminated soil range between 6.5 to 8.5 while maximum growth occurred at pH 7.5 and minimum growth at pH 5.5 and 9.5 in Mineral salt Agar medium supplemented with 5% Petrol after 7 days of incubation. All isolate namely *Streptomyces* sp.1, *Streptomyces* sp.3, *Streptomyces* sp.2, *Nocardia* sp.2, *Nocardia* sp.1 and *Rhodococcus* sp. showed highest growth at pH 7.5 (7.77 log cfu/ml, 7.71 log cfu/ml, 6.63 log cfu/ml, 6.45 log cfu/ml, 6.59 log cfu/ml and 6.68 log cfu/ml) (Table 4 and Figure 5).

Growth potential of actinomycetes

Growth of *Streptomyces sp1:* Growth of *Streptomyces sp1* was analyzed for different hydrocarbons namely Crude oil, Anthracene, Coronene, Napthacene, Acenapthene at 5%, 10% and 15% concentrations incubated for three different time intervals *viz.* 5 days, 10 days and 15 days. *Streptomyces* sp.1 had highest growth for Coronene (4.67 log cfu/ml) followed by Napthacene (4.62 log cfu/ml),

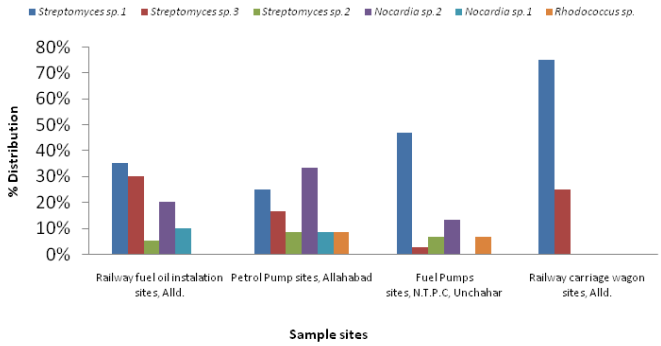


Figure 3: Percent distribution of actinomycetes isolates from different hydrocarbon contaminated soil.

Isolates	Growth of actinomycetes at 5% crude oil concentration cfu/ml				
	Incubation Temperature °C				
	10°C	20°C	**30°C**	40°C	50°C
Streptomyces sp1	2.27	3.36	**6.49**	6.35	4.32
Streptomyces sp3	1.34	3.35	**5.52**	5.36	4.34
Streptomyces sp2	2.37	4.37	**6.48**	5.41	3.37
Nocardia sp2	2.32	4.32	**6.56**	5.29	3.23
Nocardia sp1	1.38	4.44	**6.63**	4.43	4.32
Rhodococcus fascians	2.37	3.46	**6.67**	5.45	3.37

Table 3: Effect of temperature on growth of actinomycetes at 5% hydrocarbon (Crude oil).

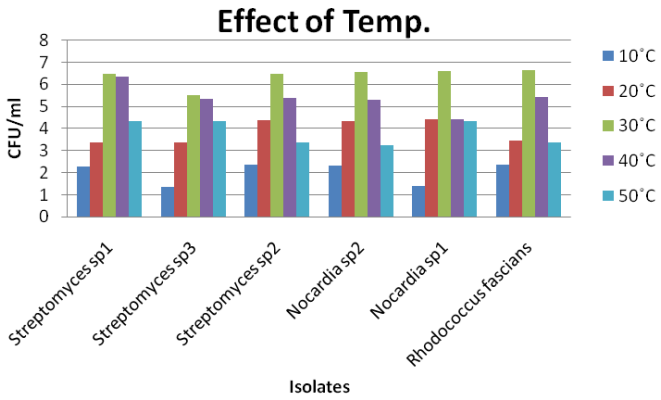


Figure 4: Effect of temperature on growth of actinomycetes at 5% hydrocarbon (Crude oil).

Anthracene (3.69 log cfu/ml), Crude oil (3.60 log cfu/ml) and least was shown by Acenapthene (3.59 log cfu/ml) at 10% and 10 days incubation respectively (Table 5 and Figure 6).

Growth of *Streptomyces sp2:* Growth of *Streptomyces sp2* was analyzed for different hydrocarbons namely Crude oil, Anthracene, Coronene, Napthacene, Acenapthene at 5%, 10% and 15% concentrations incubated for three different time intervals *viz.* 5 days, 10 days and 15 days. *Streptomyces* sp.2 had highest growth for Anthracene (7.69 log cfu/ml) followed by Crude oil (7.60 log cfu/ml), Acenapthene (7.59.59 log cfu/ml), Coronene (7.24 log cfu/ml), and least was shown by Napthacene (6.62 log cfu/ml) at 10% and 10 days incubation (Table 6 and Figure 7).

Growth of *Streptomyces sp3:* Growth of *Streptomyces sp3* was analyzed for different hydrocarbons namely Crude oil, Anthracene, Coronene, Napthacene, Acenapthene at 5%, 10% and 15% concentrations incubated for three different time intervals *viz.* 5 days, 10 days and 15 days. Highest growth of *Streptomyces* sp.3 was recorded at 10% concentration for Crude oil (7.80 log cfu/ml) followed by Anthracene (7.79 log cfu/ml), Napthacene (7.68 log cfu/ml), Acenapthene (7.59 log cfu/ml) and least was shown by Coronene (7.24 log cfu/ml) at 10% and 10 days incubation respectively (Table 7 and Figure 8).

Growth of *Nocardia sp1:* Growth of *Nocardia sp1*was analyzed for different hydrocarbons namely Crude oil, Anthracene, Coronene, Napthacene, Acenapthene at 5%, 10% and 15% concentrations incubated for three different time intervals *viz.* 5 days, 10 days and 15 days. *Nocardia* sp.1 had highest growth for Crude oil (7.68 log cfu/ml) followed by Anthracene (7.39 log cfu/ml), Coronene (7.37 log cfu/ml), Napthacene (7.36 log cfu/ml) and least was shown by Acenapthene (7.15 log cfu/ml) at 10% and 10 days of incubation respectively (Table 8 and Figure 9).

Growth of *Nocardia sp2:* Growth of *Nocardia sp2* was analyzed for different hydrocarbons namely Crude oil, Anthracene, Coronene, Napthacene, Acenapthene 5%, 10% and 15% concentrations incubated for three different time intervals *viz.* 5 days, 10 days and 15 days. Growth of *Nocardia* sp.2 was analyzed for different hydrocarbons had highest growth for Crude oil (7.80 log cfu/ml) followed by Anthracene (7.79 log cfu/ml), Coronene (7.68 log cfu/ml), Napthacene (7.63 log cfu/ml) and least was shown by Acenapthene (7.59 log cfu/ml) at 10% and 10 days incubation (Table 9 and Figure 10).

Growth of *Rhodococcus sp:* Growth of *Rhodococcus sp.* was analyzed for different hydrocarbons namely Crude oil, Anthracene, Coronene,

Isolates	Growth of actinomycetes at 5% crude oil concentration cfu/ml				
	pH				
	5.5	6.5	**7.5**	8.5	9.5
Streptomyces sp1	4.44	6.63	**7.77**	6.60	4.59
Streptomyces sp2	2.45	7.48	**7.71**	6.51	5.15
Streptomyces sp3	3.36	6.39	**7.63**	5.36	6.26
Nocardia sp2	3.40	6.42	**7.45**	7.47	5.30
Nocardia sp1	3.39	7.40	**7.59**	5.43	5.21
Rhodococcus fascians	3.46	6.46	**7.68**	5.47	5.32

Table 4: Effect of pH on growth of actinomycetes at 5% hydrocarbon (Crude oil).

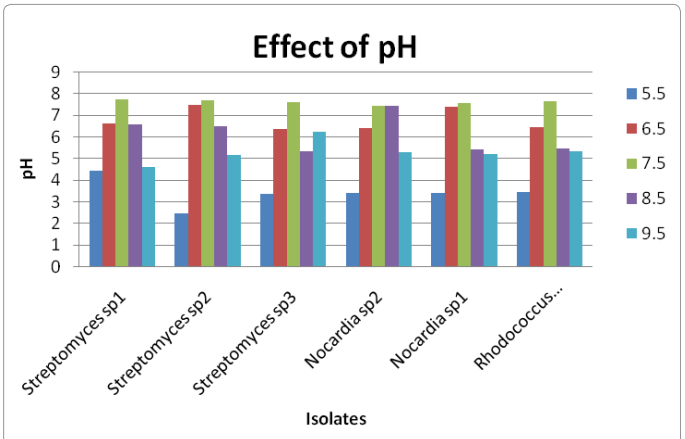


Figure 5: Effect of pH on growth of actinomycetes at 5% hydrocarbon (crude oil).

Growth of *Streptomyces sp1* Log cfu/ml												
Hydrocarbon	**0 days**			**5 days**			**10 days**			**15 days**		
	5%	10%	15%	5%	10%	15%	5%	**10%**	15%	5%	10%	15%
Crude Oil	0.6	1.77	1.84	1.91	2.48	1.44	2.12	**3.60**	2.01	1.98	2.19	1.97
Anthracene	0.84	0.77	1.77	2.07	2.53	1.98	2.16	**3.69**	2.22	2.08	2.27	2
Coronene	1.6	0.77	1.77	2.06	2.56	1.83	2.06	**4.67**	2.24	2.97	2.25	2.08
Napthacene	0.90	0.90	0.95	2.07	2.46	2.30	2.04	**4.62**	2.15	2.01	2.25	2.04
Acenapthene	0.6	1.6	1.6	1.92	2.48	1.89	2.99	**3.59**	2.25	2.12	2.26	1.98

Table 5: Growth of *Streptomyces sp1.*

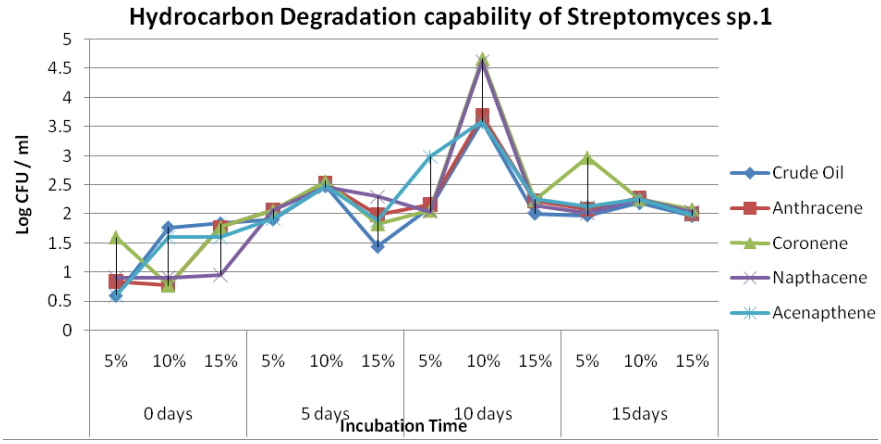


Figure 6: Growth of *Streptomyces sp1.*

Growth of *Streptomyces sp2* Log cfu/ml												
Hydrocarbon	**0 days**			**5 days**			**10 days**			**15 days**		
	5%	10%	15%	5%	10%	15%	5%	**10%**	15%	5%	10%	15%
Crude Oil	1.8	1.77	1.84	2.91	3.48	2.44	4.12	**7.60**	6.01	3.98	5.19	2.97
Anthracene	0.94	1.77	2.77	3.07	3.53	2.98	4.16	**7.69**	6.22	4.08	5.27	3
Coronene	0.9	1.77	2.77	3.06	4.56	2.83	3.06	**7.24**	6.67	4.97	4.25	3.08
Napthacene	0.90	0.90	0.95	3.07	4.46	2.30	4.04	**6.62**	6.15	5.01	4.25	2.04
Acenapthene	1.6	1.6	2.6	2.92	3.48	2.89	3.99	**7.59**	6.25	5.12	3.26	3.98

Table 6: Growth of of *Streptomyces sp2.*

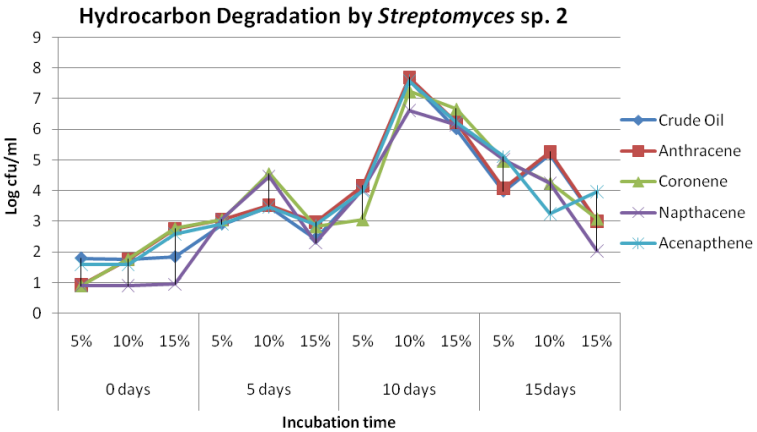


Figure 7: Growth of *Streptomyces sp2.*

Napthacene, Acenapthene at 5%, 10% and 15% concentrations incubated for three different time intervals *viz.* 5 days, 10 days and 15 days. *Rhodococcus* sp. had highest growth for Crude oil (7.50 log cfu/ml) followed by Anthracene (7.39 log cfu/ml), Coronene (7.38 log cfu/

Growth of *Streptomyces sp3* Log cfu/ml												
Hydrocarbon	**0 days**			**5 days**			**10 days**			**15 days**		
	5%	10%	15%	5%	10%	15%	5%	**10%**	15%	5%	10%	15%
Crude Oil	1.5	1.87	1.84	3.91	4.48	2.44	4.12	**7.80**	6.01	3.98	5.19	2.97
Anthracene	0.94	1.97	2.77	3.07	4.53	2.98	4.16	**7.79**	6.22	4.08	5.27	3.54
Coronene	0.96	1.67	2.77	3.06	4.56	2.83	3.06	**7.24**	6.68	4.97	4.25	3.08
Napthacene	0.93	0.95	1.95	3.07	4.46	2.30	4.04	**7.68**	6.15	5.01	4.25	2.04
Acenapthene	1.7	1.7	2.6	3.92	3.98	2.89	3.99	**7.59**	6.25	5.12	3.26	3.98

Table 7: Growth of of *Streptomyces sp3*.

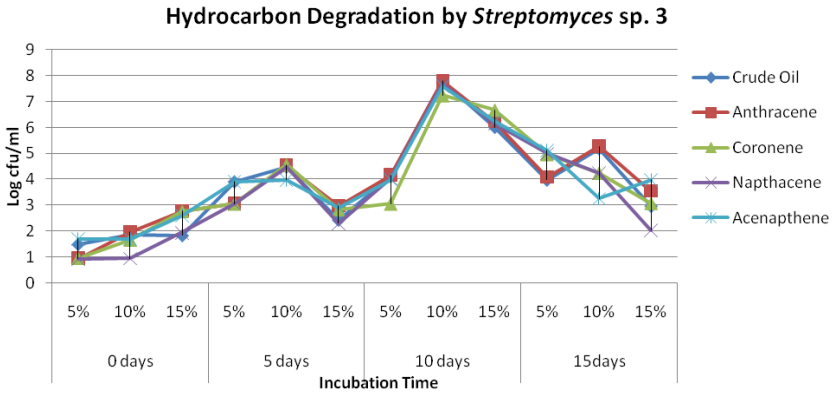


Figure 8: Growth of *Streptomyces sp3*.

Growth of *Nocardia sp1* Log cfu/ml												
Hydrocarbon	**0 days**			**5 days**			**10 days**			**15 days**		
	5%	10%	15%	5%	10%	15%	5%	**10%**	15%	5%	10%	15%
Crude Oil	0.9	1.67	1.84	3.91	4.58	2.44	4.22	**7.68**	6.01	3.98	5.19	2.97
Anthracene	1.04	1.8	2.77	3.37	4.83	2.98	4.36	**7.39**	6.22	4.08	5.27	3.54
Coronene	1.16	1.77	2.67	3.46	4.56	2.83	5.46	**7.37**	7.24	4.97	5.25	3.08
Napthacene	0.99	0.95	1.85	3.37	4.96	2.30	4.54	**7.36**	6.15	5.01	4.25	2.04
Acenapthene	1.07	1.67	2.16	3.92	3.98	2.89	3.99	**7.15**	6.25	5.12	5.26	3.98

Table 8: Growth of *Nocardia sp1*.

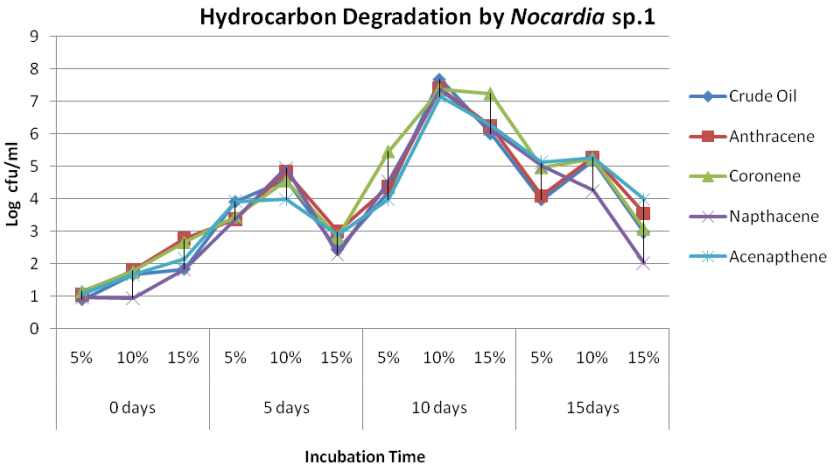


Figure 9: Growth of *Nocardia sp1*.

ml), Napthacene (7.29 log cfu/ml) and least was shown by Acenapthene (7.17 log cfu/ml) at 10% and 10 days incubation respectively (Table 10 and Figure 11).

Summary and Conclusion

For the investigation ten soil samples from four different hydrocarbon contaminated sites. The soil from four sites were used

Hydrocarbon utilizing capacity of *Nocardia sp2* Log cfu/ml												
Hydrocarbon	**0 days**			**5 days**			**10 days**			**15 days**		
	5%	10%	15%	5%	10%	15%	5%	**10%**	15%	5%	10%	15%
Crude Oil	0.98	1.98	1.84	3.91	4.58	3.44	6.22	**7.80**	6.01	3.98	5.49	3.97
Anthracene	0.94	1.84	1.97	3.67	5.83	2.98	6.36	**7.79**	6.22	4.08	5.47	3.54
Coronene	0.96	1.77	2.64	3.36	5.56	3.83	6.46	**7.68**	6.24	4.97	5.25	3.08
Napthacene	0.99	1.35	1.95	3.11	5.96	3.30	6.54	**7.63**	6.15	5.01	4.25	3.04
Acenapthene	1.17	1.57	2.16	3.33	4.98	2.89	6.99	**7.59**	6.25	5.12	5.46	3.98

Table 9: Growth of *Nocardia sp2*.

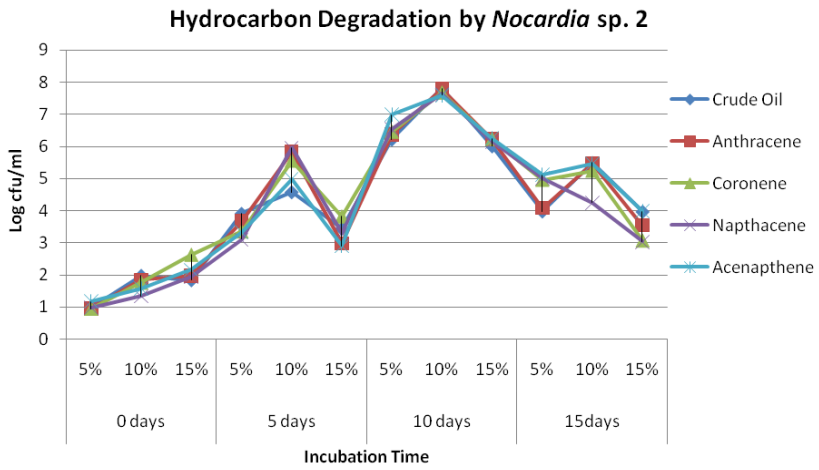


Figure 10: Growth of *Nocardia sp2*.

Growth of *Rhodococcus sp.* Log cfu/ml												
Hydrocarbon	**0 days**			**5 days**			**10 days**			**15 days**		
	5%	10%	15%	5%	10%	15%	5%	**10%**	15%	5%	10%	15%
Crude Oil	0.78	1.98	1.94	3.91	5.58	4.44	6.22	**7.50**	6.41	3.98	5.49	3.87
Anthracene	1.04	1.74	1.96	4.67	5.83	3.98	6.36	**7.39**	6.42	4.48	5.47	3.54
Coronene	1.06	1.77	2.04	4.36	5.56	3.83	5.46	**7.38**	6.24	4.97	5.25	3.78
Napthacene	1.09	1.65	1.99	4.11	5.96	4.30	6.45	**7.29**	6.54	5.01	4.25	3.74
Acenapthene	1.07	1.47	2.16	4.33	5.98	3.89	6.33	**7.17**	6.47	5.12	5.46	3.78

Table 10: Growth of *Rhodococcus sp.*

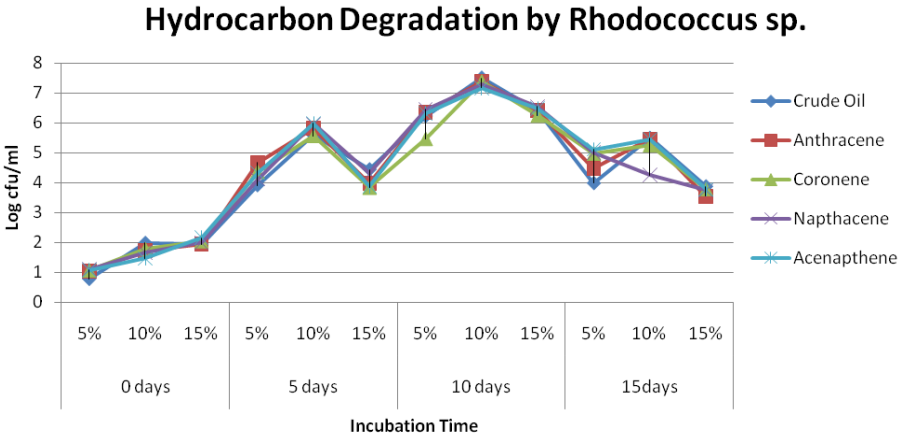


Figure 11: Growth of *Rhodococcus sp.*

for isolation and enumeration of actinomycetes after which the morphologically different colonies were purified and screened for hydrocarbon utilization as sole source of carbon and energy at 5% concentration of Crude oil (hydrocarbon). The isolate found positive

for growth on screened plates were identified on the basis of cultural, morphological and biochemical analysis and were further assessed for their growth potential for selected hydrocarbons at varying concentrations (5%, 10%, and 15%) incubated for three different time intervals *i.e.* 5 days, 10 days, and 15 days.

The present investigation revealed that indigenous actinomycetes isolated from hydrocarbon contaminated sites could be used for *in situ* bioremediation purpose. Among the 134 isolates screened, 51 efficient oil (crude oil) degraders, out of which *Streptomyces* sp.1 39% *followed by Streptomyces* sp.3 25%, *Streptomyces* sp.2 5.5%, *Nocardia* sp.2 5.8%, *Nocardia* sp.119.6%, and *Rhodococcus* sp. 3.9% found from four different sites namely Mathura Oil Refinery, Mathura, HP Petrol Pump, Lucknow, Bharat Petrol Pump, Lucknow and Railway carriage wagon, Lucknow were able to grow efficiently on model hydrocarbons *i.e.* Crude oil, Anthracene, Coronene, Napthacene, Acenapthene. Upon analyzing the growth potential of isolates at different concentration (5%, 10%, and 15%) of hydrocarbons and different time of incubation (5, 10 and 15 days). It was found that the actinomycetes isolates had efficient growth at 10% hydrocarbon concentration after prolonged incubation for 15 days. The study highlighted the potential of actinomycetes isolated from hydrocarbon contaminated soil for bioremediation of hydrocarbon polluted area, spills as it offers effective degradation of various fractions of hydrocarbons at wide range of concentration and time duration. Therefore, bioremediation of toxicant hydrocarbons in soil or spill have a better option of environmentally adopted microflora that effect detoxification and stabilization of processes of biological degradation with low economical expenses and of no danger for environment.

References

1. Young CC, Lin TC, Yeh M, Shen FT, Chang JS (2005) Identification and kinetic characteristics of an indigenous diesel-degrading Gordonia alkanivorans strains, World Journal of Microbiology and Biotechnology 2: 1409-1414.
2. Gurielidze M, Berishvili T, Cholokava N, Pataraya D, Nutosubidze N (2009) Oil destructing extremophilic actinomycetes isolated from various type of soil of Gerogian. Bulletin of the Georgian National Academy of Sciences 3: 213-217.
3. Rahman KS, Rahman T, Lakshmanaperumalsamy P, Banat IM (2002) Occurrence of crude oil degrading bacteria in gasoline and diesel station soils. J Basic Microbiol 42: 284-291.
4. Sharma, S. L. and Pant, A. 2001, Crude oil degradation by a marine actinomycete Rodococcus sp. Indian Journal of Marine Sciences 4: 245-247.
5. Baniasadi F, Shadi GH, Kariminik A (2009) In vitro petroleum decomposition by actinomycetes isolated from petroleum contaminated soils. American Eurasian Journal of Agriculture and Environmental Science 6: 268-270.
6. Thampayak I, Cheetah N, Pathom-Aree W, Leelapornpisid P, Lumyong S (2008) Isolation and identification of Biosurfactant producing actinomycetes from soil. Research Journal of Microbiology 3: 499-507.
7. Barahona LM, Varquez RR, Valasco MH, Jarquin CV, Pervez OZ, et al. (2004) Diesel removal from contaminated soil by biostimulation and supplementation with crop residues. Elsevier.
8. Ensign JC (1992) Introduction to the Actinomycetes. In: The Prokaryotes., A handbook on the biology of bacteria: Ecophysiology, Isolation, Identification, Applications. Eds: Ballows, A, Trüper, H. G., Dworkin, M., Harder W. and Schleifer, K. H. Springer-Verlag. New York. Pp: 811-815.
9. Rapp P, Bock H, Wray V, Wagner F (1979) Formation, isolation and characterization of trehalose dimycolates from Rhodococcus erythropolis grown on n-alkanes. Journal of General Microbiology 115: 491-503.
10. Kretschmer A, Bock H, Wagner F (1982) Chemical and Physical Characterization of Interfacial-Active Lipids from Rhodococcus erythropolis Grown on n-Alkanes. Appl Environ Microbiol 44: 864-870.
11. Singer ME, Finnerty WR (1990) Physiology of biosurfactant synthesis by Rhodococcus species H13-A. Can J Microbiol 36: 741-745.
12. Morikawa M, Daido H, Takao T, Murata S, Shimonishi Y, et al. (1993) A new lipopeptide biosurfactant produced by Arthrobacter sp. strain MIS38. J Bacteriol 175: 6459-6466.
13. Neu TR (1996) Significance of bacterial surface-active compounds in interaction of bacteria with interfaces. Microbiol Rev 60: 151-166.
14. Westerberg K, Elväng AM, Stackebrandt E, Jansson JK (2000) Arthrobacter chlorophenolicus sp. nov., a new species capable of degrading high concentrations of 4-chlorophenol. Int J Syst Evol Microbiol 50 Pt 6: 2083-2092.
15. Strong LC, Rosendahl C, Johnson G, Sadowsky MJ, Wackett LP (2002) Arthrobacter aurescens TC1 metabolizes diverse s-triazine ring compounds. Appl Environ Microbiol 68: 5973-5980.
16. Bhadbhade BJ, Sarnaik SS, Kanekar PP (2002) Bioremediation of an industrial effluent containing monocrotophos. Curr Microbiol 45: 346-349.
17. Durães Sette L, Mendonça Alves Da Costa LA, Marsaioli AJ, Manfio GP (2004) Biodegradation of alachlor by soil streptomycetes. Appl Microbiol Biotechnol 64: 712-717.
18. Kästner M, Breuer JM, Mahro B (1994) Enumeration and characterization of the soil micro flora from hydrocarbon-contaminated soil sites able to mineralize polycyclic aromatic hydrocarbons (PAH). Applied Microbiology and Biotechnology 41: 267-273.
19. Iwahori K, Wang M, Taki H, Fujita M (1995) Comparative studies on utilization of fatty acids and hydrocarbons in Nocardia amarae and Rhodococcus spp. Journal of Fermentation and Bioengineering. 79: 186-189.
20. Whyte LG, Slagan SJ, Pietrantonio F, Bourbonniere L, Koval SF, et al. (1999) Physiological adaptations involved in alkane assimilation at a low temperature by Rhodococcus sp. strain Q15. Applied and Environmental Microbiology 65: 2961-2968.
21. Johnsen AR, Winding A, Karlson U, Roslev P (2002) Linking of microorganisms to phenanthrene metabolism in soil by analysis of (13)C-labeled cell lipids. Appl Environ Microbiol 68: 6106-6113.
22. Milic JS, Beskoski VP, Ilic MV, Ali SAM, Vrvic MM (2009) Bioremediation of soil heavily contaminated with crude oil and its products: compostion of the microbial consortium. Journal of Serbian Chemical Society 74: 455-460.
23. Gomes EB, Soriano AU, Miranda RC, Mendonça SM, Vieira Q, et al. (2009) Biodegradation of stored jet fuel by a Nocardia sp. isolated from contaminated soil Brazilian Archives of Biology and Technology, An International Brazilian Journal 52: 1279-1284.
24. Mills AL, Breuil C, Colwell RR (1978) Enumeration of petroleum-degrading marine and estuarine microorganisms by the most probable number method. Can J Microbiol 24: 522-527.
25. Jayabarath J, Musfira SA, Giridhar R, Sundar SS, Arulmurugan R (2010) Biodegradation of carbofuran pesticide by saline soil actinomycetes. International Journal of Biotechnology and Biochemistry 6: 187-192.