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Study of Biodegradation Pathway of Hydrocarbons by Bacillus sp. E87

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ABSTRACT

Present study was focused on hydrocarbon degradation potential and detection of enzymes involved in degradation pathway of selected hydrocarbons by Bacillus sp. E87. GC analysis were used as evaluation experiment to check the degradation potential of potent isolate in presence of tested hydrocarbons at 100ppm (Benzene, diesel, hexane, petrol, toluene, xylene) and chromatograms found with different biodegradation efficiency in different supplemented conditions, which indirectly reflects Bacillus sp.E87 can be applied against the wide range of hydrocarbon like aromatic, aliphatic and complex form of hydrocarbons as well. Protein profile of Bacillus sp. E87 expressed some new proteins in hydrocarbon supplemented condition as compared to control, revealed strong induction by hydrocarbons in bacterial cell for the synthesis of desired proteins responsible for their degradation. On the basis of protein profiling it has been cleared Bacillus sp.E87 followed salicylate pathway for the biodegradation of selected hydrocarbons. On the basis of this information Bacillus sp. E87 could be considered as a powerful approach in bioremediation technology for hydrocarbon contaminated soil.

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Introduction

Petroleum hydrocarbon continues to be used as the principle source of energy. Extensive scale production, transport, use and disposal of petroleum globally have made it a major contaminant in both prevalence and quantity in the environment [1]. Contamination of soil through the oil refineries, accidents of oil tankers during transportation of crude oil, anthropogenic and pilferage activities [2]. Soil contamination with hydrocarbons causes extensive damage of local ecosystems since accumulation of pollutants in animal and plants tissues may cause progeny's death or mutation [3]. Acute exposure to petroleum hydrocarbons and its components benzene, toluene, and xylene has been associated with eye, nose, and throat irritation; headaches, loss of coordination, nausea; damage to liver, kidney, and central nervous system, and effects on the respiratory system [4].

Biodegradation by natural populations of microorganisms represents one of the prime mechanisms by which petroleum and other hydrocarbon pollutants can be removed from the environment and is cheaper than other remediation technologies. The native microbial strain of each environment are more efficient than the others because of their adaptability with the environmental conditions of the area such as temperature, pH, salinity and etc. [5,6]. To direct the assess of bioremediation efficacy, monitoring the disappearance rate of hydrocarbons from the environment (soil) through the GC. GC is a technique used for rapid semi quantitative assessments of the decomposition of hydrocarbon materials originating from oil under natural conditions or historical pollution [7].

In order to make bioremediation effective towards the broad range of hydrocarbons like aliphatic, aromatic and complex compounds as well, it is important that these organisms should be metabolically active and should carry different enzymes required for the biodegradation. Bacteria have a regulatory system that directs the synthesis of desired proteins, which required for the degradation of selected hydrocarbon. Thus, for an organism with the genetic information for utilizing benzene as carbon source, the desired protein for degradation of benzene is induced when benzene reaches the bacterial environment [8]. The change in the substrate in the media induced protein due to which different banding pattern was formed in protein profiling. Bacterial isolate PM 102 showed the five extra protein band in presence of TCA as a carbon source comparison to peptone [9].

The present study focused on to check the biodegradation efficiency of Bacillus towards benzene, xylene, hexane, petrol, diesel, and toluene and to route out degradation pathway followed by bacteria to degrade these hydrocarbons. A number of literatures are available on the protein profiling of bacteria in presence of poly-aromatic hydrocarbons but no earlier finding was reported on benzene, xylene, hexane, petrol, diesel and toluene.

Materials and Methods

Bacillus sp.E87 was isolated from the soil sample of oil refinery, Guwahati. Bacterial strain was grown in mineral salts medium contains (MSM) KH_2PO_4 (1.3gl^{-1}), (K_2HPO_4 (1.8 gl^{-1}); NH_4Cl (4 gl^{-1}); $MgSO_4.7H_2O$ (0.2 gl^{-1}); yeast extract (0.2 gl^{-1}) at 35°C. To check the degradation potential of Bacillus sp.E87 yeast extract was replaced by selected hydrocarbons (Benzene, diesel, hexane, petrol, toluene, xylene) as a carbon source [10].

To trace the metabolic pathway (metabolic intermediates compounds) followed by Bacillus E87 for degradation of hydrocarbon GC analysis of extracted hydrocarbons was carried out. For the extraction 3 flasks containing 50ml MSM with 4ml of overnight grown culture supplemented with single hydrocarbon (100 ppm) were subjected to treatment for 24, 48 and 72 hrs incubated in incubator shaker at 35°C at 100 rpm. For extraction of hydrocarbon intermediates, culture broth was mixed with petroleum ether: acetone (1:1) in a separating funnels and shaken vigorously. Moisture has removed by passing the extract through anhydrous sodium sulphate, petroleum ether and acetone was evaporated on rotator evaporator [11]. 5µl of the extracted hydrocarbons was injected by direct injection into a gas chromatograph that was connected to FID. The gas chromatograph was equipped with PE-FFA8 fused silica capillary column (length 30 m, inner diameter 250 um, film thickness 0.25um) with nitrogen as the carrier gas .The injection and the detector were maintained at 230° C, and the oven temperature was programmed to rise from 10° C/min to 210° C/min and to hold at 210° C for 10 min [12].

To find out the enzyme responsible for the degradation, protein profiling was done with selected hydrocarbons supplemented conditions. For the extraction of protein bacterial cultures were grown in MSM till log phase and centrifuged at 10,000 rpm for 10 min at 4˚C. Pellet was washed thrice with cold phosphate buffer, resuspended in 1M Tris-HCl, pH 6.8 (1 g/5 ml) and sonicated for 6 - 30 sec pulse with 2 min cooling interval. The sonicated cells were centrifuged at 6000 rpm for 30 min at 4˚C. The pellet was discarded and the supernatant was collected and about 4 times volume of ice chilled acetone was added and incubated at - 20˚C for 1-2 hrs. The precipitated protein was pelletted by centrifugation at 10,000 rpm. The pellet was air dried and dissolved in 500 µl of 1M Tris-HCl. Protein samples were analyzed by one dimensional, discontinuous vertical SDS- PAGE with 12-14% gel system consisting of separating-stacking gels (150V constant voltage). Gels were stained with Coomassie blue R-250 (0.2% w/v in the fixing solution)-10% acetic acid in methanol and destained in 10% methanol- 7% acetic acid in distilled water. Molecular weight of protein was determined by medium range marker (Bangalore Genei, India) [13].

Results and Discussion

On the basis of GC chromatogram, BE% of Bacillus sp. E87 towards benzene (Fig.1), toluene (Fig.2) and xylene (Fig.3) found to be 92%, 94%, 67% respectively. It may therefore the mono-aromatic hydrocarbon especially single benzene ring bearing compounds are most suitable for microbial attack due to their low hydrophobicity [14]. The degradation percentage of xylene is found to lower as compare to other aromatic hydrocarbon due to less solubility in water (<.1gl^{-1}) (http://www.chemicalbook.com/). Degradation of toluene may be due to the production of enzyme catalase, toluene metabolites may cause oxidative DNA damage, which lead to the death of strains that can be prevented by action of enzyme catalase as earlier proved by Abari et al.,who worked on Bacterium Ex-DG 74 that showed 79% BE towards toluene [15].

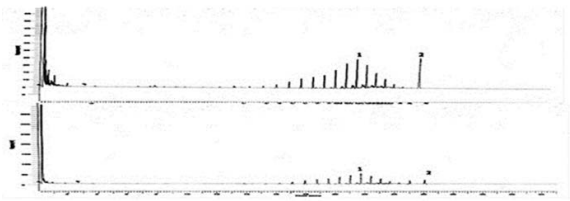

Figure 1: GC profiles of benzene extracted from the aqueous phase of the medium after 72 hrs of incubation with 0.01% of substrate with and without inoculation of Bacillus sp. E 87

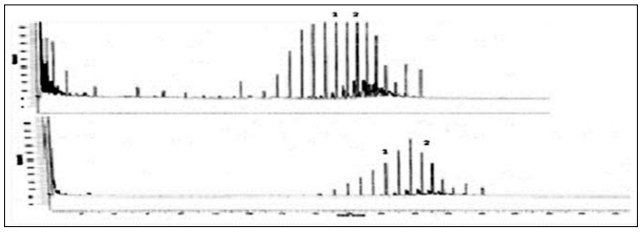

Figure 2: GC profiles of toulene extracted from the aqueous phase of the medium after 72 hours of incubation with 0.01% of substrate with and without inoculation of Bacillus sp. E 87

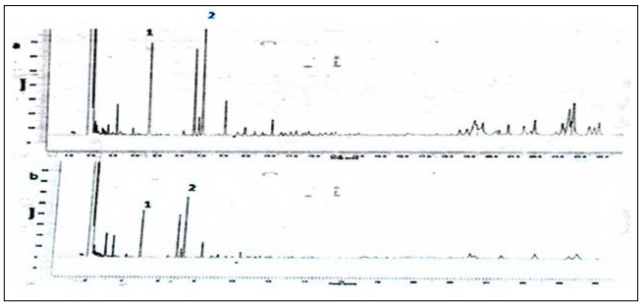

Figure 3: GC profiles of xylene extracted from the aqueous phase of the medium after 72 hours of incubation with 0.01% of substrate with and without inoculation of Bacillus sp. E 87

GC results concluded that Bacillus sp. E14 degrade the constituents of petrol 90% (Fig.4), diesel 93% (Fig.5), difference in BE% expressed variation in bioavailability of compound by bacteria, diesel is a complex mixture of high boiling point hydrocarbons due to which chance of evaporation was less. Dussan and Numpaque, reported that bacterial strain TRI, PRIII and TRIV isolated from coal mine had high BE values of 95%, 70% and 69%, respectively, followed by TRIII with a BE of 52%, and TRII with the lowest BE of 10% for the biodegradation of Diesel in 43 days.[16] On the comparison with previous reported work, time taken by Bacillus sp. E14 to degrade diesel is very less i.e.72 hrs.

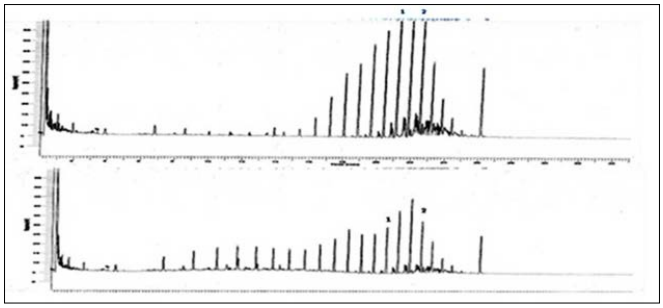

Figure 4: GC profiles of petrol extracted from the aqueous phase of the medium after 72 hours of incubation with 0.01% of substrate with and without inoculation of Bacillus sp. E 87

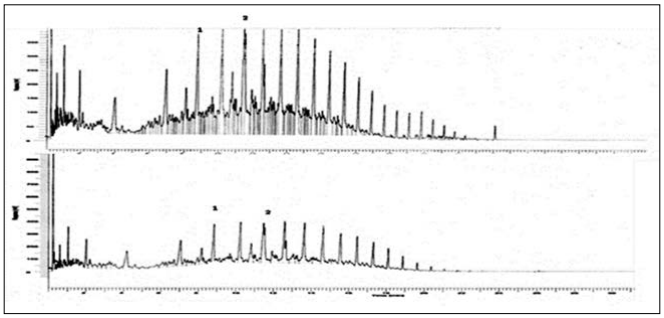

Figure 5: GC profiles of diesel extracted from the aqueous phase of the medium after 72 hours of incubation with 0.01% of substrate

with and without inoculation of Bacillus sp. E 87

Bacillus sp. E14 degrade hexane 85%, in 72 hours (Fig.6), the degradation of water-soluble short chain alkanes such as pentane, hexane, heptane and octane,which are toxic for the environment, is less frequent [17]. There is no work still reported on degradation of hexane by Bacillus sp.. Previous studies reported the hexane degradation by pseudomonas [18] reported the long chain alkane degradation by pseudomonas and Rhodococcus this may be due to the existence enzyme alkane hydroxylases [19].

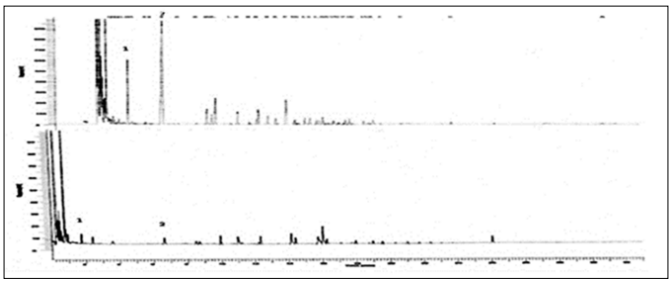

Figure 6: GC profiles of deisel extracted from the aqueous phase of the medium after 72 hours of incubation with 0.01% of substrate with and without inoculation of Bacillus sp. E 87.

Few studies have reported on the roles of Bacillus spp [20-22]. in hydrocarbon bioremediation; although there are several reports of bioremediation of pollutants by the action of Bacillus spp. occurring in extreme environments. Ijah and Antai reported Bacillus spp. as being the predominant isolate of all the crude oil utilizing bacteria characterized from highly polluted soil samples (30 and 40% crude oil) [21]. It was postulated that Bacillus spp. are more tolerant to high levels of hydrocarbons in soil due to their resistant endospores. There is growing evidence that isolates belonging to the Bacillus sp. could be effective in clearing oil spills [23].

Protein profile of Bacillus sp. E14 expressed different protein band pattern in treated conditions as compare to control indicated the presence of enzyme which is expressed by the bacteria for the degradation of hydrocarbons (Fig.7). Gh15 expressed the protein with the molecular weight of 200Kda in presence of all the substrate, but absent in control condition which is resembled to enzyme salicylaldehyde dehydrogenase. This enzyme is involved in the catechol branch of the 3-oxoadipate pathway, therefore Gh15 degrade hydrocarbons via the salicylate pathway by forming catechol, and then mineralizing this to CO2 via the TCA cycle [24].

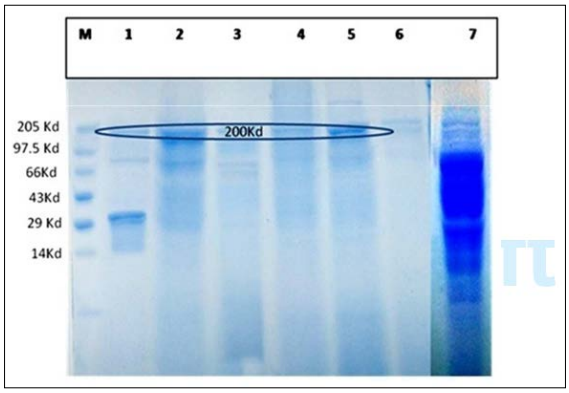

Plate 1: The protein profile modifications of Bacillus sp. E87 in the presence hydrocarbons Lane M : Protein Marker (14Kda to 205 Kda); Lane 1: Benzene; Lane 2: Xylene ; Lane 3: Toulene; Lane 4: Hexane; Lane 5: Petrol; Lane 6: Diesel and Lane 7: Control.

Similar findings were reported by Lazaroaie in which Pseudomonas aeruginosa was grown in the same media with different hydrocarbons inducing expression of different proteins as compare to control, which help to survive the strains to that environment [25]. Pseudomonas putida DOT-T1E has been provoked drastic changes in the protein pattern in toluene supplemented condition, indicating a complex response which alters the proportion of cyotoplasmic protein of bacteria [26]. Due to accumulation of these lipophilic (hydrocarbon) compounds inside the bacterial cell structural damage of cell membrane takes place, to counteract this damage new proteins involved in hydrocarbon tolerance were synthesized and expression of some preexisting proteins were increased [27-29].

Conclusions

Bacillus sp. E14 was able to tolerate and also to use aliphatic (n-hexane), aromatic (benzene, toluene, xylene), complex hydrocarbons (petrol and Diesel) as single source of carbon. The BE% of Bacillus sp. E14 towards hydrocarbons degradation depended on the substrate nature and concentration of hydrophobic substrate, and on the culture conditions, with values between 42.25% and 94%. The protein expression studies showed the presence of few new proteins that were found absent in bacteria growing in normal conditions.

These special stress protein expressions can be further manipulated by using genetic engineering and other biotechnology approaches and an effective fighting strategy can be developed (such as biofilters, ecopiling, electrobioremediation) using, these bacteria, against petroleum contamination.

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