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Novel Bacterial Strains *Pseudomonas* sp. and *Bacillus* sp. Isolated from Petroleum Oil Contaminated Soils for Degradation of Flourene and Phenanthrene

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ABSTRACT: Flourene and phenanthrene are organic compounds with high hydrophobicity and toxicity. Being recalcitrant in nature they are accumulating in the environment at an alarming concentration, posing serious threat to living beings. Thus in the present study, microorganisms were screened for their ability to degrade these contaminants at high concentrations in least period of time. Two out of fifteen isolates screened showed growth in basal medium containing 25 mg/l of fluorene/phenanthrene as the only carbon source. These selected isolates were acclimatised with step wise increased concentrations of flourene/phenanthrene for 165 days in basal medium. The acclimatised strains were identified and characterised on the basis of their morphological and biochemical characteristics and 16S rRNA gene sequence analysis. Results showed close relatedness of the isolates to *Pseudomonas aeruginosa* sp. and *Bacillus safensis* sp. Biodegradation studies carried out with these acclimatised strains at optimum conditions (pH 7 and temperature 30°C) showed 62.44% degradation of fluorene and 54.21% of phenanthrene in 10 days by *Pseudomonas* sp. VB92, whereas, *Bacillus* sp. JK17 degraded 43.64% of fluorene and 59.91% of phenanthrene in 12 days, at an initial concentration of 200 mg/l, as determined by HPTLC. During fluorene degradation by *Pseudomonas* sp. VB92, one metabolite was identified as fluorene,1,4-dihydro. An anionic biosurfactant (emulsification index of 80%) produced by strain VB92 during growth with PAHs, improved its degradation rate. This showed strong potential of the acclimatised strains for bioremediation and reclamation of polyaromatic hydrocarbon contaminated sites.

Keywords: Acclimatisation, Biodegradation, Flourene, Phenanthrene.

INTRODUCTION

PAHs are non-polar organic compounds having low solubility in water, low vapour pressure and high melting and boiling point (ASTDR, 1995; Haritash & Kaushik, 2009; Waigi et al., 2015). These are composed of two or more fused aromatic rings arranged in linear, angular or cluster form (Seo et al., 2009; Abdel-Shafy & Mansour, 2016). PAH tend to rapidly adsorb to particulate organic matter in sediments or soots and remain in the environment for years due to high hydrophobicity and lipophilicity (Kasumba & Holmen, 2018; Bamforth, 2005). Due to their formation during incomplete combustion of fossil fuels, there is migration of PAHs in various ecosystems. These are categorised into two classes, viz. low molecular weight PAHs (two or three aromatic rings such as phenanthrene, fluorene and naphthalene) and high molecular weight PAHs (four or more than

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four aromatic rings such as pyrene, fluoranthene and benzo[b]fluoranthene). PAHs are known for their carcinogenic, mutagenic and neurotoxic nature and cause acute or chronic effects on human health (ASTDR, 2009). PAHs cause severe toxic effects on living beings through different routes of exposure such as inhalation, food chain bioaccumulation, direct contact with PAH contaminated sites and through pharmaceutical products that are applied to the skin. Being lipid soluble, they readily get absorbed through skin, nasal and gastrointestinal routes in mammals and get distributed in tissues with a marked tendency for localization in body fat (Abdel-Shafy & Mansour, 2016). Adverse health effects of PAHs are cardiovascular diseases, immune-suppression and cancer in various tissues such as prostate, breast, pancreatic and cervical (Chupungars et al., 2009; Williams et al., 2013; Marchand et al., 2017).

Biodegradation through microorganisms remains the most effective approach for removal and elimination of PAHs from the environment because microbial metabolism provides a safer, more efficient, and less expensive alternative to other chemical methods, which are least effective (Rodrigues et al., 2015; Varjani & Upasani, 2016). Previous studies done revealed that bacterial strains isolated from PAHs contaminated sites have ability to degrade PAHs to some extent when the polyaromatic compound was present at a very low concentration of 50-100 µg/l (Seo et al., 2009; Ma et al., 2012). The strains belonging to the genus of *Pseudomonas, Sphingomonas, Rhodococcus, Mycobacteria, Nocardioides, Arthrobacter* and *Burkholderia* have been reported in literature but there is no report on efficient and fast removal of these toxicants from the environment (Chen et al., 2015; Drainas & Koukkou, 2007; Oyehan & Al-thukair, 2017). Thus aim of the present study was to isolate and develop organisms for efficient and faster degradation of flourene and phenanthrene. These are major constituent of fossil fuels and coal derivatives and get produced as a primary product during combustion of organic materials. These possess potential mutagenic and carcinogenic properties, as reported by U.S. EPA (Abdel-Shafy & Mansour, 2016; Tasi et al., 2009). Therefore, in the present study, the isolated strains were manipulated in the lab for higher degradation efficiency and evaluated for their enhanced capability for phenanthrene and fluorene degradation.

MATERIAL & METHODS

For the isolation of phenanthrene and flourene degrading bacteria, soil samples were collected from petroleum oil contaminated soil of Guru Gobind Singh Refinery (Bathinda), scooter markets, petrol pumps and crude oil workshops of Gurdaspur, Punjab, India.

One gram of petroleum contaminated soil was dispensed in 50 ml of nutrient broth (NB) and incubated for 24-48 h at 37°C on rotary shaker at 100 rpm. After growth, serial dilutions were made and 50 µl of the aliquot was surface-spread on nutrient agar plates and incubated at 37°C for 48-72 h. On the basis of morphological differences, the bacterial colonies were picked and streaked on basal medium agar plates containing phenanthrene and flourene (25 mg/l). Out of these, the best grown isolates were selected and were maintained in basal medium containing (g/l): K_2HPO_4 0.5, KH_2PO_4 0.5, $(NH_4)_2Cl$ 1.0, $MgCl_2.6H_2O$ 0.33, $CaCl_2.2H_2O$ 0.05, NaCl 10.0; phenanthrene 25 mg/l and flourene 25 mg/l, as sole source of carbon at pH 7.0.

Selected isolates were characterised by their morphology on nutrient agar and FE-SEM was done using Hitachi S-4800. Gram staining and morphological characterisation were done according to Cappuccino and Sherman, (2010). Additional biochemical characterization and motility tests were performed using

microbes from exponential phase of growth according to Bergey's manual of Determinative Bacteriology (Holt et al., 1994). For 16S rRNA gene sequencing, the methodology adopted was as described by Gupta et al. (2016). The obtained sequences were submitted to NCBI for GenBank accession number.

Growth of the isolates under different pH (5–10) and temperature (25–40°C) was investigated. Cultures were incubated at different pH (5, 6, 7, 8, 9 and 10) and growth was determined after 48 h of incubation. For temperature optimisation, the experiment was performed at their optimised pH and growth was determined spectrophotometrically by taking OD values at 600nm.

Growth was checked in basal medium at 50 mg/l concentration of fluorene ($C_{13}H_{10}$) and phenanthrene ($C_{14}H_{10}$), separately. From the stock of fluorene and phenanthrene (5 mg/ml) in ethyl acetate, different volumes were transferred into Erlenmeyer flask to obtain different concentrations (100 and 200 mg/l) of PAHs. After complete evaporation of ethyl acetate, 20 ml of basal salt medium with adjusted pH was added to the flask and inoculated with 2% inoculum of each isolate separately (except the control). Flasks with 100 and 200 mg/l concentrations of PAHs were incubated at 30°C and 150 rpm for 10 and 12 days, respectively. Bacterial growth was determined by the method of counting CFUs. Culture broth of each flask was serially diluted upto 10^5-10^6 times and 50ul of each diluted sample was spread on nutrient agar plates. All the plates were then incubated for 24-48hrs at optimum temperatures. Number of colonies on each agar plate was counted and results expressed as cfu/ml of culture.

Residual concentration of fluorene and phenanthrene was determined using HPTLC. High performance thin layer chromatography (HPTLC) is a cheap, effective and time saving analysis method as compared to other analytical methods viz. HPLC, GC, TLC etc. 4 µl of the organic phase was analysed using CAMAG-HPTLC. Different concentrations of standard of pyrene and anthracene (20, 40, 60, 80 and 100 mg/l) were spotted onto the chromatographic plate, separately. Chromatograms were observed under illumination at λ_{max} of fluorine (263 nm) and phenanthrene (254 nm). Concentration of pyrene/anthracene in test samples was calculated by comparing the peak area of sample with the peak area of standard/control.

$$\text{Concentration of PAH in the sample } (\text{mg/l}) = \frac{(\text{Peak area of chromatogram of sample})}{(\text{Peak area of chromatogram of standard PAH})} \text{ x Concentration of standard PAH}$$

Identification of metabolites was done using GCMS-QP2010 Plus. Capillary column used in the GC was Rtx-TMS (30 m x 0.25mm ID x 0.25 µm df). GC column oven temperature was programmed for an initial hold of 1 min at 100°C; then increased at 10°C/ min to 200°C; then upto 260°C at the rate of 15°C/min; followed to 300°C at the rate of 3°C/min and held at 300°C for 2 min. The gas flow rate was 10 ml/min in split less mode with injection temperature of 270°C. For MS measurement: MS ion source was set at 200°C, MS interface temperature at 250°C, electron impact ionisation (EI) at 70 eV, selective ion monitoring mode with dwell time 30 min, solvent delay: 3.5 min. Chromatographic data were collected and recorded by GC-MS real time analysis software (Akdogan & Pazarlioglu, 2011).

For biosurfactant extraction, bacterial cells of *Pseudomonas* sp. VB92 after growth with PAH were removed by centrifugation at 10,000 rpm for 10 min and supernatant was collected. pH of supernatant was adjusted at 2.0 using 6 M HCl and was kept at 4°C overnight for precipitation. Precipitates were collected and biosurfactant was extracted with ethyl acetate. Biosurfactant analysis were done by dropping 100 µl of crude oil on the surface of 20 µl water into the petri dish,

then dropped 100 µl of a test solution onto the surface of oil. Occurrence of a clear zone indicates presence of biosurfactant.

Emulsification index (E_{24}) test was performed by adding 2 ml of petrol to the same volume of seventh day culture filtrate, then vortexed vigorously for 2 min. The emulsified mixture was allowed to stand undisturbed at room temperature for 24 h to separate the aqueous and oil phases. The emulsification index (E_{24}) was calculated as the percentage of height (cm) of the emulsion layer divided by the total height (cm). For CTAB test, 50 µl of the cell-free culture supernatant was loaded into each well on CTAB agar plates and incubated at 30°C for 24-48 h and then stored in the refrigerator for at least 24 h. Appearance of dark blue halo zone around the well on CTAB agar plate was considered positive (+) for anionic biosurfactant production (Nie et al., 2010; Kuppusamy et al., 2017; Aparna et al., 2012).

RESULTS AND DISCUSSION

A total of fifteen bacterial isolates were tested for their ability to grown on basal medium with 25 mg/l fluorene/phenanthrene as described in section 2.2. Among them, two isolates designated as VB92 and JK17 showed growth with fluorene/phenanthrene. The isolates (VB92 and JK17) were maintained in glycerol stocks at -20°C for further studies.

The morphological characteristics for the isolates are shown in Table 3.1 and Fig. 3.1-3.2. The isolated strain VB92 was greenish in colour, opaque with rough surface and had irregular margins [Fig. 3.1] whereas strain JK17 was circular in shape with smooth surface and creamy-whitish colour [Fig. 3.2]. The gram staining, motility test and FE-SEM analysis shows that the isolate VB92 was Gram-negative, short rod and motile while isolate JK17 was Gram-positive, long rod and non-motile, Table 3.1. Biochemical characteristics of the isolates VB92 and JK17 showed positive result for catalase, oxidase, gelatine hydrolysis and citrate. Isolate VB92 showed positive results for H_2S production, lipase, pectinase, amylase and cellulase and motility however JK17 showed negative results. On the basis of morphological and biochemical results, isolates VB92 and JK17 showed close relatedness to the genus of *Pseudomonas* and *Bacillus,* respectively, same results were reported by (Pedetta et al., 2013; Marco-Urrea et al., 2015; Fooladi et al., 2016; Mojarad et al., 2016).

The isolates (VB92 and JK17) were studied for their growth at different pH (5–9) and temperature (25, 30, 32, 37 and 40°C). As shown in Fig. 3.3–3.6, highest growth was observed at pH 7.0 and 30°C temperature for both the isolates. Drastic fall in OD values with increase in pH from 8 to 10 was observed for both the isolates [Fig. 3.3–3.4]. pH could affect the physiological and biochemical properties of microbes thus affecting biotransformation of PAH. A report by Ma et al., 2012, showed significant effect of pH on PAH degradation when studied over a range of 4–9, with maximum achieved within a pH range of 5.5–7.5.

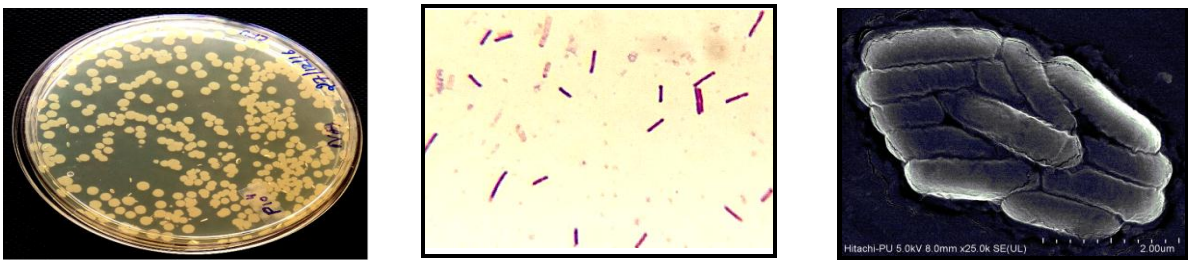
When studied at different temperatures, maximum growth of the isolate VB92 was observed at 30°C ($O.D_{600}$ 2.63), with a decrease in growth at low (25°C) and high (40°C) temperature. Similar results were observed with isolate JK17, maximum growth at 30°C with $O.D_{600}$ value of 1.25 and a decrease was seen at low and high temperatures [Fig. 3.5–3.6]. Effect of temperature on PAH degradation with bacterial strains *Pseudomonas aeruginosa*, *Agrobacterium sp. and Bacillus sp.* was reported by (Chauhan et al., 2008; Zhang et al., 2009; Masakorala et al., 2013).

Table 1. Morphological characterisation of bacterial strains

Isolate	Gram strain	Shape	Motility	Colour	Pigment
VB92	Negative	Rod	+	Green	+
JK17	Positive	Rod	-	Cream/white	-

A **B** **C**

Fig. 1. Bacterial isolate VB92 on nutrient agar plate (A); Gram staining (B); FE-SEM images of isolate VB92 at 30.0k magnifications (C).



A **B** **C**

Fig. 2. Bacterial isolate JK17 on nutrient agar plate (A); Gram staining of isolate JK17 (B); FE-SEM images of isolate JK17 at 25.0k magnifications (C).

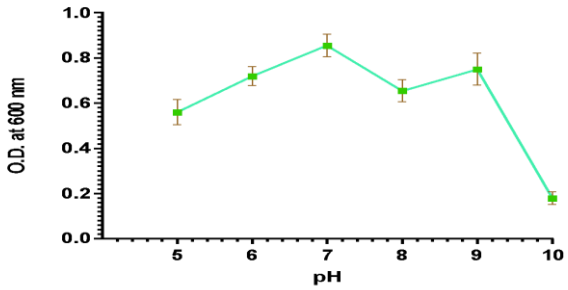


Fig. 3. Growth of the isolate VB92 at different pH.

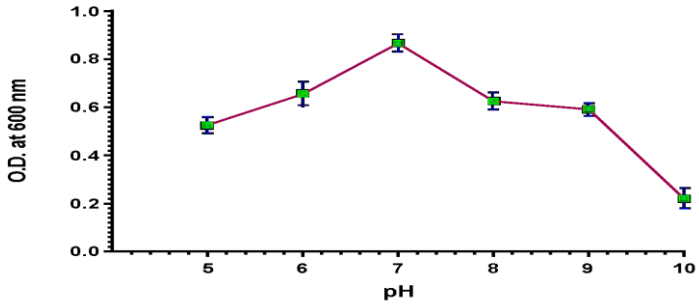


Fig. 4. Growth of the isolate JK17 at different pH.

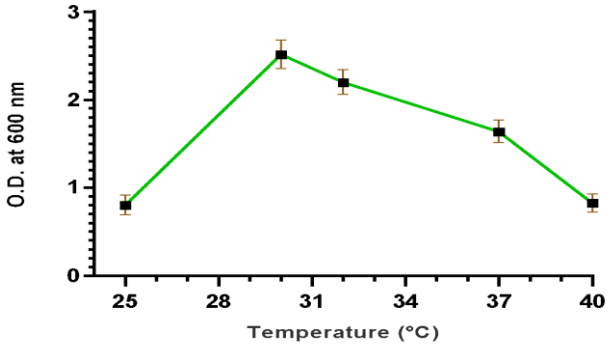


Fig. 5. Growth of the isolate VB92 at different temperatures.

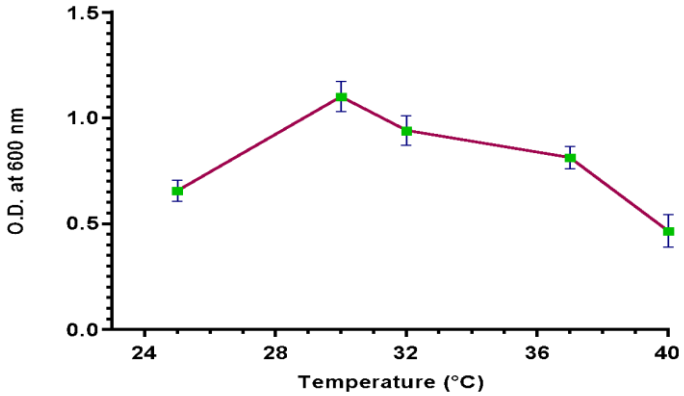


Fig .6. Growth of the isolate JK17 at different temperatures.

Growth of the isolates was checked with 50 mg/l concentration of fluorene/phenanthrene in basal medium, when present as the only carbon source. 100 fold hike in growth was observed in first 30 days of incubation, reaching to a CFU value of 2.3 x 10^7 and 2.8 x 10^7 when incubated with phenanthrene, and with flourene attained CFU value was 2.2 x 10^7 and 1.5 x 10^7, for the isolate VB92 and JK17, respectively, [Fig. 3.7–3.8]. Maximum growth for the isolate VB92 was observed after 90 days of incubation when grown with phenanthrene, whereas, the same isolate showed maximum growth with flourene after 120 days of incubation. CFU value obtained with the isolate JK17 was maximum after 105 days of incubation when studied with phenanthrene as well as flourene, as shown in Fig. 3.7–3.8.

Biodegradation of fluorene/ phenanthrene was checked at 100 mg/l concentration with the selected isolates. The test samples were extracted after 10 days of incubation and analysed for the residual concentration of fluorene and phenanthrene, using HPTLC. High performance thin layer chromatography (HPTLC) is a cheap, effective and time saving analysis method as compared to other analytical methods viz. HPLC, GC, TLC etc. Degradation of fluorene after 10 days of incubation was 60.67%, and of phenanthrene was 14.59%, with isolate VB92, Table 3.2. On the contrary, isolate JK17 degraded 38.63% flourene and 38.08% of phenanthrene, in 10 days, as shown in Table 3.3. This showed more adaptability of isolate VB92 with flourene as compared to phenanthrene, and more adaptability of isolate JK17 with phenanthrene than flourene.

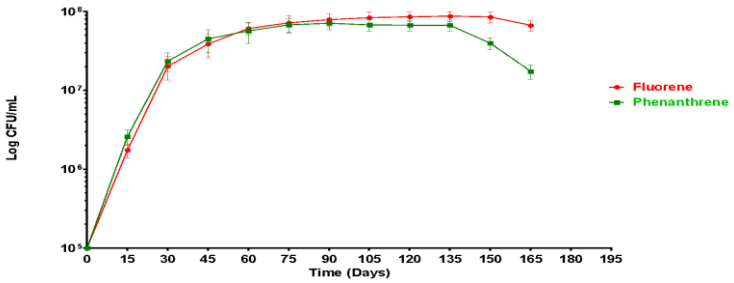


Fig. 7. Growth of the isolate VB92 with fluorene and phenanthrene at 50 mg/l in basal medium at 30°C.

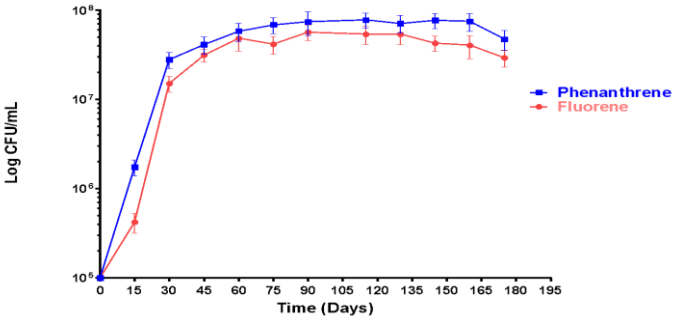


Fig. 8. Growth of the isolate JK17 with fluorene and phenanthrene at 50 mg/l in basal medium at 30°C.

Table 2. Biodegradation efficiency (BE) of the isolate VB92 with fluorene and phenanthrene

Test samples	Initial concentration (C_O)	Final concentration (C_e)	BE (%) of the isolate VB92
Fluorene			
At 100 mg/l	100 mg/l	39.32 mg/l	60.67
Phenanthrene			
At 100 mg/l	100 mg/l	85.40 mg/l	14.59

Table 3. Biodegradation efficiency (BE) of the isolate JK17 with fluorene and phenanthrene

Test samples	Initial concentration (C_O)	Final concentration (C_e)	BE (%) of the isolate JK17
Fluorene			
At 100 mg/l	100 mg/l	61.36 mg/l	38.63
Phenanthrene			
At 100 mg/l	100 mg/l	mg/l	

After obtaining growth and degradation with the selected isolates at high concentrations (50 and 100 mg/l) of PAHs, the isolates were characterised on molecular basis. From 16S rRNA gene sequencing of the isolate VB92 and JK17, 1574 and 1564 bp long sequence was obtained for the respective isolate. Their phylogenetic analysis revealed that the isolate VB92 is closely related (98%) to *Pseudomonas aeruginosa* strains as shown in Fig. 3.9 and isolate JK17 showed 99% homology to 16S rRNA sequence of *Bacillus safensis* as shown in Fig. 3.10. The nucleotide sequences obtained by 16S rRNA gene sequencing of isolate *Bacillus* sp. JK17 and *Pseudomonas* sp.VB92 have been deposited in the GenBank database under accession number MF942411 and MF785088, respectively.

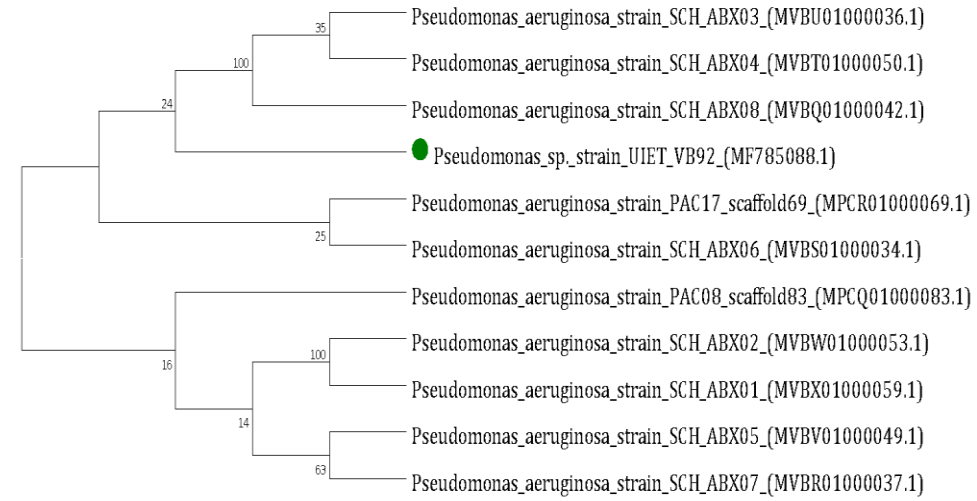


Fig. 9. Neighbour-joining phylogenetic tree of bacterial isolate UIET VB92 based on 16S rRNA gene sequences.

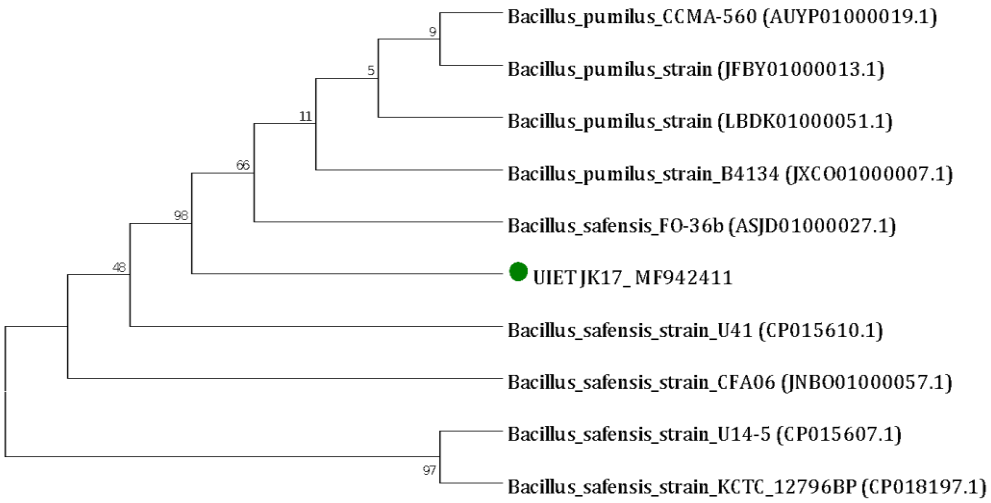


Fig. 10. Neighbour-joining phylogenetic tree of bacterial isolate UIET JK17 based on 16S rRNA gene sequences.

100 fold upsurge in growth was attained in 30 days for both the isolates when studied with 50 mg/l concentration of flourene/phenanthrene. After acclimatisation of the isolates with the compounds separately for 165 days, both the acclimatised strains showed 100 fold rise in CFU count within 2 days when inoculated with an initial cell count of 10^5, even at high concentration of 200 mg/l [Fig. 3.11–3.12]. Increase in growth from 10^5 to 10^7 and 10^8 CFU/ml was observed within four days of incubation with the strain *Bacillus* sp. JK17 and *Pseudomonas* sp. VB92, respectively, Fig. 3.11–3.12. Maximum bacterial count with fluorene and phenanthrene for strain *Pseudomonas* sp. VB92 was found to be 7.1 x 10^8 and 5.3 x 10^8 CFU/ml respectively, whereas for strain *Bacillus* sp. JK17 it was 4.7 x 10^7 and 8.35 x 10^7 CFU/ml respectively, on 8^{th} day of incubation, as shown in Fig. 3.11–3.12.

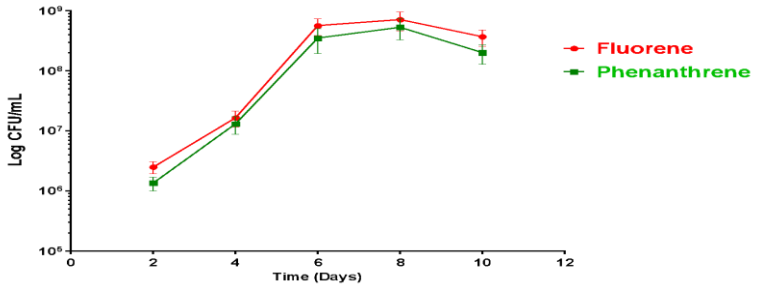


Fig. 11. Growth of *Pseudomonas* sp. VB92 with flourene and phenanthrene at 200 mg/l concentration for 10 days of incubation at 30°C.

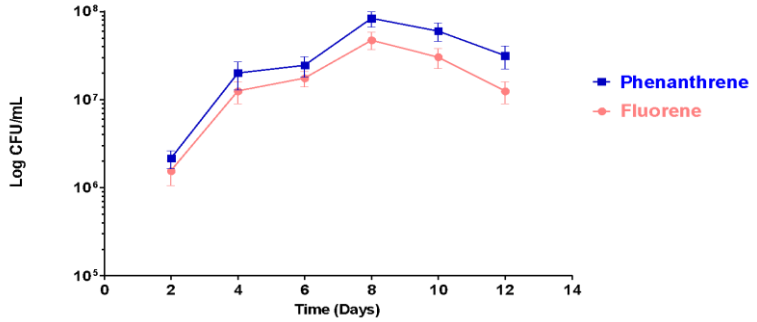


Fig. 12. Growth of *Bacillus* sp. JK17 with fluorene and phenanthrene at 200 mg/l concentration for 12 days at 30°C.

The HPTLC analysis showed that both the strains (*Pseudomonas* sp. VB92 and *Bacillus* sp. JK17) were able to utilise fluorene/phenanthrene (200 mg/l) as sole source of carbon in basal medium under optimised conditions. With *Pseudomonas* sp. VB92, the residual concentration of fluorene in 200 mg/l test sample on 10th day was 75.12 mg/l (62.44% degraded), where as the residual concentration of phenanthrene was 91.5 mg/l (54.21% degraded), as tabulated in Table 3.4. Other strain *Bacillus* sp. JK17 showed 20% less degradation of flourene (43.64%) in 12 days but a marginal increase in phenanthrene degradation as compared to strain VB92, degrading 59.19 % in 12 days, Table 3.5. The results indicate that at optimized conditions, strain *Pseudomonas* sp. VB92 degraded higher concentration (124.88 mg/l) of fluorene in 10 days as compared to *Bacillus* sp. JK17, whereas, with phenanthrene both the strains degraded almost equal amount. The amount of PAHs degraded were found to be highest than reported earlier in the related literature. Masakorala et al. (2013) reported 86.65% degradation of phenanthrene (100 mg/l) in 8 days using *Pseudomonas* strain and identified degradation of phenanthrene through protocatechuate metabolic pathway. Zhou et al. (2016) have reported 87.2% degradation of fluorene (100 mg/l) within 7 days at 30°C by *Sphingomonas*. In a report by Ling et al., (2011), degradation of fluorene, phenanthrene and pyrene as sole source of carbon was reported by *Bacillus* strain. Kuppusamy et al., (2017) have also reported 97.99% degradation of phenanthrene (100 mg/l) in 7 days of incubation by a *Pseudomonas* strain P2. Biodegradation rate of flourene and phenanthrene reported earlier in literature was found to be less as obtained in our study. This indicates higher potential of the acclimatised strains to degrade phenanthrene and flourene at higher concentrations in shorter duration of time.

Table 4. Biodegradation efficiency (BE) of strain *Pseudomonas* sp. VB92 with fluorene and phenanthrene

Test samples	Initial concentration (C_O)	Final concentration (C_e)	BE (%) of strain *Pseudomonas* sp. VB92
Fluorene			
At 200 mg/l	200 mg/l	75.12 mg/l	62.44
Phenanthrene			
At 200 mg/l	200 mg/l	91.57 mg/l	54.21

Table 5. Biodegradation efficiency (BE) of strain *Bacillus* sp. JK17 with fluorene and phenanthrene

Test samples	Initial concentration (C_O)	Final concentration (C_e)	BE (%) of strain *Bacillus* sp. JK17
Fluorene			
At 200 mg/l	200 mg/l	112.72 mg/l	43.64
Phenanthrene			
At 200 mg/l	200 mg/l	81.60 mg/l	

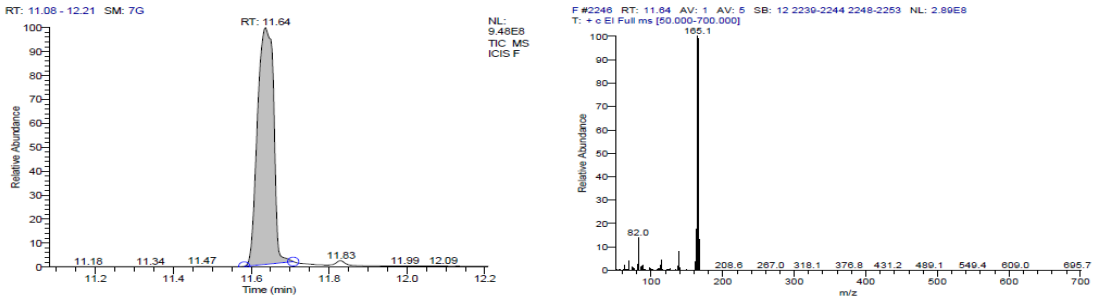


Fig. 13. Chromatogram and mass spectra obtained by GC/MS of standard of flourene.

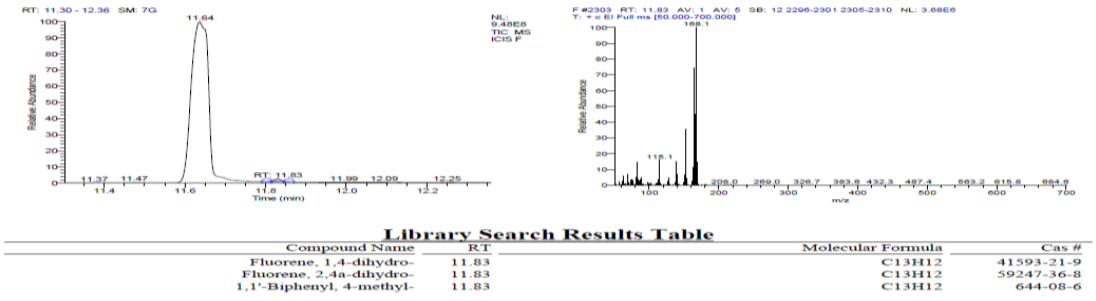


Fig. 14. Chromatogram and mass spectra obtained by GC/MS of metabolite of flourene.

Fluorene degradation products or metabolites were characterized by GC-MS analysis. In the present study, metabolite produced during fluorene degradation was identified as fluorene,1,4-dihydro (11.83 RT), as shown in Fig. 3.13-3.14.

For identification of biosurfactant produced by strain *Pseudomonas* sp. VB92; oil displacement, emulsification index and Cetyl Trimethyl Ammonium Bromide (CTAB)-methylene blue agar test was performed.

The oil displacement test was performed to measure surface activity of the surfactant against crude oil used; a larger diameter of clear zone represents a higher surface

activity of the surfactant. The diameter of clear zone formed by strain VB92 was~ 2.2 cm, Fig. 3.15. Emulsification of petrol by strains *Pseudomonas* sp.VB92 was observed to be 80% [Fig 3.16].

Formation of dark blue halos around the wells confirmed the secretion of anionic surfactant by *Pseudomonas* sp. VB92, Fig. 3.17. The dark blue halos are due to the formation of an insoluble ion pair of the secreted anionic surfactants with the cationic surfactant CTAB and the basic dye methylene blue.

Crude oil drop **Clear zone produced by VB92**

Fig. 15. Oil displacement test to determine the surface activity of biosurfactant

Fig. 16. Emulsification layer produced by strain VB92.

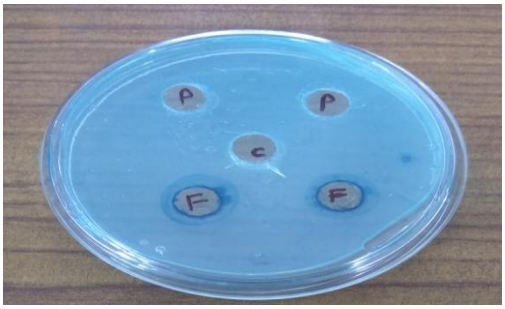


Fig. 17. CTAB agar test for presence of biosurfactant by strain VB92.
(C - Control, P- Phenanthrene, F- Flourene)

CONCLUSION

Two flourene and phenanthrene degrading bacteria isolated from petroleum oil contaminated soil samples were identified as *Pseudomonas* sp. VB92 (accession no. MF942411) and *Bacillus* sp. JK17 (accession no. MF785088). Degradation studies carried out with these isolates after acclimatising with step wise increased concentrations of PAHs for 165 days, showed degradation of 60.67 mg/l of fluorene and 14.59 mg/l of phenanthrene in 10 days, with strain VB92. Other strain JK17 degraded higher concentration of phenanthrene (61.91 mg/l) in 10 days but less of flourene (38.63 mg/l). Further increase in degradation rate was observed by acclimatising these strains with higher

concentrations (100 and 200 mg/l) of flourene and phenanthrene. Strain *Pseudomonas* sp. VB92 degraded 157.12 mg/l of flourene and 108.20 mg/l of phenanthrene in 10 days and *Bacillus* sp. JK17 degraded 87.26 mg/l of fluorene and 161.80 mg/l of phenanthrene in 12 days. To the best of our knowledge, this is the highest rate of degradation reported with phenanthrene and flourene. During growth with PAH in basal medium strain VB92 produces biosurfactant, which improves its degradation rate by increasing bioavailability of the compounds. From these results we can conclude that acclimatised strains (*Pseudomonas* sp. VB92 and *Bacillus* sp. JK17) possess great potential for bioremediation of poly aromatic hydrocarbons contaminated sites and it can further be exploited in natural environment.

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