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Phenanthrene degradation using *Streptomyces variabilis* strain RD5 isolated from marine ecosystem

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ABSTRACT

Streptomyces variabilis strain RD5, a marine actinomycete was isolated from Gulf of Khambhat and was employed to degrade, a model polycyclic aromatic hydrocarbon, phenanthrene. *S. variabilis* strain RD5 degraded (50 mg/L phenanthrene within 6 d) and the degradation was confirmed using the different analytical techniques. The phenanthrene biodegradation resulted in the formation of carbonochloridic acid ethyl ester, ethanol and acetone which were found in the mass spectral profile of the recovered degradation products. The plant seeds of *Phaseolus mungo* did not exhibit any growth inhibition when irrigated with the products of degradation.

Keywords: Biodegradation; Gulf of Khambhat; Phenanthrene; Polycyclic aromatic hydrocarbon; *Streptomyces variabilis*

1. Introduction

The point source of marine pollution caused by the polycyclic aromatic hydrocarbons (PAHs) originates from the petrochemical industry and spillage of petroleum products from ships [1].The presence of these PAHs in the marine environments may create irreversible changes to the public health and marine life owing to their hydrophobicity, toxicity, mutagenicity and carcinogenicity [2]. PAHs are persistent environmental recalcitrant compounds and resist degradation due to the thermodynamic stability offered by the complex structural and functional moieties which makes them to categorize under the priority environmental pollutants [3]. Phenanthrene among the PAH is often used as a model for understanding the metabolic fate of carcinogenic PAHs and is widely distributed as an environmental contaminant causing unfavourable biological effects [4]. Despite being expensive, the physicochemical techniques fail to eliminate the toxicity of phenanthrene contaminated water [5].

In industries, reducing not only the pollution levels but also the concentration of specific pollutants will be obligated in the near future. The use of biological processes is of particular attention for the treatment of effluents from individual sources, especially if a well-defined chemical compound had to be degraded instead of a complex or even unknown mixture of pollutants. The pollutants are degraded using the well-adapted biomass, wherein the adapted cells metabolizes the pollutants at a faster rate without producing any harmful byproducts [6,7].

Bioremediation is well attracted by the environmentalists to remediate the problems of recalcitrants in the environment [8].The microbial degradation represents a trustworthy route to remediate the phenanthrene pollution in the water and species belonging different genus are known to be efficient [9–11]. PAHs biodegradation involves the enzymatic catalysis to form *cis*-dihydrodiol and form dihydroxylated

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intermediates which furthermore undergoes breakdown [1,11]. The low molecular weight PAHs such as naphthalene, anthracene and phenanthrene are more volatile, water soluble and are easily biodegraded when compared with the high molecular weight PAHs such as pyrene and benzo[a] pyrene [12].

Actinomycetes utilize a wide range of carbon sources to degrade complex polymers in a lesser time when compared with the fungal treatment, and they represent a dominant group effective in remediating diverse environmental pollutants [13–16]. Various researchers have reported the degradation of distinct pollutants such as pesticides and PAHs by diverse actinomycetes [17–20]. An isolate, *Rhodococcus* sp. from contaminated river sediment could utilize 31% of phenanthrene as source of carbon [21]. The strains of *Streptomyces* sp. are known to be potentially viable degrader either in the form of single strain and/or with the combination with other microorganisms [22]. *Amycolatopsis tucumanensis* DSM 45259 and *Streptomyces* sp. A12 could degrade 36.2% and 20% phenanthrene, respectively [23]. A consortium of *S. flavovirens* and *Agmenellum quadruplicatum* PR-6 was effective in degrading phenanthrene by inducing mono-oxygenase epoxide hydrolase rather than dioxygenase [24]. Pizzul et al. [25] demonstrated that *Rhodococcus* sp. DSM 44126 and *Rhodococcus wratislaviensis* would degrade ~80% phenanthrene after 14 d. *Rhodococcus* sp. P14 exhibited 34% phenanthrene degradation [26].

The objective of this research is to screen and identify potential marine actinomycete isolated from Gulf of Khambhat (GoK) for the phenanthrene degradation. The degradation mechanisms were to be studied using different spectral and chromatographic techniques. To characterize the degraded products using gas chromatography with mass spectrometry (GC-MS) and to assess the toxicity of phenanthrene and their products of degradation using phytotoxicity tests.

2. Material and methods

2.1. Chemicals

Phenanthrene (molecular weight, MW = 178.234 g/mol and maximum wavelength, λ_{max} = 310 nm) and 2,6-dimethoxy phenol (2,6-DMP) were purchased from Sigma-Aldrich (Mumbai, India), Luria-Bertani (LB) medium was purchased from Hi-Media, (Mumbai, India). Dimethyl formamide was used for dissolving the phenanthrene throughout the study, and the stock solution was stored in the dark at room temperature. All the chemicals were of highest purity and analytical grade.

2.2. Screening of phenanthrene degrading marine indigenous actinomycete

The marine sediment samples were collected from coastal areas of GoK near a ship scraping industry (21°24′35.85″N, 72°11′54.1″E) and introduced into Gause's synthetic medium containing the following (g/L): starch, 20.0; KNO_3, 1.0; NaCl, 0.5; K_2HPO_4, 0.5; $MgSO_4.7H_2O$, 0.5; $FeSO_4$, 0.01; agar, 20.0 and 0.01% of potassium dichromate (pH 7.3 ± 0.2) was added into the medium to prevent bacterial and fungal growth [19]. After suitable dilution, the different morphological actinomycetes were isolated and preserved. The isolated different cultures were inoculated in LB containing the following (g/L): casein enzymic hydrolysate, 10.0; yeast extract, 5.0; sodium chloride, 10.0 (pH 7.5 ± 0.2) with 50 mg/L of phenanthrene and incubated at 30°C under shaking condition (120 rpm). The pure strain which were able to survive in 50 mg/L phenanthrene was subcultured, and the stocks were stored at –80°C in glycerol stock.

2.3. Identification of phenanthrene degrading actinomycete

2.3.1. Morphological characterization

The strain was characterized on the basis of their morphological (macroscopic and microscopic) features. The macroscopic characters include colony morphology with respect to size, nature of colony, pigmentation and presence or absence of aerial mycelium. The microscopic features include the substrate and aerial mycelium fragmentation and spore chain morphology. The culture was slowly released at the intersection of the medium and sterile cover slip which was incubated at 28 ± 2°C for 5–8 d [27]. After incubation, the cover slip was removed and placed on slide for phase contrast microscopy and on stab for scanning electron microscopic (SEM) analysis on JSM 7100F, field emission SEM, JEOL, USA. Simultaneously, a thin smear of the culture was made on a clean glass slide and heat fixed followed by Gram's staining and examined under the microscope.

2.3.2. Biochemical characterization

The biochemical characterization of the isolated pure culture was done using Biolog (BIOLOG MicroStation™ System, Hayward, California, USA) and fatty acid methyl ester (FAME) profiling. Biolog's test panel (GEN III) contains 71 carbon sources and 23 chemical sensitivity assays. In FAME analysis, the selected isolates were inoculated in soya bean casein digest medium and incubated at 30°C for 72–96 h. The cell pellet was saponified, methylated and extracted for fatty acids as described by the microbial identification system (MIDI). The extracted samples were analyzed with Agilent GC6850 (gas chromatograph), and the profiles were compared with the Sherlock TSBA Library 6.0 version (Microbial ID, MIDI Inc.).

2.3.3. Molecular identification

The cells were harvested from 50 mL of grown culture which was used for isolating the genomic DNA and was quantified for its purity. A total of 16S rRNA genes of these isolates were amplified using the primer set of 27F and 1492R [28]. Initially the forward and reverse sequences were aligned using Bio-Edit software to make a full length of 16S rRNA gene sequences and BLAST were done using BLASTn program of National Center for Biotechnological Information (NCBI) as well as RDP II databases. MEGA 7.0 was used for constructing the phylogenetic tree [29]. The sequences displaying similarities >97% with known species were identified as corresponding species and the sequences were submitted to NCBI to obtain accession number [30].

2.4. Analysis of phenanthrene degradation

Aliquot (5 mL) of the media was withdrawn after particular reaction time and centrifuged at 7,000 rpm at 10°C and the supernatant was used to analyze the degradation using UV-Vis spectrophotometer (Shimadzu Corp., UV-1800, Japan). UV-Vis spectra of the abiotic control (without microorganisms) and the culture medium were scanned, compared at λ_{max} of phenanthrene (310 nm). All the experiments were performed in triplicate, and the mean value was calculated.

2.5. Recovery and characterization of phenanthrene degradation products

Based on maximum degradation, the grown culture with the phenanthrene containing medium was extracted using equal volume of ethyl acetate, dried over anhydrous sodium sulphite and evaporated to dryness. The functional groups in the fingerprint region (1,500–500 cm^{-1}) of phenanthrene and the degraded products were compared by recording the Fourier transform infrared (FT-IR) spectra using a GX-FT-IR system (PerkinElmer, USA). The samples were mixed with KBr and made as pellets, then placed on the sample holder for spectral analyses in the frequency range of 4,000–400 cm^{-1}. High-performance liquid chromatography (HPLC) was operated at 35°C with mobile phase as HPLC grade methanol at a flow rate of 1.0 mL/min for 10 min run time. The analytes (phenanthrene and products) were detected using the UV-Vis detector at their respectively wavelength. The chromatograms were recorded using Shimadzu Corporation RF-10 AxL instrument equipped with C_{18} column. The mass spectral analyses of the degradation product was recorded on Shimadzu QP 2010 system using helium as the carrier gas and the compounds were identified based on the standards available in the NIST library.

2.6. Analysis of manganese peroxidase activity

Manganese peroxidase (MnP) activity was measured spectrophotometrically the oxidation of 2,6-DMP at 37°C at 468 nm [31]. The assay mixture comprised of 200 μL of 250 mM sodium malonate (pH 4.5), 50 μL of 20 mM 2,6-DMP, 50 μL of 20 mM $MnSO_4$, 50 μL of the crude extract and 550 μL of water. The reaction was initiated by adding 100 μL of 4 mM H_2O_2. One unit of MnP activity is defined as the amount of enzyme releasing 1 μmol of the product during the oxidation of 2,6-DMP per min.

2.7. Analysis of phenanthrene detoxification

The extracted products of phenanthrene degradation were dissolved in sterile distilled water. *Phaseolus mungo* seeds were placed on the filter paper, and irrigated with 5 mL of phenanthrene and the products individually [32,33]. The control set (seeds irrigated with distilled water) was also included simultaneously. The parameters assessing the seedling's growth such as percentage germination, length of plumule and radicle, and the chlorophyll content were reported after 7 d [34,35].

3. Results and discussion

3.1. Screening of phenanthrene degrading marine actinomycetes

In this investigation, an indigenous actinomycete was screened from the petrochemical spillages exposed in and around the ship scraping industry, Sosiya Ship Breaking Yard, Alang (Gujarat). The morphologically different isolates were screened on the basis of growth in medium surviving with 50 mg/L of phenanthrene. The single screened culture was tested for their ability to survive in LB broth supplemented with 50 mg/L of phenanthrene and degraded phenanthrene after 6 d of incubation at pH 7.8 and 30°C. A 72-h-old grown culture of *Streptomyces variabilis* strain RD5 with the phenanthrene containing medium was extracted and analysed further for the degradation.

3.2. Identification of the potential degrader

The culture was further streaked to yield a consistent colony, and its characteristics were observed from plate cultures after 7 d (Fig. 1(a)) while the preliminary investigation on the morphological, physiological and biochemical characteristics revealed that the isolate was Gram positive and had fungal-like branched networks of hyphae and short branches are formed that usually produce several spores Fig. 1(b), (c). The metabolic profiles of the isolate from Biolog GENE III microplate indicated that the strain could significantly utilise 75 of 95 carbon sources comprised of polymers, sugar derivatives, carboxylic acids, methyl esters, alcohol, nucleosides,

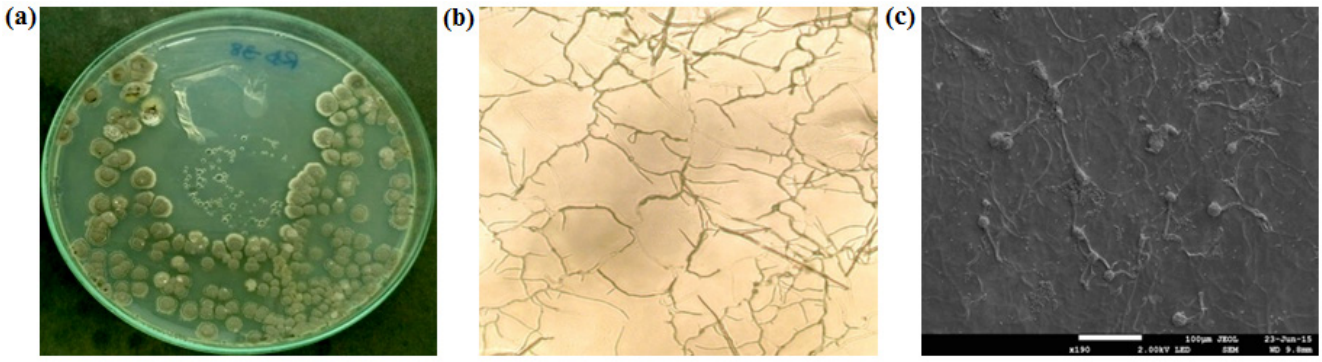

Fig. 1. Phenotypic identification of the marine isolates: (a) morphological appearance, (b) spore chain arrangement observed at 1000X in compound microscope and (c) SEM images of spore chains.

nucleotides and sugar phosphates. The strain could grow in the presence of rifamycin, vancomycin, linkomycin and minocycline describing the resistivity against antibiotics. FAME analysis of the selected strain showed 96.56% in the profile and had 27 fatty acids, the predominant cellular fatty acid of the strain were 13:0 anteiso (38.3%), 15:0 iso (15.4%), 16:0 (12.9%) and 17:0 anteiso (12.7%) which closely matched with *Streptomyces* [36].

16S rRNA gene sequence data having 1,300 bp were analysed according to BLASTn to identify the isolate and found as *S. variabilis* [37,38]. The sequences were submitted to NCBI with the accession number KT588655 (*S. variabilis* strain RD5). The phylogenetic analysis was undertaken by comparing 16S rRNA gene sequences of tree representatives of closely related type strains of actinomycetes (Fig. 2).

3.3. Analyses of phenanthrene degradation

3.3.1. UV-Vis spectrophotometric analyses

UV-Vis spectral analyses were employed for understanding the phenanthrene degradation by comparing the spectral behaviour of the phenanthrene and degraded products. UV-Vis absorbance peak of phenanthrene had a peak λ_{max} of 310 nm, whereas, in case of the products the λ_{max} shifted to 250 nm explaining the phenanthrene degradation by the cellular metabolism of *S. variabilis* strain RD5 either by phthalic acid and proto-catechuic acid pathway [39,40].

3.3.2. FT-IR spectroscopic analyses

FT-IR spectra of phenanthrene displayed peaks at 3,806, 3,754, 3,430, 3,051, 1,601 and 997 cm^{-1} for the NH stretch of amino bond and the ammonium ions (Fig. 3(a)). The presence of the aromatic compounds was found at 1,348 cm^{-1} for the CH stretch of phenols and the peak at 2,365 cm^{-1} refers to the CN stretching of the alkyl nitrile. The peaks at 1,835 and 1,915 cm^{-1} refers to the CH stretch of the CH_3 and CH_2 groups, respectively. The peak at 3,655 cm^{-1} describes the OH stretching of alcohols while 1,684 and 1,760 cm^{-1} describes the C=O stretch of carboxylic acids. C=C stretch was evidenced at 1,449 cm^{-1} whereas by 614, 544 and 494 cm^{-1} refers to the alkanes. The peak 1,139 cm^{-1} stands for the NO_2 stretch of the aromatic amines, and the presence of benzene ring were confirmed by the peaks at 869, 816 and 731 cm^{-1}, explaining the aromaticity of phenanthrene [41].

FT-IR spectra of the degraded products had peaks at 3,451 and 1,632 cm^{-1} representing the strong primary amines supported by 2,364 cm^{-1} for the NH stretch of charged amines. The peak at 1,932 cm^{-1} explains the CH bend, confirming the aromaticity disruption (Fig. 3(b)). The presence of 972 cm^{-1} represents CH bend supported by the peak at 631 cm^{-1}. The distorted peak positions in the fingerprint region of the products described the phenanthrene degradation by *S. variabilis* strain RD5.

3.3.3. Chromatographic analyses

HPLC chromatograms of phenanthrene and products of degradation were recorded by detecting the samples at 310 and 250 nm, respectively, using UV-Vis detector. The HPLC elution profiles had peaks at varying retention time (R_t). The phenanthrene had R_t at 7.66 min with many shoulder peaks at 1.84, 2.13, 2.35, 2.48 and 7.49 min whereas, the degraded products by *S. variabilis* strain RD5 had a new peak at R_t = 2.13 min.

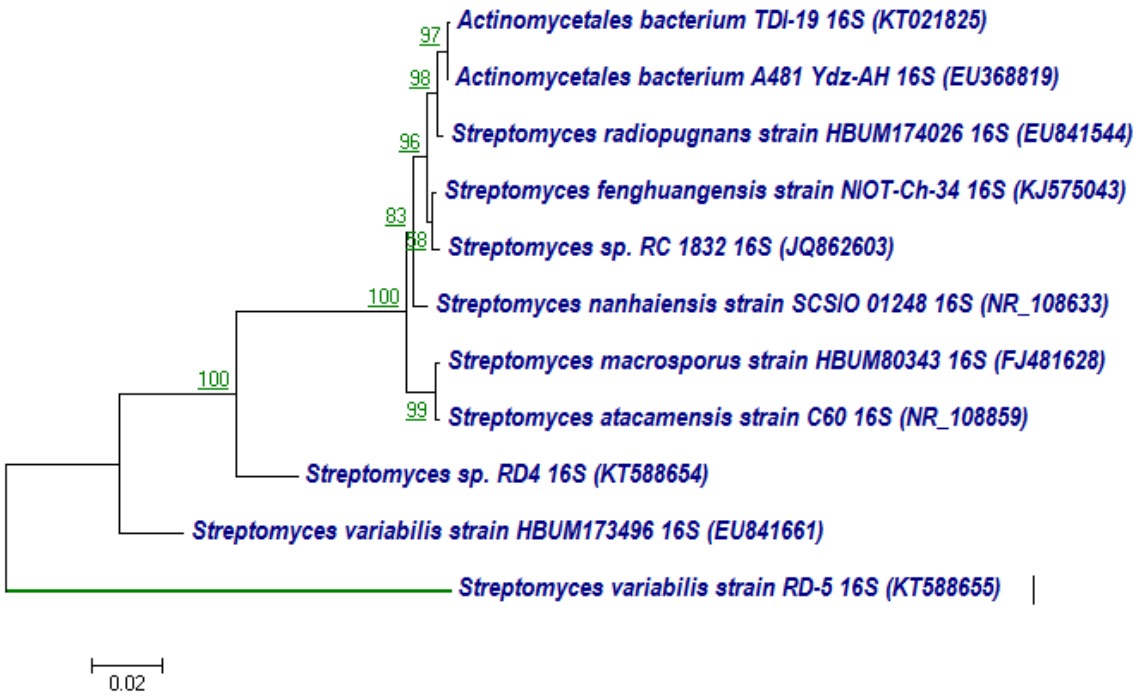

Fig. 2. Neighbour-joining tree based on nearly complete 16S rRNA gene sequences showing relationships between *Streptomyces variabilis* strain RD5 and closely related members of the genus *Streptomyces*.

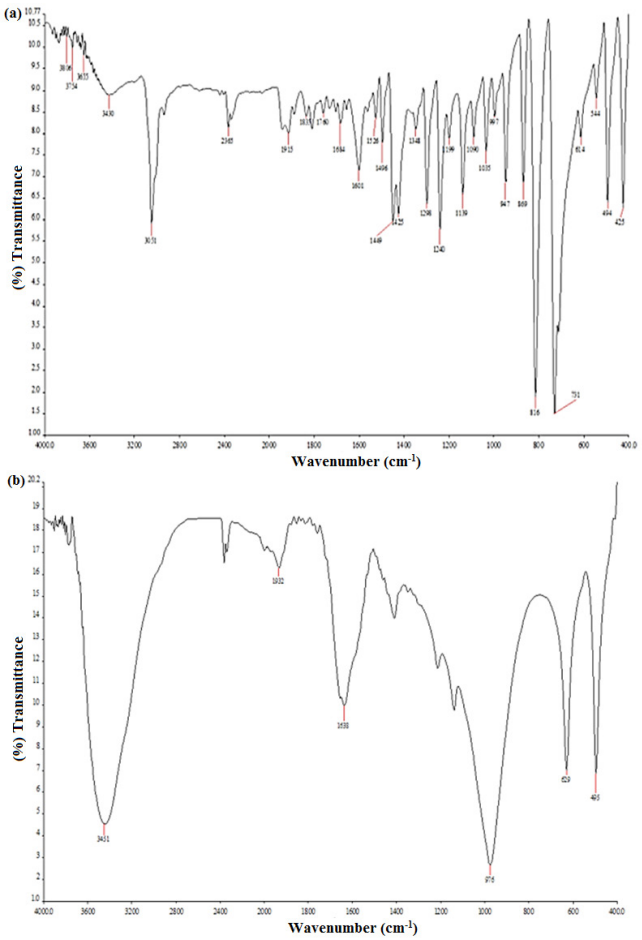

Fig. 3. FT-IR spectra of phenanthrene (a) before and (b) after treatment with *S. variabilis* strain RD5.

3.3.4. Mass spectral product identification

GC-MS analysis of phenanthrene degradation products revealed that phenanthrene was bio transformed by *S. variabilis* strain RD5 into 1-(2-methoxyethoxy)-2-methyl-2-propanol, methyl ether and 3-isopropoxy-1,1,1,7,7,7-hexamethyl-3,5,5-tris (trimethylsiloxy) tetrasiloxane eluting out at R_t = 15.725 and 16.849 min, respectively. The results suggest that the phenanthrene was degraded into simpler compounds by *S. variabilis* strain RD5 mediated by the extracellular MnP. The MnP activities in the control and phenanthrene treated with *S. variabilis* strain RD5 were 0.0017 ± 0.003 and 0.091 ± 0.019 (U/min/mg of protein), respectively, which had catalysed the degradation of phenanthrene [42–45].

3.4. Phenanthrene detoxification

P. mungo seeds did not germinate when supplied with phenanthrene whereas the seeds germinated with the degraded products did not show any inhibition of growth, and the test values are presented in Table 1. The increase in the plumule and radicle length with increase in chlorophyll content explained that *S. variabilis* strain RD5 had degraded and detoxified phenanthrene considerably [46–48].

Table 1
Phyto-toxicity of phenanthrene and their degradation products on *Phaseolus mungo* seeds

Growth assessors	Control	Phenanthrene	Product
Germination (%)	100	85	100
Plumule length (cm)	17.0 ± 0.45	1.0 ± 0.10	16.0 ± 0.15
Radicle length (cm)	12.0 ± 0.25	–	10.0 ± 0.20
Chlorophyll (mg/g tissue)	3.075	ND	2.428

ND, not detected.

4. Conclusions

S. variabilis strain RD5 utilized 50 mg/L phenanthrene and degraded them into simpler detoxified metabolites at pH 7.8, 30°C. The induction of extracellular MnP by *S. variabilis* strain RD5 described the enzyme aided cleavage of the aromatic rings of phenanthrene, thereby degrading the complex structural moieties. Thus, *S. variabilis* strain RD5 would serve as a potential choice to mitigate the vicinities contaminated by the petrochemical recalcitrant compounds.

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References

[1] R.A. Kanaly, S. Harayama, Biodegradation of high-molecular-weight polycyclic aromatic hydrocarbons by bacteria, J. Bacteriol., 182 (2000) 2059–2067.
[2] J.P. Meador, J.E. Stein, W.L. Reichert, U. Varanasi, Bioaccumulation of polycyclic aromatic hydrocarbons by marine organisms, Rev. Environ. Contam. Toxicol., 143 (1995) 79–165.
[3] J. Komal, M.A. Kumar, K.V. Thiruvengadaravi, D. Nilavunesan, M.P. Premkumar, V.V. Kumar, S. Sivanesan, Indigenously acclimatized bacterial consortium for anthracene biotransformation, Energy Sources Part A, 39 (2017) 528–537.
[4] S.K. Samanta, O.V. Singh, R.K. Jain, Polycyclic aromatic hydrocarbons: environmental pollution and bioremediation, Trends Biotechnol., 20 (2002) 243–248.
[5] Y.G. Cho, S.K. Rhee, S.T. Lee, Effect of soil moisture on bioremediation of chlorophenol-contaminated soil, Biotechnol. Lett., 22 (2000) 915–919.
[6] C. Seignez, V. Mottier, C. Pulgarin, N. Adler, P. Peringer, Biodegradation of xenobiotics in a fixed bed reactor, Environ. Prog. Sustainable Energy, 12 (1993) 306–311.
[7] J. Liu, J. Ren, R. Xu, B. Yu, J. Wang, Biodegradation of partially hydrolyzed polyacrylamide by immobilized bacteria isolated from HPAM-containing wastewater, Environ. Prog. Sustainable Energy, 35 (2016) 1344–1352.
[8] N. Joshi, R.N. Dholakiya, M.A. Kumar, K.H. Mody, Recycling of starch processing industrial wastewater as a sole nutrient source for the bioflocculant production, Environ. Prog. Sustainable Energy, 36 (2017) 1458–1465.
[9] H. Kiyohara, K. Nagao, R. Nomi, Degradation of phenanthrene through *o*-phthalate by an *Aeromonas* sp., Agr. Biol. Chem., 40 (1976) 1075–1082.
[10] M.S. Reddy, B. Naresh, T. Leela, M. Prashanthi, N.Ch. Madhusudhan, G. Dhanasri, P. Devi, Biodegradation of phenanthrene with biosurfactant production by a new strain of *Brevibacillus* sp., Bioresour. Technol., 101 (2010) 7980–7983.

[11] S. Mallick, J. Chakraborty, T.K. Dutta, Role of oxygenases in guiding diverse metabolic pathways in the bacterial degradation of low-molecular weight polycyclic aromatic hydrocarbons: a review, Crit. Rev. Microbiol., 37 (2011) 64–90.
[12] J. Pannu,, A. Singh, O. Ward, Influence of peanut oil on microbial degradation of polycyclic aromatic hydrocarbons, Can. J. Microbiol., 49 (2003) 508–513.
[13] A.J. McCarthy, S.T. Williams, Actinomycetes as agents of biodegradation in the environment – a review, Gene, 115 (1992) 189–192.
[14] M. Kästner, M.B. Jammali, B. Mahro, Enumeration and characterization of the soil microflora from hydrocarbon-contaminated soil sites able to mineralize polycyclic aromatic hydrocarbons (PAH), Appl. Microbiol. Biotechnol., 41 (1994) 267–273.
[15] L.Y. Wick, A.R. de Munain, D. Springael, H. Harms, Responses of *Mycobacterium* sp. LB501T to the low bioavailability of solid anthracene, Appl. Microbiol. Biotechnol., 58 (2002) 378–385.
[16] A.R. Johnsen, A. Winding, U. Karlson, P. Roslev, Linking of microorganisms to phenanthrene metabolism in soil by analysis of 13C-labeled cell lipids, Appl. Environ. Microbiol., 68 (2002) 6106–6113.
[17] K. Iwahori, M. Wang, H. Taki, M. Fujita, Comparative studies on utilization of fatty acids and hydrocarbons in *Nocardia amarae* and *Rhodococcus* spp., J. Biosci. Bioeng., 79 (1995) 186–189.
[18] L.G. Whyte, S.J. Slagman, F. Pietrantonio, L. Bourbonniere, S.F. Koval, J.R. Lawrence, W.E. Inniss, C.W. Greer, Physiological adaptations involved in alkane assimilation at a low temperature by *Rhodococcus* sp. strain Q15, Appl. Environ. Microbiol., 65 (1999) 2961–2968.
[19] L.C. Strong, C. Rosendahl, G. Johnson, M.J. Sadowsky, L.P. Wackett, ArthrobacteraurescensTC1 metabolizes diverse s-triazine ring compounds, Appl. Environ. Microbiol., 68 (2002) 5973–5980.
[20] B.J. Bhadbhade, S.S. Sarnaik, P.P. Kanekar, Bioremediation of an industrial effluent containing monocrotophos, Curr. Microbiol., 45 (2002) 346–349.
[21] D. Dean-Ross, J.D. Moody, J.P. Freeman, D.R. Doerge, C.E. Cerniglia, Metabolism of anthracene by a *Rhodococcus* spp., FEMS Microbiol. Lett., 204 (2001) 205–211.
[22] L.D. Sette, L.A.M.A. Costa, A.J. Marsaioli, G.P. Manfio, Biodegradation of alachlor by soil *Streptomycetes*, Appl. Microbiol. Biotechnol., 64 (2004) 712–717.
[23] N. Bourguignon, P. Isaac, H. Alvarez, M.J. Amoroso, M.A. Ferrero, Enhanced polyaromatic hydrocarbon degradation by adapted cultures of actinomycete strains, J. Basic Microbiol., 54 (2014) 1288–1294.
[24] M.L. Narro, C.E. Cerniglia, C.V. Baalen, D.T. Gibson, Metabolism of phenanthrene by the marine cyanobacterium *Agmenellum quadruplicatum* PR-6, Appl. Environ. Microbiol., 58 (1992) 1351–1359.
[25] L. Pizzul, M.P. Castillo, J. Stenstro, Characterization of selected actinomycetes degrading polyaromatic hydrocarbons in liquid culture and spiked soil, Biodegradation, 18 (2006) 745–752.
[26] X. Song, Y. Xu, G. Li, Y. Zhang, T. Huang, Z. Hu, Isolation, characterization of *Rhodococcus* sp. P14 capable of degrading high-molecular-weight polycyclic aromatic hydrocarbons and aliphatic hydrocarbons, Mar. Pollut. Bull., 62 (2011) 2122–2128.
[27] T.G. Pridham, C.W. Hesseltine, R.G.Benedict, A guide for the classification of *Streptomycetes* according to selected groups. Placement of strains in morphological sections, Appl. Microbiol., 6 (1958) 52–79.
[28] D.J. Lane, 16S/23S rRNA Sequencing, E. Stackebrandt, M. Goodfellow, Eds., Nucleic Acids Techniques in Bacterial Systematics, John Wiley & Sons, Chichester, 1991, pp. 115–147.
[29] A.K. Passari, P. Chandra, Zothanpuia, V.K. Mishra, V.V. Leo, V.K. Gupta, B. Kumar, B.P. Singh, Detection of biosynthetic gene and phytohormone production by endophytic actinobacteria associated with *Solanumlycopersicum* and their plant growth promoting effect, Res. Microbiol., 167 (2016) 692–705.
[30] K. Tamura, J. Dudley, M. Nei, S. Kumar, MEGA4: molecular evolutionary genetics analysis (MEGA) software version 4.0, Mol. Biol. Evol., 24 (2007) 1596–1599.
[31] G. Eibes, T. Cajthaml, M.T. Moreira, G. Feijoo, J.M. Lema, Enzymatic degradation of anthracene, dibenzothiophene and pyrene by manganese peroxidase in media containing acetone, Chemosphere, 64 (2006) 408–414.
[32] M.A. Kumar, S. Poonam, V.V. Kumar, G. Baskar, M. Seenuvasan, D. Anuradha, S. Sivanesan, Mineralization of aromatic amines liberated during the degradation of a sulfonated textile colorant using *Klebsiella pneumoniae* strain AHM, Process Biochem., 57 (2017) 181–189.
[33] M.A. Kumar, P.A. Zamana, V.V. Kumar, P. Baskaralingam, K.V. Thiruvengadaravi, T. Amudha, S. Sivanesan, *Achromobacter xylosoxidans* strain APZ for phthalocyanine dye degradation: chemo-metric optimization and canonical correlation analyses, J. Water Process Eng., 18 (2017) 73–82.
[34] V. Karthikeyan, M.A. Kumar, P. Mohanapriya, T. Amudha, D. Thiruselvi, P. Karthik, S. Sivanesan, Biodegradation of Remazol Brilliant Blue R using isolated bacterial culture (*Staphylococcus* sp. K2204), Environ. Technol., 2017. doi: http://dx.doi.org/10.1080/09593330.2017.1369579.
[35] M.A. Kumar, D.K. Harthy, V.V. Kumar, K.G. Balashri, M. Seenuvasan, D. Anuradha, S. Sivanesan, Detoxification of a triphenylmethane textile colorant using acclimated cells of *Bacillus mannanilyticus* strain AVS, Environ. Prog. Sustainable Energy, 36 (2017) 394–403.
[36] S. Sengupta, A. Pramanik, A. Ghosh, M. Bhattacharyya, Antimicrobial activities of actinomycetes isolated from unexplored regions of Sundarbans mangrove ecosystem, BMC Microbiol., 15 (2015) 1–16.
[37] B.J. Tindall, R. Rosselló-Móra, H.J. Busse, W. Ludwig, P. Kämpfer, Notes on the characterization of prokaryote strains for taxonomic purposes, Int. J. Syst. Evol. Microbiol., 60 (2010) 249–266.
[38] D.N. Riddhi, N. Joshi, M.A. Kumar, K.H. Mody, Production and characterization of melanin from *Streptomyces cavourensis* strain RD8 using response surface optimization, Environ. Pollut. Prot., 2 (2017) 168–178.
[39] M.T. Bhatti, M. Ali, G.N. Shahid, M. Saleh, Characterization of phenanthrene single crystals, Turk. J. Phys., 24 (2000) 673–679.
[40] S. Chatterjee, T.K. Dutta, Complete degradation of butyl benzyl phthalate by a defined bacterial consortium: role of individual isolates in the assimilation pathway, Chemosphere, 70 (2008) 933–941.
[41] M.L. Wu, M.Q. Nie, X.C. Wang, J.M. Su, W. Cao, Analysis of phenanthrene biodegradation by using FTIR, UV and GC–MS, Spectrochim. Acta, Part A, 75 (2010) 1047–1050.
[42] J.A. Rosales-Castillo, K. Oyama, M.S. Vázquez-Garcidueñas, R. Aguilar-Romero, F. García-Oliva, G. Vázquez-Marrufo, Fungal community and ligninolytic enzyme activities in *Quercus deserticola* Trel. Litter from forest fragments with increasing levels of disturbance, Forests, 9 (2018) 1–18.
[43] Q. Hussain, Peroxidase mediated decolorization and remediation of wastewater containing industrial dyes: a review, Rev. Environ. Sci. Biotechnol., 9 (2010) 117–140.
[44] M.A. Kumar, V.V. Kumar, M.P. Premkumar, P. Baskaralingam, K.V. Thiruvengadaravi, D. Anuradha, S. Sivanesan, Chemometric formulation of bacterial consortium-AVS for improved decolorization of resonance-stabilized and hetero-polyaromatic dyes, Bioresour. Technol., 123 (2012) 344–351.
[45] U. Sack, M. Hofrichter, W. Fritsche, Degradation of polycyclic aromatic hydrocarbons by managanese peroxidase of *Nematoloma frowardii,* FEMS Microbiol. Lett., 152 (1997) 227–234.
[46] M.A. Kumar, V.V. Kumar, R. Ponnusamy, F.P. Daniel, M. Seenuvasan, C.D. Anuradha, S. Sivanesan, Concomitant mineralization and detoxification of acid red 88 by an indigenous acclimated mixed culture, Environ. Prog. Sustainable Energy, 34 (2015) 1455–1466.
[47] M.A. Kumar, G. Vigneshwaran, M.E. Priya, M. Seenuvasan, V.V. Kumar, D. Anuradha, S. Sivanesan, Concocted bacterial consortium for the detoxification and mineralization of azoic-cum-sulfonic textile mill effluent, J. Water Process Eng., 16C (2017) 199–205.
[48] M.A. Kumar, R. Priyadarshini, M. Seenuvasan, V.V. Kumar, D. Nilavunesan, D. Anuradha, S. Sivanesan, Biotransformation and detoxification of a greater tinctorial textile colorant using an isolated bacterial strain, J. Env. Biol., 37 (2016) 1497–1506.