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ORIGINAL PAPER

Biodegradation of benzo(a)pyrene mediated by catabolic enzymes of bacteria

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Abstract An investigation was carried out to compare the ability of two bacteria *Pseudomonas aeruginosa* PSA5 and *Rhodococcus* sp. NJ2 isolated from petroleum sludge for degradation of benzo(a)pyrene [B(a)P], a HMW PAH compound in MSM. During 25 days of incubation, 50 ppm B(a)P was degraded by 88 and 47 % by *P. aeruginosa* PSA5 and *Rhodococcus* sp. NJ2, respectively. Besides, involvement of different catabolic enzymes, that is, salicylate hydroxylase, 2-carboxybenzaldehyde dehydrogenase, catechol 1,2-dioxygenase and catechol 2,3-dioxygenase, was also examined to identify their differential role in B(a)P degradation. Among these enzymes, the highest induction of 2-carboxybenzaldehyde dehydrogenase (773.5 nmol mg^{-1} protein) was recorded in *P. aeruginosa* PSA5, while salicylate hydroxylase was highly expressed (839.6 nmol mg^{-1} protein) in *Rhodococcus* sp. NJ2. Both the bacteria were found biosurfactant (glycolipid) producing, and role of biosurfactant in PAH degradation was also ascertained by reduced surface tension, higher emulsification index and increased cell surface hydrophobicity.

Keywords Biosurfactant · Cell surface hydrophobicity · Degradative enzymes · Surface tension

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Introduction

Polycyclic aromatic hydrocarbons (PAHs) are released from various anthropogenic sources like crude oil spillage, waste solid incineration, incomplete combustion of fossil fuels, etc. (Juhasz and Naidu 2000). Hence, they are ubiquitous in the soil environment and tend to accumulate in the top soil mainly around industrial and urban activities (Johnsen and Karlson 2007). Persistence of high molecular weight (HMW) PAHs in the environment mainly due to dense clouds of π-electrons on both sides of the ring structures is a serious environmental concern which needs to be addressed for ensuring human health (Long et al. 2008).

Among PAHs, benzo[a]pyrene is known for carcinogenic, genotoxic and cytotoxic properties (Hsu et al. 2005). Although several reports are available for microbial biodegradation of HMW PAH compounds (Sayara et al. 2010), their degradation still continues to be unreliable due to non-availability of potential soil microbes with appropriate catabolic pathways and their low bioavailability (Cerniglia 1992). However, benzo(a)pyrene [B(a)P] degradation has been reported by *Sphingomonas yanoikuyae* JAR02 and white rot fungus *Armillaria* sp. F022 (Rentz et al. 2008; Hadibarata and Kristani 2012).

At times, degradation of HMW PAHs by microbe is inhibited by the presence of more water-soluble PAHs via competition at active sites of oxygenases or through the accumulation of toxic products following co-metabolism of other PAHs (Bouchez et al. 1995). Initial dioxygenase is a key enzyme for attacking the aromatic ring structure of PAHs under aerobic condition (Juhasz and Naidu 2000). Dioxygenase genes, such as *NahAc*, *phnAc*, *NidA* and *pdoB,* were used to detect PAH-degrading Gram-negative and Gram-positive bacteria (Widada et al. 2002; Sho et al. 2004).

Bacteria secrete many other degradative enzymes, growth factors and biosurfactants for fast utilization of PAHs (Mukherjee and Das 2005). Biosurfactants produced by microorganisms are amphiphilic in nature which reduce surface tension, critical micelle concentration (CMC) and interfacial tension (IFT) in both aqueous solutions and hydrocarbon mixtures and thus facilitate PAH degradation (Maier 2003; Desai and Banat 1997).

In the present investigation, a comparison of intrinsic ability of two bacterial strains PSA5 and NJ2 isolated from petroleum sludge was made for degradation of B(a)P in MSM. Besides, differential expression of catabolic enzymes such as salicylate hydroxylase, 2-carboxybenzaldehyde dehydrogenase, catechol 1,2-dioxygenase and catechol 2,3-dioxygenase was also studied to investigate their role in B(a)P degradation. A link between nature and extent of biosurfactant production was explored with cell surface hydrophobicity, surface tension and emulsification index which affect bioavailability of PAHs. This study was carried out at NBRI during June–July, 2012.

Materials and methods

Chemicals and media

Benzo(a)pyrene (with >97.0 % purity) and HPLC grade Acetonitrile were procured from (Sigma Aldrich, USA), while mineral salt medium (MSM) was obtained from Hi media.

Bacterial isolation and screening

Bacteria were isolated from oily sludge collected from Barauni oil refinery, Barauni (Bihar, India), through enrichment in 20 ml MSM supplemented with 1 g of oily sludge in 100 ml Erlenmeyer flask incubated in an orbital shaker for 7 days set at 37 °C and 150 rpm. Subsequently, isolated bacterial strains were screened on the basis of degradation of B(a)P (25–200 ppm) in MSM as a sole carbon source after incubation for 7 days in an orbital shaker set at 37 °C and 150 rpm. Out of 12 bacterial strains, two were selected as potential degraders of B(a)P and designated as PSA5 and NJ2.

Experimental setup

Preparation of bacterial inoculum

Bacteria were grown in 30 ml NB (nutrient broth) with 50 ppm B(a)P to attain the exponential growth phase. Cell density of the suspension at this stage was found to be OD = 1 at 600 nm as measured by UV–Vis spectrophotometer (Perkin Elmer Lambda 35). After this, the cells were harvested by centrifugation at 10,000 rpm for 10 min, washed with sterile MSM and concentrated in 10 ml of MSM to be used as inoculum.

Biodegradation of benzo(a)pyrene [B(a)P]

In order to measure the potential of bacterial isolates (PSA5 and NJ2) for B(a)P degradation in MSM, 200 µl inoculum of each isolate was added separately to 10 ml MSM (pH 7.2) with 50 ppm B(a)P as a substrate in 100 ml flasks. For reference, a control was also set up in the same way without bacterial inoculum. These flasks were incubated at 37 °C in an orbital shaker at 150 rpm for 25 days. After every 5 days, residual B(a)P in MSM was extracted thrice with 10 ml DCM to ensure complete extraction. The extracts were concentrated to 2 ml by evaporation and quantified by injecting 20 µl of the samples to HPLC (Dionex ultimate 3000) with a reverse-phase ODS-C18 column (5 µm, 250 × 4.6 mm) and PDA detector (254 nm) using a gradient mixture of H_2O and acetonitrile (50:50–85:50) in mobile phase at a flow rate of 1.5 ml min^{-1}. The biodegradation efficiency (BE) of the bacterial strains was calculated by using the following formula:

$$\text{BE}\,\% = (C_0 - C_e)/C_0 \times 100$$

where C_0 is initial concentration of PAH (µg g^{-1}) and C_e is equilibrium concentration of PAH (µg g^{-1}).

Bacterial growth and protein analysis

The growth of bacterial strains (PSA5 and NJ2) was monitored at 600 nm by UV–Vis spectrophotometer during the degradation of B(a)P in sterile MSM after every 5 days interval of incubation. For the estimation of protein, cells of the bacterial isolates (PSA5 and NJ2) grown in MSM with 50 ppm B(a)P were harvested, suspended and washed in potassium phosphate buffer, pH 7. Subsequently, cells were sonicated and centrifuged at 20,000 rpm at 4 °C for 25 min to remove the cell debris. The supernatant was then stored at 2 °C, and the protein estimation of the cell extract was carried out following the Lowry et al. (1951) method using bovine serum albumin (BSA) as a standard at 660 nm using UV–Vis spectrophotometer.

Enzyme extraction

For the extraction of enzymes after every 5 day intervals, the cells were harvested and washed twice with 2 ml of

50 mM sodium phosphate buffer (pH-7) and resuspended in the same buffer (pH-7) and then homogenized by sonicator for 5 min. Sonicated sample was centrifuged for 25 min at 20,000 rpm at 4 °C. After removal of the cell debris by centrifugation, the supernatant was stored at 2 °C and was further used for different enzyme assays.

Enzyme assays

Activities of ortho-cleaving catechol 1,2-dioxygenase (decycling) (EC 1.13.11.1) and meta-cleaving enzyme catechol 2,3-dioxygenase (decycling) (EC 1.13.11.2) were measured by following the methods of Ngai et al. (1990), Sala-Trepat and Evans (1971), respectively. Further, the activity of 2-carboxybenzaldehyde dehydrogenase was determined by the method of Kiyohara and Nagao (1978) while salicylate hydroxylase [EC 1.14.13.1] activity was measured as described by Yamamoto et al. (1965).

Screening of bacteria for biosurfactant

Bacterial isolates were tested for glycolipid production using the method of Siegmund and Wagner (1991). Subsequently, biosurfactant was extracted in chloroform and methanol (2:1) following the method of Rahman et al. (2003) and then quantified.

Presence of moiety in biosurfactant

Sugar moiety of biosurfactant was tested by molish reagent using the method described by Nelson and Cox (2005) while protein moiety in biosurfactant was tested by ninhydrin, following the method Noudeh et al. (2005). Similarly, for the presence of lipid moiety in biosurfactant, the culture supernatant and water was mixed in 1:1 ratio in a test tube and shaken gently. Then, 3 drops of 2 % Sudan III stain solution (in ethanol) were added, and formation of red layer on the upper surface of the supernatant indicated presence of lipid moiety.

Quantitative estimation of carbohydrate (anthrone method)

Quantitative analysis of carbohydrate is based on the reaction of glucose with anthrone (9,10-dihydro-9-oxoanthracene) in the presence of a strong acid. The carbohydrate content of the isolated biosurfactant was determined by the anthrone reagent by UV–Vis spectrophotometer at 620 nm as described by Spiro (1966).

Surface tension and surface activity

Surface tension of the MSM was measured by stalagmometer before and after biodegradation of B(a)P. The following formula was used for the calculation of surface tension and surface activity.

$$\sigma = \sigma_{H_2O} \cdot m/m_{H_2O}$$

where σ = surface tension of MSM with bacterial culture, σ_{H_2O} = surface tension of water, m = the mass of MSM with bacterial culture, m_{H_2O} = mass of the water and surface activity = surface tension of uninoculated medium − surface tension of inoculated medium.

Cell surface hydrophobicity (CSH)

The BATH test was used to determine changes in CSH during bacterial growth in liquid MSM media with benzene as a carbon source. This test was carried out using the method of Rosenberg et al. (1980).

Emulsification index

Emulsification index of culture samples with kerosene, hexadecane, xylene, olive oil, petrol, diesel and benzene was measured using the method as described by Cooper and Goldenberg (1987).

Estimation of emulsification activity

Emulsification activity of biosurfactant with different hydrocarbons like phenanthrene, anthracene, fluoranthene, pyrene, fluorene, B(a)P and chrysene was measured as described by Rosenberg et al. (1979).

Detection of meta-dioxygenase assay

In order to know whether meta-cleavage pathway was involved in B(a)P degradation, a method of Kim and Zylstra (1995) was followed.

Identification of bacterial strains

These strains were identified as *Pseudomonas aeruginosa* PSA5 and *Rhodococcus* sp. NJ2 on the basis of their homology (>99 %) of DNA sequence with NCBI databases of bacteria following 16s ribosomal DNA technology (Chromous Biotech, Bangalore, India) which have been reflected as supplementary data.

Results and discussion

Biodegradation of B(a)P

Degradation study reveals that *P. aeruginosa* PSA5 and *Rhodococcus* sp. NJ2 degraded B(a)P by 19 and 16 %, respectively, in 5 days of incubation despite its recalcitrant nature. Beyond this period also, degradation continued to increase with the incubation period. After 25 days of incubation, *P. aeruginosa* PSA5 degraded B(a)P by 88 %, while *Rhodococcus* sp. NJ2 could degrade only 47 % (Fig. 1a). This clearly indicated an intrinsic difference in the ability of two bacteria in B(a)P degradation. However, in control, 13 % degradation of B(a)P was observed due to abiotic factor. Thus, *P. aeruginosa* PSA5 showed more BE than *Rhodococcus* sp. NJ2.

Luo et al. (2009) have observed 20.98 % of B(a)P degradation by *Ochrobactrum* sp. BL01 strain and 44.07 % by *Pseudomonas fluorescens* BL03 strain after 14 days of incubation. When a comparison was made about the degradation ability of our strains with *Mycobacterium vanbaalenii* PYR-1, it was found that B(a)P (50 μg ml^{-1}) was degraded by 88 and 47 % by *P. aeruginosa* PSA5 and *Rhodococcus* sp. NJ2, respectively, during 25 days of incubation against 24.7 % degradation by *M. vanbaalenii* PYR-1 in 14 days of incubation period. This clearly indicated that PSA5 and NJ2 strains have higher degradation ability than *M. vanbaalenii* PYR-1 as reported by Heitkamp and Cerniglia (1988).

Moody et al. (2004) have elucidated the mechanism of B(a)P degradation by *M. vanbaalenii* PYR-1 which initiates its attack on B(a)P by dioxygenation or monooxygenation at C-4, 5, C-9, 10 and C-11, 12. Different metabolites, formed during B(a)P degradation by *M. vanbaalenii* PYR-1, have been identified as benzo(a)pyrene-11,12-epoxide, benzo(a)pyrene-*cis*-4,5-dihydrodiol, ben-

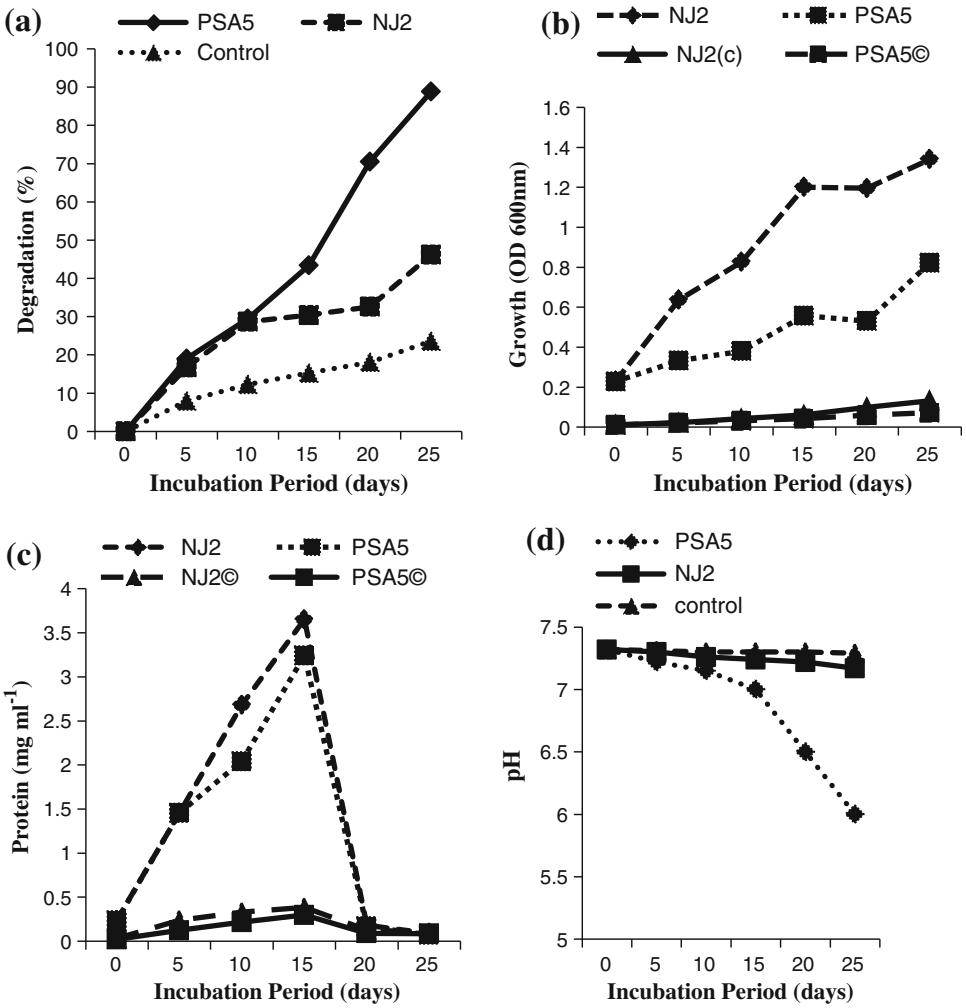

Fig. 1 **a** Biodegradation of 50 ppm B(a)P, **b** growth of different bacterial strains (PSA5 and NJ2), **c** protein content of different bacterial strains (PSA5 and NJ2), **d** pH of media during 50 ppm B(a)P degradation

zo(a)pyrene-*cis*-11,12-dihydrodiol, benzo(a)pyrene-*trans*-11,12-dihydrodiol, 11,12-dihydroxybenzo(a)pyrene, hydroxymethoxybenzo(a)pyrene, dimethoxybenzo(a)pyrene, 4,5-dihydroxybenzo(a)pyrene, 4-formylchrysene-5-carboxylic acid, 4,5-chrysene-dicarboxylic acid, chrysene-4 or 5 carboxylic acid, *cis*-4-(8-hydroxypyren-7yl)-2-oxobut-3-enoic acid and 10-oxabenzo(def)chrysene-9-one.

Growth kinetics

Cells of *P. aeruginosa* PSA5 and *Rhodococcus* sp. NJ2 continued to multiply in MSM with 50 ppm B(a)P during incubation period. However, the growth of *Rhodococcus* sp. NJ2 was recorded faster than *P. aeruginosa* PSA5 (Fig. 1b). However, in control, the bacterial growth was not significant due to nonavailability of B(a)P as a carbon source.

Cell protein in both the bacteria continued to increase with cell multiplication, attaining maximum value of protein 3.62 and 3.2 mg ml^{-1} in *Rhodococcus* sp. NJ2 and *P. aeruginosa* PSA5, respectively, after 15 days of incubation (Fig. 1c).

Degradative enzymes

In B(a)P degradation, many enzymes may be involved, but in this study activities of only four enzymes salicylate hydroxylase, 2-carboxybenzaldehyde dehydrogenase, catechol 1,2-dioxygenase and catechol 2,3-dioxygenase actually involved in degradation process were investigated.

Salicylate hydroxylase

It was observed that salicylate hydroxylase was induced during B(a)P degradation in both the bacteria, but at different incubation periods. *Rhodococcus sp.* NJ2 exhibited maximum induction of salicylate hydroxylase (839 nmol mg^{-1} protein) after 10 days of incubation, while *P. aeruginosa* PSA5 showed a peak induction of this enzyme (662.28 nmol mg^{-1} protein) after 15 days of incubation period (Fig. 2a). This indicates that salicylate hydroxylase was induced more in *Rhodococcus* sp. NJ2, than in *P. aeruginosa* PSA5 during B(a)P degradation.

Salicylate hydroxylase is mainly involved in the conversion of salicylate to catechol in B(a)P degradation and forms a reduced enzyme substrate complex in the presence of NADH. Subsequently, molecular oxygen binds to the complex for production of catechol, CO_2 and H_2O.

There are two pathways for the microbial degradation of naphthalene, in which salicylate is formed as an intermediate. In the classical pathway, as studied extensively in *Pseudomonas* sp., salicylate undergoes oxidative decarboxylation by salicylate 1-hydroxylase to produce catechol (Yen and Serdar 1998). However, an alternative pathway was reported in *Rhodococcus* sp., which involves a salicylate 5-hydroxylase yielding gentisate for naphthalene degradation (Allen et al. 1997).

2-Carboxybenzaldehyde dehydrogenase (2-CBD)

This enzyme was more expressed in *P. aeruginosa* PSA5 than in *Rhodococcus* sp. NJ2. The activity of 2-CBD continued to increase with incubation period attaining a peak (973.5 nmol mg^{-1} protein) after 15 days of incubation in *P. aeruginosa* PSA5, while in *Rhodococcus* sp. NJ2, highest 2-CBD activity was recorded 775.9 nmol mg^{-1} protein after 20 days (Fig. 2b).

Induction of the 2-CBD activity indicates the formation of phenanthrene in degradation pathway of B(a)P. Krishnan et al. (2004) reported two major pathways for phenanthrene degradation in *Pseudomonas* PP2 strain. Both pathways generate 1-hydroxy 2-naphthoic acid (1-H-2-NA) via 3,4-dihydroxyphenanthrene which is further oxidized by two different routes. Route 1 leads to complete mineralization of 1-H-2-NA to TCA cycle intermediates via 1,2-dihydroxynaphthalene, salicylate and catechol, while route 2 partially degrades 1-H-2-NA to 2-carboxybenzapyruvate and 2-carboxybenzaldehyde and finally to o-phthalic acid.

Catechol 1,2-dioxygenase (C12O)

As compared to salicylate hydroxylase and 2-CBD, C12O was less induced, and its peak induction was recorded after 15 days of incubation period in both the bacteria. The highest induction of this enzyme was found to be 280.9 nmol mg^{-1} protein in *P. aeruginosa* PSA5 and 174.1 nmol mg^{-1} protein in *Rhodococcus* sp. NJ2 (Fig. 2c).

Ring-cleaving dioxygenases play an important role in the degradation of aromatic compounds by incorporating O_2 into the aromatic nucleus which results oxidation of aromatic ring (Goyal and Zylstra 1996). A terminal dioxygenase has been found to be an important factor in PAH degradation in *M. vanbaalenii's* pathway to transform B(a)P into benzo[a]pyrene-*cis*-4R,5S-dihydrodiol and benzo[a]pyrene cis-4S,5R-dihydrodiol.

Schneider et al. (1996) found *Mycobacterium* sp. Strain RJGII-135 highly capable of transforming benzo[*a*]pyrene

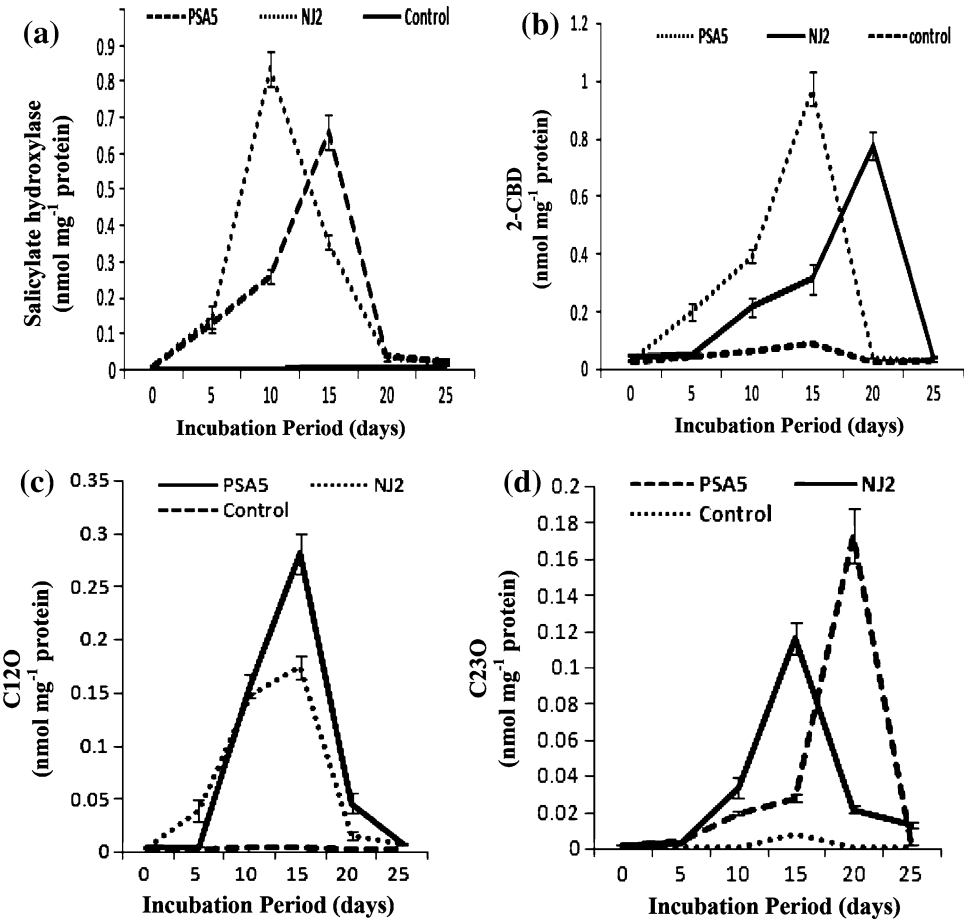

Fig. 2 Enzyme activities of salicylate hydroxylase (**a**), 2-carboxybenzaldehyde dehydrogenase (2-CBD) (**b**), catechol 1,2-dioxygenase (C12O) (**c**) and catechol 2,3-dioxygenase (C23O) (**d**) during B(a)P degradation in MSM (mean of 3 replicates)

to initial ring oxidation and ring cleavage products by initial enzymatic attack at C-4, 5, C-7, 8 and/or C-9, 10 of benzo[*a*]pyrene. However, only benzo[*a*]pyrene-*cis*-7,8-dihydrodiol was identified. It was suggested that the formation of 4,5-chrysene-dicarboxylic acid is the *ortho*-cleavage ring fission product of benzo[*a*]pyrene-*cis*-4,5-dihydrodiol.

Catechol 2,3-dioxygenase (C23O)

During B(a)P degradation, an induction of C23O was not as high as C12O in both the bacteria. In *P. aeruginosa* PSA5, a maximum induction of this enzyme was found to be 172.9 nmol mg^{-1} protein after 20 days of incubation period, while in *Rhodococcus* sp. NJ2, its highest activity (116.3 nmol mg^{-1} protein) was observed after 15 days of incubation (Fig. 2d).

Schneider et al. (1996) observed the transformation of B(a)P through meta-cleavage by *Mycobacterium* sp. Strain RJGII-135. During *meta*-cleavage of B(a)P, they suggested formation of either *cis*-4-(8-hydroxypyren-7-yl)-2-oxobut-3-enoic acid or *cis*-4-(7-hydroxypyren-8-yl)-2-oxobut-3-enoic acid and either 7,8-dihydropyrene-7-carboxylic acid or 7,8-dihydropyrene-8-carboxylic acid as intermediates. The meta-fission products were formed after initial dioxygenation and subsequent dehydrogenation at C-7, 8 and/or C-9, 10.

It was observed from the activity of degradative enzymes that out of four, three enzymes, 2-CBD, C12O and C23O, were highly expressed in *P. aeruginosa* PSA5 and salicylate hydroxylase in *Rhodococcus* sp. NJ2, in B(a)P degradation after 25 days of incubation. The genes for degradation of HMW PAH such as pyrene and chrysene were found to be inducible and chromosomally located (Kim et al. 2006, 2007), while genes responsible for degradation of low molecular weight PAH such as naphthalene, phenanthrene and anthracene occur on plasmids (Mallick et al. 2007). This observation was further con-

firmed by Lily et al. (2010) in relation to B(a)P degradation by *Bacillus subtilis* BMT4i (MTCC 9447).

pH of the media

No significant change in pH of the media was recorded during B(a)P degradation by *Rhodococcus* sp. NJ2. However, in *P. aeruginosa* PSA5, the medium pH was changed to acidic side (6.0) (Fig. 1d).

Role of biosurfactant

Both bacteria were found glycolipid producing. Although *P. aeruginosa* PSA5 produced rhamnolipid, but *Rhodococcus* sp. NJ2 did not produce rhamnolipid as evidenced by the formation of a blue halo around the colony of *P. aeruginosa* which indicated the presence of an anionic surfactant, that is, rhamnolipid. When moieties of biosurfactant produced by bacteria were examined, both indicated sugar and lipid moieties confirming glycolipid production. The carbohydrate content was found to be 650.63 mg ml^{-1} in *P. aeruginosa* PSA5 and 215.06 mg ml^{-1} in *Rhodococcus* sp. NJ2. It is possible that higher degradability of B(a)P by *P. aeruginosa* PSA5 is linked to high production of rhamnolipid evidenced by enhanced production of carbohydrates.

Several species of *P. aeruginosa* have been reported to produce rhamnolipid, which increases the solubility and bioavailability of petroleum hydrocarbons for enhanced biodegradation of diesel contamination in soil (Kojima et al. 1961). Different trehalose containing glycolipids are produced by several other microorganisms belonging to mycolates group, such as *Arthrobacter*, *Nocardia*, *Rhodococcus* and *Gordonia*.

Surface tension and surface activity

When surface tension and surface activity of the MSM inoculated with two bacteria were measured, it was observed that surface tension decreased with the incubation period, while the surface activity increased. As evident from Fig. 3a, b, the surface tension was decreased from

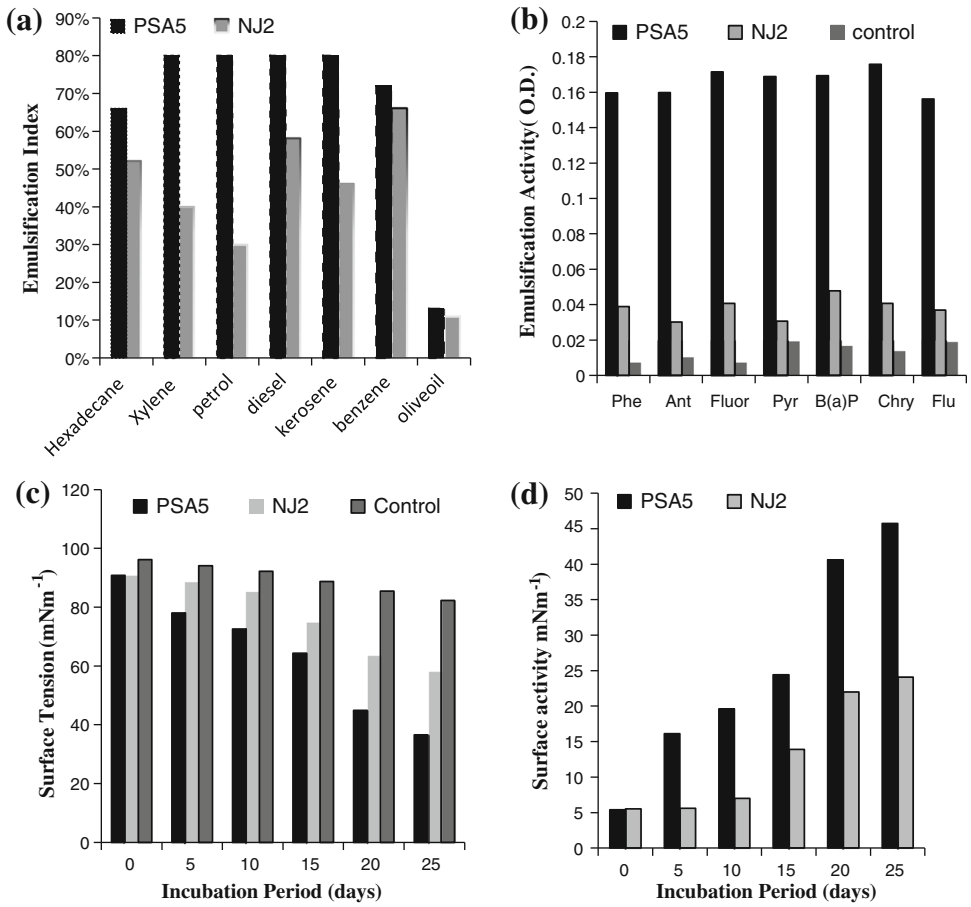

Fig. 3 Changes in emulsification index (**a**), emulsification activity (**b**) surface tension (**c**) and surface activity (**d**) caused by biosurfactant produced by bacteria

90.8 to 36 mN m^{-1} in *P. aeruginosa* PSA5, while in *Rhodococcus* sp. NJ2, it declined from 90.7 to 58.2 mN m^{-1} as compared to control (82.3 mN m^{-1}). Similarly, Noudeh et al. (2010) also observed a sharp decline in surface tension from 60 to 35 mN m^{-1} in 24 h fermentation by *P. aeruginosa* PTCC 1561. However, surface activity increased with incubation period in both the bacteria, showing an inverse relationship with surface tension.

Low molecular weight biosurfactants (e.g., glycolipids and lipopeptides) having high surface activity are able to reduce the surface tension of water to 25–30 mN m^{-1}, which facilitates the uptake of hydrocarbons into bacterial cells for enzymatic degradation (Franzetti et al. 2010).

Cell surface hydrophobicity (CSH)

Cell surface hydrophobicity is an important factor in determining hydrocarbon adhesion to cell surface. When cell surface hydrophobicity of bacterial cultures was measured, *P. aeruginosa* PSA5 showed a maximum hydrophobicity of 51 %, while *Rhodococcus* sp. NJ2 exhibited hydrophobicity of 47 % for benzene.

The ability of different microorganisms to access hydrocarbons depends on their cell surface hydrophobicity. Microorganisms with high CSH directly contact oil drops and solid hydrocarbons, while low CSH permits the adhesion of microbial cells to the micelles or emulsified oils, formed due to the presence of extracellular biosurfactants or bioemulsifiers (Van Hamme et al. 2003).

Chang et al. (2009) demonstrated that the cell surface hydrophobicity was enhanced by the accumulation of different fatty acids on the cell surface during growth of *Rhodococcus erythropolis* NTU-1 on hydrocarbon. Zhong et al. (2007) showed that the adsorption of dirhamnolipid biosurfactants on cells of *Bacillus subtilis*, *P. aeruginosa* and *Candida lipolytica* affected the cell surface hydrophobicity depending on the rhamnolipid concentration and the physiological state of the cell.

Emulsification activity and emulsification index

When emulsification activity of 7 different solid hydrocarbons phenanthrene, fluoranthene, fluorene, anthracene, pyrene, chrysene and B(a)P and emulsification index of hexadecane, xylene, olive oil, kerosene, petrol and diesel were measured, it was observed that *P. aeruginosa* PSA5 showed higher emulsification activity and emulsification index than *Rhodococcus* sp. NJ2 (Fig. 3c, d).

Emulsification of the hydrocarbons by biosurfactant helps in microbial degradation. Van Hamme and Ward (2001) observed that hydrocarbon-grown *Rhodococcus* sp. Strain F9-D79 cells had high surface-active and emulsification properties. Anyanwu and Chukwudi (2010) reported that biosurfactants produced by *P. aeruginosa* LS1 were effective in producing good emulsification with kerosene. Obayori et al. (2009) have also observed high emulsification activity of engine oil, crude oil and diesel by *Pseudomonas* strain sp. LP1.

Thus, both the bacteria were found to be potential degraders of B(a)P as mediated by catabolic enzymes and facilitated by the biosurfactant production. Hence, these bacteria can be recommended for use in in situ bioremediation of soil contaminated with PAHs.

Conclusion

It may be concluded from this investigation that both the bacteria *P. aeruginosa* PSA5 and *Rhodococcus* sp. NJ2 showed differentiated ability to degrade B(a)P in MSM, mediated by catabolic enzymes, that is, salicylate hydroxylase, 2-CBD, C12O and C23O. These enzymes were differentially expressed in both the bacteria, showing their involvement in B(a)P degradation at different stages. A clear link between B(a)P degradation and glycolipid production was also found.

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