Bioresource Technology 133 (2013) 293–300

Contents lists available at SciVerse ScienceDirect

Bioresource Technology

journal homepage: www.elsevier.com/locate/biortech

Bacterial degradation of pyrene in minimal salt medium mediated by catechol dioxygenases: Enzyme purification and molecular size determination

S.N. Singh [a,*], Babita Kumari [a], Santosh Kumar Upadhyay [b], Shweta Mishra [a], Dileep Kumar [c]

[a] Environmental Science Division, CSIR-National Botanical Research Institute, Lucknow 226001, UP, India
[b] Plant Molecular Biology and Genetic Engineering Division, CSIR-National Botanical Research Institute, Lucknow 226001, UP, India
[c] Microbial Biotechnology, CSIR-North-East Institute of Science and Technology, Jorhat, India

HIGHLIGHTS

- ▶ Microbial degradation of pyrene in MSM.
- ▶ Involvement of catechol 1,2 dioxygenase and catechol 2,3 dioxygenase in pyrene degradation.
- ▶ Purification and molecular size determination of catechol 1,2 dioxygensae.

ARTICLE INFO

Article history:
Received 12 November 2012
Received in revised form 11 January 2013
Accepted 12 January 2013
Available online 4 February 2013

Keywords:
Pyrene degradation
Bacterial strains
Catechol dioxygenases
Enzyme purification
Molecular size determination

ABSTRACT

In vitro degradation of pyrene was studied in MSM by three bacterial strains individually, designated as BP10, NJ2 and P2. Among these strains, NJ2 was the highest degrader (60%) of pyrene, followed by BP10 (44%) and the least was P2 (42%) in MSM with pyrene (50 $\mu g\ ml^{-1}$) in 8 days. During pyrene degradation, catechol 1,2 dioxygenase (C12O) activity was induced by 13 folds in BP10 and 17 folds in P2 as compared to catechol 2,3 dioxygenase (C23O). However, in NJ2, C23O activity was augmented 1.3 times more than C12O. This clearly indicated that C12O played a major role in pyrene degradation by BP10and P2, while in NJ2, C23O contributed more to degradation process than C12O. Molecular weight of highly inducible C12O was determined as ~64 kDa by size exclusion chromatography and as ~32 kDa on denaturing SDS PAGE in BP10 which indicated dimeric nature of the enzyme.

© 2013 Elsevier Ltd. All rights reserved.

1. Introduction

Polycyclic aromatic hydrocarbons (PAHs) are ubiquitous contaminants in the environment, mainly originating from anthropogenic activities like burning of fossil fuels, coal liquefaction and gasification process, oil seepage and accidental spillage of hydrocarbons and petroleum industries (Guerin and Jones, 1988; Juhasz and Naidu, 2000). PAHs are a group of compounds mainly composed of two or more fused aromatic rings in linear, angular and clustered arrangements. They are classified as low molecular weight (LMW) as well as high molecular weight (HMW) PAHs depending upon the number of aromatic rings. LMW PAHs are characterized by higher volatility, solubility and greater ease of degradation than HMW PAHs which are thermodynamically stable and hydrophobic in nature. Hence, HMW PAHs strongly bind to the soil particles and therefore, become recalcitrant to microbial degradation. Besides, due to their high toxicity and/or mutagenic, tetratogenic and carcinogenic properties (Abd-Elsalam et al., 2009; Abou-Arab et al., 2010), PAHs pose a serious health risk to human beings on persistent exposure.

Possible fates of PAHs in the environment are volatilization, photooxidation, chemical oxidation, bioaccumulation and adsorption to soil particles. But, microbial transformation and degradation are projected as an environmentally benign and public acceptable potential strategy to address PAH contamination in the soils (Gibson et al., 1975). However, a major constraint for biodegradation of PAHs is their low availability to bacteria due to their high hydrophobicity and strong adsorption to soil (Thomas et al., 1986; Volkering et al., 1998). A lot of reports are available on bacterial degradation of PAHs (Cerniglia, 1992; Wilson and Jones, 1993), but in most of the cases, the organisms have been isolated from the oil sludge contaminated soil or crude oil spills with the likelihood that they could have developed the catabolic capability

* Corresponding author. Tel.: +91 522 2297823; fax: +91 522 2205839.
E-mail address: drsn06@gmail.com (S.N. Singh).

0960-8524/$ - see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.biortech.2013.01.068

to degrade PAHs as a result of selective pressure of the contaminated site on the organisms as compared to uncontaminated sites. However, there are reports also available for occurrence of PAH degrading bacteria from uncontaminated sites (Juhasz and Naidu, 2000; Kanaly et al., 2000). Several species of bacterial genera *Pseudomonas*, *Alcaligenes*, *Mycobacterium*, *Rhodococcus*, *Sphingomonas* have been found highly capable of degrading PAHs (Cerniglia, 1992; Mueller et al., 1997). Lease et al. (2011) compared the PAH mineralization ability of *Mycobacterium* isolates from both contaminated and uncontaminated sites.

Among the HMW PAHs, pyrene is an important compound which is recalcitrant for microbial degradation due to their high hydrophobicity which restricts its cellular uptake by microbes (Seo et al., 2009). The first report of the pyrene degradation as a sole source of carbon and energy was demonstrated by *Rhodococcus* sp. strain UWI (Walter et al., 1991). Liang et al. (2006) studied the catabolic enzymes involved and metabolites formed during pyrene degradation by *Mycobacterium* sp. strain KMS. Interestingly, Khan et al. (2009) reported more pyrene degradation by the rhizospheric bacteria than non-rhizospheric bacteria and identified the catabolic genes *nahAc* and *pdo 1* responsible for PAH degradation. Krivobok et al. (2003) studied involvement of two ring hydroxylating dioxygenases and also identified pyrene induced proteins in *Mycobacterium* sp. Besides, an enteric bacterium *Leclercia adecarboxylate* PS4040 was reported by Sarma et al. (2010) for pyrene degradation. Recently, Ceyhan (2012) identified a novel strain *Proteus vulgaris* 4Bi which degraded pyrene more effectively with the formation of non-toxic and non-persistant metabolites in nature.

In this study, pyrene was chosen as a model compound of HMW PAHs for degradation in isolation by three bacterial strains (BP10, P2 and NJ2) isolated from the oil-contaminated soil to examine their differential degradation ability and to investigate the involvement of catechol 1,2 dioxygenase and catechol 2,3 dioxygenase in pyrene degradation. Molecular size of highly expressed catechol 1,2 dioxygenase in BP10 was also determined after enzyme purification and the co-factor with oxidation state was identified.

2. Methods

2.1. Materials

Catechol, folin and pyrene were purchased from Sigma–Aldrich while Na_2HPO_4, NaH_2PO_4, EDTA, NaOH, ammonium potassium tartrate, $CuSO_4{\cdot}5H_2O$, hexane, benzene etc. were procured from Fisher Scientific and culture media like MSM without dextrose, nutrient agar and nutrient broth were all procured from Himedia.

2.2. Isolation and screening of bacterial strains

2 g of crude oil, collected from Barauni Oil Refinery, Barauni (Bihar), India was added to 100 ml sterilized minimal salt medium (MSM) (composition: 7 g dipotassium phosphate, 2 g monopotassium phosphate, 0.5 g sodium citrate, 1 g ammonium sulfate, 0.1 g magnesium sulfate in 1 l medium pH 7.0 ± 0.2) and then incubated in an orbital shaker set at 37 °C and 150 rpm for the enrichment of petroleum hydrocarbon degrading bacterial strains. After 1 week of incubation, 1 ml of active inoculum was transferred to the flasks having fresh sterile MSM supplemented with petroleum oil (2% w/v). After 3 transfers in MSM (100 ml) with 2% (w/v) of petroleum oil, the active inoculum was used for the isolation of TPH (total petroleum hydrocarbon) degrading bacteria by serial dilution method and spreading over the nutrient agar (NA, composition: 10 g peptic digest of animal tissue, 5 g beef extract, 5 g sodium chloride and 15 g agar in 1 l medium pH 7.4 + 0.2) plates. Separate colonies were picked up for the purification of bacteria by streaking method on MSM agar plates supplemented with 50 mg l^{-1} pyrene.

Bacterial strains isolated from petroleum oil and NJ2 (supplied by NIEST, Jorhat, India) were first screened on the basis of the utilization of pyrene (50 mg l^{-1}) in MSM agar. Three strains out of 10 bacterial isolates designated as BP10, P2 and NJ2 showed faster growth on MSM agar plates compared to other strains. Therefore, these strains were selected for further study of pyrene degradation.

2.3. Pyrene degradation

Three selected bacterial strains (BP10, P2 and NJ2) were enriched separately in 100 ml of nutrient broth (NB) (composition: 5 g of peptic digest of animal tissue, 5 g of sodium chloride, 1.5 g of beef extract and 1.5 g of yeast extract in 1 l of media) in 250 ml flasks in an orbit shaker set at 150 rpm and 35 °C till OD_{600} was equivalent to 1. Bacterial cells were harvested by centrifugation at 5000*g* at 4 °C for 5 min. Harvested cells were resuspended in MSM. Degradation of pyrene by individual strains was monitored in 100 ml flask containing 20 ml MSM with 50 mg l^{-1} of pyrene as a source of carbon and energy. For this, sterilized MSM with 50 mg l^{-1} of pyrene was inoculated separately with 1 ml of active inoculum of each bacterial strain. Uninoculated sterilized medium was treated as control. Each treatment was prepared in 6 sets, of which 3 sets were used for the extraction of remaining amount of pyrene after degradation and another 3 sets for analysis of other parameters like pH, protein, enzyme etc. to prevent any loss of pyrene during cell harvesting. All flasks were incubated in an orbital shaker set at 150 rpm and 37 °C. Samples were harvested every day after 24 h intervals from both uninoculated and inoculated flasks for 8 days.

2.4. Extraction and analysis of pyrene

Residual amount of pyrene after degradation was extracted from MSM by liquid–liquid extraction (1:1 v/v MSM: Benzene). Samples were extracted three times and pooled together. Extraction efficiency was 98%. Extract was evaporated under a gentle nitrogen hood and residue was dissolved in 2 ml acetone. Samples were analyzed by the gas chromatograph (Agilent GC model 7890A) with FID using capillary BP-5 column (5% phenyl methyl polysiloxane column, 30 m × 0.32 mm × 0.25 μm). Both injection and detector temperatures were maintained at 280 °C. The initial oven temperature was kept 80 °C for 2 min and then increased to 300 °C with 10 °C increase per min. The injection volume (5 μl) of sample was taken for GC analysis.

2.5. Bacterial growth

Bacterial growth was determined by CFU counting. CFU ml^{-1} of bacteria was determined by counting colonies on NA plates incubated at 37 °C for 24 h through serial dilution method.

2.6. Protein extraction and analysis

Ten ml of sample was centrifuged at 5000*g* for 10 min and the cell pellet was washed with 0.01 M phosphate buffer (pH 7.0). Again the cell pellet was resuspended in the same buffer, sonicated (for 5 min at 0 °C) and then centrifuged at 20,000*g* for 25 min at 4 °C. Supernatant was used for the protein estimation and enzyme assays. Protein was determined following the standard method of Lowry et al. (1951).

Download English Version:

https://daneshyari.com/en/article/7084365

Download Persian Version:

https://daneshyari.com/article/7084365

Daneshyari.com