北京大学学报(自然科学版) 第 54 卷 第 1 期 2018 年 1 月
Acta Scientiarum Naturalium Universitatis Pekinensis, Vol. 54, No. 1 (Jan. 2018)
doi: 10.13209/j.0479-8023.2017.049

Isolation, Identification, and Biodegradation Characteristics of a Benzo[a]pyrene-degrading Bacterium *Acinetobacter* sp. Bap30

ZHU Tingting[1,†], NI Jinren[2], PENG Shenghua[1]

1. State Environmental Protection Key Laboratory of Drinking Water Source Management and Technology, Shenzhen Key Laboratory of Drinking Water Source Safety Control, Shenzhen Key Laboratory of Emerging Contaminants Detection and Control in Water Environment, Shenzhen Academy of Environmental Sciences, Shenzhen 518001; 2. Department of Environmental Engineering, Peking University, The Key Laboratory of water and Sediment Science (MOE), Beijing 100871;
† E-mail: zttszhky@sina.com

Abstract An enrichment method was used to enrich functional bacteria in continuous fluid using porous carrier, which simulated dynamic environments. This method was developed based on the theory of enrichment culture and medium retain manner technique. Four bacteria strains were isolated from sewer using benzo[a]pyrene (BaP) to provide energy and carbon, among them a single strain of bacteria isolated, identified as *Acinetobacter* sp. Bap30 according to 16S rRNA gene sequence and identification of its physiological and biochemical characteristics, grew in mineral salt medium with 40 mg/L BaP and degraded 28.7% BaP after 20 days of incubation. The BaP-degradation function of Acinetobacter genus has not been previously reported in the literature. Addition of extra carbon sources (in particular sucrose) and phenanthrene (a low molecular weight polycyclic aromatic hydrocarbon) influenced the degradation rate, which was effectted by the type and concentration. The present study is of practical importance in environmental engineering, given that BaP is a highly toxic polycyclic aromatic hydrocarbon (PAH) commonly found in petroleum-polluted soil and industrial discharges such as coking wastewater.

Key words benzo[a]pyrene (BaP); *Acinetobacter* sp. Bap30; biodegradation; carbon source; co-metabolism

苯并[a]芘降解菌 *Acinetobacter* sp. Bap30 菌株的分离、鉴定及降解特性研究

朱婷婷[1,†] 倪晋仁[2] 彭盛华[1]

1. 国家环境保护饮用水水源地管理技术重点实验室, 深圳市饮用水水源地安全保障重点实验室, 深圳市水环境中新型污染物检测与控制重点实验室, 深圳市环境科学研究院, 深圳 518001; 2. 北京大学环境工程系, 水沙科学教育部重点实验室, 北京 100871;
† E-mail: zttszhky@sina.com

摘要 提出一种新的方法, 用于富集苯并[a]芘耐受菌株。该方法使用多孔介质脱脂棉作为载体, 从连续流动的流体——下水道污水中富集菌株。利用介质截留法、富集培养法等理论, 为富集目标微生物提供了最佳条件。以苯并[a]芘为唯一碳源和能源, 从下水道沉积物中分离、筛选出 4 株苯并[a]芘耐受菌株, 其中一株能在 20 天内将 40 mg/L 的苯并[a]芘降解 28.7%。通过 16S rRNA 基因序列分析和部分生理生化特征分析, 鉴定该菌株为 *Acinetobacter* sp. Bap30。这是不动杆菌可降解苯并[a]芘的首次报道。添加其他碳源和低分子量多环芳烃——菲作为共代谢底物, 研究菌株的共代谢作用。研究结果对石油污染的土壤或者焦化废水等工业污水中的高分子量多环芳烃——苯并[a]芘具有非常重要的实践意义。

深圳市科技研发资金(JCYJ20160531114757157, JCYJ20130402094730839)资助
收稿日期: 2016–10–21; 修回日期: 2016–11–26; 网络出版日期: 2016–12–14

关键词 苯并[a]芘; *Acinetobacter* sp. Bap30; 微生物降解; 碳源; 共代谢
中图分类号 X703

Benzo[*a*]pyrene (BaP) is a PAH with a five-ring structure, is very stable, and has low solubility in water. It is sufficiently toxic that the US Environmental Protection Agency[1] categorized BaP as a priority pollutant because of its damaging effects on human health as a poison, carcinogen, and agent for genetic mutation.

Many investigations have been carried out into the bacterial degradation of BaP. According to Juhasz et al.[2], the majority of studies before 2000 concentrated on the biodegradation of BaP in the presence of a carbon source. Previous studies have reported the biodegradation of PAHs by microorganisms, but most of them showed relatively low degradation efficiency, which constrains its potential applications. Thus, isolation of efficient degrading-bacteria has attracted increasing interests, particularly for biodegradation of biorefractory pollutants. For example, Schuler et al.[3] characterized a ring-hydroxylating dioxygenase from phenanthrene-degrading *Sphingomonas* sp. strain LH-128 able to oxidize benz[a]anthracene. Moreover, addition of proper carbon sources could enhance the decomposition of BaP. The presence of low-molecular weight PAHs may promote the degradation of a complex mixture by inducing enzymatic systems that cometabolize the high-molecular-weight, recalcitrant structures. Toyama et al.[4] discussed accelerated biodegradation of pyrene and benzo[a]pyrene in the Phragmites australis rhizosphere by bacteria-root exudate interactions.

The present paper aims to identify a single strain of bacteria capable of utilizing BaP as the sole source of carbon and energy. One such bacterial strain has been isolated from sewer bacteria enriched by use of porous cotton carrier in continuous fluid, and named *Acinetobacter* sp. Bap30. This strain is found to be particularly effective at degrading BaP. The paper also investigates the co-metabolisms of the isolate in the presence of additional carbon sources (such as sucrose) and another PAH, phenanthrene (Phe).

1 Materials and Methods

1.1 Chemicals and reagents

98.7% BaP and Phe, and 99.9% standard BaP samples were purchased from Accustandard Inc., USA. The agar, beef extract, peptone, staining reagent, tryptone, and yeast extracts were provided by the Lanyi Chemical Reagent Company, China. The mineral salt medium (MSM) consisted of 1 g/L of $(NH_4)_2SO_4$, 2 g/L of K_2HPO_4, 0.5 g/L of $MgSO_4 \cdot 7H_2O$, 0.1 g/L of NaCl, 0.5 g/L of $FeCl_3$, and 0.5 g/L of $CaCl_2$. The initial pH of MSM was adjusted to 7.0±0.2 using dilute HCl and NaOH. All these chemicals and other solvents, used for high performance liquid chromatography (HPLC) analysis, were of analytical grade and obtained from the Bailingwei Chemical Reagent Beijing Co. Ltd, China.

1.2 Bacteria cultivation and isolation

5 g cotton carrier was soaked in a BaP acetone solution (20 mg/L) for 12 hours to make sure the acetone has been vaporized completely, and then placed in the sewer of families residential areas in China Agricultural University for 20 days.

The cotton carrier provided suitable place and conditions for microbial growth and metabolic activities. First, the cotton carrier played an important role in intercepting and protecting the microorganism cells, since the specific surface area of the carrier was large enough to allow more microorganisms to be adsorbed and grow efficiently. Second, the continuous flowing effluent in sewer would crash air bubbles, and reduce the transport resistance of oxygen, so the oxygen transfer rates could increase. Third, continuous fluid could bring more microorganisms resources, which provided more original material for our experiments. Fourth, the hydrodynamic shear stress made it easy to carry away the impurities on the carrier surface based on the cross-flow filtration principle, which could be helpful for more microorganisms gathering in the surface and interior of the carrier. BaP

would adsorb on the surface of the insoluble cotton carrier because of its hydrophobicity, so BaP would not be carried away by the water flow. Overall, this method could significantly increase the contact area and chance between the bacteria and BaP, and thus enrich functional bacteria efficiently.

The cotton sample of mass 5 g was transferred into a 50 mL MSM solution containing 40 mg/L BaP, and incubated for 7 days at 37°C and 160 rpm in a 250 mL conical flask before being transferred to fresh liquid medium, and then incubated under the same condition. This process was repeated about five times. Then the culture samples were collected and spread onto MSM plates containing 200 mg/L BaP. The plates were incubated for 3–7 days at 37°C. The BaP-degrading bacteria colonies were screened out and purified using the streak plate method. This procedure was repeated three times. A bacterial strain with relatively high growth rate on the MSM plate was obtained and preserved as strain Bap30 in the BaP-containing liquid MSM.

1.3 Identification and characterization of the BaP-degrading bacteria

Genomic DNA was extracted from bacteria grown overnight in nutrient broth at 37°C and 160 rpm using a polymerase-chain-reaction (PCR) mixture (50 μL) provided by the Tiangen Co. Ltd. (Beijing, China), composed of 10 × 5 μL PCR buffer, 0.25 μL Taq polymerase, 1 μL DNA template, 4 μL dNTP (10 mmol/L), 1 μL of each primer (20 μmol/L) and sterile water to volume. The 16S rRNA gene fragment was amplified by PCR using a thermal cycler (Bio-Rad, USA), under the following conditions: initial denatura tion of the template DNA for 5 min at 94°C, followed by 26 cycles for a total of 1 min at 94°C, annealing for 1 min at 56°C, and elongation for 2 min at 72°C, followed by a final extension for 10 min at 72°C, and thereafter kept at a constant temperature of 4°C. The PCR products were later sequenced by the Yingjun Co. Ltd. (Shanghai, China). The sequence of a 1390 bp fragment of the partial 16S rRNA gene of Bap30 was then compared against the GenBank sequence database using the BLAST program provided by the National Center for Biotechnology Information (NCBI), US National Library of Medicine, Bethesda MD, USA. The sequence was deposited at GenBank, NCBI (Accession No. JF682491). A phylogenetic tree was constructed by the MEGA 4.0 software[5]. Strain Bap30 was also characterized according to the *Manual of Systematic Identification of Common Bacteria*[6]. The strain was saved with the method of slant culture at 4°C as collection number CGMCC No. 4586 in the China General Microbiological Culture Center, Institute of Microbiology, Chinese Academy of Sciences, Beijing, China.

1.4 Biodegradation of BaP

The inoculum was prepared by placing each isolated strain in a 10 mL nutrient broth and incubating for 16 hours at 37°C and 160 rpm. The biodegradation experiments were conducted using at least twenty 50 mL conical flasks as batch reactors. A 0.5 mL inoculum suspension and a 10 mL MSM solution supplemented with 40 mg/L BaP were added into each flask. All the flasks were sealed and shaken at 37°C and 160 rpm for 20 days. Every 4 days, a flask was extracted and its contents was analyzed. The biodegradation experiments were carried out in triplicate, and conducted in the dark in order to prevent photodecomposition of BaP. After pretreatment of the samples[7], the BaP concentrations were determined by an Agilent 1100 Series HPLC System (Agilent Technologies, USA) including a UV-Vis detector and Zorbax SB-C18 column (150 mm × 4.6 mm, 5 μm). The mobile phase comprised 90/10 (*v*/*v*) acetonitrile/water solution at a flow rate of 1 mL/min. BaP was detected at 264 nm wavelength. Cell growth was monitored by measurement of optical density (OD_{600}) against time using a UV-1800 spectrophotometer (Bio-Rad, USA). Glucose (40 mg/L), sucrose (10–500 mg/L), maltose (40 mg/L), sodium acetate (40 mg/L), soluble starch (40 mg/L), and Phe (20–100 mg/L) were respectively added into the MSM besides BaP (40 mg/L) in order to investigate the effects of extra carbon sources and an alternative PAH on BaP biodegradation by Bap30. The Phe concentration was determined by HPLC. The mobile phase comprised 80/20 (*v*/*v*) methanol/water

solution at a flow rate of 1 mL/min. Phe was detected at 250 nm wavelength. All other processes and operations were the same as previously mentioned.

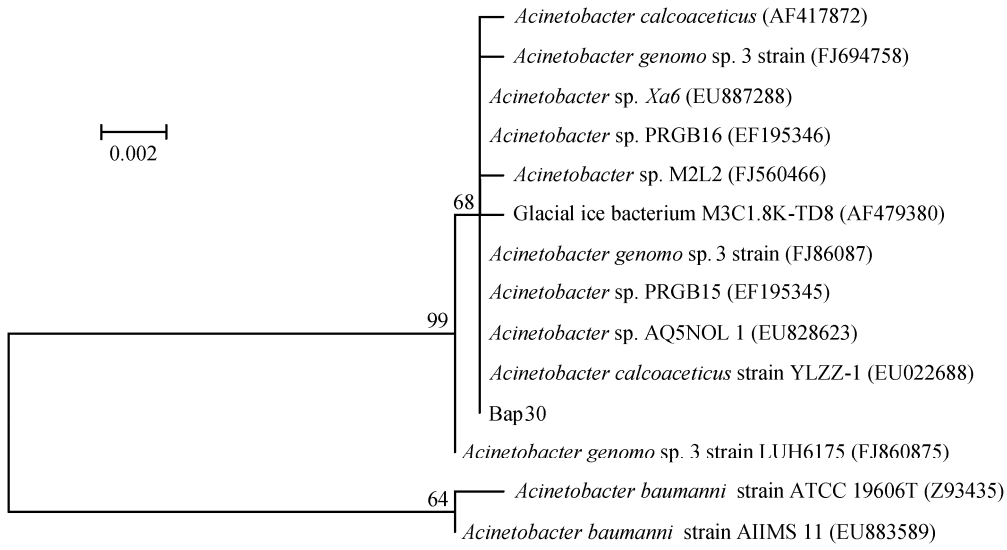
2 Results

2.1 Isolation and identification of strain Bap30

Several BaP-degrading bacterial strains were isolated from sewer sediment with BaP providing the source of carbon and energy. Strain Bap30 was found to be the most effective at degrading BaP, and so was selected. Fig. 1 shows the phylogenetic tree related to *Acinetobacter* sp. Bap30, based on comparison of gene sequences. It can be seen that the partial 16S rRNA sequence of the Bap30 is 99% identical to that of *Acinetobacter* sp. AQ5NOL 1 (Accession No. EU-828623). All the closest members belong to the genus of *Acinetobacter*. Table 1 lists the morphological and physiological characteristics of strain Bap30. Hence, we identify Bap30 as *Acinetobacter* sp.

2.2 Biodegradation of BaP

Fig. 2 plots the time history of the degradation of BaP by strain Bap30, and shows that about 18% and 29% BaP were removed by the ends of day 8 and day 20. Fig. 2 also depicts the bacterial cell growth during



Numbers at branch nodes indicate percentages of bootstrap support for the clades. The scale corresponds to the mean number of nucleotide substitutions per site. Numbers in parentheses relate to the accession index of each strain in GenBank, NCBI

Fig. 1 Phylogenetic tree illustrating the similarity of *Acinetobacter* sp. Bap30 to its closest relatives

Table 1 Biochemical and physiological characteristics of strain Bap30

Item	Results	Item	Results
shape and arrangement	short, bacilliform	carbohydrate fermentation	
colonies	yellow, nummular	a. lactose	negative
gram staining	negative	b. sucrose	positive
margins	satin	c. glucose	positive
motility	immotile	d. maltose	positive
oxidase	negative	starch hydrolysis	negative
catalase	positive	indole production	negative
urease	negative	citrate utilization	negative

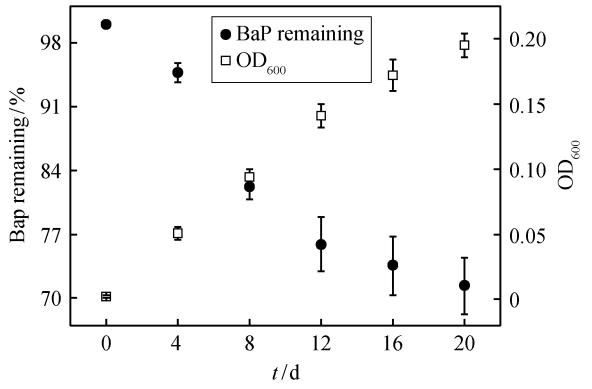


Fig. 2　Degradation of BaP by *Acinetobacter* sp. Bap30 in MSM with 40 mg/L BaP

the incubation period, which corresponds to the biodegradation of *Acinetobacter* sp. Bap30. It should be noted that the results in Fig. 2 show the mean values joined as lines, along with the maximum and minimum data values as bars to give a measure of the experimental error.

2.3　Effects of extra carbon sources on BaP degradation

40 mg/L glucose, sucrose, maltose, sodium acetate, and soluble starch were respectively added into the MSM as extra carbon sources for BaP biodegradation by strain Bap30. Table 2 lists the BaP removal and the biomass of strain Bap30 measured as OD_{600} after 7 days incubation. Except for soluble starch, the addition of extra carbon increased the degradation of BaP and the cell growth of strain Bap30. The addition of sucrose had the most positive effect, with BaP removal increased to 23.7% and the production of eight times more biomass than in the control case.

Table 3 indicates the effect of initial sucrose concentration on BaP degradation by strain Bap30 after 20 days incubation. For initial sucrose concentrations in the range from 10 to 100 mg/L, there is an obvious trend of increased BaP removal with increased sucrose concentration. However, when the initial sucrose concentration is 500 mg/L, the BaP removal dropped to 13.4% to a value less than half of that obtained for the control. The biomass of strain Bap30 increased monotonically with additional sucrose within the range of initial concentrations considered. From the trend of the results, it appears that the biomass production would saturate at a higher initial sucrose concentration.

Table 2　BaP removal and biomass production by *Acinetobacter* sp. Bap30 in the MSM for different kinds of extra carbon sources after 7 days incubation

Extra carbon source	BaP degradation/%	OD_{600}
sucrose	23.7±3.3	0.632±0.037
glucose	19.2±3.0	0.541±0.029
maltose	22.0±3.1	0.553±0.024
sodium acetate	15.3±2.9	0.142±0.013
soluble starch	14.1±2.6	0.090±0.011
blank	14.9±1.3	0.071±0.004

Note: BaP=40 mg/L, Extra carbon source=40 mg/L, pH=7.0, 160 rpm, 37°C. Mean and standard deviation values of the triplicates are listed.

Table 3　BaP removal and biomass production by *Acinetobacter* sp. Bap30 in the MSM for different concentrations of sucrose after 20 days incubation

Sucrose concentration/ ($mg \cdot L^{-1}$)	BaP degradation/%	OD_{600}
0	28.7±2.5	0.202±0.023
10	31.3±4.7	0.451±0.041
40	37.5±5.1	0.935±0.057
100	40.9±4.3	1.120±0.069
500	13.4±3.3	1.353±0.073

Note: BaP=40 mg/L, pH=7.0, 160 rpm, 37°C. Mean and standard deviation values of the triplicates are listed.

2.4　Co-metabolism of BaP and Phe

Table 4 lists the data on BaP removal and biomass production when Phe is present as a co-metabolism substrate during BaP degradation by strain Bap30. Phe was added into the MSM containing 40 mg/L BaP. For initial Phe concentrations of 20 and 40 mg/L, the removal of BaP significantly improved to 38.1% and 48.9%, respectively. However, at higher Phe concentrations of 60 and 100 mg/L, the BaP degradation became inhibited compared to the control, and the biomass of strain Bap30 also gradually decreased.

Table 4 BaP removal and biomass production by *Acinetobacter* sp. Bap30 in the MSM for different concentrations of Phe after 20 days incubation

Phe concentration /(mg · L^{-1})	BaP degradation/%	OD_{600}
0	28.7±2.5	0.202±0.023
20	38.1±5.0	0.285±0.025
40	48.9±5.3	0.352±0.036
60	22.4±3.1	0.313±0.029
100	17.3±2.2	0.261±0.031

Fig. 3 illustrates the results in terms of degradation kinetics. Again the mean values are plotted as points, with maximum and minimum values as bars. It can be seen that the rate of BaP degradation was similar to that of the growth of biomass of strain Bap30. The degradation rate was 20.2% higher than that of incubation with BaP only. Meanwhile, Phe was concurrently degraded with BaP. However, Phe degraded at a much faster rate than BaP, with over 95% Phe removed after 8 days of incubation. No Phe was detected by the end of the experiment (day 20). Fig. 3 also indicates that the increment of cell growth corresponded to the biodegradation of BaP and Phe.

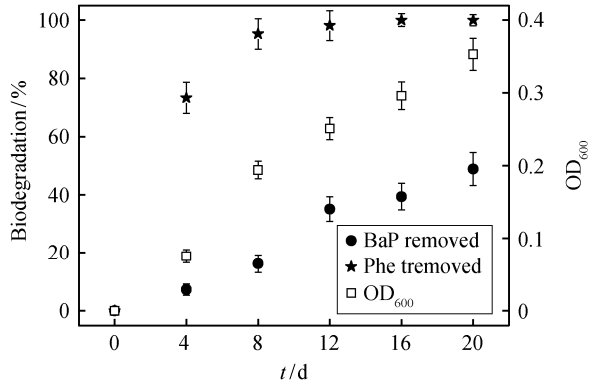


Fig. 3 Time-dependent co-metabolism of BaP and Phe by *Acinetobacter* sp. Bap30

3 Discussion

In the present study, a bacterial strain Bap30, able to utilize BaP as the sole source of carbon and energy, has been isolated from sewer sediment and identified as *Acinetobacter* sp. Using Bap30, 28.7% of BaP (40 mg/L) was degraded after 20 days incubation. To the authors' knowledge, such a function of *Acinetobacter* sp. and high level of BaP degradation has not been previously reported in the literature.

In Table 5, comparison of the BaP degradation efficiency between the present and other bacterial species was made, from which the isolated bacteria Bap30 was proved to be able to degrade BaP of high concentration in a shorter time.

As a genus of microorganisms for bioremediation, *Acinetobacter* sp. is already known to have broad substrate specificity towards organic pollutants, such as phenol[14], swainsonine[15], pentachlorophenol[16], benzene, toluene, ethylbenzene[17], and phthalate isomers[18].

It is well established that extra carbon sources can enhance the biodegradation of PAHs by various bacterial strains[19]. The present study similarly shows

Table 5 Bacterial species of benzo[a]pyrene degradation

Organism	Growth substrate	Initial BaP concentration/ (mg · L^{-1})	Removal rate/%	Incubation time/d	References
Mycobacterium sp. RGJII-135	yeast extract, peptone, starch	0.4	40	32	[8]
Sphingom paucimobilis	yeast extract	10	38	14	[9]
Burkholderia cepacia	pyrene	50	20–30	63	[10]
Rhodanobacter sp. Strain BPC1	diesel fuel	10	33–65	14	[11]
Ochrobactrum sp. BAP5		50	20	30	[7]
Thalassospira sp. strain TSL5-2	yeast powder and peptone	8	38.2	25	[12]
Ochrobactrum sp.G2	pyrene	10	24.8	7	[13]
Acinetobacter sp. Bap30		40	28.7	20	this paper

that the presence of glucose, sucrose, maltose, and sodium acetate can significantly improve BaP degradation by strain Bap30. These carbon sources all have simple chemical structures compared to BaP, and so could have been easily and preferentially assimilated by strain Bap30. As a result, biomass growth was promoted, benefiting biodegradation. However, utilization of the extra carbon sources for biomass growth was always accompanied by consumption of other essential elements in the system, such as nitrogen and phosphorus. Depletion of these essential elements inhibited BaP degradation when too much extra carbon was added.

Co-metabolism is the primary mechanism for the biodegradation of high molecular weight PAHs, especially those with more than 4 rings. Hence, addition of low molecular weight PAHs such as Phe can provide a co-metabolic substrate to enhance the biodegradation of high molecular weight PAHs[20]. The present study has demonstrated that Phe is a suitable hydrocarbon supplement for BaP degradation by strain Bap30. Moreover, BaP biodegradation by strain Bap30 in the presence of 40 mg/L Phe was even better than for sucrose, whereas the biomass was much lower. This implies that the presence of Phe stimulates the activity of strain Bap30 regarding BaP degradation. In general, the bay-region of Phe is double hydroxylated by a dioxygenase enzyme to form cis-3,4-phenanthrenedihydrodiol. The dihydrodiol undergoes meta-cleavage and subsequently converted to 3,4-dihydroxyphenanthrene by dihydrodiol ehydrogenase. The ring-cleavage product is then converted to 1-hydroxy-2-naphthoic acid. Similar to the metabolic pathway of Phe, the degradation of BaP is also initiated by involving two oxygen atoms into the Benzene ring to form carboxylic acid by a dioxygenase enzyme. A possible explanation could be that a dioxygenase enzyme is produced by strain Bap30 during Phe degradation, which initiated the degradation of BaP through hydroxylation and ring cleavage[21]. Further research is underway at Peking University to investigate this aspect.

In conclusion, *Acinetobacter* sp. Bap30 has been identified as a single strain of bacteria that degrades BaP, a highly toxic PAH that is damaging to human health. It is found that addition of a limited amount of extra carbon, especially sucrose, can accelerate the degradation process. Moreover, the addition of a limited amount of Phe, a low molecular weight PAH, also enhances degradation through co-metabolism. *Acinetobacter* sp. Bap30 naturally occurs in contaminated sediment in sewers, and so could have considerable potential for the bioremediation of BaP or other PAHs found in polluted soil and wastewater effluent, such as petroleum-polluted soil and coking wastewater.

Acknowledgments The authors gratefully acknowledge advice and access to the help provided by Professor Alistair Borthwick at the Department of Engineering Science, University of Oxford in UK.

References

[1] Chu M, Chen C. Evaluation and estimation of potential carcinogenic risks of polynuclear aromatic hydrocarbons (PAH). Washington DC: US EPA, 1985

[2] Juhasz A L, Britz M L, Stanley G A. Degradation of Benzo[a]pyrene, Dibenz[a,h]anthracene and Coronene by *Burkholderia cepacia*. Water Science and Technology, 1997, 36(10): 45–51

[3] Schuler L, Jouanneau Y, Chadhain S M, et al. Characterization of a ring-hydroxylating dioxygenase from phenanthrene-degrading *Sphingomonas* sp. strain LH128 able to oxidize benz[a]anthracene. Applied Microbiology and Biotechnology, 2009, 83(3): 465–475

[4] Toyama T, Furukawa T, Maeda N, et al. Accelerated biodegradation of pyrene and benzo[a]pyrene in the *Phragmites australis* rhizosphere by bacteria-root exudate interactions. Water Resaerch, 2011, 45(4): 1629–1638

[5] Tamura K, Dudley J, Nei M, et al. MEGA4: molecular evolutionary genetics analysis (MEGA) software version 4.0. Molecular Biology and Evolution, 2007, 24(8): 1596–1599

[6] Dong X Z, Cai M Y. Manual of systematic identification of common bacteria. Beijing: Beijing Science Press, 2001

[7] Wu Y R, He T T, Zhong M Q, et al. Isolation of

marine benzo[*a*]pyrene-degrading *Ochrobactrum* sp. BAP5 and proteins characterization. Journal of Environmental Sciences, 2009, 21(10): 1446–1451

[8] Schneider J, Grosser R, Jayasimhulu K, et al. Degradation of pyrene, benz[*a*]anthracene, and benzo-[*a*]pyrene by *Mycobacterium* sp. strain RJGII-135, isolated from a former coal gasification site. Applied and Environmental Microbiology, 1996, 62(1): 13–19

[9] Ye D Y, Siddiqi M A, Maccubbin A E, et al. Degradation of polynuclear aromatic hydrocarbons by *Sphingomonas paucimobilis*. Environmental Science Technology, 1996, 30(1): 136–142

[10] Juhasz A L, Naidu R. Bioremediation of high molecular weight polycyclic aromatic hydrocarbons: a review of the microbial degradation of benzo[a]pyrene. International Biodeterioration and Biodegradation, 2000, 45(1/2): 57–88

[11] Kanaly R A, Bartha R, Watanabe K, et al. Rapid mineralization of benzo[*a*]pyrene by a microbial consortium growing on diesel fuel. Applied and Environmental Microbiology, 2000, 66(10): 4205–4211

[12] Wang H, Zhou H Y, Huan G Y, et al. Isolation and degradation characteristics of a HMW-PAHs degrading halophilic strain *Thalassospira* sp. Tsinghua Science and Technology, 2015, 55(1): 87–92

[13] Gao Y M, Yang X L, Li F M, et al. Isolation of heavy PAH-degrading bacteria and their characteristics of degradation. Chinese Journal of Ecology, 2016, 35(6): 1539–1546

[14] Wang Y, Tian Y, Han B, et al. Biodegradation of phenol by free and immobilized *Acinetobacter* sp. strain PD12. Journal of Environmental Sciences, 2007, 19(2): 222–225

[15] Zhao X H, He X, Wang J N, et al. Biodegradation of Swainsonine by *Acinetobacter calcoaceticus* strain YLZZ-1 and its isolation and identification. Biodegradation, 2009, 20(3): 331–338

[16] Sharma A, Thakur I S, Dureja P. Enrichment, isolation and characterization of pentachlorophenol degrading bacterium *Acinetobacter* sp. ISTPCP-3 from effluent discharge site. Biodegradation, 2009, 20(5): 643–650

[17] Kim J M, Jeon C O. Isolation and characterization of a new benzene, toluene, and ethylbenzene degrading bacterium, *Acinetobacter* sp B113. Current Microbiology, 2009, 58(1): 70–75

[18] Vamsee-Krishna C, Mohan Y, Phale P S. Biodegradation of phthalate isomers by *Pseudomonas aeruginosa* PP4, *Pseudomonas* sp. PPD and *Acinetobacter lwoffii* ISP4. Applied Microbiology and Biotechnology, 2006, 72(6): 1263–1269

[19] McLellan S L, Warshawsky D, Shann J R. The effect of polycyclic aromatic hydrocarbons on the degradation of benzo[*a*]pyrene by *Mycobacterium* sp. strain RJGII-135. Environmental Toxicology and Chemistry, 2002, 21(2): 253–259

[20] Kanaly R A, Harayama S. Biodegradation of high-molecular-weight polycyclic aromatic hydrocarbons by bacteria. Journal of Bacteriology, 2000, 182(8): 2059–2067

[21] Capotorti G, Digianvincenzo P, Cesti P, et al. Pyrene and benzo[*a*]pyrene metabolism by an *Aspergillus terreus* strain isolated from a polycylic aromatic hydrocarbons polluted soil. Biodegradation, 2004, 15(2): 79–85