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Degradation of Di-*n*-butyl Phthalate by Newly Isolated *Ochrobactrum* sp.

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Abstract A gram negative isolate designated JDC-41 was obtained from river sludge using mixtures of phthalate esters as the sole source and energy. The isolate was identified as *Ochrobactrum* sp. based on its 16S rRNA gene sequence. Over 87% of supplied di-*n*-butyl phthalate (DBP) was degraded by JDC-41 in a pH neutral mineral salts medium at 30°C within 48 h. Increased DBP (50–500 mg/L) in the culture correspondingly increased degradation half-life from 3.83 to 18.12 h. DBP induced cells more rapidly degraded DBP.

Keywords Biodegradation · Di-*n*-butyl phthalate · Kinetics · *Ochrobactrum* sp.

Phthalate esters (PAEs) are anthropogenic compounds widely-used in plastics, clothing, building materials, home furnishings, transportation components, cosmetics, surface lubricants, and medical product packaging (Vamsee-Krishna et al. 2006). They readily migrate into the ecosystem or wastewater during their use and after disposal, particularly from plastic products (Psillakis et al. 2004). Even at very low concentrations, their effects on the endocrine system can interfere with reproduction and behavior in humans and wildlife (Lottrup et al. 2006), and can damage hemocytes and influence the defense mechanism of giant freshwater prawns (Sung et al. 2003).

The common plasticizer di-*n*-butyl phthalate (DBP) has been detected in diverse environmental samples including groundwater, ocean water, soil, and sediment from freshwater and marine environments (Wu et al. 2009), principally due to microbial metabolically related degradation (Sun and Shan 2007). Although several strains from different genera capable of DBP biodegradation have been isolated (Chang et al. 2004; Hashizume et al. 2002; Patil et al. 2006), little is known concerning the biodegradation of DBP by the genus *Ochrobactrum* (Ogawa et al. 2009), a ubiquitous, aerobic gram-negative bacillus. In the present study, we report the biodegradation of DBP by *Ochrobactrum* sp. and the influence of DBP induction on the degradation rate.

Materials and Methods

Di-n-octyl phthalate (DOP) and di-isononyl phthalate (DINP) with 99% purity were purchased from Aldrich-Sigma Chemicals (St. Louis, Missouri, USA). Dimethyl phthalate (DMP), diethyl phthalate (DEP), and diisooctyl phthalate (DIOP), all with 99.5% purity, were obtained from China National Medicine Group. All other chemical reagents were of analytical grade and all solvents were of HPLC grade.

The organism was isolated from river sludge collected from Guilin, Guangxi Province, China. The initial enrichment culture was mineral salts medium (MSM: 5.8 g/L K_2HPO_4, 4.5 g/L KH_2PO_4, 2.0 g/L $(NH4)_2SO_4$, 0.16 g/L $MgCl_2$, 0.02 g/L $CaCl_2$, 0.24 mg/L $Na_2MoO_4.2H_2O$, 0.18 mg/L $FeCl_3$, 0.15 mg/L $MnCl_2.2H_2O$) supplemented with

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100 mg/L mixtures of phthalate esters (each 50 mg/L for DMP, DEP, DBP and DOP) as the sole source of carbon and energy. 1.0 mL of the enrichment culture was serially transferred to freshly medium with gradually increasing concentration of mixtures of phthalate esters (100–500 mg/L). After seven subcultures (30°C, 7 day), the final pure culture was designated JDC-41. The pure culture was characterized using 16S rRNA gene sequencing.

Degradation of DBP in MSM was carried using 20 mL medium in 50 mL flasks shaken at 180 rpm. The degradation rate was measured by high performance liquid chromatography (HPLC). The effect of pH (4, 5, 6, 7, 8, 9, 10), temperature (15, 20, 25, 30, 35, 40, 45) and DBP concentration (50, 100, 200, 300, 400, 500 mg/L) on degradation was assessed to determine the optimal degradation conditions. All the experiments were carried out in triplicate.

Ochrobactrum sp. JDC-41 was cultured in liquid MSM supplemented with 200 mg/L of DMP, DEP, DBP, DOP, DIOP or DINP as the sole source of carbon and energy. Cell growth was monitored turbidometrically at 600 nm after 60 h incubation. In the other experiment, DBP non-induced cells were cultured in LB medium and DBP induced cells were cultured in MSM containing DBP (500 mg/L) at 30°C for 60 h. Cells were collected (rpm 12,000) and washed three times with 0.05 mol/L potassium phosphate buffer, prior to inoculation into fresh MSM containing DBP (500 mg/L). DBP degradation was determined in samples withdrawn periodically as described above. Each determined was conducted in triplicate.

Results and Discussion

Isolate JDC-41 was identified as *Ochrobactrum* sp. by 16S rRNA gene sequence analysis (GenBank accession GU 248309). *Ochrobactrum* are widespread in nature and can be obtained from various sources such as wastewater, soil and activated sludge (Zhang et al. 2006). To date, most *Ochrobactrum* species have been demonstrated to be able to degrade a variety of pollutants. Recently, isolation of two *Ochrobactrum anthropi* strains capable of utilizing DBP as the sole source of carbon and energy was reported (Ogawa et al. 2009). However, detailed characterization of DBP degradation by *O. anthropi* was not investigated. The present report is, thus, the first detailed report on DBP degradation by *Ochrobactrum* sp.

The optimal conditions for the degradation of DBP by JDC-41 were found to be a temperature of 30°C and pH of 7.0 (Table 1), consistent with previous reports describing an optimum temperature is 30–38°C and optimum pH of 6–8 (Xu et al. 2005).

Table 1 Effect of pH and temperature on degradation of 500 mg/L by *Ochrobactrum* sp. JDC-41

pH	Degradation (%)[a]	Temperature (°C)	Degradation (%)[a]
4	2 ± 1	15	3 ± 1
5	35 ± 2	20	23 ± 2
6	79 ± 2	25	53 ± 2
7	87 ± 3	30	86 ± 4
8	70 ± 1	35	66 ± 2
9	39 ± 2	40	13 ± 1
10	5 ± 1	45	7 ± 2

[a] Degradation was determined from the mean of the average relative value of three replications

DBP biodegradation by *Ochrobactrum* sp. JDC-41 was assumed to fit the Monod first-order kinetic equation as follows:

$$\ln C = -Kt + A \quad (1)$$

where C is DBP concentration, t expresses time, K is the first order rate constant and A is a constant. The half-life of DBP biodegradation by JDC-41 could be calculated according to the equation:

$$t_{1/2} = \ln 2/K. \quad (2)$$

The half-life value increased from approximately 3.83 to 18.12 h as the substrate concentration in the culture medium increased from 50 to 500 mg/L (Table 2). Obviously, the degradation rate was markedly higher than previous reports using pure cultures (Chao et al. 2006; Ogawa et al. 2009). Xu et al. (2005) reported that about 75% of DBP (10 mg/L) was degraded by *Pseudomonas fluorescens* B-1 after 48 h incubation. In another research, it was reported that less than 40% of DBP (100 mg/L) was degraded by *Ochrobactrum* sp. strains after incubation in dark for 2 weeks (Ogawa et al. 2009).

Substrates utilization testing indicated that phthalates with shorter ester chains were readily biodegraded, whereas those with longer ester chains were only marginally degraded (Table 3), entirely consistent with previous studies (Liang et al. 2008). Figure 1 shows that DBP induced cells rapidly degraded DBP (500 mg/L) to an undetectable level in the culture medium within 70 h. On the contrary, DBP non-induced cells required about 150 h to completely degrade the same concentration of DBP. Esterase-mediated de-esterification is the primary step in the catabolic pathway of PAEs (Liang et al. 2008). DBP induction improves the esterase activity to activate the utilization of DBP through hydrolysis of the diester into intermediates. Similar results on DBP biodegradation have been reported previously (Zhou et al. 2005).

In conclusion, a strain *Ochrobactrum* sp. JDC-41 was isolated from river sludge. The half-life value increased

Table 2 Kinetics of aerobic DBP degradation by *Ochrobactrum* sp. JDC-41 at 30°C and pH 7

Initial concentration (mg/L)	Kinetic equations	Half life (h)	R^2
50	$\ln C = -0.18091x + 3.90855$	3.83	0.99486
100	$\ln C = -0.08995x + 4.6131$	7.71	0.99553
200	$\ln C = -0.06119x + 5.30069$	11.33	0.99463
300	$\ln C = -0.05845x + 5.73286$	11.86	0.99168
400	$\ln C = -0.04112x + 6.06242$	16.86	0.93586
500	$\ln C = -0.03826x + 6.28766$	18.12	0.94327

Table 3 Growth profile of *Ochrobactrum* sp. JDC-41 on various PAEs

Substrates[a]	DMP	DEP	DBP	DOP	DIOP	DINP
Growth	++	++	++	−	−	−

[a] DMP = dimethyl phthalate, DEP = diethyl phthalate, DBP = di-*n*-butyl phthalate, DOP = di-n-octyl phthalate, DIOP = diisooctyl phthalate, DINP = di-isononyl phthalate, ++ vigorous growth, − no growth

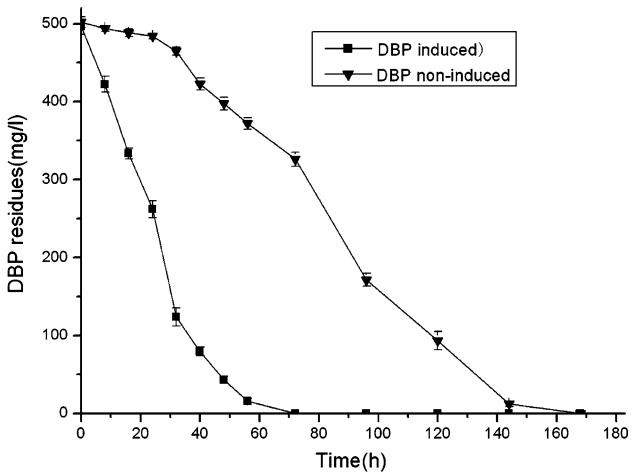

Fig. 1 DBP degradation by DBP induced and non-induced bacterial cells of *Ochrobactrum* sp. JDC-41. *Square* DBP degradation with DBP induction; *Triangle* DBP degradation without DBP induction. The DBP non-induced cells were cultured in LB medium, whereas the DBP induced cells were cultured in MSM containing DBP (500 mg/L)

with the concentrations of DBP increased from 50 to 500 mg/L. This study also showed that the substrate induction could influence the degradation rate of DBP. To our knowledge, this is the first detailed report on DBP degradation by strain from the genus *Ochrobactrum*. In particular, JDC-41 was much more efficient in degrading DBP than previous reports.

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