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Complete degradation of di-*n*-octyl phthalate by biochemical cooperation between *Gordonia* sp. strain JDC-2 and *Arthrobacter* sp. strain JDC-32 isolated from activated sludge

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ABSTRACT

Two bacterial strains were isolated from activated sludge using mixtures of phthalic acid esters (PAEs) as the sole source of carbon and energy. One of the isolates was identified as *Gordonia* sp. strain JDC-2 and the other as *Arthrobacter* sp. strain JDC-32, mainly through 16S rRNA gene sequence analysis. *Gordonia* sp. strain JDC-2 rapidly degraded di-*n*-octyl phthalate (DOP) into phthalic acid (PA), which accumulated in the culture medium. *Arthrobacter* sp. strain JDC-32 degraded PA but not DOP. The co-culture of *Gordonia* sp. strain JDC-2 and *Arthrobacter* sp. strain JDC-32 degraded DOP completely by overcoming the degradative limitations of each species alone. The biochemical pathway of DOP degradation by *Gordonia* sp. strain JDC-2 was proposed based on the identified degradation intermediates. The results suggest that DOP is completely degraded by the biochemical cooperation of different microorganisms isolated from activated sludge.

1. Introduction

Phthalic acid esters (PAEs) are a group of synthetic compounds predominantly used as plasticizers in polyvinyl chloride (PVC) plastics. For over half a century, they have been used to increase flexibility of some polymeric materials. Because phthalates are mixed with the raw materials of plastic production without chemically binding to them, they easily leach out during the products' lifetime [1]. Therefore they have been detected in various environments across China such as sediment, natural bodies of water, soils, and even in the atmosphere [2–5]. In recent years, phthalates have become great environmental concerns because of their suspected carcinogenic, estrogenic, and endocrine-disrupting properties [6]. Some of the PAEs including dimethyl phthalate (DMP), di-*n*-butyl phthalate (DBP), and di-*n*-octyl phthalate (DOP) have been listed as priority pollutants by the China National Environmental Monitoring Center [7] and the US Environmental Protection Agency [8].

DOP is the straight chain isomer of di-2-ethylhexyl phthalate (DEHP), one of the most commonly utilized plasticizers, and has eight carbons in its alkyl-chain. Considerable research has been conducted on the biodegradability of PAEs over the last few decades [9]. It is generally acknowledged that phthalates with shorter ester chains like dimethyl phthalate (DMP), diethyl phthalate (DEP), and dibutyl phthalate (DBP) can be more readily biodegraded and mineralized than those with longer ester chains such as dioctyl phthalate (DOP) and di-2-ethylhexyl phthalate (DEHP) [10–13]. Although many studies have been conducted on DEHP degradation under various conditions [14–19], only a few have been conducted on the biodegradability of DOP [7,20–22]. Moreover, these studies found that the degradation rate of DOP is relatively slow. Therefore, it is of great significance to isolate bacterial strains that can degrade DOP completely.

Although some individual microbes are capable of completely mineralizing phthalate esters, more efficient metabolism appears to result from mixed microbial populations in the environment [1]. To date, there have been several reports about the complete mineralization of specific PAEs by a mixed culture consortium, in contrast to partial utilization by a single strain [23–26]. However, most of the previous studies on DOP degradation were centered on primary degradation by pure cultures [20–22]. Therefore, no research has been published on complete degradation of DOP by a consortium of selected microorganisms. Moreover, the characterization of the biochemical pathway of DOP is not currently in the literature.

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The present work described the isolation and characterization of two PAEs-degrading bacterial strains from activated sludge. A consortium of these two isolates was found to degrade DOP completely. In addition, the pathway of DOP degradation was proposed on the basis of identified possible intermediates, and the contribution of each organism to the degradation was established.

2. Materials and methods

2.1. Chemicals

DOP and di-isononyl phthalate (DINP) with 99% purity were purchased from Aldrich–Sigma Chemicals (St. Louis, MO). Dimethyl phthalate (DMP), diethyl phthalate (DEP), di-*n*-butyl phthalate (DBP), and di-isooctyl 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.

2.2. Source of microorganisms

The bacteria used in the degradation experiment were isolated from two samples (A and B). Sample A was from river sludge collected in Dingcheng District, Changde City, in Hunan Province, whereas B was sewage sludge sampled from the Qujiang River of Yongxin City in Jiangxi Province. The sludge samples were stored in plastic bags and kept at 4 °C for a week before experiments.

2.3. Enrichment and isolation of bacteria

The minimum salt medium (MSM) [27] used in all experiments including enrichment experiments contained (g/l): K_2HPO_4 5.8 g, KH_2PO_4 4.5 g, $(NH_4)_2SO_4$ 2.0 g, $MgCl_2$ 0.16 g, $CaCl_2$ 0.02 g, $Na_2MoO_4{\cdot}2H_2O$ 0.0024 g, $FeCl_3$ 0.0018 g, and $MnCl_2{\cdot}2H_2O$ 0.0015 g. The pH of MSM was adjusted to 7.0 with HCl or NaOH and then sterilized by autoclaving for 20 min at 121 °C. The agar plates were prepared by adding 18 g of agar to 1 l of the MSM.

The enrichment procedure was similar to that described by Chao et al. [27] with some modifications. Initially, 5.0 g of sludge was added to a 500-ml Erlenmeyer flask containing 200 ml of MSM solution amended with a mixture of PAEs (200 mg/l) each 50 mg/l for DMP, DEP, DBP and DOP. The suspension was incubated for 7 days in the dark at 30 °C on a rotary shaker operated at 175 rpm. Subsequently, 2 ml of the enrichment culture was serially transferred four times to fresh medium (each time containing a higher concentration of PAEs (240–500 mg/l)) and incubated under the same conditions. Then the final enrichment was streaked onto MSM agar (18 g/l) plates supplemented with a mixture of PAEs (500 mg/l) and incubated 1 week at 30 °C. Presumptive colonies were picked on the basis of differences in colony morphology and coloration and re-streaked onto MSM agar plates amended with the mixture of PAEs. The bacterial isolates were further purified by streaking on LB Nutrient Agar plates and then re-streaked onto MSM agar plates with and without PAEs to confirm their degradation abilities. Isolates showing growth in the presence of PAEs but not in their absence were selected for further study.

2.4. Identification and characterization of bacteria

Gram reaction and cell morphology were determined by observing stained cells under a light microscope. Biochemical tests for the identification of bacteria were performed using standard methods. The bacterial isolates of pure cultures were further characterized using 16S rRNA gene sequencing methods. To extract the genomic DNA of bacterial strains for 16S rRNA gene amplification, 2 ml of bacterial culture grown in liquid MSM amended with PAEs was collected and centrifuged. The cell pellet was washed three times with phosphate buffer saline (PBS, 0.05 mol/l, pH 7.4) and then re-suspended in 200 μl of Tris/EDTA (TE) buffer (pH 8.0). Genomic DNA was extracted using an EZ-10 Spin Column Genomic DNA Minipreps Kit (Bio Basic Inc., Markham, Ontario, Canada) according to the manufacturer's instructions. Then the 16S rRNA gene from genomic DNA was amplified with bacterial universal primers F27 and R1492. The PCR products were purified using an E.Z.N.A.® Gel Extraction Kit (Omega Bio-Tek, Inc., Doraville, GA) according to manufacturer's instructions, and then they were sequenced (Sangon, Shanghai, China). The 16S rRNA gene sequences of the bacteria were aligned and compared with the bacterial gene sequences in the GenBank. The closest references were retrieved from NCBI GenBank and aligned with CLUSTALW with all parameters set at their default values. Phylogenetic trees were then generated by a neighbour-joining algorithm and Kimura II parameter model of base substitution as implemented in MEGA4.1. The trees were validated using bootstrap analysis performed with 1000 replicates.

2.5. Substrate utilization tests

Strains JDC-2 and JDC-32 were tested for their ability to grow on a variety of carbon sources. Liquid MSM was supplemented with either protocatechuate (PCA), phthalic acid (PA), DMP, DEP, DBP, DOP, DIOP, or DINP (200 mg/l) as carbon sources to examine the ability of PAEs-degrading bacterial strains to utilize these compounds. PA and PCA were dissolved in chloroform and sterilized by filtering through 0.22 μm membranes. Others were added to MSM medium directly and autoclaved. The microbial growth in culture flasks was determined by optical density measurements at 600 nm (OD_{600}) using a UV-9200 spectrophotometer (Rayleigh Analytical Instrument Corp., Beijing, China) and microscopic observation.

2.6. Degradation experiments

Grown in the MSM supplemented with PAEs at 30 °C on a rotary shaker operated at 150 rpm, the cells were harvested after 36 h, washed three times with 0.05 mol/l potassium phosphate buffer (pH 7.5) under sterile conditions, re-suspended in the same phosphate buffer to an OD_{600} of 0.2.

Degradation of DOP (500 mg/l) by each strain (1 ml of suspension) or the consortium (1 ml of each strain) was studied in 20 ml of sterilized MSM in 50-ml Erlenmeyer flasks incubated at 30 °C on a rotary shaker operated at 150 rpm in the dark. At 6-h intervals, 20 ml of dichloromethane was added directly to individual flasks, which were vigorously shaken 3 min. The resulting heavy emulsion was centrifuged (5000 rpm, 5 min) and the aqueous phase was extracted twice. The dichloromethane phase was evaporated to dryness and dissolved in 10 ml of methanol for further analysis.

At the same time, the degradation of PA (500 mg/l) by each strain (1 ml of suspension) was studied in 200 ml of sterilized MSM in 500-ml Erlenmeyer flasks incubated under the same conditions. Aqueous samples (1 ml) were periodically withdrawn by sterile syringe and preserved at −20 °C for optical density measurement and further HPLC analysis.

All experiments were performed in triplicate. Samples and sterile controls (non-inoculated MSM) were periodically analyzed in similar way. The microbial biomass in culture flasks was determined by measuring optical density (OD_{600}) as described previously.

2.7. Analysis of residual substrates by HPLC

The frozen aliquots were thawed and filtered through a 0.22-μm membrane filter before injection (20 μl) into the HPLC system

Table 1
Phenotypic characteristics of strain JDC-2 and strain JDC-32.

Characteristic	Strains		Characteristic	Strains	
	JDC-2	JDC-32		JDC-2	JDC-32
Gram reaction	+	+	Glucose	+	+
Spore formation	–	–	Sucrose	+	+
Motility	–	–	Fructose	+	+
Catalase	+	+	Galactose	–	+
Oxidase	–	–	Lactose	–	+
Urease	+	+	Mannose	+	+
Gelatinase	–	–	Maltose	+	+
Starch hydrolysis	–	–	Citrate	–	–
Nitrate reduction	+	+	L-Histidine	–	+
Indole test	–	–	L-Phenylalanine	–	+
Methyl red test	–	–	L-Glutamine	+	+
Voges–Prosauker test	–	–	L-Tyrosine	–	+

(+) positive; (–) negative.

(Elite series; Elite, Dalian, China) equipped with a UV230+ UV-Vis detector and a Hypersil BDS-C18 (200 mm × 4.6 mm, 5 μm) chromatography column. The mobile phase for detecting DOP was methanol:water (90:10, v/v), the flow rate was 1 ml min^{-1}, and the UV wavelength was 254 nm. The mobile phase for separating PA was methanol:1% acetic acid water (40:60, v/v), the flow rate was 0.5 ml min^{-1}, and the UV wavelength was 228 nm. The calibration curve was linear for the range 12.5–500 mg/l.

2.8. Analysis of DOP-derived degradation products by GC/MS

To detect minor intermediate products during the course of degradation, the samples extracted above were concentrated to 1 ml and then subjected to GC/MS analysis using a ThermoFinnigan Trace GC2000 equipped with a PolarisQ ion trap mass spectrometer (GC-MS, Thermo Electron Corporation, Austin, TX). Gas chromatography was performed on a 30-m DB-5MS elastic quartz capillary column (0.25 μm film thickness, 0.25 mm inner diameter; temperature maintained at 60 °C for 3 min, increased from 5 °C min^{-1} to 280 °C for 15 min, then cooled rapidly to 60 °C) using ultra-high purity helium as carrier (flow rate, 1 ml min^{-1}). For MS spectra collected in the positive electron ionization (EI) mode, the source temperature was 250 °C with an electron ionization energy of 70 eV, the mass range was 40–500 mu, and 1 μl of the extracted sample was injected in split mode (1:10). The NIST Mass Spectral Search Program (National Institutes of Standard and Technology, Gaithersburg, MD) was used to identify signals by comparison to the retention time and mass spectra of authentic compounds.

3. Results

3.1. Isolation and characterization

Two bacterial isolates (JDC-2 and JDC-32) were obtained that could utilize mixtures of PAEs as sole source of carbon and energy both on agar plates and in liquid cultures. Strain JDC-2 was a Gram-positive, rod-shaped aerobe that formed round and red colonies on LB agar plates. Strain JDC-32 was also a Gram-positive, obligate aerobe that showed rod-coccus cycles and formed yellow glossy circular colonies on LB agar plates. Table 1 summarizes some of the phenotypic characteristics of these two isolates. Based on BLAST analysis of its 16S rRNA gene sequence, JDC-2 belonged to the genus *Gordonia* (GenBank accession no. FJ851356), whereas JDC-32 seemed to be a species of *Arthrobacter* (FJ851358). Fig. 1 illustrates the phylogenetic relationship of JDC-2 and JDC-32 to their close relatives.

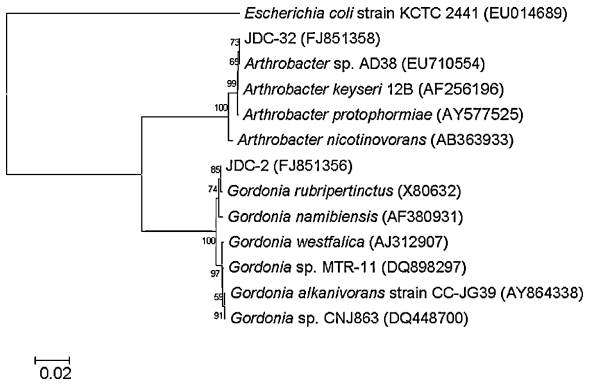


Fig. 1. 16S rRNA gene sequence-generated phylogenetic tree showing the relationship of *Gordonia* sp. strain JDC-2, *Arthrobacter* sp. strain JDC-32, and close relatives. The numbers at the branch nodes are bootstrap values based on 1000 re-samplings. Accession numbers of the bacterial isolates in this study and others are shown in brackets.

3.2. Substrate utilization tests

MSM supplemented with each substrate (200 mg/l) was used to test substrate range. The strains differed in their ability to degrade phthalate esters (Table 2).

JDC-32 rapidly degraded PAEs with shorter alkyl-chains such as DMP, DEP, and DBP, but not PAEs with longer alkyl-chains like DOP, DIOP, and DINP. At the same time, JDC-32 utilized PA and PCA as the sole source of carbon and energy for growth. By contrast, JDC-2 degraded all six PAEs but not PA and PCA. These findings suggested that PAEs with longer alkyl-chains might be completely degraded by biochemical cooperation of these two strains.

3.3. Degradation of DOP and PA by Gordonia sp. strain JDC-2

Gordonia sp. strain JDC-2 completely transformed DOP (500 mg/l; used as the sole source of carbon and energy) in 60 h (Fig. 2). At the same time, biomass increased (OD_{600} values reaching 0.352) after 48 h of incubation. An intermediate product was detected in the culture medium at 12 h and gradually increased from 3.8327 mg/l to 93.2646 mg/l. Unexpectedly, it remained undegraded even after 30 days of incubation. Both the retention time on HPLC and UV–vis absorption spectrum of the intermediate matched those of the authentic standard of PA. In a parallel experiment, *Gordonia* sp. strain JDC-2 did not degrade PA added as the sole source of carbon and energy, and PA failed to support growth even after 30 days of incubation. The above results collectively suggest that *Gordonia* sp. strain JDC-2 transforms DOP to PA, but cannot cleave the aromatic ring structure of PA. In sterile controls, DOP and PA were not transformed and no growth occurred under the same conditions (data not shown).

3.4. Degradation of PA and DOP by Arthrobacter sp. strain JDC-32

The growth of the strain *Arthrobacter* sp. strain JDC-32 and the amount of PA (500 mg/l of MSM) consumed are presented in Fig. 3,

Table 2
Growth characteristics of the strains JDC-2 and JDC-12 on various phthalates and possible intermediates.

Strains	Substrates							
	DINP	DIOP	DOP	DBP	DEP	DMP	PA	PCA
JDC-2	+	+	+	++	++	++	–	–
JDC-32	–	–	–	++	++	++	++	++

(++) vigorous growth; (+) growth; (–) no growth.

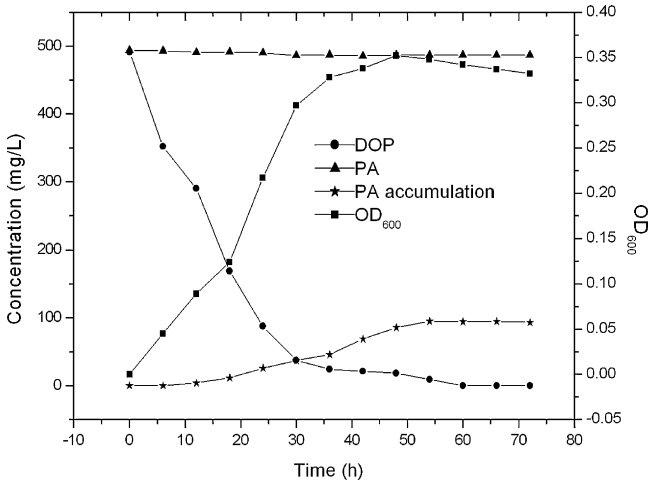


Fig. 2. Time course of microbial growth (OD_{600}) and degradation of DOP by *Gordonia* sp. strain JDC-2.

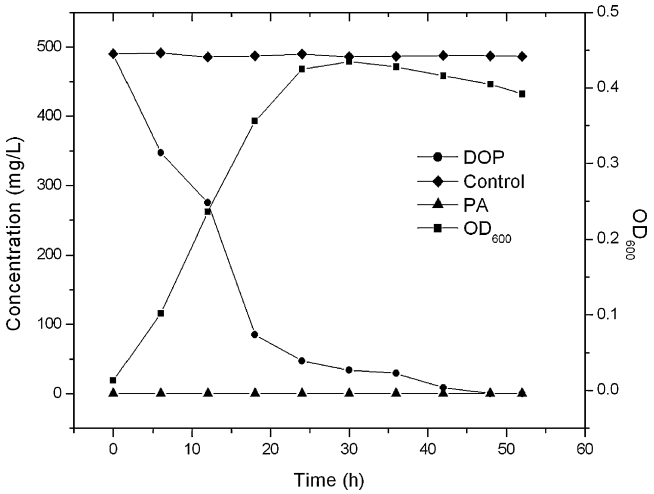


Fig. 4. Time course of microbial growth (OD_{600}) and degradation of DOP by the co-culture of *Gordonia* sp. strain JDC-2 and *Arthrobacter* sp. strain JDC-32.

which shows strain JDC-32 was highly efficient in degrading PA. The concentration of PA decreased slowly in the first 12 h and then decreased more rapidly after 12 h. After 20 h of incubation, PA was completely degraded and OD_{600} had increased from 0.012 to 0.441. In the parallel experiment, strain JDC-32 degraded DOP only slightly and did not grow even after 30 days of incubation. At the same time, the abiotic control had no effect on DOP or PA concentration and failed to grow (no increase in OD_{600}). Thus, *Arthrobacter* sp. strain JDC-32 could degrade PA but not DOP. These findings implied that *Arthrobacter* sp. strain JDC-32 with *Gordonia* sp. strain JDC-2 might be able to degrade DOP completely.

3.5. Complete degradation of DOP by a co-culture of Gordonia sp. strain JDC-2 and Arthrobacter sp. strain JDC-32

HPLC monitoring of the degradation of DOP by a co-culture of *Gordonia* sp. strain JDC-2 and *Arthrobacter* sp. strain JDC-32 in MSM revealed that DOP was completely hydrolyzed over time (Fig. 4), and microbial biomass increased over time. The consortium degraded DOP a little more efficiently than did strain JDC-2 alone (Fig. 4). The initial concentration of DOP (500 mg/l) was depleted by the consortium in 48 h and by *Gordonia* sp. strain JDC-2 in 60 h. No PA was detected in the culture medium by HPLC monitoring

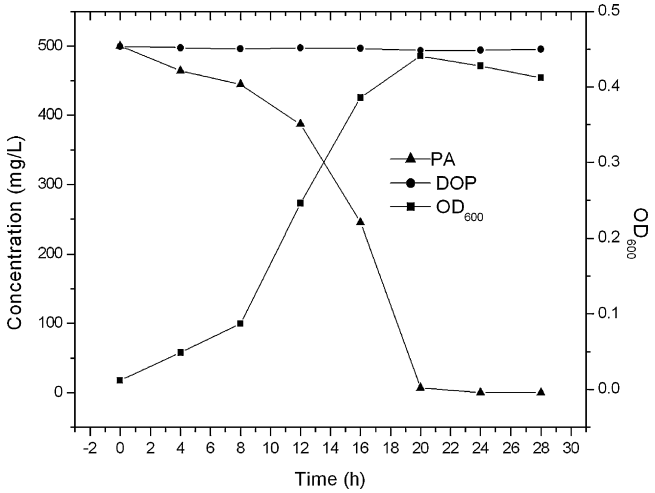


Fig. 3. Time course of microbial growth (OD_{600}) and degradation of PA by *Arthrobacter* sp. strain JDC-32.

under the same conditions during the whole course of incubation. It is suggested that PA produced by the strain JDC-2 from DOP was quickly utilized by *Arthrobacter* sp. strain JDC-32. No significant decrease in DOP concentration was observed in the control during the experiment.

3.6. GC/MS analysis of DOP-derived degradation products

To determine the metabolites or intermediates formed by *Gordonia* sp. strain JDC-2 in the pathway to PA, the concentrated samples were analyzed by GC/MS. Surprisingly, five short alkyl side-chain phthalates (butyl octyl phthalate [BOP], dibutyl phthalate [DBP], diethyl phthalate [DEP], monomethyl phthalate [MMP], and 1,3-isobenzofurandione [IBF]) were detected over the course of degradation by comparing the mass spectrum at a particular retention time with the published mass spectra from National Institute of Standards and Technology (NIST) Database (Table 3 and Fig. 5). At the same time, GC/MS detected OBP, DBP, and DEP (in amounts lower than that formed by strain JDC-2 alone) but not PA and MMP as the intermediates of DOP degradation by the consortium.

4. Discussion

In the present study, two indigenous bacterial strains *Gordonia* sp. JDC-2 and *Arthrobacter* sp. JDC-32 were successfully isolated from polluted river sludge using mixtures of PAEs as the sole source of carbon and energy. Further study revealed that strain JDC-32 rapidly degraded and utilized PA and PAEs with short alkyl-chains (DMP, DEP, and DBP) but not with long alkyl-chains (DOP, DIOP, and DINP) while JDC-2 utilized all six tested PAEs but not PA as growth substrate (Table 2). Similar results (i.e., PA as the end product during phthalate degradation) have also been shown by several other researchers [28–30]. The above findings therefore suggested that PAEs with longer alkyl-chains might be completely degraded by biochemical cooperation of both strains. Previous studies have demonstrated that complete degradation of complex organic compounds like phthalates can be achieved in natural environments by syntrophic consortia [31]. However, few reports describe the mechanism of cooperation. In one such report by Vega and Bastide [23], *Arthrobacter* sp. transformed DMP to MMP, and then *Sphingomonas paucimobilis* hydrolyzed MMP to PA. Another study demonstrated that *Klebsiella oxytoca* Sc rapidly transformed dimethyl isophthalate to monomethyl isophthalate, which was further converted to isophthalic acid by *Methylobacterium mesophilicum* Sr [25]. Using

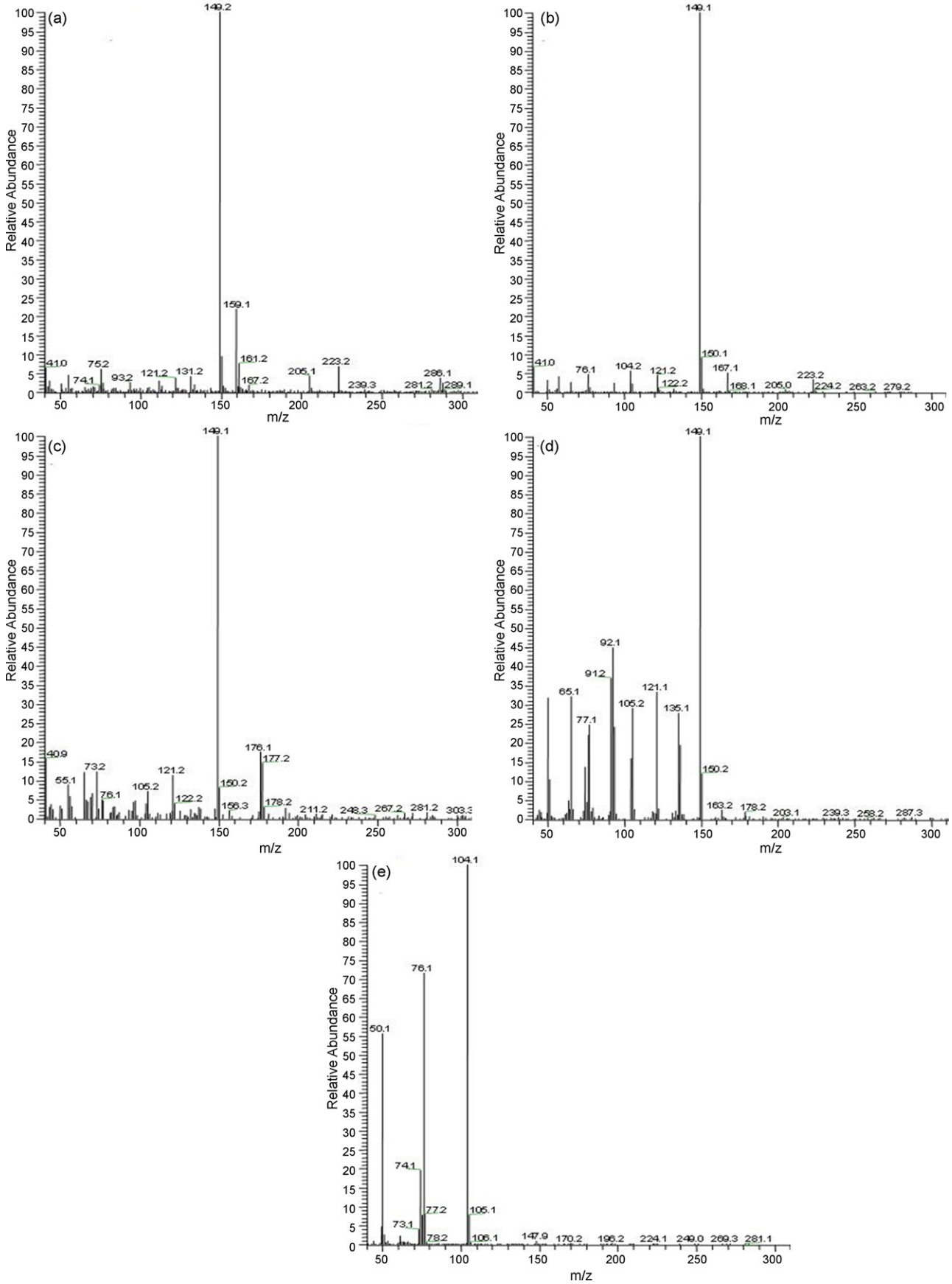
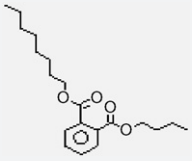
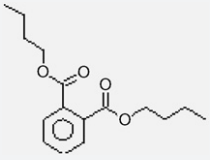
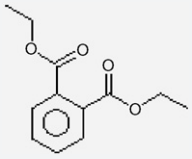
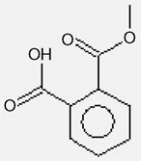
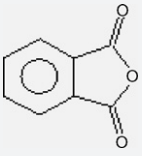


Fig. 5. MS spectra of the intermediates of DOP by *Gordonia* sp. strain JDC-2: (a) BOP, (b) DBP, (c) DEP, (d) MMP, and (e) IBF.

Table 3
Characterization of degradation products of DOP by *Gordonia* sp. strain JDC-2 determined by GC/MS analysis.

Name	Chemical formula	Structure	Retention time (min)	Major peaks (*m*/*z*)
BOP	$C_{20}H_{30}O_4$		39.07	41, 55, 76, 149, 150, 223
DBP	$C_{16}H_{22}O_4$		31.71	41, 76, 104, 149, 150, 205, 223
DEP	$C_{12}H_{14}O_4$		25.73	76, 149, 150, 176, 177
MMP	$C_9H_8O_4$		24.76	50, 75, 76, 77, 105, 149,
IBF	$C_8H_4O_3$		18.79	50, 75, 76, 77, 105, 148

DOP as the test compound, it was found that strain JDC-2 can effectively degrade DOP in 60 h with PA as the end product (Fig. 2). However, if strain JDC-32 was added, results indicated that co-culture of the two strains can degrade DOP completely in 48 h without leaving any residue of PA (Fig. 4). Furthermore, the results also indicated that degradation of DOP (500 mg/l) was also more efficient by the consortium than by the single strain JDC-2 (Fig. 2; 48 h *versus* 60 h). The reduction of degradation efficiency observed when JDC-2 was used alone was probably due to the accumulation of PA which in turn inhibits the degradation of DOP to some degree. The results of the current investigation suggest that strain JDC-2 initially transformed DOP rapidly to PA, while strain JDC-32 then carried out the degradation of PA. This finding may have broader ecological significance for a wide spectrum of pollutants and natural organic compounds [25].

In the current study, the products of DOP degradation by strain JDC-2 and the consortium were determined by GC/MS method. Unexpectedly, the degradative pathway was different from the most common de-esterification pathway that transforms di-esters to PA via monoesters [1]. Five different short alkyl side-chain phthalates (BOP, DBP, DEP, MMP, and IBF) were detected over the course of degradation (Fig. 5 and Table 3). The existence of BOP, DBP, and DEP suggests that the biodegradation of DOP by strain JDC-2 might be through β-oxidation. PAEs with longer side chains than DEP are occasionally converted to PAEs with shorter chains by β-oxidation, which removes one ethyl group each time [32]. Attack on the side chains of DOP could result in the production of BOP and then DBP. DOP transformation to DBP may occur by sequential removal of ethyl groups, before conversion to DEP. According to the literature, DEP is further converted to PA by de-esterification and

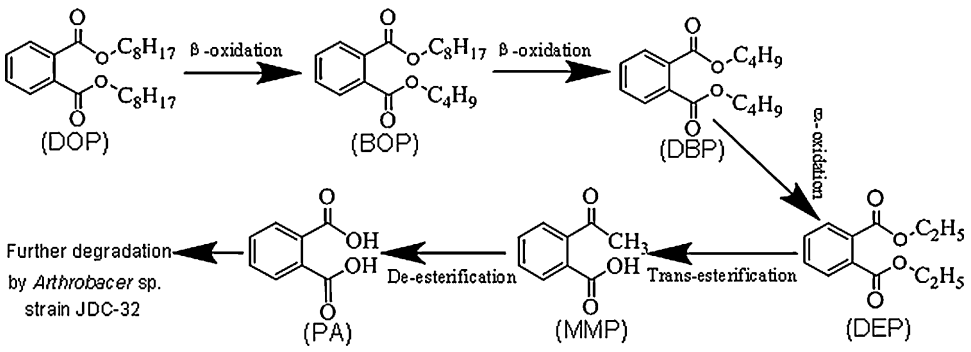


Fig. 6. Proposed biochemical pathway for biodegradation of DOP by *Gordonia* sp. strain JDC-2.

trans-esterification. In trans-esterification, proposed by Cartwright et al. [33], DEP is degraded by two replacements of an ethyl group with a methyl group, thereby producing ethyl–methyl phthalate and DMP, which is further degraded by de-esterification through MMP to PA. The production of MMP by strain JDC-2 implies that DEP might be trans-esterified to PA. The transitory intermediates, EMP and DMP, in our study were below detection limits. Furthermore, PA was notably not detected even though it was the end product of degradation determined by HPLC previously. IBF, which is structurally very similar to PA, was detected. Under similar conditions, GC/MS detected IBF among the products of DEHP [16], DBP [34], and DPrP [35] degradation by *Fusarium oxysporum*. In the present study, PA might have been transformed spontaneously into IBF through oxo-bridge formation since PA is known to decompose into water and IBF at temperatures over 210 °C. Nevertheless, the intermediate (PA) under the same GC/MS conditions had the same retention time and mass spectrum as the authentic PA standard. These results suggest that *Gordonia* sp. strain JDC-2 degrades DOP via a complex, metabolically versatile biochemical pathway (proposed pathway in Fig. 6). GC/MS analysis of the intermediates of DOP degradation by the consortium revealed DBP, OBP, and DEP (but not PA and MMP) as major products in amounts that were much less than that produced by a monoculture of strain JDC-2 suggesting that strain JDC-32 degrades the intermediates produced by strain JDC-2. The metabolites of PA due to ring-cleavage were not detected in this study possibly because only very minor amounts are formed and they are unstable in air.

5. Conclusion

Two bacterial strains, identified as *Gordonia* sp. strain JDC-2 and *Arthrobacter* sp. strain JDC-32, degraded DOP completely in 48 h. BOP, DBP, DEP, MMP, and PA were detected in the course of DOP degradation by *Gordonia* sp. strain JDC-2. We propose that *Gordonia* sp. strain JDC-2 transforms DOP sequentially to BOP, DBP, and DEP by β-oxidation and then converts DEP to PA through trans-esterification and de-esterification.

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