Combined genomic and transcriptomic analysis of Dibutyl phthalate metabolic pathway in Arthrobacter sp. ZJUTW

Tengfei Liu[1], Jun Li[1], Lequn Qiu[1], Fuming Zhang[2], Robert Linhardt[2], and Weihong Zhong[1]

[1]Zhejiang University of Technology
[2]Rensselaer Polytechnic Institute

May 5, 2020

Abstract

Dibutyl phthalate (DBP) is an environmental pollutant that can threaten human health. The strain Arthrobacter sp. ZJUTW, isolated from the sludge of river of Hangzhou city, can efficiently degrade DBP. Its genomic and transcriptomic differences when cultivated with DBP and with glucose revealed specific DBP metabolic pathways in the ZJUTW strain. The degrading gene clusters distribute separately on a circular chromosome and a plasmid pQL1. Genes related to the initial steps of DBP degradation from DBP to phthalic acid (PA), the pehA gene and pht gene cluster, are located on the plasmid pQL1. While pca gene cluster related to the transforming of protocatechuic acid (PCA) to acetyl-CoA, is located on the chromosome. After homologous alignment analysis with the reported gene clusters, we found that there were a series of double copies of homologous genes in pht and pca gene clusters that contribute to the efficient degradation of DBP by ZJUTW. In addition, transcriptomic analysis showed a synergistic effect between pht and pca clusters, which also favor ZJUTW allowing it to efficiently degrade DBP. Combined genomic and transcriptomic analyses affords the complete DBP metabolic pathway in Arthrobacter sp. ZJUTW that is different from that of reported other Arthrobacter strains. After necessary modification based on its metabolic characteristics, Arthrobacter sp. ZJUTW or its mutants might represent promising candidates for use in the bioremediation of DBP pollution.

1. INTRODUCTION

Phthalic acid esters (PAEs) are important synthetic organic compounds that are often added as plasticizers in plastics and plastic products. Because PAEs combine with plastics in the form of non-covalent bonds,PAEs can dissociate from plastic products and slowly release into the environment when the plastic products are exposed over time to physical factors such as light, weathering, and mechanical forces. As esters with poor aqueous solubility, PAEs accumulate in large quantities in soil and water systems (J. Wu, Liao, Yu, Wei, & Yang, 2013), causing serious environmental pollution. Studies have shown that PAEs have toxic effects such as carcinogenicity, teratogenicity, mutagenicity and developmental toxicity. Their accumulation in the environment may cause potential substantial harm to human health (Matsumoto, Hirata-Koizumi, & Ema, 2008; Benjamin et al., 2017).

PAEs including DBP, dimethyl phthalate (DMP), diethyl phthalate (DEP), di-n-octyl phthalate (DOP), benzyl butyl phthalate (BBP) and di-(2-ethylhexyl) phthalate (DEHP), have been listed as priority pollutants and environmental endocrine disruptors by the U.S Environmental Protection Agency (X. Y. Zhang et al., 2014). DBP is most widely and frequently used of the PAEs (Cheng, Liu, Wan, Yuan, & Yu, 2018). In China, the concentrations of soil accumulated DBP ranged from 0.04 mg/kg to 29.4 mg/kg (He et al., 2015; Niu, Xu, Xu, Yun, & Liu, 201). This accumulated DBP may enter plants and eventually the human body through the food chain. Therefore, the elimination of DBP in the environment is crucial for human health.

Removal strategies for pollutants, such as PAEs, usually include hydrolysis, photolysis, and microbial degradation. Recently, the utilization of microorganisms to degrade environmental PAEs has been considered one of the most effective ways for PAEs removal and has resulted in increased attention. For example, Gavala et al. introduced mesophilic anaerobic digestion for treatment of sludge containing PAEs via a process of biodegradation(Gavala, Yenal, & Ahring, 2004; Gavala, Alatriste-Mondragon, Iranpour, & Ahring, 2003).

An increasing number of PAEs degrading strains belonging to genera*Pseudomonas* , *Gordonia* , *Rhodococcus* , and*Sphingomonas* , have been isolated (Ren, Lin, Liu, & Hu, 2018; Liang, Zhang, Fang, & He, 2008; Benjamin, Pradeep, Sarath Josh, Kumar, & Masai, 2015). However, few DBP degrading strains belonging to genus*Arthrobacter* have been reported. Only a few DBP-degrading *Arthrobacter* stains, *A. keysery* 12B (Eaton, 2001),*Arthrobacter*sp. C21 (Wen, Gao, & Wu, 2014) and*Arthrobacter*sp. ZH2v (Y. Wang, Miao, Hou, Wu, & Peng, 2012b) have been isolated from the environment. The DBP degradation efficiency of these*Arthrobacter* strains is not sufficiently high for their commercial application. Thus, additional novel strains having higher DBP degrading ability are still required.

Recently, a possible metabolic pathway for the biodegradation of PAEs was proposed and divided into three steps (Figure 1): (1) PAEs are converted to PA by the action of a hydrolase; (2) PA is converted to PCA by series of enzymes encoded by the *pht* gene cluster; (3) PCA is transformed into acetyl-CoA, and then enters the tricarboxylic acid (TCA) cycle, and converted to CO_2 and H_2O (Ren et al., 2018). However, a complete BDP metabolic pathway is still required for the discovery of new and improved strains.

In our previous study, a novel DBP-degrading strain *Arthrobacter*sp. ZJUTW was isolated from the sludge of river of Hangzhou City, China (Chu, Liu, Zhang, & Qiu, 2017). This organism could degrade and grow on DBP, DEP, and DMP as the sole carbon source under the optimal conditions, 30 °C and pH 7.0-8.0. Resting cells could completely degrade 1200 mg/L of DBP. Thus, we concluded that ZJUTW exhibited a higher capacity of DBP degradation than organisms described in past publications. However, the mechanism of DBP metabolism needs to be elucidated before the potential application of this organism and its genetic modification to further improve its DBP degrading capacity. Therefore, this study was undertaken to combine comparative genomic and transcriptomic analysis to explore the genes and gene clusters involved in DBP biodegradation and to uncover the complete degradation mechanisms for*Arthrobacter* sp. ZJUTW degradation of DBP.

2. MATERIALS AND METHODS

2.1 Chemicals, bacterial strains and growth conditions

DBP,DMP,DEP,DEHP were purchased from Yonghua Chemical Co., Ltd. (Jiangsu, China), with a purity greater than 99.5%. Methanol was high-performance liquid chromatography (HPLC) grade and was purchased from Tianjin Siyou Fine Chemicals Co., Ltd. All other reagents were pure analytical grade.

Arthrobacter sp. ZJUTW, isolated from sludge of river of Hangzhou City, China had been deposited with the China Center of Typical Culture Collection (CCTCCM2012246).

Luria-Bertani (LB) medium was applied for bacterial enrichment. A modified basic inorganic salt medium (BSM) used for the degradation tests consisted of: $K_2HPO_4{\cdot}3H_2O$ 1.0 g,NaCl 1.0 g,$(NH_4)_2SO_4$ 0.5 g,$MgSO_4$*$7H_2O$ 0.4 g,$CaCl_2$ 0.0755 g,and $FeCl_3$ 0.0143 g.

2.2 DBP degradation by *Arthrobacter*sp. ZJUTW

Arthrobacter sp. ZJUTW strain was inoculated into LB medium, cultured at 30 degC for 12 h at 180 rpm. Bacterial cells were harvested by centrifuging at 4000 rpm for 10 min after the OD_{600} reached about 0.6. Pellets were washed three times with 0.1 mM phosphate buffered saline (PBS, pH = 7.2). The resuspended cells in the inorganic saline liquid medium were inoculated into BSM containing different initial DBP concentrations (50 mg/L,125 mg/L, 250 mg/L, 500 mg/L, and 1000 mg/L, respectively), and then

incubated under the optimum conditions (pH = 7.0-8.0, 30 degC, and 180 rpm). All experiments were performed in triplicate.

The supernatant was collected by centrifugation at 12000 rpm for 10 min after 48 h of incubation, then mixed with the equal volume of dichloromethane with violently oscillation. The aqueous phase was transferred to a rotary evaporator for drying. The dried substances were dissolved in 2 ml methanol and after filtering through a 0.22 μm membrane filter, the DBP concentration in methanol was measured using an Agilent 1260 HPLC (USA) under the following conditions: Diamonsil C18 column, 250 mm × 4.6 mm, 5 μm; the UV wavelength 235 nm; the mobile phase contained 90% (vol) methanol and 10% (vol) water; the flow rate,1.0 ml/min.

2.3 Genomic sequencing

After *Arthrobacter* sp. ZJUTW was cultured to the mid-exponential phase in LB medium, cells were harvested by centrifugation (10000 rpm, 10 min, 4 °C). Genomic DNA was extracted using an EasyPure Bacteria Genomic DNA Kit (TransGenBiotech, Beijing, China). The whole-genome sequencing of the strain ZJUTW was performed using PacBio SMRT. The raw reads were polished by using the SMRT Analysis workflow from Pacific Biosciences to obtain clean reads. The genome of the strain ZJUTW was re-sequenced by Illumina Hiseq to correct the PacBio results. The complete genome was obtained by sequence assembly using software canu, SPAdes (Bankevich et al., 2012). If there was a certain length of overlap at both ends of the final assembly sequence, the sequence was looped and one of the overlap sequences was truncated. Finally, the complete chromosome and plasmid sequences were obtained.

2.4 Genome annotations

After sequencing, gene prediction of the chromosome was carried out using Glimmer 3.02 software (*http://ccb.jhu.edu/software/glimmer/index.shtml*) and the GeneMarkS (*http://opal.biology.gatech.edu/GeneMark/*) was used for gene prediction of the plasmid. The predicted gene sequences were performed by sequence alignment with NR (Non-Redundant Protein Database), Swiss-Prot (*https://web.expasy.org/docs/swiss-prot_guideline.html*), GO (Gene Ontology) and KEGG (Kyoto Encyclopedia of Genes and Genome databases) to obtain functional annotation information. CGview software was used to implement circular genome visualization (Stothard & Wishart, 2005). The rRNA and tRNA contained in the genome were predicted using Barrnap 0.4.2 and tRNAscan-SE v1.3.1 software. Tandem Repeats Finder was used to predict tandem repeats. Gene Island (GI) was directed using soft IslandViewer (Bertelli et al., 2017). PHAST (Zhou, Liang, Lynch, Dennis, & Wishart, 2011) and Minced (Bland et al., 2007) were used to find phages and CRISPR-Cas.

2.5 Comparative genomic analysis

A total of 25 *Arthrobacter* strains chromosome sequences have been fully sequenced and were downloaded from NCBI. The strain ZJUTW was used as a reference genome and its genome's circular comparison with 25 strains were performed and visualized using BLAST Ring Image Generator (BRIG, version 0.95) (Alikhan, Petty, Ben Zakour, & Beatson, 2011). The specific fragments of the strain ZJUTW were identified based on the alignment results.

The phylogenetic position of ZJUTW among the 26 *Arthrobacter* strains was validated by using CVTree3 (tlife.fudan.edu.cn/cvtree). According to the phylogenetic tree of 26 strains of the genome, four strains with close evolutionary relationship were obtained. Their orthologous gene alignment analysis was based on protein sequence alignment. The BLAST software was used for comparison and extraction (E- value $< 10^{-5}$) of homologous gene pairs. Finally, the orthologous genes and the specific genes in the ZJUTW strain were displayed using a Venn diagram.

2.6 Mining of genes and gene clusters involved in PAE metabolism

Ester hydrolase and dioxygenase play a key role in PAE degradation. Ester hydrolase is responsible for removing the side chain of PAEs and the ring-cleavage process of aromatic compounds results from the action of dioxygenase. Based on the genome sequence obtained and the annotation results, all dioxygenase coding sequences and hydrolase coding sequences were retrieved and manually screened to identify dioxygenases involved in the catabolism of aromatic compounds. The genes of interest (dioxygenase and hydrolase genes) were located by the Basic Local Alignment Search Tools (BLAST) of National Center for Biotechnology Information (NCBI). In addition, the upstream and downstream sequences of the target genes were analyzed to identify possible gene clusters. Moreover, to obtain identified genes and gene clusters related to PAE degradation, we reviewed a large body of relevant literature. The proposed genes and gene clusters were also further analyzed by blast-2.4.0+ to show their similarity with reported gene clusters.

2.7 Total RNA isolation and sequencing

For transcriptomic analysis, *Arthrobacter* sp. ZJUTW was inoculated in BSM, supplemented with 0.1% glucose and 400 mg/L (w/v) DBP, respectively. After incubation at 30 °C and 180 rpm until the broth OD_{600} reached 0.4, the cells were harvested by centrifugation (10000 rpm, 10 min, 4 °C) and immediately mixed with RNA isolater from Vazyme Biotech stored at 80 °C for RNA extraction. The quality of extracted RNA was determined by the ND-5000 spectrophotometer (USA) and gel electrophoresis. The extracted RNA was sent to Beijing Nuohe Zhiyuan Technology Co., Ltd for RNA sequencing. The transcriptome sequencing of *Arthrobacter* sp. ZJUTW was performed based on the Illumina sequencing platform. The transcriptomic data was subjected to parametric analysis using*Arthrobacter* sp. ZJUTW as a reference genome. The clean reads were compared to the reference genome and reference gene sequences using the HISAT2 software and the Bowtie2 software, respectively. Quantitative analysis using RSEM (RNASeq by Expectation Maximization) software based on the alignment results and calculates the expression level of the gene. The analysis of differential expression focuses on finding genes that are differentially expressed between samples and conducting an in-depth analysis of these genes. The differentially expressed genes were screened for GO and KEGG enrichment by hypergeometric analysis.

3. RESULTS

3.1. DBP degrading ability of*Arthrobacter* sp. ZJUTW

Strain ZJUTW was capable of rapidly degrading low concentrations of DBP (Figure 2). When DBP concentration in BSM increased to 1000 mg/L, strain ZJUTW could still grow rapidly and degrade more than 90% of DBP within 18 h. The DBP degradation rate of strain ZJUTW could reach 50 mg/L/h. Other reported strains that can efficiently degrade DBP such as,*Rhodococcus* sp. JDC-11 could completely degrade 1 g/L DBP within 24 h with a degradation rate of 21.33 mg/L/h, *Bacillus* sp. (NCIM 5220) could degrade 2.783 g/L of DBP within 72 h with a degradation rate of 38.61 mg/L/h, and *Gordonia* sp. JDC2 could degrade 96% of 400 mg/L DBP within 18 h with a degradation rate of 21.33 mg/L/h. Thus, the ZJUTW strain exhibited the highest degradation rate among all reported DBP-degrading strains (Table 1).

3.2 Genomic analysis of *Arthrobacter*sp. ZJUTW

The sequencing results showed that the *Arthrobacter* sp. ZJUTW genome contained a chromosome and a plasmid pQL1 (Fig. S1). Its genomic nucleotide sequences were submitted to the GenBank databases under accession number CP043624 (chromosome) and CP043625 (plasmid). Genome features for *Arthrobacter* sp. ZJUTW are provided in Table 2. Using the IslandViewer for analysis, a total of seven gene islands including 125 hypothetical proteins, nine transposase genes, and some other functional proteins were predicted. Using Pre-phage prediction of PHAST, a total of 45 genes were predicted, including 36 putative proteins, one

esterase gene and 8 other functional proteins. The samples were analyzed by Minced software, and a total of six CRISPR-Cas structures with a total of 32 sequences were predicted.

3.3 Comparative genomics analysis between ZJUTW and other 25 *Arthrobacter*strains

Twenty-five *Arthrobacter* strains were selected for whole-genome comparison with the strain ZJUTW (Fig. S2). A total of 218 specific genes, accounting for 5.96% of all genes, were only found in*Arthrobacte* sp. ZJUTW. Among the 218 specific genes, 118 gene functions were undetermined and the others genes were transposase genes, molybdenum absorption and transformation related genes (*moaE* ,*moaA* ,*modA* ,*modB* ,*moeA*), benzoic acid metabolism-related genes (*xylX* , *xylY* ,*xylZ* , *xylL*), catechol metabolism-related genes (*catR* ,*catB* , *catC* , *catA*), and other functional genes. These specific genes suggest *Arthrobacter* sp. ZJUTW is capable of degrading benzoic acid, catechol and is capable of absorbing molybdate.

The phylogenetic tree analysis results of the whole-genome sequence of 26 strains (Figure 3A) showed that *Arthrobacter* sp. LS16,*Arthrobacter* sp. YC-RL1, and *Arthrobacter* sp. 7749 are evolutionally closer to ZJUTW than the other 22 strains. LS16 is capable of metabolism of phenolic compounds (Hassan et al., 2016), YC-RL1 can efficiently degrade p-xylene, naphthalene, phenanthrene, biphenyl, p-nitrophenol, and bisphenol (Ren et al., 2018). *Arthrobacter* sp. 7749 can oxidize phenylethanol derivatives (Sastre, Santos, Kagohara, & Andrade, 2017). The protein sequences of LS16, YC-RL1, and 7749 were obtained from NCBI and were subjected to pairwise alignment using local blast-2.4.0+ software (E-value $< 10^{-5}$). A Venn diagram (Figure 3B) shows 2167 homologous genes in the four*Arthrobacter* strains and 558 specific genes were found only in stain ZJUTW and not found in other 3 *Arthrobacter* strains. However, 400 of the 558 specific genes are pseudogenes, the remaining 158 genes are functionally annotated as 1 esterase, 7 ABC transporters,11 MFS transporters, 9 transposases, transcriptional regulators (GntR, PaaX, HxlR, MerR), and other functional enzymes. The related data are presented in Supplementary Table S1 and Table S2.

3.4 Genes and gene clusters involved in DBP metabolism

Based on the *Arthrobacter* sp. ZJUTW genome annotation results, there are 25 hydrolase genes, 9 esterase genes, and four dioxygenase genes involved in PAE metabolism. Some gene clusters related closely to biodegradation of DBP, including *pehA* ,*pht*cluster, and *pca* gene cluster, were identified in this study. The*pehA* encodes α/β hydrolase, which can convert DBP to PA. The*pht* cluster encodes phthalic acid catabolic enzymes that catalyze the conversion of PA to PCA. The *pca* gene cluster encodes the enzymes catalyzing the transformation of PCA into acetyl-CoA

3.4.1 Characteristics of *pht* gene cluster on ZJUTW plasmid pQL1

In the genome of ZJUTW, a *pht* gene cluster was found located on plasmid pQL1. The *pht* gene clusters have been also found in the genomes of other strains including *A. keyseri* 12B (Eaton, 2001),*Gordonia s* p. YC-JH1 (Fan et al., 2018), *Gordonia* sp. HS-NH1 (Li et al., 2016), *Arthrobcter* sp. 68b, *Terrabacter*sp. DBF63 (Habe et al., 2003), and *Mycobacterium vanbaaleniii*PYR-1 (Stingley, 2004). However, the gene architecture of the *pht*cluster in *Arthrobacter* sp. ZJUTW is different from that in*Gordonia* sp. YC-JH1 and *A. keyseri* 12B. Each gene of the*pht* gene cluster is adjacent to one another and is aggregated in*A.* keyseri 12B plasmid pRE1 and *Gordonia* sp. YC-JH1. There are *phtAa* , *phtAb* , *phtAc* , *phtAd* encoded for 3, 4-phthalate dioxygenase on the ZJUTW plasmid pQL1, the *phtAb* ,*phtAc* , *phtAd* have two copies. The positions of gene cluster *phtAb1Ac1Ad1* and *phtAaAb2Ac2Ad2* on the plasmid are far away from one another, 12113bp, and both of these have the same transcription direction. The *phtB* and *phtC* genes are transcribed in the same direction, at a distance of 25325bp. The transcriptional orientation of gene *phtB* and *phtC* is opposite to that of the two 3, 4-phthalate dioxygenase genes mentioned above. The amino acid sequence of the *pht* cluster of *Arthrobacter* sp. ZJUTW was done homology alignment analysis with known reports and the identities they share with each other are shown in (Figure 4A). The gene cluster*phtAaAbAdBC* found in ZJUTW was significantly different from the*pht* gene clusters present in other bacteria.

The genes for the initial hydrolysis of DBP are indispensable on plasmid pQL1 (52 kb) of ZJUTW. For example, the putative phthalate (PA) degrading genes encode the necessary enzymes for the conversion of phthalic acid to protocatechuic acid, are found on pQL1. PA degradation genes also found on other plasmids including pASPHE302 (94 kb) of*Arthrobacter phenanthrenivorans* Sphe3 (Vandera, Samiotaki, Parapouli, Panayotou, & Koukkou, 2015), pJ30-114 (98 kb) of *Arthrobacter* sp. J3-40A, p2MP (112 kb) of *Arthrobacter* sp. 68b (Stanislauskienė et al., 2011), and plasmid 2 (115 kb) of *Arthrobacter* sp. FB24. A synteny comparison analysis between the above-mentioned four plasmids and the plasmid pQL1 showed that pQL1 is significantly different from the other five plasmids. It is the smallest one among the five plasmids and poorly correlated with other plasmids (Figure 4B).

In addition, according to the annotation results of plasmid nucleic acid sequences, we found that there are 12 sequences related to gene transfer on the plasmid, ten of which are annotated as transposase genes and the other two are resolvase, some transposases are very close to the genes in the *pht* gene cluster (Figure 4A). These many mobile genetic components are most likely to participate in the shift ingression of the PA decomposition metabolic module, resulting in gene rearrangement, and in complex mosaic gene structures.

Moreover, the *pehA* is also located on the plasmid pQL1, and it is close to *phtR2* . The PehA has been successfully expressed exogenously in BL21 and the enzymatic properties have been determined. It can hydrolyze monoesters and diesters and is a bifunctional enzyme. The gene *pehA* serves an indispensable role in DBP-degrading.

In summary, the whole *pht* gene cluster present in the plasmid pQL1 is very different from all other reported *pht* gene clusters. Elimination of plasmid was performed by exposing the grown culture to sodium dodecyl sulfate (SDS), to obtain a mutant strain of ZJUTW without plasmid to further confirm the critical role of the *pehA* and*pht* gene cluster from the strain ZJUTW plasmid pQL1 during DBP degradation. The DBP degradation ability and cell growth of wild-type and mutant strains was evaluated from its growth curve (Figure 4C). The plasmid-eliminated strain was kept in a stagnant state where DBP was its sole carbon source. Wild type grows more rapidly with an OD_{600} reaching 0.4 in 12 h. This suggests that this strain ZJUTW could no longer degrade DBP after plasmid elimination. In addition, the GC content of the strain ZJUTW chromosome was 61.86%, and that of plasmid pQL1 was 57.23% (Table 2). The plasmid might result from a possible horizontal gene transfer.

3.4.2 Characteristics of the *pca* gene cluster on ZJUTW chromosome

The gene cluster *pcaHGBCDIJF,* which is involved in the protocatechuic acid (PCA) branch of the 3-ketoadipate pathway, is located on the chromosome of the ZJUTW strain. Specifically, genes *pcaI* , *pcaJ* , and *pcaF* in this cluster have two copies (i.e. *pcaI1* and *pcaI2,pcaJ1* and *pcaJ2, pcaF1* and *pcaF2*) that encode the enzymes catalyzing the transformation of protocatechuic acid into acetyl-CoA. Some aromatic compound degrading strains, for example,*Streptomyces* sp. 2065 (Iwagami, Yang, & Davies, 2000),*Gordonia* sp. YC-JH1 (Fan et al., 2018), *A. keyseri*12B, *Arthrobacter* sp.YC-RL1 (Ren et al., 2018) and*Rhodococcus opacus* 1CP (Eulberg, Lakner, Golovleva, & Schlömann, 1998), also carry PCA degradation-related genes in their genomes.

The *pca* gene cluster located on the ZJUTW strain chromosome displays major differences from all other *pca* gene clusters mentioned above. As shown in Figure 4D, the *pca* genes form up to three parts in the chromosome of the ZJUTW strain, and the three parts are far apart from each other. Gene cluster *pcaHGBL* is 289334 bp away from *pcaI1J1F1* , and *pcaI1J1F1* is 2571339 bp apart from *pcaI2J2F2* . The gene cluster *pcaI1J1F1* is closer to gene cluster *pcaHGBCD* compared with the gene cluster*pcaI2J2F2* . The amino acid sequence of the *pca* cluster of*Arthrobacter* sp. ZJUTW shares an identity of 36%-71% and 38%-62% with that of *Gordonia* sp. YC-JH1 and *Sreptomyces*sp. 2065, respectively. However, the gene cluster responsible for PCA degradation in *A. keyseri* 12B genome is called the*pcm* gene cluster, which has the same function to *pca* gene cluster, while carrying different genes. The *pcm* gene cluster harbors five key genes: *pcmA* (encoding protocatechuic acid 4,5-dioxygenase), *pcmB* (encoding 2-hydroxy-4-carboxymuconic semialdehyde dehydrogenase), *pcmC* (encoding 2-pyrone-4,6-dicarboxylate hydrolase), *pcmD* (encoding 4-oxalomesaconate

hydratase) and *pcmE* (4-oxalocitramalate aldolase). Moreover, there is no homology between the *pca* gene clusters in the ZJUTW strain and the *pcm* gene cluster, as analyzed using Blastp.

3.5 Differential transcriptional profile of ZJUTW under DBP and glucose

The transcriptional profile of *Arthrobacter* sp. ZJUTW grown on DBP and glucose was analyzed using RNA-seq. Among total 2908 genes detected (including that on chromosome and plasmid), 677 genes were up-regulated, 416 genes were down-regulated, and 1815 genes did not change significantly (Figure 5A). The gene expression level changed under growth on DBP and glucose carbon sources as shown in Figure 5B. It was also found that most of *pca*cluster genes fall in the up-regulated fields. Among 677 up-regulated and 416 down-regulated genes, 126 and 126 genes were significantly up-regulated (log2FoldChange [**?**] 2.0, p-value $<$ 0.05) and down-regulated (log2FoldChange $<$ 2.0, p-value $<$ 0.05), respectively (Supplementary Table S3).

Among the total of 558 specific genes in the *Arthrobacter* sp. ZJUTW genome, 60 genes were up-regulated (Supplementary Table S4), 23 genes were down-regulated (Supplementary Table S5), and 475 genes did not change significantly (Figure 3B). It is notable that not all specific areas exhibit significant responses to DBP. Only three genes (gene 2924, gene 3435, and gene 3504) out of 126 significantly up-regulated genes and six genes (gene 0928, gene 0929, gene 1081, gene 1082, gene 1083, and gene 3144) out of 126 significantly down-regulated genes, belong to 558 specific genes. Three significantly up-regulated specific genes of the ZJUTW strain, are annotated as hypothetical proteins (gene 2924 and gene 3435) and α-ketoglutarate transporter (gene 3504), respectively. The up-regulation of alpha-ketoglutarate transporter (gene 3504) may be a response of ZJUTW to DBP environment. Gram-positive bacteria have cell walls that contain high levels of peptidoglycans, approximately reaching 90%. When*Arthrobacter* sp. ZJUTW is grown in a BSM with high concentration DBP, its cell wall may be damaged. Peptidoglycan synthesis related genes will be induced to express to adapt to environmental pressure of DBP. Peptidoglycan consists of three parts: disaccharide unit, tetrapepitide side chain and peptide interbridge. The tetrapeptide side chain consists of four amino acids, and they are connected to each other by the L-type and D-type alternately. Because α-ketoglutarate is involved in most L-form amino acid transformations, alpha-ketoglutarate transporter (gene 3504) is crucial for the process. Therefore, about 5-fold upregulation of gene 3504 was detected under DBP stress (Supplementary Table S3).

Among the top ten genes that are most up-regulated, three are chaperone protein genes (GrpE, DnaK, and GroEL), two genes encode ClpB protein, one gene encodes ArsR family transcriptional regulator, one gene encodes anti-sigma factor, and the other three genes encode the MFS (major facilitator superfamily) transporter, flavin-dependent oxidoreductase and NADPH-dependent FMN reductase, respectively (Supplementary Table S6). According to previous publications (Thomas, Ayling, & Baneyx, 1997; Arnau, Sorensen, Appel, Vogensen, & Hammer, 1996; Hartke, Frère, Boutibonnes, & Auffray, 1997), it is known that when cells are exposed to extreme conditions, such as extreme temperatures and arsenite, a series of high-level expressions of hot shock proteins (HSPs),including chaperone proteins, such as GrpE, DnaK, GroEL and ClpB, are induced. PAEs are toxic to cells, when the ZJUTW strain is grown in BSM medium at high concentrations DBP, the permeability of the cell membrane will be changed, causing damage to the cells, causing some functional proteins to fail to fold properly. These factors induce the up-regulation of MFS (major facilitator superfamily) transporter and a series of chaperone protein (GrpE, DnaK, GroEL, ClpB). Significantly up-regulated expression of the MFS transporter gene can be correlated with DBP efflux. GrpE, DnaK belongs to the HSP70 protein family, GroEL belongs to the HSP60 protein family, and two ClpB belong to the HSP100 protein family. These chaperone proteins fold the newly synthesized peptide chain correctly, repair of misfolded polypeptides, degrade the inactive protein and enable the cells to grow normally and metabolize. Expression of GrpE, DnaK, GroEL chaperone proteins regulated by the σ32 factor. When intracellular stress response was reduced, the anti-sigma factor binds and sequester σ32, terminating the sustained transcription of these chaperone proteins. This may be the reason for the expression level of the anti-sigma factor is significantly up-regulated. A series of stress responses in the cells is caused by the high concentration of DBP,these biochemical reactions involve many intracellular redox reactions. We conclude

that a significant up-regulation of flavin-dependent oxidoreductases and NADPH-dependent FMN reductase genes may be associated with this.

3.6 Transcription level changes of DBP degrading related genes

The transcription levels of genes located on the *pht* gene cluster and *pca* cluster and *pehA* were measured to obtain a comprehensive understanding of the metabolic process of DBP. As mentioned above, when *Arthrobacter* sp. ZJUTW was cultured in BSM with DBP as the sole carbon source, a total of 126 genes are significantly up-regulated (Supplementary Table S3).

As shown in Supplementary Table S3, the expression level of the*pehA* is upregulated by 2.66-fold compared to the control group. Among *pht* gene cluster, which comprises *phtAb1* ,*phtAc1* , *phtAc1* , *phtR1* , *phtAa* , *phtAb2* ,*phtAc2* , *phtAd2* , *phtB* , and *phtC* , the expression level of the *phtAa* , *phtAb1* , *phtAb2* ,*phtAc2, phtAc1,* and *phtB* were dramatically upregulated with 3.05-, 3.52-, 3.47-, 5.12-, 4.25- and 5.02-fold, respectively. However, the expression level of *phtAc1* and*phtAd2* did not change significantly, while the transcription level of *phtC* is unknown. In summary, these results clearly indicate that the *pehA* and *pht* gene clusters play a significant role in DBP metabolism.

PcaH (the protocatechuic acid 3,4-dioxygenase β-subunit, gene 0406), and PcaG (the protocatechuic acid 3,4-dioxygenase α-subunit, gene 0407) are protocatechuic acid 3,4-dioxygenases,catalyzing the ring cleavage and the transformation of protocatechuic acid into 3-carboxy-cis, cis-muconate through ortho-cleavage. Compared to the control group, the expression levels of PcaH and PcaG were up-regulated by 6.17-fold, 5.64-fold, respectively. The PcaB (3-carboxy-cis, cis-muconate cycloisomerase, gene0405), catalyzing conversion of 3-carboxy-cis, cis-muconate to 4-carboxymuconolactone, was not detected using RNA-seq in transcriptomic analysis. The PcaC (4-carboxymuconolactone decarboxylase, gene0403) catalyzes the 4-carboxymuconolactone decarboxylation to form 3-oxoadipate enol-lactone. Its expression is increased by 3.31-fold. The PcaD (gene 0404) for the beta-ketoadipate enol-lactone hydrolase can hydrolyze 3-oxoadipate enol-lactone to form the 3-oxoadipate. Its expression level is up-regulated by 3.28-fold. Next, the 3-oxoadipate transformed into acetyl-CoA associated with two key enzymes. The one is 3-oxoadipate CoA-transferase, composed by PcaI1 (3-oxoadipate CoA-transferase subunit A) and PcaJ1 (3-oxoadipate CoA-transferase subunit B), 3-oxoadipate is converted to 3-oxoadipyl-CoA by this enzyme. The other one is PcaF1 (acetyl-CoA acetyltransferase), catalyzing the conversion of 3-oxoadipyl-CoA to acetyl-CoA. The expression of *pcaF2* , *pcaI12* and *pcaJ* 2 was not detected in the transcriptome. This result suggests that *pcaF1* ,*pcaI1* , and *pcaJ1* play a leading role in the 3-oxoadipate transformation process.

RT-qPCR analysis on some of the DBP degradation-related genes was performed to confirm the transcriptomic analysis. The RT-qPCR results also show that the expression level of *pehA* , *phtAa* ,*pcaD* , *pcaG* , *pcaF1* and *pcaJ1* , were up-regulated, consistent with transcriptome data (Figure 6). Combining the results of genomic analysis on the genes and gene clusters involved in DBP degradation and transcriptomic analysis, a possible complete metabolic pathway of DBP in strain ZJUTW could be proposed (Figure 7). In this specific pathway, two ester bonds of DBP are hydrolyzed by α, β-hydrolase (*pehA* encoded) to form PA. PA is then converted to PCA by a series of enzymes encoded by the *pht* gene cluster. Finally, PCA is transferred to acetyl-CoA through related enzymes (*pca* gene cluster encoded). Thus far, only one complete metabolic pathway for DBP in *A. keyseri* 12B has been reported among all*Arthrobacter* strains. In *A. keyseri* 12B,PCA is catalyzed by some enzymes encoded by the gene cluster *pcm* . The *pca*and *pcm* gene clusters encode completely different enzymes (Eaton, 2001). In addition, some key genes in the *pht* and *pca* gene clusters have double copies. Overall, the metabolic pathway of DBP in strain ZJUTW is distinct from the pathway in *A. keyseri* 12B.

4. DISCUSSION

4.1 Specific genetic architecture may contribute to efficiently degradation of DBP by ZJUTW

A large number of DBP-degrading strains have been isolated from the natural environment. For example, *Delftia tsuruhatensis* TBKNP-05 can tolerate and completely degraded 2783 mg/L DBP in 120 h (Patil, Kundapur, Shouche, & Karegoudar, 2006), *Bacillus* sp. NCIM:5220 entrapped in alginate gels can completely degrade 2783 mg/L DBP in 72 h (Patil & Karegoudar, 2005), and *Gordonia* sp. JDC2,*Gordonia* sp. JDC13, and *Gordonia* sp. JDC33 were obtained from activated sludge and showed a good ability to degrade DBP. JDC2 could degrade 96% of 400 mg/L DBP in 18 h. JDC13 could degrade 98% in 30 h and JDC33 could degrade 78% in 48 h (X. Wu, Wang, Dai, Liang, & Jin, 2011). *Pseudomonas* sp. V21b, isolated from soil, could degrade 57% of when the initial concentration of DBP was 1997 mg/L DBP, within 192 h (Kumar, Sharma, & Maitra, 2017). When the ZJUTW strain was cultured in BSM containing 1000 mg/L DBP, it could degrade more than 90% of DBP within 18 h. The DBP degrading rate of the ZJUTW strain was the highest among the reported DBP degrading strains (Table 1).

The highly efficient DBP degradation ability of *Arthrobacter* sp. ZJUTW may be related to the specific genetic architecture of its genome. According to the results of genome sequencing, we found that DBP degradation related gene *pehA* , and gene clusters *pht* and*pca* exhibit a favorable coexisting pattern. As shown in Figure 4A and 4D, the *pehA* is close to the *pht* gene cluster. In addition, a series of double copy genes were found both in the*pht* and *pca* gene clusters. To our knowledge, this is first reported that there are double copy genes in the *pht* gene cluster. The above two aspects of genetic architecture may play a role in the efficient degradation of DBP by ZJUTW.

> There is only one report of a complete metabolic pathway of DBP in genus*Arthrobacter* for *A. keysery* 12B (Eaton, 2001). However, its corresponding gene clusters are *pht* and *pcm*. The *pht* gene cluster in *A. keysery* 12B is homologous to that in strain ZJUTW, while the *pcm* gene cluster in strain 12B is not homologous to the *pca* gene cluster in the ZJUTW strain. Thus, the DBP metabolic pathway for ZJUTW is distinctly different from that found in *A. keysery* 12B.

4.2 Synergistic effect of the *pht* and*pca* gene clusters may also contribute to efficient degradation of DBP

Arthrobacter sp. ZJUTW exhibited highly efficient degradation of DBP. This may be closely related to the activity of enzymes encoding by*pht* and *pca* gene cluster, the number of copies of key genes, and the location of the two gene clusters in the genome. Some PAEs or PA degrading strains exhibit a different distribution of*pht* and *pca* gene clusters in their genomes. For example, in *A. keyseri* 12B, both of the *pht* gene cluster and gene cluster *pcmDECABF* responsible for PCA metabolism, are located on plasmid pRE1 (Eaton, 2001). In *Mycobacterium vanbaalenii* PYR-1, both the *phtRAaAbAcAd* gene cluster and the*pcaHGBLIJ* gene cluster are located on the chromosome (Stingley, 2004). In *Terrabacter* sp. strain DBF63, the *pht* gene cluster is located on its chromosome while the *pca* gene cluster is unmentioned (Habe et al., 2003). In *Rhodococcus* sp. RHA1, the PA degradation gene clusters are located two plasmids pRHL1 and pRHL2. (Hara, Stewart, & Mohn, 2010), while its PCA degradation gene cluster is located on the chromosome (Hara et al., 2010). In *Gordonia*sp.YC-JH1, both of the gene cluster *pcaRGHBLIJF* and*phtRAaAbAcAdBC* are located on the chromosome (Fan et al., 2018). In *A. phenanthrenivorans* Sphe3, two clusters that possibly constitute a phthalic acid operon and share an 87% identity with each other, are found on the two plasmids pASPHE301(190 kb) and pASPHE302 (94 kb) (Vandera et al., 2015). Through these comparisons, we show that the distribution of *pht* and *pca* gene cluster on the strain ZJUTW genome is specific to these PAE-degrading strains and each has its own particularity. However, the phenomenon of the two gene clusters, constituting complete metabolic pathway of a substance, distributed on a chromosome and a plasmid is not a special case in aromatic-degrading strains.

Based on the results of the transcriptome, we compared the transcription level between the genes of the*pht* gene cluster located on the plasmid pQL1 and the genes of the*pca* gene cluster located on the chromosome (Figure 7). The genes related to DBP degradation detected in the transcriptome show different degrees of up-regulation. The expression levels of the genes in the*pht* gene cluster were up-regulated range from 2.66- to 5.02-fold, and the expression levels of the genes in the*pca* gene cluster were up-regulated range from 2.34- to 6.17-fold. The related data are shown in Supplementary Table S3. Overall, the expression level of the *pht* gene cluster is higher than the*pca* gene cluster. We speculate that a series of DBP degradation related enzymes encoded by the *pht* gene cluster and gene*pehA* have high enzyme activity and that these enzymes can transform the initial substrate and metabolic intermediates rapidly, resulting in the accumulation of protocatechuic acid in the cells. The enzyme activity of protocatechuic acid degradation related enzymes encoded by the *pca* gene cluster may be lower than the enzymes encoded by the *pht* gene cluster. Therefore, to transform the accumulated protocatechuic acid in time, more key enzymes are needed to participate in the metabolic reaction, causing a significant increase in the transcription level of genes in the *pca* gene cluster. For DBP to be efficiently degraded and to reduce the consumption of energy and certain nutrients, as much as possible, there may be some regulatory mechanism in cells to regulate the transcription of key enzyme genes in *pht* gene cluster and*pca* gene cluster based on the amount of intracellular substrate and the accumulation of intermediate products.

5. CONCLUSION

The strain *Arthrobacter* sp. ZJUTW exhibited high degradation efficiency towards DBP. Based on the results of the genome sequencing and the transcriptome, we found the key gene *pehA* and the*pht* gene cluster and *pca* gene cluster involved in DBP degradation. The *pehA* and *pht* gene cluster are located in plasmid pQL1, and the *pca* gene cluster is in the chromosome. The particularity of the *pht* and *pca* gene cluster is determined by homology comparison analysis with reported PAEs-degradation gene clusters. The plasmid was successfully eliminated by using SDS from strain ZJUTW, and the plasmid elimination the strain ZJUTW could not grow in BSM with DBP was as the sole carbon source. The result demonstrates that plasmid pQL1 is critical for the strain ZJUTW to degrade DBP. To our best knowledge, we proposed a new complete metabolic pathway from DBP to Acetyl-CoA in the genus *Arthrobacter* for the first time.

ACKNOWLEDGEMENTS

This study was supported by the Zhejiang Provincial Natural Science Foundation of China under Grant No. LY15C010002, for that the authors are grateful.

CONFLICTS OF INTEREST

The authors declare no conflict of interest.

REFERENCES

Ahuactzin-Pérez, M., Tlecuitl-Beristain, S., García-Dávila, J., Santacruz-Juárez, E., González-Pérez, M., Gutiérrez-Ruíz, M. C., & Sánchez, C. (2018). Mineralization of high concentrations of the endocrine disruptor dibutyl phthalate by Fusarium culmorum. *3 Biotech* , *8* (1), 1–10. https://doi.org/10.1007/s13205-017-1065-2

Alikhan, N.-F., Petty, N. K., Ben Zakour, N. L., & Beatson, S. A. (2011). BLAST Ring Image Generator (BRIG): simple prokaryote genome comparisons. *BMC Genomics* , *12* (1), 402. https://doi.org/10.1186/1471-2164-12-402

Arnau, J., Sorensen, K. I., Appel, K. F., Vogensen, F. K., & Hammer, K. (1996). Analysis of heat shock gene expression in Lactococcus lactis MG1363. *Microbiology* , *142* (7), 1685–1691. https://doi.org/10.1099/13500872-142-7-1685

Bankevich, A., Nurk, S., Antipov, D., Gurevich, A. A., Dvorkin, M., Kulikov, A. S., ... Pevzner, P. A. (2012). SPAdes: A New Genome Assembly Algorithm and Its Applications to Single-Cell Sequencing. *Journal of Computational Biology* , *19* (5), 455–477. https://doi.org/10.1089/cmb.2012.0021

Benjamin, S., Masai, E., Kamimura, N., Takahashi, K., Anderson, R. C., & Faisal, P. A. (2017). Phthalates impact human health: Epidemiological evidences and plausible mechanism of action. *Journal of Hazardous Materials* , *340* , 360–383. https://doi.org/10.1016/j.jhazmat.2017.06.036

Benjamin, S., Pradeep, S., Sarath Josh, M., Kumar, S., & Masai, E. (2015). A monograph on the remediation of hazardous phthalates. *Journal of Hazardous Materials* , *298* , 58–72. https://doi.org/10.1016/j.jhazmat.2015.05.004

Bertelli, C., Laird, M. R., Williams, K. P., Lau, B. Y., Hoad, G., Winsor, G. L., & Brinkman, F. S. (2017). IslandViewer 4: expanded prediction of genomic islands for larger-scale datasets. *Nucleic Acids Research* , *45* (W1), W30–W35. https://doi.org/10.1093/nar/gkx343

Bland, C., Ramsey, T. L., Sabree, F., Lowe, M., Brown, K., Kyrpides, N. C., & Hugenholtz, P. (2007). CRISPR Recognition Tool (CRT): a tool for automatic detection of clustered regularly interspaced palindromic repeats. *BMC Bioinformatics* , *8* (1), 209. https://doi.org/10.1186/1471-2105-8-209

Cheng, J., Liu, Y., Wan, Q., Yuan, L., & Yu, X. (2018). Degradation of dibutyl phthalate in two contrasting agricultural soils and its long-term effects on soil microbial community. *Science of The Total Environment* , *640 –641* , 821–829. https://doi.org/10.1016/j.scitotenv.2018.05.336

Chu Jiaoyan, Liu Tengfei, Zhang Hanyu, & Qiu Lequan. (2017). Screening and characterization of a dibutyl phthalate-degrading bacterium. *Microbiology China* , *44* (7), 1555–1562. https://doi.org/10.13344/j.microbiol.china.170127

Eaton, R. W. (2001). Plasmid-Encoded Phthalate Catabolic Pathway in Arthrobacter keyseri 12B. *Journal of Bacteriology* ,*183* (12), 3689–3703. https://doi.org/10.1128/JB.183.12.3689-3703.2001

Eulberg, D., Lakner, S., Golovleva, L. A., & Schlömann, M. (1998). Characterization of a protocatechuate catabolic gene cluster from Rhodococcus opacus 1CP: evidence for a merged enzyme with 4-carboxymuconolactone-decarboxylating and 3-oxoadipate enol-lactone-hydrolyzing activity. *Journal of Bacteriology* ,*180* (5), 1072–1081.

Fan, S., Wang, J., Li, K., Yang, T., Jia, Y., Zhao, B., & Yan, Y. (2018). Complete genome sequence of Gordonia sp. YC-JH1, a bacterium efficiently degrading a wide range of phthalic acid esters. *Journal of Biotechnology* , *279* (January), 55–60. https://doi.org/10.1016/j.jbiotec.2018.05.009

Fand, Y., Zhang, L., Wang, J., Zhou, Y., & Ye, B. (2017). Biodegradation of Phthalate Esters by a Newly Isolated Acinetobacter sp. Strain LMB-5 and Characteristics of Its Esterase. *Pedosphere* ,*27* (3), 606–615. https://doi.org/10.1016/S1002-0160(17)60355-2

Feng, L., Liu, H., Cheng, D., Mao, X., Wang, Y., Wu, Z., & Wu, Q. (2018a). Characterization and Genome Analysis of a Phthalate Esters-Degrading Strain *Sphingobium yanoikuyae* SHJ. *BioMed Research International* , *2018* , 1–8. https://doi.org/10.1155/2018/3917054

Feng, N.-X., Yu, J., Mo, C.-H., Zhao, H.-M., Li, Y.-W., Wu, B.-X., Wong, M.-H. (2018b). Biodegradation of di-n-butyl phthalate (DBP) by a novel endophytic *Bacillus megaterium* strain YJB3. *Science of The Total Environment* , *616 –617* , 117–127. https://doi.org/10.1016/j.scitotenv.2017.10.298

Gavala, H. N., Alatriste-Mondragon, F., Iranpour, R., & Ahring, B. K. (2003). Biodegradation of phthalate esters during the mesophilic anaerobic digestion of sludge. *Chemosphere* , *52* (4), 673–682.

https://doi.org/10.1016/S0045-6535(03)00126-7

Gavala, H. N., Yenal, U., & Ahring, B. K. (2004). Thermal and enzymatic pretreatment of sludge containing phthalate esters prior to mesophilic anaerobic digestion. *Biotechnology and Bioengineering* ,*85* (5), 561–567. https://doi.org/10.1002/bit.20003

Habe, H., Miyakoshi, M., Chung, J., Kasuga, K., Yoshida, T., Nojiri, H., & Omori, T. (2003). Phthalate catabolic gene cluster is linked to the angular dioxygenase gene in Terrabacter sp. strain DBF63. *Applied Microbiology and Biotechnology* , *61* (1), 44–54. https://doi.org/10.1007/s00253-002-1166-6

Hara, H., Stewart, G. R., & Mohn, W. W. (2010). Involvement of a Novel ABC Transporter and Monoalkyl Phthalate Ester Hydrolase in Phthalate Ester Catabolism by Rhodococcus jostii RHA1. *Applied and Environmental Microbiology* , *76* (5), 1516–1523. https://doi.org/10.1128/AEM.02621-09

Hartke, A., Frère, J., Boutibonnes, P., & Auffray, Y. (1997). Differential Induction of the Chaperonin GroEL and the Co-Chaperonin GroES by Heat, Acid, and UV-Irradiation in Lactococcus lactis subsp. lactis. *Current Microbiology* , *34* (1), 23–26. https://doi.org/10.1007/s002849900138

Hashizume, K., Nanya, J., Toda, C., Yasui, T., Nagano, H., & Kojima, N. (2002). Phthalate esters detected in various water samples and biodegradation of the phthalates by microbes isolated from river water.*Biological and Pharmaceutical Bulletin* , *25* (2), 209–214. https://doi.org/10.1248/bpb.25.209

Hassan, I., Eastman, A. W., Weselowski, B., Mohamedelhassan, E., Yanful, E. K., & Yuan, Z.-C. (2016). Complete Genome Sequence of Arthrobacter sp. Strain LS16, Isolated from Agricultural Soils with Potential for Applications in Bioremediation and Bioproducts. *Genome Announcements* , *4* (1), 4–5. https://doi.org/10.1128/genomeA.01586-15

He, L., Gielen, G., Bolan, N. S., Zhang, X., Qin, H., Huang, H., & Wang, H. (2015). Contamination and remediation of phthalic acid esters in agricultural soils in China: a review. *Agronomy for Sustainable Development* , *35* (2), 519–534. https://doi.org/10.1007/s13593-014-0270-1

Hu, L. R. & Z. L. & H. L. & H. (2018). Bacteria-mediated phthalic acid esters degradation and related molecular mechanisms. *Applied Microbiology and Biotechnology* , *102* (3), 1085–1096. https://doi.org/10.1007/s00253-017-8687-5

Iwagami, S. G., Yang, K., & Davies, J. (2000). Characterization of the Protocatechuic Acid Catabolic Gene Cluster from Streptomyces sp. Strain 2065. *Applied and Environmental Microbiology* , *66* (4), 1499–1508. https://doi.org/10.1128/AEM.66.4.1499-1508.2000

Jin, D. C., Liang, R. X., Dai, Q. Y., Zhang, R. Y., Wu, X. L., & Chao, W. L. (2010). Biodegradation of Di-n-butyl phthalate by rhodococcus sp. JDC-11 and molecular detection of 3,4-phthalate dioxygenase gene.*Journal of Microbiology and Biotechnology* , *20* (10), 1440–1445. https://doi.org/10.4014/jmb.1004.04034

Jin, D., Kong, X., Cui, B., Bai, Z., & Zhang, H. (2013). Biodegradation of di-n-butyl phthalate by a newly isolated halotolerant Sphingobium sp.*International Journal of Molecular Sciences* , *14* (12), 24046–24054. https://doi.org/10.3390/ijms141224046

Kumar, V., & Maitra, S. S. (2016). Biodegradation of endocrine disruptor dibutyl phthalate (DBP) by a newly isolated Methylobacillus sp. V29b and the DBP degradation pathway. *3 Biotech* , *6* (2). https://doi.org/10.1007/s13205-016-0524-5

Kumar, V., Sharma, N., & Maitra, S. S. (2017). Comparative study on the degradation of dibutyl phthalate by two newly isolated Pseudomonas sp. V21b and Comamonas sp. 51F. *Biotechnology Reports* ,*15* (April), 1–10. https://doi.org/10.1016/j.btre.2017.04.002

Lee, S.-M., Lee, J.-W., Koo, B.-W., Kim, M.-K., Choi, D.-H., & Choi, I.-G. (2007). Dibutyl phthalate biodegradation by the white rot fungus,Polyporus brumalis. *Biotechnology and Bioengineering* ,*97* (6), 1516–1522. https://doi.org/10.1002/bit.21333

Li, D., Yan, J., Wang, L., Zhang, Y., Liu, D., Geng, H., & Xiong, L. (2016). Characterization of the phthalate acid catabolic gene cluster in phthalate acid esters transforming bacterium-Gordonia sp. strain HS-NH1.*International Biodeterioration and Biodegradation* , *106* , 34–40. https://doi.org/10.1016/j.ibiod.2015.09.019

Liang, D. W., Zhang, T., Fang, H. H. P., & He, J. (2008). Phthalates biodegradation in the environment. *Applied Microbiology and Biotechnology* , *80* (2), 183–198. https://doi.org/10.1007/s00253-008-1548-5

Liu, T. F., Zhang, H. Y., Chu, J. Y., & Qiu, L. Q. (2017). Biodegradation of di-n-butyl phthalate ester by newly isolated Raoultella sp . ZJY. *Bulgarian Chemical Communications* ,*49* , 104–108.

Matsumoto, M., Hirata-Koizumi, M., & Ema, M. (2008). Potential adverse effects of phthalic acid esters on human health: A review of recent studies on reproduction. *Regulatory Toxicology and Pharmacology* ,*50* (1), 37–49. https://doi.org/10.1016/j.yrtph.2007.09.004

Nahurira, R., Ren, L., Song, J., Jia, Y., Wang, J., Fan, S., ... Yan, Y. (2017). Degradation of Di(2-Ethylhexyl) Phthalate by a Novel Gordonia alkanivorans Strain YC-RL2. *Current Microbiology* ,*74* (3), 309–319. https://doi.org/10.1007/s00284-016-1159-9

Niu, L., Xu, Y., Xu, C., Yun, L., & Liu, W. (2014). Status of phthalate esters contamination in agricultural soils across China and associated health risks. *Environmental Pollution* , *195* , 16–23. https://doi.org/10.1016/j.envpol.2014.08.014

Patil, N. K., & Karegoudar, T. B. (2005). Parametric studies on batch degradation of a plasticizer di-n-butylphthalate by immobilized Bacillus sp. *World Journal of Microbiology and Biotechnology* ,*21* (8–9), 1493–1498. https://doi.org/10.1007/s11274-005-7369-0

Patil, N. K., Kundapur, R., Shouche, Y. S., & Karegoudar, T. B. (2006). Degradation of a Plasticizer, di-n-Butylphthalate by Delftia sp. TBKNP-05. *Current Microbiology* , *52* (3), 225–230. https://doi.org/10.1007/s00284-005-0258-9

Ren, L., Jia, Y., Ruth, N., Qiao, C., Wang, J., Zhao, B., & Yan, Y. (2016). Biodegradation of phthalic acid esters by a newly isolated Mycobacterium sp. YC-RL4 and the bioprocess with environmental samples.*Environmental Science and Pollution Research* , *23* (16), 16609–16619. https://doi.org/10.1007/s11356-016-6829-4

Ren, L., Jia, Y., Zhang, R., Lin, Z., Zhen, Z., Hu, H., & Yan, Y. (2018). Insight Into Metabolic Versatility of an Aromatic Compounds-Degrading Arthrobacter sp. YC-RL1. *Frontiers in Microbiology* , *9* (OCT), 1–15. https://doi.org/10.3389/fmicb.2018.02438

Sastre, D. E., Santos, L. P., Kagohara, E., & Andrade, L. H. (2017). Draft Whole-Genome Sequence of Psychrotrophic Arthrobacter sp. Strain 7749, Isolated from Antarctic Marine Sediments with Applications in Enantioselective Alcohol Oxidation. *Genome Announcements* ,*5* (43), 16–17. https://doi.org/10.1128/genomeA.01197-17

Stanislauskienė, R., Rudenkov, M., Karvelis, L., Gasparacitute, R., Meskiene, R., Časaite, V., & Messkys, R. (2011). Analysis of phthalate degradation operon from Arthrobacter sp. 68b. *Biologija* ,*57* (2), 45–54. https://doi.org/10.6001/biologija.v57i2.1828

Stingley, R. L. (2004). Novel organization of genes in a phthalate degradation operon of Mycobacterium vanbaalenii PYR-1.*Microbiology* , *150* (11), 3749–3761. https://doi.org/10.1099/mic.0.27263-0

Stothard, P., & Wishart, D. S. (2005). Circular genome visualization and exploration using CGView. *Bioinformatics* , *21* (4), 537–539. https://doi.org/10.1093/bioinformatics/bti054

Sun, R., Wang, L., Jiao, Y., Zhang, Y., Zhang, X., Wu, P., Yan, L. (2019). Metabolic process of di-n-butyl phthalate (DBP) by Enterobacter sp. DNB-S2, isolated from Mollisol region in China. *Environmental Pollution* , *255* , 113344. https://doi.org/10.1016/j.envpol.2019.113344

Thomas, J. G., Ayling, A., & Baneyx, F. (1997). Molecular chaperones, folding catalysts, and the recovery of active recombinant proteins fromE. coli. *Applied Biochemistry and Biotechnology* ,*66* (3), 197–238. https://doi.org/10.1007/BF02785589

Vandera, E., Samiotaki, M., Parapouli, M., Panayotou, G., & Koukkou, A. I. (2015). Comparative proteomic analysis of Arthrobacter phenanthrenivorans Sphe3 on phenanthrene, phthalate and glucose. *Journal of Proteomics* , *113* , 73–89. https://doi.org/10.1016/j.jprot.2014.08.018

Wang, J., Zhang, M. Y., Chen, T., Zhu, Y., Teng, Y., Luo, Y. M., & Christie, P. (2015). Isolation and identification of a di-(2-ethylhexyl) phthalate-degrading bacterium and its role in the bioremediation of a contaminated soil. *Pedosphere* , *25* (2), 202–211. https://doi.org/10.1016/S1002-0160(15)60005-4

Wang, Y., Miao, B., Hou, D., Wu, X., & Peng, B. (2012a). Biodegradation of di-n-butyl phthalate and expression of the 3,4-phthalate dioxygenase gene in Arthrobacter sp. ZH 2 strain. *Process Biochemistry* ,*47* (6), 936–940. https://doi.org/10.1016/j.procbio.2012.02.027

Wang, Y., Miao, B., Hou, D., Wu, X., & Peng, B. (2012b). Biodegradation of di-n-butyl phthalate and expression of the 3,4-phthalate dioxygenase gene in Arthrobacter sp. ZH2 strain. *Process Biochemistry* ,*47* (6), 936–940. https://doi.org/10.1016/j.procbio.2012.02.027

Wen, Z. D., Gao, D. W., & Wu, W. M. (2014). Biodegradation and kinetic analysis of phthalates by an Arthrobacter strain isolated from constructed wetland soil. *Applied Microbiology and Biotechnology* ,*98* (10), 4683–4690. https://doi.org/10.1007/s00253-014-5568-z

Wu, J., Liao, X., Yu, F., Wei, Z., & Yang, L. (2013). Cloning of a dibutyl phthalate hydrolase gene from Acinetobacter sp. strain M673 and functional analysis of its expression product in Escherichia coli. *Applied Microbiology and Biotechnology* , *97* (6), 2483–2491. https://doi.org/10.1007/s00253-012-4232-8

Wu, X. L., Wang, Y. Y., Liang, R. X., Dai, Q. Y., & Chao, W. L. (2010). Degradation of di-n-butyl phthalate by newly isolated Ochrobactrum sp. *Bulletin of Environmental Contamination and Toxicology* ,*85* (3), 235–237. https://doi.org/10.1007/s00128-010-0080-3

Wu, X., Wang, Y., Dai, Q., Liang, R., & Jin, D. (2011). Isolation and characterization of four di-n-butyl phthalate (DBP)-degrading Gordonia sp. strains and cloning the 3,4-phthalate dioxygenase gene. *World Journal of Microbiology and Biotechnology* , *27* (11), 2611–2617. https://doi.org/10.1007/s11274-011-0734-2

Yuan, L., Cheng, J., Chu, Q., Ji, X., Yuan, J., Feng, F., ... Yu, X. (2019). Di-n-butyl phthalate degrading endophytic bacterium Bacillus amyloliquefaciens subsp. strain JR20 isolated from garlic chive and its colonization in a leafy vegetable. *Journal of Environmental Science and Health - Part B Pesticides, Food Contaminants, and Agricultural Wastes* , *0* (0), 1–9. https://doi.org/10.1080/03601234.2019.1633211

Zeng, F., Cui, K., Li, X., Fu, J., & Sheng, G. (2004). Biodegradation kinetics of phthalate esters by Pseudomonas fluoresences FS1. *Process Biochemistry* , *39* (9), 1125–1129. https://doi.org/10.1016/S0032-9592(03)00226-7

Zhang, J., Zhang, C., Zhu, Y., Li, J., & Li, X. (2018). Biodegradation of seven phthalate esters by Bacillus mojavensis B1811. *International Biodeterioration and Biodegradation* ,*132* (April), 200–207. https://doi.org/10.1016/j.ibiod.2018.04.006

Zhang, X. Y., Fan, X., Qiu, Y. J., Li, C. Y., Xing, S., Zheng, Y. T., & Xu, J. H. (2014). Newly identified thermostable esterase from Sulfobacillus acidophilus: Properties and performance in phthalate ester degradation. *Applied and Environmental Microbiology* ,*80* (22), 6870–6878. https://doi.org/10.1128/AEM.02072-14

Zhao, H. M., Du, H., Feng, N. X., Xiang, L., Li, Y. W., Li, H., ... Mo, C. H. (2016). Biodegradation of di-n-butylphthalate and phthalic acid by a novel Providencia sp. 2D and its stimulation in a compost-amended soil. *Biology and Fertility of Soils* ,*52* (1), 65–76. https://doi.org/10.1007/s00374-015-1054-8

Zhao, H. M., Hu, R. W., Chen, X. X., Chen, X. Bin, Lü, H., Li, Y. W., ... Wong, M. H. (2018). Biodegradation pathway of di-(2-ethylhexyl) phthalate by a novel Rhodococcus pyridinivorans XB and its bioaugmentation for remediation of DEHP contaminated soil. *Science of the Total Environment* , *640 –641* , 1121–1131. https://doi.org/10.1016/j.scitotenv.2018.05.334

Zhou, Y., Liang, Y., Lynch, K. H., Dennis, J. J., & Wishart, D. S. (2011). PHAST: A Fast Phage Search Tool. *Nucleic Acids Research* ,*39* (suppl), W347–W352. https://doi.org/10.1093/nar/gkr485

Hosted file

`Image.docx` available at https://authorea.com/users/305216/articles/435875-combined-genomic-and-transcriptomic-analysis-of-dibutyl-phthalate-metabolic-pathway-in-arthrobacter-sp-zjutw