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Analysis of phthalate degradation operon from *Arthrobacter* sp. 68b

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Bacterial strain 68b was isolated from contaminated soil. According to 16S rDNA analysis it belongs to genus *Arthrobacter*. This strain is capable to utilise phthalic acid as a sole carbon source. This ability was proved by physiological and biochemical tests. By using resting cells, it was found out that *Arthrobacter* sp. 68b cells could use phthalic acid or convert quinolinic acid if they were pre-grown in the presence of phthalic acid. While analysing the results of a partially sequenced genome, the putative phthalate degradation operon (*pht*) was detected. It consisted of eight genes; seven genes could code the conversion of phthalate to protocatechuate. It was determined that the gene (*pehA*) of putative phthalate ester hydrolase is located upstream of *pht* operon. Genes of putative phthalate degradation operon were re-sequenced and their sequences fully corresponded to the *de novo* sequencing data. The homology search of genes revealed that all gene products are most similar to phthalate degradation proteins from other *Arthrobacter* spp. strains and confirmed that the strain 68b converts phthalate to protocatechuate by 3,4-dioxygenase pathway.

Key words: *Arthrobacter* sp., phthalic acid, 3,4-phthalate dioxygenase, quinolinic acid

INTRODUCTION

Phthalate (1,2-dicarboxybenzene) is a key metabolic intermediate in the aerobic degradation of phthalate esters and some polycyclic aromatic hydrocarbons such as pyrene, phenanthrene, fluorene and fluoranthene [1]. Phthalate esters (PEs) are industrial chemicals, widely used as plasticizers for polyvinyl chloride resins and as cellulose coatings. Their industrial application also includes the manufacturing of a variety of consumer and health care products [2]. Due to their widespread use, PEs have been found in sediments, natural waters, soils and aquatic organisms [2, 3].

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The metabolism of PEs by bacteria is considered a major fate of these widespread pollutants. It is believed that the metabolism of PEs is initiated in bacteria by their hydrolysis to phthalate and two alcohols [4, 5]. Two catabolic pathways have been identified for the aerobic degradation of phthalate (Fig. 1). Gram-negative bacteria (*Burkholderia cepacia*, *Pseudomonas* spp.) transform phthalate through oxygenation and dehydrogenation at carbons 4 and 5 to form 4,5-dihydroxyphthalate, followed by decarboxylation to yield protocatechuate, while gram-positive bacteria (*Arthrobacter keyseri*, *Rhodococcus* spp., *Terrabacter* spp., *Mycobacterium vanbaalenii*) initially oxidize phthalate to 3,4-dihydro-3,4-dihydroxyphthalate (phthalate dihydrodiol), which is subsequently dehydrogenated and decarboxylated to form protocatechuate [1, 4].

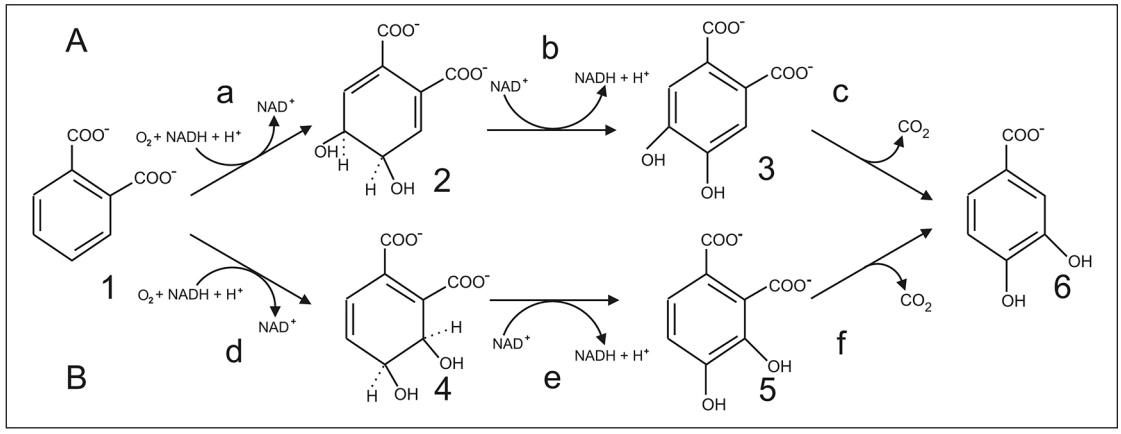

Fig. 1. General pathways for the initial degradation of phthalate by Gram-positive (A) and Gram-negative (B) bacteria. Chemical designations: (1) phthalate; (2) *cis*-4,5-dihydroxy-4,5-dihydrophthalate; (3) 4,5-dihydroxyphthalate; (4) *cis*-3,4-dihydroxy-3,4-dihydrophthalate; (5) 3,4-dihydroxyphthalate; (6) protocatechuate. Enzymes: (a) phthalate 4,5-dioxygenase; (b) *cis*-4,5-dihydroxy-4,5-dihydrophthalate dehydrogenase; (c) 4,5-dihydroxyphthalate-2-decarboxylase; (d) phthalate 3,4-dioxygenase; (e) *cis*-3,4-dihydroxy-3,4-dihydrophthalate dehydrogenase; (f) 3,4-dihydroxyphthalate-2-decarboxylase

The genus *Arthrobacter* are gram-positive, aerobic, nonmotile soil bacteria that occur in a wide variety of environmental niches. Phylogenetically the genus belongs to a group of GC-rich bacteria within the order of *Actinomycetales* that includes *Gordonia*, *Nocardia*, *Mycobacterium*, *Rhodococcus*. Various strains of *Arthrobacter* genus are able to gain energy from the degradation of a large variety of natural or man-made organic compounds present in their surroundings; therefore, they do not need any growth factors [6]. Recently, these microorganisms have received considerable attention because of their potential use in pesticides, aromatic and *N*-heterocyclic compounds and other industrial product waste detoxification. It has been shown that *Arthrobacter* spp. bacteria are capable of degrading nicotine [7], nitrophenols [8, 9], phtalates [4], carbaryl [10], atrazine [11, 12], phenmedipham [13], duron [14], pyridines [15–17] and other compounds.

To date, several microorganisms belonging to *Arthrobacter* spp. and capable to degrade phthalate are known. *Arthrobacter keyseri* 12B harbour the plasmid pRE1 which encodes a complete catabolism of phthalate [4]. Recently, *Arthrobacter* sp. strain WY capable to utilize butyl benzyl phthalate as well as phthalic and protocatechuic acids as sole carbon and energy source has been described [2].

At present, there are increasing demands for new chemical products that are used in environmentally friendly technologies and adopt sustainable approaches. These are driving the search for new biocatalysts for the development of new bioprocesses. Environmental microbes are considered to be the main source of new enzymatic activities owing to their enormous metabolic capability and diversity, much of which currently remains unexplored [18]. The new range of enzymes, especially the ones involved in various biodegradation pathways and enantiopure bioproducts, including di-, tri- and tetra-oxygenated metabolites resulting from tandem dioxygenase-catalysed oxidations of arene substrates, open exciting possibilities for the synthesis of new compounds [19, 20].

Various *N*-heterocyclic compounds and their derivatives are converted into useful chemicals by microbial or enzymatic oxidation [21–25]. It is known that cells of a *Pseudomonas putida* strain adapted for phthalate could convert 2,3-dicarboxypyridine (quinolinic acid) to a hydroxylated product. Phthalate 4,5-dioxygenase and 4,5-dihydro-4,5-dihydroxyphthalate dehydrogenase are involved in the initial steps of phthalate degradation by this bacteria [26]. However, it is not shown that quinolinic acid could be converted by the microorganisms possesing the phthalate 3,4-dioxygenase.

In this study, *Arthrobacter* sp. 68b strain, utilizing phthalate as a sole carbon and energy source by phthalate 3,4-dioxygenase pathway, is described. Details on the phthalate degradation operon from this bacteria and data on the bioconversion of 2,3-dicarboxypyridine (quinolinic acid) by phthalate-induced *Arthrobacter* sp. 68b cells are presented.

MATERIALS AND METHODS

Bacterial strains and plasmids. Bacterial strains *Escherichia coli* DH5α (*F*– (φ*80d*Δ (*lacZ*)*M15*) *recA1 endA1 gyrA96 thi1 hsdR17* (*rk*–*mk*+) *supE44 relA1 deoR* Δ (*lacZYA*–*argF*) *U169* (Pharmacia)) and *Arthrobacter* sp. 68b (capable to utilize phthalic acid as a sole carbon and energy source (laboratory strain)) were used in this study. Plasmid

pTZ57R / T (Ap^R, *lacZ*, f1, 2.9 kb, 3'-ddT overhangs at both ends (JSC "Fermentas", Lithuania)) was used for fragment cloning and sequencing.

E. coli DH5α bacteria were cultivated on NA (nutrient agar 28 g/L) (Oxoid) plates at 37 °C or in NB (nutrient broth 13 g/L) (Oxoid) aerobically at 30 °C. Ampicillin (Fluka) was added to the media at the final concentrations of 50 μg/ml. X-Gal and IPTG (JSC "Fermentas", Lithuania) were used at 10 mM final concentration. *Arthrobacter* sp. 68b bacteria were grown in mineral media EFA (10 g/L K_2HPO_4, 4.0 g/L KH_2PO_4, 1.0 g/L $(NH_4)_2SO_4$, 0.5 g/L yeast extract, 0.4 g/L $MgSO_4 \times 7H_2O$, 10 ml/L salt solution (2.0 g/L $CaCl_2 \times 2H_2O$, 1.0 g/L $MnSO_4 \times 4H_2O$, 0.5 g/L $FeSO_4 \times 7H_2O$ dissolved in 0.1N HCl)) at 30 °C with shaking (180 rpm) or on EFA agar plates at 30 °C.

Evaluation of optimal phthalic acid growth concentration. To perform the growth curves, 1 mL of *Arthrobacter* sp. 68b bacteria night culture was transferred to 20 mL of mineral media EFA containing 0.1–1% of phthalic acid. Media without carbon source was used as control. Optical density was measured by spectrophotometer at 600 nm length wave in the cuvette of 1 cm width every 24 hours.

DNA isolation and manipulation. Total DNA from *Arthrobacter* sp. 68b was isolated by the method proposed by Woo et al. [27]. Plasmid DNA from *E. coli* was isolated by the alkaline lysis method [28]. Restriction endonucleases, phosphotase (FastAP), T4 DNA ligase were purchased from "Fermentas" (Lithuania) and used as recommended. DNA for sequencing was purified using ZYMO Plasmid MiniPREP (Zymo Research). *E. coli* cells were prepared for electroporation by the method of Sharma & Schimke [29]. Electroporation into 100 μl of the cells was performed using the electroporator 2510 (Eppendorf, Germany) with the electrical conditions selected as 2.0 kV/cm and impulse duration of 5.0–5.6 ms. Transformed *E. coli* bacteria were incubated at 37 °C for 1 hour, thereafter cells were spread on solid NA with ampicillin.

DNA sequencing and analysis. Total DNA from *Arthrobacter* sp. 68b was applied for 454 pyrosequencing (Macrogen, South Korea). The BLAST software was used for the analysis of contigs [30]. Primers for phthalic acid degradation operon genes were designed using DNA from a partially sequenced genome (Table 1). PCR products were cloned in pTZ57R / T and re-sequenced using standard sequencing primers M13 (R and F). 16S rRNA encoding gene (1.5 kb fragment) was amplified using universal primers w001 and w002. The PCR product was purified with a DNA purification kit and cloned into a pTZ57R / T plasmid. The sequencing was performed at Macrogen (South Korea). VectorNTI 9.0 [31] was used for the DNA and protein analysis. The nearest homologues for the phylogenetic analysis of proteins were picked by using the BLAST family programmes (NCBI) [30]. The evolutionary history was inferred using the neighbour-joining method [32]. The evolutionary distances were computed using the Poisson correction method [33] and were found in the units of the number of amino acid substitutions per site. The phylogenetic analyses were carried out by MEGA 5 [34].

Detection of phthalate 4,5-dioxygenase activity. To detect phthalate 4,5-dioxygenase activity, a diazotized *p*-nitroaniline was used as described by Nomura et al. [26]. The reagent was prepared by mixing 50 volumes of a 0.3% (w/v) solution of *p*-nitroaniline in 0.8 N HCl and 3 volumes of a 5% $NaNO_2$ solution immediately before use. *Arthrobacter* sp. 68b bacteria were cultivated 48 hours in EFA medium containing 0.5% phthalic acid at 30 °C by shaking. Cells were harvested and washed with 20 mM Tris-HCl buffer (pH 8.0) and suspended in the same buffer containing 2.5 mM of quinolinic acid. The cell suspension was incubated at 30 °C and the samples were taken after 1 and 2 hours. Cells were eliminated by centrifugation and the supernatant was added with 2 μL of diazotized *p*-nitroaniline reagent per mL. The absorption spectrum was read over the range of 300 to 700 nm.

Resting cell reaction. Cells were grown in 20 mL of EFA containing 0.5% phthalic acid for 48 hours, harvested at 10 °C by centrifugation at 3.220 g for 10 min, washed two times with 20 mM potassium phosphate buffer (pH 7.5), suspended in 10 mL and used as the resting cells. Cells from 1.5 mL culture broth were incubated in 20 mM

Table 1. **Primers used for the amplification of phthalate degradation and *pehA* genes**

Primer name, amplified region	Primer sequence
FEH (F) phthalic ester hydrolase	gacatccgaacagtttaggaagagg
FEH (R) phthalic ester hydrolase	gtatatctaggcgggcatctctag
HFDH (F) 3,4-dihydroxy-3,4-dihidrophthalate dehidrogenase	ctagagatgcccgcctagatatac
HFDH (R) 3,4-dihydroxy-3,4-dihidrophthalate dehidrogenase	ggttctggtatgaagagaatgga
FOXG (F) phthalate dioxygenase large and small subunits	gagatccgtccatgactgaccac
FOXG (R) phthalate dioxygenase large and small subunits	gtcctcttcttcgccttcccatt
FOXGS (F) ferredoxin and reductase subunits	gaggacgagcagtcgccccagaaca
FOXGS (R) ferredoxin and reductase subunits	ctgcctttgctcttcgtgggccac
FDK (F) 3,4-dihidroxyphthalate-2-decarboxylase	cagcgtgcaggaactccgggacaaac
FDK (R) 3,4-dihidroxyphthalate-2-decarboxylase	cgcatgctttgtatcgtacccagcg

potassium phosphate buffer (pH 7.5) with an appropriate dicarboxylic acid in a 1 mL reaction mixture. The reaction was carried out in an orbital shaker (350 rpm) at 30 °C temperature.

The nucleotide sequences. The nucleotide sequences, determined in this study, were deposited in the EMBL-Bank under the accession numbers AJ879122 (16S rDNA) and JN381019 (*pht* operon).

RESULTS

The 68b strain was isolated from the contaminated soil. The analysis of 16S rDNA sequence revealed that it belonged to the genus *Arthrobacter*. The partial sequencing of the *Arthrobacter* sp. 68b genome was carried out. While analysing the sequencing results, the putative phthalate degradation operon was detected. To confirm the *de novo* sequencing data, a set of primers was chosen according to the DNA sequence (Table 1). All PCR reactions using these primer pairs and total DNA from *Arthrobacter* sp. 68b were positive (data not shown). Moreover, the amplified DNA fragments were re-sequenced and their sequences fully corresponded to the *de novo* sequencing data.

The ability to utilize phthalic acid was proved by cultivating bacteria on media containing phthalic acid as a sole carbon source (Fig. 2). The optimal phthalic acid growth concentration for *Arthrobacter* sp. 68b was determined as described in "Materials and methods". The best bacteria growth after 24 hours was observed when phthalic acid concentrations ranged from 0.5–1% (Fig. 2). After 72 hours, the decrease of optical density was observed in all cultures. Bioconversion of phthalic acid by resting cells was additionally analysed to confirm that cells of *Arthrobacter* sp. 68b could consume this substrate (Fig. 3). It was found that *Arthrobacter* sp. 68b cells could use phthalic acid only if they were pre-grown in the presence of this substrate (Fig. 3, B). The cells cultivated in the presence of succinic acid were not able to consume phthalic acid (Fig. 3, A). Hence, phthalate utilization is an inducible process in the *Arthrobacter* sp. 68b cells.

A more detailed analysis of the phthalate degradation operon (*pht*) from *Arthrobacter* sp. 68b showed that it consisted of eight open reading frames (ORF) (Fig. 2, Table 2), all transcribed in the same direction. Seven genes arranged in the order *phtBAaAbAcAdCR* could code the conversion of phthalate to protocatechuate by 3,4-dioxygenase pathway (Fig. 1, B).

Comparison of the organization of genes, involved in phthalate metabolism in *Arthrobacter* sp. 68b and *Arthrobacter keyseri* 12B, revealed that the genes of both operons are arranged in the same order and transcribed in the same direction (Fig. 4). However, *Arthrobacter* sp. 68b operon has an additional gene *orf4* that is inserted between the genes

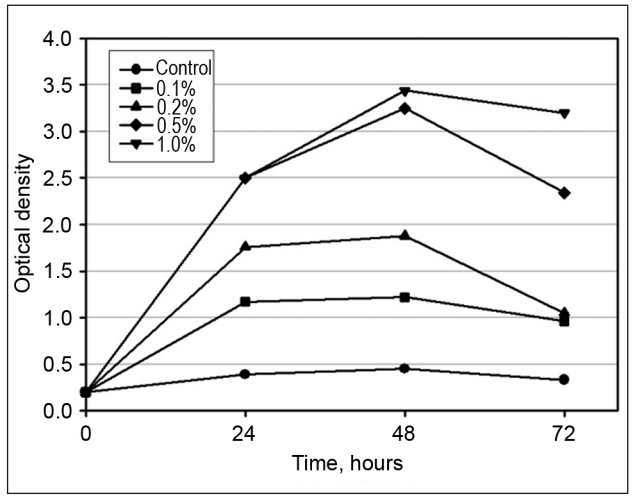

Fig. 2. Growth of *Arthrobacter* sp. 68b in EFA medium containing various concentrations of phthalic acid. Control EFA medium without phthalic acid

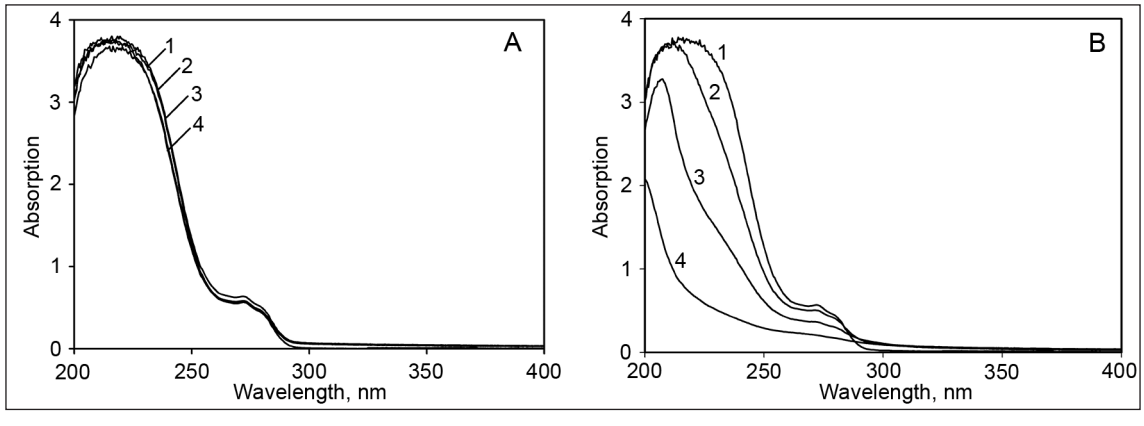

Fig. 3. Spectral changes during aerobic conversion of phthalic acid by resting cells of *Arthrobacter* sp. 68b pre-grown with succinate (A) and phthalic acid (B) in 20 mM potassium phosphate buffer (pH 7.5) at 30 °C. Initial substrate concentration was 1 mM. The reaction mixture after centrifugation (16.000 × g, 1 min) was scanned at 1, 2, 3, 4 hours

Table 2. **Genes and gene products**

ORF	Gene	Protein length (aa)	Homology	GenBank accession No.	E value	Reference
ORF1	*phtB*	287	3,4-dihydroxy-3,4-dihydrophthalate dehydrogenase, *Arthrobacter keyseri*	AF331043_13	8e-126	[4]
ORF2	*phtAa*	474	phthalate dioxygenase large subunit, *Arthrobacter keyseri*	AF331043_14	0	[4]
ORF3	*phtAb*	202	phthalate 3,4-dioxygenase subunit beta, *Arthrobacter phenanthrenivorans* Sphe3	YP_004243272	5e-97	[38]
ORF4	*orf4*	111	hypothetical protein Arth_4365, *Arthrobacter* sp. FB24	YP_829383	5e-46	Unpublished
ORF5	*phtAc*	65	ferredoxin, *Arthrobacter phenanthrenivorans* Sphe3	YP_004243274	1e-24	[38]
ORF6	*phtAd*	411	phthalate 3,4-dioxygenase, ferredoxin reductase subunit, *Arthrobacter phenanthrenivorans* Sphe3	YP_004243275	0	[38]
ORF7	*phtC*	249	3,4-dihydroxyphthalate decarboxylase, *Arthrobacter phenanthrenivorans* Sphe3	YP_004243276	7e-111	[38]
ORF8	*phtR*	264	PhtR family transcriptional regulator, *Arthrobacter phenanthrenivorans* Sphe3	YP_004243277	3e-121	[38]

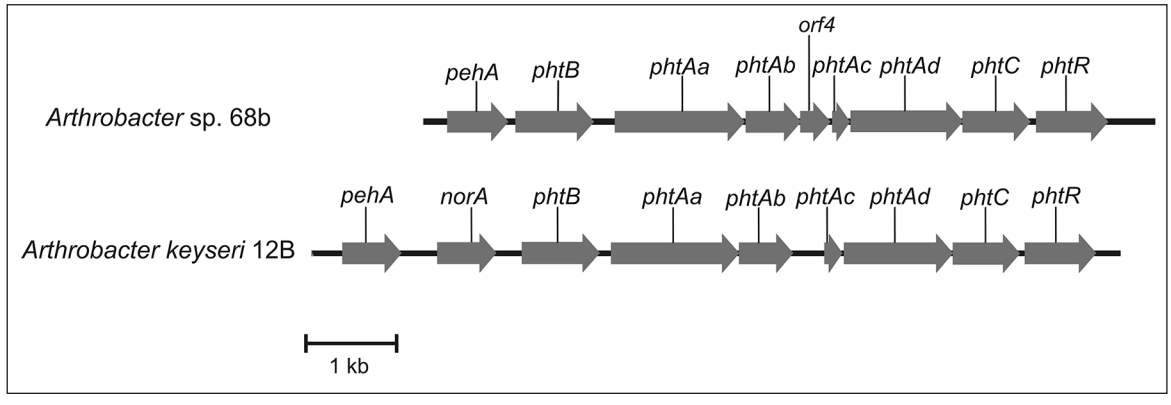

Fig. 4. Phthalate degradation operons and the organization of genes in *Arthrobacter* sp. 68b and *Arthrobacter keyseri* 12B

phtAb and *phtAc*. The *orf4* overlaps with the gene *phtAb* by four nucleotides, while the start codons of genes *phtC* and *phtAd* overlap with the stop codon of the preceding gene by one nucleotide. The gene *pehA,* encoding a putative phthalate ester hydrolase, is located upstream of *pht* operon in both cases. This gene is transcribed in the same direction as genes of phthalate degradation operon. An additional gene (*norA*, a fragment of antibiotic resistance transporter) is located between the gene *pehA* and *pht* operon in the case of *A. keyseri* 12B, however, none homologous gene was found in *Arthrobacter* sp. 68b.

As the organization of both operons was similar, genes of the phthalate operon from *Arthrobacter* sp. 68b were named in the manner of those of *A. keyseri* 12B. The homology search of genes revealed that all gene products were most similar to the phthalate degradation proteins from other *Arthrobacter* spp. strains (Table 2).The genes *phtAaAbAcAd* encode a phthalate 3,4-dioxygenase. Gene *phtAa* encodes protein (53.7 kDa) that is most homologous to *A. keyseri* 12B phthalate dioxygenase large subunit. The most similar proteins are phthalate 3,4-dioxygenase large subunits or ring hydroxylating dioxygenase alpha subunits generally from *Rhodococcus* sp. and *Mycobacterium* sp. *Arthrobacter* sp. 68b *phtAa* product together with *A. keyseri* 12B and *Arthrobacter* sp. FB24 proteins make a discrete group in the phylogenetic tree (Fig. 5, A). The product (22.8 kDa) of gene *phtAb* is analogous to the phthalate 3,4-dioxygenase subunit beta or a small subunit and the 3-phenylpropionate dioxygenase subunit beta from various *Arthrobacter* sp., *Rhodococcus* sp. and *Mycobacterium* sp. strains. As the phylogenetic tree presents the *Arthrobacter* sp. 68b protein, it shares the same branch with the proteins from *Arthrobacter* sp. FB24, *A. keyseri* 12B and *A. phenanthrenivorans* Sphe3 (Fig. 5, B). The *phtAc* polypeptide (7.0 kDa) is related to the electron transfer protein ferredoxin. Its homologues are proteins from various *Actinomycetales* bacteria species.

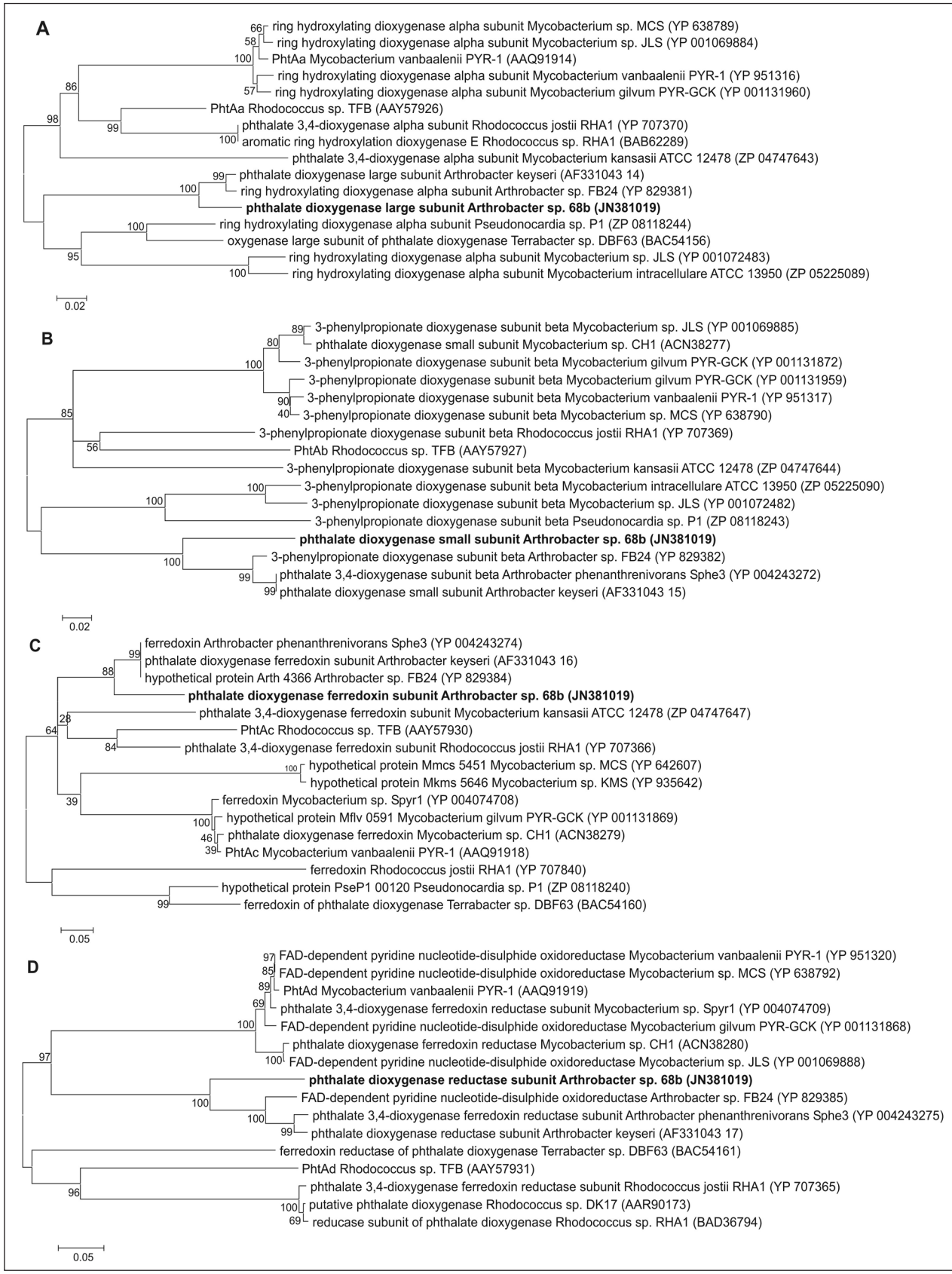

Fig. 5. The phylogenetic tree of phthalate 3,4-dioxygenase from *Arthrobacter* sp. 68b. A – large subunit, B – small subunit, C – ferredoxin subunit, D – reductase subunit. The numbers at the branches show how many times the group to the right of the branch occurred among the 100 trees generated in a bootstrap analysis. Scale bar represents the expected amino acid substitutions per position. The GenBank accession number is indicated for each protein. Details of phylogenetic method are given in Materials and methods

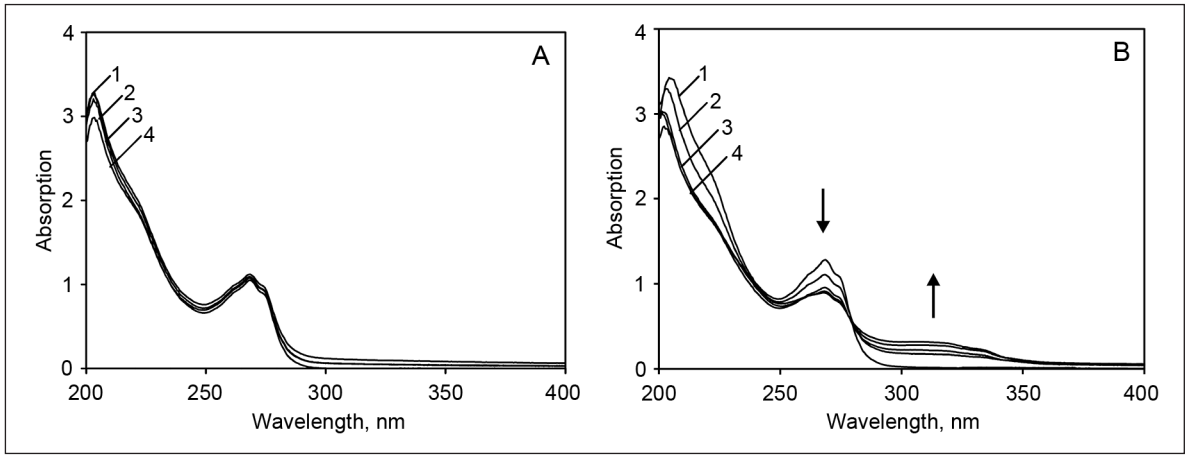

Fig. 6. Spectral changes during aerobic conversion of quinolinic acid by resting cells of *Arthrobacter* sp. 68b pre-grown with succinate (A) and phthalic acid (B) in 20 mM potassium phosphate buffer (pH 7.5) at 30 °C. Initial substrate concentration was 0.4 mM. The reaction mixture after centrifugation (16.000 × g, 1 min) was scanned at 1, 2, 3, 4 hours. Arrows show changes in absorption during bioconversion

PhtAc is the most similar to proteins from *Arthrobacter* sp. FB24, *A. keyseri* 12B and *A. phenanthrenivorans* Sphe3. Together, they make a discrete branch in the phylogenetic tree (Fig. 5, C). The *phtAd* encoded protein (43.7 kDa) is similar to the phthalate 3,4-dioxygenase ferredoxin reductase subunit, FAD-dependent pyridine nucleotide-disulphide oxidoreductase. The phylogenetic analysis of the protein revealed that it is most related to the proteins from *Arthrobacter* spp. and form together a separate phylogenetic branch (Fig. 5, D).

The phtB product (31.3 kDa) is homologous to aldo/keto reductases, 2,5-didehydrogluconate reductases, 2,5-diketo-D-gluconate reductases and oxidoreductases from various *Actinomycetales* bacteria and several proteobacteria. PhtB is the most similar to 3,4-dihydroxy-3,4-dihydrophthalate dehydrogenase from *A. keyseri* 12B. The product (26.2 kDa) of *phtC* is homologous to 3,4-dihydroxyphthalate decarboxylase, class II aldolase / adducin family protein from *Arthrobacter* spp., *Rhodococcus* spp. and *Mycobacterium* spp. *phtR* encodes 29.2 kDa protein that is similar to the transcriptional regulators of PhtR or IclR families from *Actinomycetales* bacteria. In the *N*-terminus of PhtR protein, a HTH motif was detected. It is typical to IclR family proteins that can act as a transcriptional activators or repressors.

It was known that cells of a *Pseudomonas putida* strain, harbouring both phthalate 4,5-dioxygenase and 4,5-dihydro-4,5-dihydroxyphthalate dehydrogenase and adapted for phthalic acid, could also convert quinolinic acid to a hydroxylated product [26]. The formation of this compound was visualized by the reaction with diazotized *p*-nitroaniline with which a red compound with the absorbtion maximum at 512 nm was produced [26]. The phthalic acid induced cells of *Arthrobacter* sp. 68b were tested on their ability to form a similar product under the same conditions, however, no formation of the red compound was observed. Bioconversion of quinolinic acid by resting cells was tested to determine if phthalate-induced cells of *Arthrobacter* sp. 68b could consume quinolinic acid. It was found that *Arthrobacter* sp. 68b cells converted quinolinic acid if they were pre-grown in the presence of phthalic acid. The changes in the UV-VIS spectrum during a typical biotransformation are illustrated in Fig. 6 (B). The cells, cultivated in the presence of succinic acid, were not able to use quinolinic acid (Fig. 6, A). Hence, an induction of phthalate catabolic genes was necessary for the utilization of quinolinic acid by *Arthrobacter* sp. 68b cells. Moreover, the absorbance increase in the region 300–350 nm during the bioconversion procedure was observed.

DISCUSSION

Partial *de novo* sequencing of *Arthrobacter* sp. 68b genome shows that this microorganism harbours the genes encoding phthalic acid degradation. The organization of genes in *Arthrobacter* sp. 68b and *A. keyseri* 12B phthalate degradation operons is similar, though it differs from the organizations of *pht* operons in other *Actinomycetales*, for example, phthalate degradation genes are arranged in order *phtRAaAbBAcAdC* in *Rhodococcus* sp. DK17 [35] and *Rhodococcus* sp. TFB [36] strains. The decarboxylase encoding gene is not presented in *pht* operon of *Mycobacterium vanbaalenii* PYR-1, hence, genes are set in *phtRAaAbBAcAd* order [37]. All genes of the operons are transcribed in the same direction, except the regulatory protein gene (*phtR*), which is located upstream of the operon and is transcribed from the opposite strand in these strains [35–37]. Phylogenetic analysis of *pht* genes

encoding proteins shows that *Arthrobacter* sp. 68b biodegradation of phthalic acid has to proceed through the 3,4-dioxygenation step (Fig. 1, B).

Physiological and biochemical tests confirm the capability of *Arthrobacter* sp. 68b to utilize phthalic acid. Moreover, that is an inducible process.

It should be noted that the phthalate degradation genes of *A. keyseri* 12B are situated on the plasmid pRE1 and form an operon [4]. *Arthrobacter phenanthrenivorans* Sphe3 plasmid pASPHE301 contains genes that could be involved in phthalate degradation [38]. The previous findings that phthalic acid degradation genes in various *Actinomycetales* (*Arthrobacter* and *Rhodococcus* spp.) are plasmid located [35, 36, 39] raise the question if a catabolic plasmid encoding analogous genes exist in *Arthrobacter* sp. 68b cells. Further experiments have to be carried out to elucidate this possibility.

Bioconversion of quinolinic acid by phthalate induced *Arthrobacter* sp. 68b cells is rather interesting, since it is generally believed that only phthalate 4,5-dioxygenase and 4,5-dihydro-4,5-dihydroxyphthalate dehydrogenase harbouring microorganisms are capable of such transformation [26]. During the quinolinic acid biotransformation by resting *Arthrobacter* sp. 68b cells, the UV absorption maximum shifted into the longer wavelength area (Fig. 6, B), indicating the introduction of a hydroxyl group into the heterocyclic ring [40]. There are several possibilities to form the hydroxylated derivatives of quinolinic acid. The first, quinolinic acid is initially attacked by phthalate 3,4-dioxygenase. Then, the 4,5-dihydroxy-4,5-dihydroquinolinic acid is oxidized by 3,4-dihydroxy-3,4-dihydrophthalate dehydrogenase to 4,5-dihydroxyquinolinic acid. The second, quinolinic acid is initially attacked by phthalate 3,4-dioxygenase. Then, the 4,5-dihydroxy-4,5-dihydroquinolinic acid is spontaneously dehydrated to 4-hydroxy or 5-hydroxyquinolinic acid. The products of both, either the first or the second bioconversions, are promising as synthons, since they are not easily obtainable by the known methods of organic chemistry. However, further experiments have to be carried out to determine the structure of the formed compound.

Concluding, it has to be noted that a new phthalate degrading microorganism belonging to *Arthrobacter* genus is characterized. Bioconversion of quinolinic acid by 68b strain bacteria opens a possibility for biocatalytic synthesis of new hydroxylated *N*-heterocyclic compounds.

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FTALIO RŪGŠTIES SKAIDYMO OPERONO IŠ *ARTHROBACTER* SP. 68B ANALIZĖ

Santrauka

68b bakterinis kamienas buvo išskirtas iš užteršto dirvožemio. Remiantis 16S rDNR analize, jis priklauso *Arthrobacter* genčiai. Šis kamienas sugeba panaudoti ftalio rūgštį kaip vienintelį anglies šaltinį – tai buvo įrodyta fiziologiniais ir biocheminiais metodais. Nustatyta, kad *Arthrobacter* sp. 68b ląstelės sugeba panaudoti ftalio rūgštį arba modifikuoti chinolino rūgštį, jei bakterijos buvo kultivuotos terpėje su ftalio rūgštimi. Nagrinėjant iš dalies nustatyto genomo seką, buvo aptiktas galimas ftalio rūgšties skaidymo operonas (*pht*), kurį sudaro aštuoni genai, septyni iš jų dalyvauja ftalio rūgšties skaidyme iki protokatechuato. Nustatyta, kad prieš *pht* operoną yra galimos ftalato esterio hidrolazės genas (*pehA*). Visi operono genai buvo individualiai padauginti PGR metodu ir nustatytos jų sekos. Sekoskaitos rezultatai visiškai atitiko *de novo* gautas sekas. Genų homologų paieškos rezultatai rodo, kad visų genų produktai yra panašiausi į kitų *Arthrobacter* genties kamienų ftalio rūgšties degradacijos baltymus, ir patvirtino, kad 68b kamienas ftalio rūgštį verčia iki protokatechuato 3,4-dioksigenazės būdu.

Raktažodžiai: *Arthrobacter* sp., ftalio rūgštis, 3,4-ftalato dioksigenazė, chinolininė rūgštis