

DIMETHYLTEREPHTHALATE CATABOLISM BY *PSEUDOMONAS* SP.

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Summary

Pseudomonas sp. strain 054 isolated from polluted soil utilizes dimethylterephthalate (DMT) as a sole carbon and energy source. The degradation of this compound starts with a two-stage hydrolysis of the ester bonds to monomethylterephthalate and terephthalic acid. In the meta-pathway for cleavage of the aromatic ring the latter is metabolized to protocatechuate. The induction of the ester hydrolysis is slightly specific, while the oxygenase mechanism is induced by substrates with aromatic structure.

Introduction

The phthalates and their derivatives are widely distributed and applied in various industrial productions. At the same time they are among the first compounds included in the list of the environmental pollutants [7]. This provokes big scientific interest to their biological degradation [4, 8, 11, 18]. The catabolic schemes of a number of phthalate derivatives have already been investigated [2, 9, 14]. However, the metabolism of the terephthalic acid (TA) and its esters is considerably less studied. The dimethylterephthalate (DMT) is the main raw material for the production of the Bulgarian fiber

Jambolen. In other countries the textile fibers Lavan, Terilen, Dacron, etc., are prepared from the same monomer. Some bacteria from the genera *Rhodococcus* [19] and *Mycobacterium* [15] and some fungi from the genus *Aspergillus* [6] are found to degrade terephthalates.

In the present paper the assimilation of DMT by *Pseudomonas* sp. strain 054 is discussed. The strain is isolated from polluted soil and adapted to utilize this aromatic compound. The stages of the metabolism of the dimethyl ester are investigated.

Materials and Methods

Microorganism and cultivation. The investigated microorganism – *Pseudomonas* sp. strain 054, was isolated from the soil by enrichment procedure [20]. The culture was grown on a minimal salt medium supplemented with DMT in concentration 1000 mg/l as a sole carbon and energy source. The cultivation was performed on a rotor shaker, 200 rpm, at 28°C for 4 days. The residual substrate amount was colorimetrically determined [20]. The biomass was estimated on the basis of the optical density and the dry mass [21].

Thin layer chromatography (TLC). The intermediate products of the DMT degradation were determined by TLC. The culture liquid (24th hour and 48th hour of cultivation) was filtrated. Concentrated HCl was applied to acidify the filtrate to pH 2. Ethylacetate was used for the extraction. The obtained extract was dried over Na₂SO₄ for 24 hours. A rotary evaporator was used for the solvent separation. The residue was applied to a Kieselgel plate 60 F 254 in a system of solvents - benzene:dioxane:acetic acid = 90:25:4 [4]. For detection of the spots UV absorption at 254 nm was used.

Preparation of the cell-free enzyme extract. The cell-free extract for the enzymological investigation was harvested by centrifugation of the culture liquid (48th hour) on Beckman centrifuge, 12 000 g for 10 min

at 4°C. The received biomass was washed twice with a buffer, pH 7.6. An ultrasound disintegrator UD-20 was used for the cell disruption at 20 kHz for 2-3 min. The intact cells and the cell debris were separated by centrifugation at 12 000 g for 3 min at 4°C [5].

Determination of the esterase activity. The esterase activity was assayed by a modified method of Poutanen [16]. The enzyme sample (with 0.05 - 0.2 mg protein content) and α -naphthylacetate in 50 mM citrate buffer, pH 5.3, were incubated in a shaker for 15 hours at 26°C. Then the absorption at $\lambda = 535$ nm was measured. One esterase unit was defined as the amount of enzyme, which produced 1 μ M α -naphthole for 1 min under the standard assay conditions.

Determination of the protocatechuate-4,5-dioxygenase activity. The enzyme activity was determined spectrophotometrically by the method of Ono [17]. The reaction mixture (3 ml) contained 1 μ M protocatechuate, 150 μ M TRIS-acetate buffer, pH 9.0, and a suitable amount of the enzyme. The accumulation of the product was measured at 410 nm. One unit of the enzyme activity was determined as that amount which grades 1 μ M protocatechuate per 1 min at 24°C.

Determination of the protein. Protein in the enzyme extract was determined by the method of Lowry using bovine serum albumine as a standard [12].

Results and Discussion

Pseudomonas sp. strain 054 utilized DMT as a sole carbon and energy source. The degradation of this aromatic compound and the bacterial growth are presented in Fig. 1. DMT was totally assimilated up to the 96th hour of the culture growth. No residual amount of the substrate was detected after this hour.

The intermediate products, which were

produced by the catabolism of the aromatic substrate, were proved by thin layer chromatography. Two intermediate products were detected for the both samples. The R_f-values of the spots 1 and 2 were identical with those of the standard monomethylterephthalate (MMT) and terephthalic acid (Table 1). As an intermediate metabolic product protocate-

chuate was not registered. These results made us suggest that the initial steps of the metabolism of this aromatic substrate included a two-stage hydrolysis through the monoester. A similar result was observed for *Rhodococcus* by Slizen [19] and for *Aspergillus niger* by Ganii [6].

Another result was reported by Kurane [10]. *Nocardia erythropolis* possessed a much higher affinity of the phthalate-hydrolysing enzyme for the monoester. It was rapidly hydrolysed to phthalic acid without accumulation of phthalate monoester.

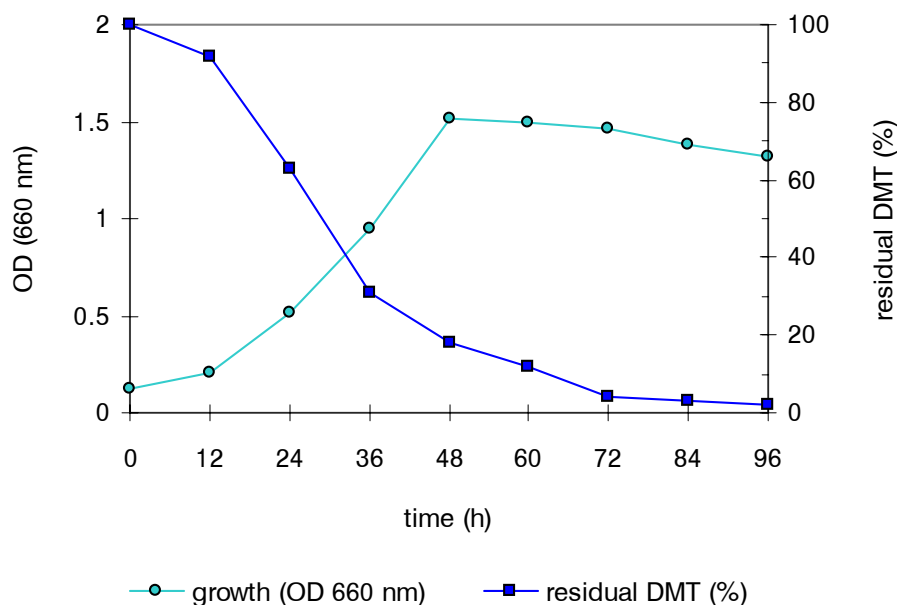


Fig. 1. Biodegradation of DMT from *Pseudomonas* sp. strain 054.

Table 1. Thin layer chromatography of the products of degradation of DMT from *Pseudomonas* sp. strain 054.

Samples	Rf-values
Spot I	0.73
Spot II	0.53
Standards	
DMT	0.82
MMT	0.73
TA	0.53
PC	0.41

For determination of the esterase activity *Pseudomonas* sp. strain 054 was cultivated on media with DMT, TA, succinate and tributyrin as sole carbon sources respectively. The results are presented in Table 2. In all the cases enzyme activity was observed which suggested that this enzyme was characterised by low substrate specificity and

constitutive synthesis. This was registered for *Pseudomonas fluorescens* [1] and *Micrococcus* sp. strain 12 B [3]. In *Nocardia* the phthalate-hydrolysing enzyme was isolated and purified by Kurane. It was defined as a new type of lipase with broad substrate specificity [10]. This enzyme had an inductive character as well as the DMT-esterase in *Aspergillus niger* [6].

We were interested in the oxygenase activity of the tested strain *Pseudomonas* sp. 054. Cell-free extracts from cells growing on DMT, TA and succinate were investigated for protocatechuate-4,5-dioxygenase activity. The obtained results are shown in Table 2. The specific enzyme activity of the cells, growing on DMT and TA was similar – 2.45 and 1.98 U/mg protein respectively. The cells growing on succinate did not show oxygenase activity. This was an indication for the inductive synthesis of these types of enzymes. This fact

Table 2. Esterase and protocatechuate-4,5-dioxygenase activities of *Pseudomonas* sp. strain 054.

Growth substrate	Specific activity (U/mg protein)	
	Esterase	Protocatechuate-4,5-dioxygenase
DMT	1.58 ± 0.011	2.453 ± 0.007
MMT		1.892 ± 0.003
TA	0.59 ± 0.005	
Succinate	0.48 ± 0.010	0
Tributyryn	1.02 ± 0.008	

was confirmed by many authors for different aromatic compounds [3,13,18]. Protocatechuate-3,4-dioxygenase activity was not registered. This proved the suggestion that the assimilation of the compound was performed by the meta-pathway cleavage of the aromatic ring.

In conclusion we should underline that *Pseudomonas* sp. 054 showed high ability to utilize DMT. For 96 hours it almost completely

assimilated DMT in concentration 100 mg/l. Our investigation shows that the metabolism of this substrate starts with hydrolysis to monomethylterephthalate and terephthalic acid. The aromatic ring is decomposed in the meta-pathway. The esterase activity is a constitutive property of the investigated strain while the oxygenase activity is induced by aromatic compounds.

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КАТАБОЛИЗЪМ НА ДИМЕТИЛТЕРЕФТАЛАТ ОТ *PSEUDOMONAS SP.*

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Резюме

Щам Pseudomonas sp. 054, изолиран от замърсена почва, усвоява диметилтерефталат (ДМТ) като единствен въглероден и енергетичен източник. Разграждането на ароматния субстрат започва с двустепенна хидролиза на естерните връзки до монометилтерефталат и терефталова киселина. Последната се метаболизира през протокатехоат по мета-пътя на разцепване на ароматното ядро. Индукцията на естерната хидролиза е слабо специфична, а оксигеназният механизъм се индуцира от субстрати с ароматна структура.