

EXTRA- AND INTRACELLULAR ESTERASES INVOLVED IN DIMETHYLTEREPHTHALATE CATABOLISM BY *PSEUDOMONAS* SP.

Lilia Tserovska^{1*}, Raycho Dimkov², Tanya Rasheva³ and Tanya Yordanova¹

¹National Bank for Industrial Microorganisms and Cell Cultures; 1113 Sofia, P.O.Box 239, Bulgaria; ²Sofia University, Faculty of Biology, Department of Hydrobiology and Ichthyology, 8 Dragan Tzankov blvd., 1164 Sofia, Bulgaria; ³Sofia University, Faculty of Biology, Department of Microbiology, 8 Dragan Tzankov blvd., 1164 Sofia, Bulgaria

*Corresponding author, e-mail: l_tserovska@abv.bg

Summary

The strain Pseudomonas sp. 054, isolated previously from polluted soil, possesses a high biodegrading activity towards the aromatic ester dimethylterephthalate. The ester hydrolyzing activity of the strain was investigated. Two types of esterases, extra- and intracellular, were detected after growth in liquid medium. The enzyme activities were proved in the culture supernatant and in the crude cell extract by the use of a native electrophoresis. The extracellular protein with an esterase activity had a molecular weight of 67 kDa, while the intracellular fraction produced four separate bands with ester hydrolyzing activity.

Key words: *Pseudomonas, dimethylterephthalate, esterases.*

Introduction

The ester hydrolyzing enzymes (esterases) are a roughly specified group of enzymes (EC 3.1.1.2) of a great importance for the primary attack of xenobiotics, including phthalic derivatives [2]. Many insoluble or hardly soluble organic substrates become biodegradable only with the aid of esterases.

The diversity of the identified catabolic pathways for the aromatic ring cleavage suggests the existence of a great natural genetic pool [6, 14]. The initial stages of degradation, before the substrate enters the central metabolism, are poorly studied. Kurane et al. have isolated and purified a phthalate ester hydrolyzing enzyme from *Nocardia erythropolis* [7]. It exhibits a wide substrate specificity but is inactive towards the phthalic acid *p*-isomers.

Our investigations were focused on the *p*-

isomer of the terephthalic acid – dimethylterephthalate (DMT). It is a main raw material and a hardly degradable waste in the polyester fibre Yambolen production. In a previous study it was found that the bacterial strain 054 utilized DMT as a sole carbon and energy source. This strain, determined as *Pseudomonas* sp., degraded completely DMT in 72 hours. The degradation of the compound included two-stage hydrolysis and cleavage of the aromatic ring at *m*-position to protocatechuate [12]. Biodegradation and biotransformation of xenobiotics with aromatic structure by *Pseudomonas* strains was recorded by many other authors [4, 11, 13].

In this work we have paid attention to the initial microbial attack of DMT and describe the existence and localization of an ester hydrolyzing activity in the strain *Pseudomonas* sp. 054.

Materials and Methods

Microorganisms and growth conditions.

The bacterial strain *Pseudomonas* sp. 054 was isolated from DMT polluted soil. It was adapted to growth in a minimal medium containing DMT as a single carbon and energy source by the use of enrichment cultures. The cultivation was performed periodically on a shaker at 200 rpm, 28 °C, as described previously [12].

Preparation of extracts for electrophoretic analysis. The extracellular extract was prepared from the supernatant of a 2-days liquid culture. The protein fraction was concentrated by precipitation with $(\text{NH}_4)_2\text{SO}_4$ and dialyzed overnight against distilled water. Crude cell extract was obtained from a culture in the late exponential phase. The cells were collected by centrifugation and disrupted in ultrasonic disintegrator UD-20 [12]. All steps were done in the cold.

Protein determination. The protein concentration in the cell extract was determined by the method of Lowry with bovine serum albumin

as a standard [9].

Sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) [8]. It was carried out in 12 % gel in 25 mM TRIS-glycine buffer, pH 8.3, containing 0.1 % SDS, at 35 V for 14 hours. Low molecular weight markers (Pharmacia) were applied: phosphorylase *b* (94 kDa), bovine serum albumin (67 kDa), ovalbumin (43 kDa), carbonic anhydrase (30 kDa), soybean trypsin inhibitor (20 kDa) and α -lactalbumin (14,4 kDa). Gels were stained with Coomassie blue.

Native PAGE. It was performed in 5 % gel at 100 V for 4 hours. The ester hydrolyzing activity was qualitatively proved by the method of Lund [10]. The gels were placed for 1 hour in 0.1 M Tris-maleic buffer, pH 6.4, and then in 1 % α -naphthylacetate in acetone-water containing Fast blue BB salt. The esterase activity led to the appearance of dark red bands on the gel which developed in 1 hour at room temperature.

Results and Discussion

In previous investigations with our isolates, utilizing DMT, high protein contents in the supernatant of the strain *Pseudomonas* sp. 054 was established (data not presented).

The concentrated protein from the supernatant showed a single band in SDS-PAGE (Fig. 1, lane 2). Other protein bands were not detected. Based on standard markers (Fig. 1, lane 1), the molecular weight of the observed protein was determined to be 67 kDa. The SDS-PAGE of the strain 054 crude cell extract produced a well distinguishable band of the same molecular weight (Fig. 1, lane 3), suggesting an extracellular secretion of the protein. The pro-

duction and secretion of microbial enzymes (esterases, lipases, laccases) in the cultural medium was also reported for bacteria, as well as for other microorganisms [1, 3, 5].

The ester hydrolyzing activity of the studied protein was proved by native PAGE and α -naphthylacetate as the indicator substrate. The extracellular protein exhibited a strong reaction in a single band (Fig. 2, A). A similar extracellular protein from *N. erythropolis* was detected by disk gel electrophoresis and its molecular weight was determined as approximately 15 kDa [7]. The enzyme possessed wide substrate specificity and was defined as either

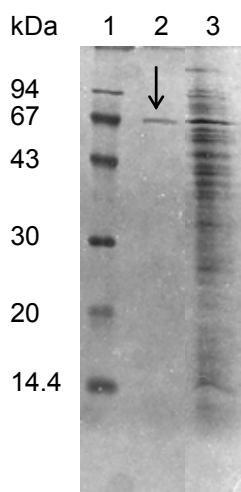


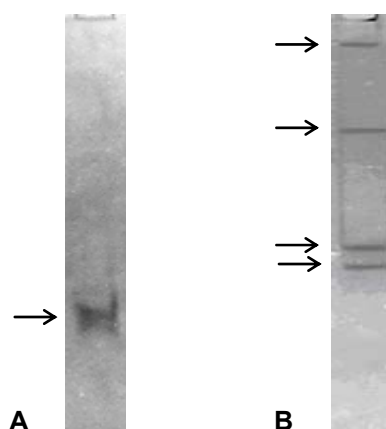
Fig. 1: SDS-PAGE of strain *Pseudomonas* sp. 054.

Lanes:

1. Molecular weight markers;
2. Extracellular protein from culture supernatant;
3. Crude extract of the strain.

a new lipase form or a newly characterized ester hydrolyzing enzyme. It, however, was inactive towards esters like DMT with *p*-position substitutes.

The esterase activity of a crude intracellular fraction of *Pseudomonas* sp. 054 was also



studied. Four separate bands with ester hydrolyzing activity were identified by native PAGE (Fig. 2, B). The patterns were active on α -naphthylacetate suggesting that these ester hydrolyzing enzymes also participate in the initial hydrolysis of the dimethylterephthalate.

Fig. 2: Native PAGE of the ester hydrolyzing enzymes of strain *Pseudomonas* sp. 054:
A. Extracellular protein;
B. Cell-free extract.

In conclusion, the strain *Pseudomonas* sp. 054 produces both extra- and intracellular esterase activities, which are important in the initial stage of biodegradation of aromatic substrates. The extracellular protein detected has an ester hydrolyzing activity and a molecu-

lar weight of 67 kDa.

Further investigations will be directed towards the biotechnological production of an enzyme designed to catalyze the aromatic ester hydrolysis in the textile production waste waters.

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ИЗВЪН- И ВЪТРЕКЛЕТЪЧНИ ЕСТЕРАЗИ, УЧАСТВАЩИ В КАТАБОЛИЗМА НА ДИМЕТИЛТЕРЕФТАЛАТ ПРИ *PSEUDOMONAS* SP.

Лилия Церовска^{1*}, Райчо Димков², Таня Рашева³, Таня Йорданова¹

Резюме

Щам *Pseudomonas* sp. 054, изолиран от замърсена почва, притежава ензимни активности за ефективно биологично разграждане на ароматния естер диметилтерефталат. Изследвана е естер-хидролазната активност на щам. Установени са извън- и вътреклетъчни естерази при растеж в течна среда. Активностите са доказани в хранителната среда и в клетъчен екстракт чрез нативна електрофореза. Регистрираният извънклетъчен белтък с естеразна активност има молекулно тегло 67 kDa. Вътреклетъчната фракция показва 4 отделни субфракции с естер-хидролазна активност.