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CHARACTERIZATION OF THREE BACTERIAL STRAINS – BIODEGRADANTS OF AROMATIC SUBSTRATES

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Summary

Three bacterial strains, isolated from contaminated soil, were adapted to degrade increasing concentrations of xenobiotic aromatic substrates. Morphological, physiological and biochemical characteristics define them as belonging to the genera Pseudomonas, Alcaligenes and Citrobacter. The strain with highest biodegradative activity is taxonomically related as the closest to Pseudomonas pseudoalcaligenes.

Introduction

Ecological problems of worldwide importance are extended every year with the accumulation of greater amounts and variety of anthropogenic contaminants. In most of the cases they resist physical and chemical influences and the biological factor is the one that could accomplish their degradation. Bacteria are the group of microorganisms with the greatest role in the biopurifying processes [4]. Their function is determined by the wide catabolic potential and adapting abilities to assimilate different xenobiotic substrates [3, 9]. Often the conditions of contaminated regions lead to phenotype and genotype changes that trouble the biodegrading strain identification. Despite that, the taxonomical determination is an important stage of the biotechnological approach in the environmental purifying.

The aim of the present work was the investigation and the taxonomical determination of three strains biodegrading aromatic substrates.

Materials and Methods

Microorganisms and cultivation. Six bacterial strains were investigated for biodegradative abilities. They were isolated and adapted for assimilation of xenobiotic substrates with aromatic structure. The adaptation was made in periodical cultivation in mineral medium with different substrates as a sole carbon and energy source – methylbenzoate and dimethyltherephthalate from 100 to 1000 mg/l [7]. The study of the substrate assimilation was carried out in liquid medium with minimal mineral composition (g/l): K_2HPO_4 – 8.25; KH_2PO_4 - 1.82; NH_4NO_3 – 1.0; $MgSO_4 \times 7H_2O$ – 0.2; $CaCl_2 \times 2H_2O$ – 0.02; $FeSO_4 \times 7H_2O$ – 0.0006; $NaMoO_4 \times 2H_2O$ – 0.06 and $MnSO_4$ – 0.06. The following substrates were added: B-ester; pT– ester; Zumpf- ester; therephthal acid (TA); dimethyltherephthalate

(DMT) and dimethylisophthalate (DMI) in working concentration 250 mg/l.

Identification of the bacterial strains. The strain determination was made by means of morphological, physiological and biochemical characteristics, according to the procedures in Bergey's manual [1]. API 20 (bioMerieux) tests were used for identification of nonfermenting Gram - negative bacteria. Two strains from CNCTC – *Pseudomonas alcaligenes* 152 and *P. pseudoalcaligenes* 160, were used as control for species defining.

Results and Discussion

Forty five microbial cultures with biodegradative activity to the limiting aromatic substrate were isolated from the contaminated biotops of textile factory "D. Dimov" purifying station - Yambol. They were adapted by increasing the substrate concentration [7]. Cultures with the following designations – 109, 112, 169, 170, 185 and 189, were selected for further studies.

The substrate specificity of the mentioned above strains was of interest, because of the arising polluting. Microorganisms were cultivated in mineral medium with addition of different aromatic compounds as a sole carbon and energy source. Substrate type and assimilation of adapted bacterial strains are presented in Table 1.

Table 1. Aromatic compounds assimilation as a sole carbon source by the bacterial strains 109, 112, 169, 170, 185 and 189.

Substrates	Strains					
	109	112	169	170	185	189
B-ester	-	-	-	+	-	-
pT-ester	+	-	-	+	-	-
Zumpf-ester	-	-	+	-	-	-
TA	+++	+	+++	++	+	++
DMT	+++	++	++	+++	++	++
DMI	+++	+	+	++	++	++

Legend: presence of growth (+); good growth (++); very good growth (+++).

Envisage the wide substrate specificity and biodegradation efficiency, the most active three strains - 109, 170 and 189 were investigated. The morphological and some physiological characteristics are shown in Table 2.

The biochemical properties of the tested strains were studied by means of specific media. The results are presented in Table 3.

The obtained data referred these strains according to Bergey's manual as follows: strain 109 – to genus *Alcaligenes,* strain 170 – to genus *Pseudomonas* and strain 189 – to genus *Citrobacter*. The presence of these bacteria [2, 5, 6] is connected to the well known adaptive and biodegradative abilities of the genera *Pseudomonas* and *Alcaligenes*. The *Citrobacter* strain is explained with the technological mixing of industrial and daily faecal wasted waters, during the purifying process. For the last strain the results from IMFIC - test were: formation of indol (-); methylrot test (+); VP - test (-); Simons' citrate (+).

Strain 170 was enlisted in group A (RNA group I, section I, 2b) of *Pseudomonas* [1]. Two control type strains 152 and 160 were investigated for a comparison and more accurate taxonomical determination. The results from the morphological

Table 2. Morphological and physiological properties of bacterial strains 109, 170, 189, 152 and 160.

Strains	Characteristics																			
	Cell morphology (μm)	Gram stain	Spore formation	Motility	Colony morphology	Growth in liquid medium	Catalase reaction	Oxidase reaction	Acid from glucose	Nitrate reduction	Urease reaction	Growth at t°C				Growth at pH			pH opt	Temp. opt (°C)
												4	30	37	41	5.5	7.0	8.0		
109	Rod 0.7 x 1.6	G-	-	+	Round, regular, flat, smooth edge, opaque, mucous, to 2 mm	Mud slurry, not forming veil and sediment	+	+	+	To nitrite	-	-	+	+	-	+	+	-	7.0	35
170	Rod 0.6 x 2.2	G-	-	+	Round, regular, flat, smooth edge, hemi-translucent, mucous, to 4 mm	Mud slurry, forming ring and sediment	+	+	-	+	ND	-	+	+	+	+	+	+	6.5	35
189	Rod 0.8 x 2.0	G-	-	+	Round, regular, low convex, smooth edge, opaque, mucous, to 3 mm	Mud slurry, not forming veil and sediment	+	-	+	To nitrite	-	-	+	+	+	-	+	+	7.2	37
152	Rod 0.5 x 2.3	G-	-	+	Round, regular, flat, wrinkled, hemi-translucent, mucous, to 2 mm	Mud slurry, without veil or ring, forming sediment	+	+	-	+	ND	-	+	+	+	ND	ND	ND	7.0	35
160	Rod 0.7 x 2.0	G-	-	+	Round, regular, flat, smooth edge, hemi-translucent, mucous, to 1 - 2 mm	Mud slurry, without veil or ring, forming sediment	+	+	-	+	ND	-	+	+	+	ND	ND	ND	7.0	35

Legend: positive reaction (+); negative reaction (-); no data (ND).

Table 3. Biochemical properties of bacterial strains 109, 170 and 189.

Strains	Characteristics																									
	Adonitol	Arabinose	Cellobiose	Citrate	Esculin	Fructose	Galactose	Glucose	Lactate	Lactose	Maltose	Mannitol	Mannose	Rhamnose	Ribose	Sorbitol	Sucrose	Trehalose	Xylose	Decarboxylation of L-lisine	Decarboxylation of L-ornitine	Dehydrolysation of L-arginine	Desamination of phenylalanine	Hydrolysis of phenylalanine	Hydrolysis of esculin	Hydrolysis of gelatin
109	-	-	+	-	-	+	+	+	+	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	+
170	-	+	-	+	ND	+	-	-	-	-	+	-	+	-	-	-	-	-	-	ND	-	-	-	-	-	-
189	-	+	+	+	-	+	+	+	ND	+	+	+	-	+	-	-	±	-	+	-	+	-	-	-	ND	-

Table 4. Biochemical properties of bacterial strains 152, 160 and 170.

Strains	Characteristics																			
	L-valine	L-serine	Alanine	Phenylalanine	L-arginine	L-glutamate	Betaine	Acetate	Citrate	Succinate	Pyruvate	Malonate	Tartaric acid	Gluconate	Ethanol	Mannitol	Ethylamin	Fructose	Sorbitol	Glycerol
152	-	-	+	-	+	+	-	-	+	+	+	-	-	-	-	-	-	-	-	-
160	-	-	+	-	-	+	+	+	-	+	+	-	-	-	+	-	+	+	-	-
170	-	-	+	-	-	+	-	+	+	+	+	+	-	-	+	-	+	+	-	-

study of the three cultures are included in Table 2 and the biochemical characteristics in Table 4.

The present results revealed, that strain 170 could not be defined correctly by the methods of classical taxonomy. Obviously it endured variety of adaptive changes (probably on a genetic base) as a result of the toxic aromatic pollution in the region. The strain could be related as the closest to *P. pseudoalcaligenes*, but distinguished by the assimilation of arabinose, manose, maltose, citrate, malonate and betaine. The investigation of Whiteley and Bailey on strains actively degrading phenol announced, that a large amount of them also belonged to *P. pseudoalcaligenes* [8].

The taxonomical determination of "wild" strains, exposed to continuous "toxic pressure" as a result of polluted environment, faces difficulties using the classical methods. The adaptive changes of these microorganisms give an advantage in evolutional aspect, but differ them from the collections strains, cultivated at optimal conditions. It is necessary to apply a number of modern molecular genetic methods for determination that will be a future task.

Regardless of not entirely correct identification, the "wild" strains are the best natural purifiers of the environment, therefore future investigations have to be continued and extended.

References

1. Bergey's Manual of Systematic Bacteriology, 1984. N. Krieg (Eds.), vol. 1, 2, Baltimore: Williams and Wilkins.
2. Hill, G., B. Milne, P. Nawrocki, 1996. *Appl. Microbiol. Biotechnol.*, **46,** 163-168
3. Johnson, R., R. Olsen, 1997. *Appl. Envir. Microbiol.*, **63** (10), 4047-4052
4. Powlowski, J., V. Shinger, 1994. *Biodegradation,* **5**, 219-235.
5. O'Reilly, K., R. Crawford, 1989. *Appl. Envir. Microbiol.*, **55** (4), 866-870.
6. Richter, M., R.-M. Wittich, 1994. *Biodegradation,* **5**, 63-69.
7. Tserovska, L., R. Dimkov, Y. Topalova, 1995. *J. Cult. Coll.*, **1**, 23-27.
8. Whiteley, A., M. Bailey, 2000. *Appl. Envir. Microbiol.*, **66** 6, 2400-2407.
9. Widada, J., H. Nojiri, T. Omori, 2002. *Appl. Microbiol. Biotechnol.*, **60**(1-2), 45-59.

ОХАРАКТЕРИЗИРАНЕ НА ТРИ БАКТЕРИАЛНИ ЩАМА – БИОДЕГРАДАНТИ НА АРОМАТНИ СУБСТРАТИ

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Резюме

Три бактериални щама, изолирани от замърсена почва, са адаптирани към разграждане на повишаващи се концентрации ксенобиотични ароматни субстрати. Морфолого-културалното и физиолого-биохимичното им охарактеризиране ги определят като представители на родовете Pseudomonas, Alcaligenes и Citrobacter. Щамът с най-висока биодеградационна активност е отнесен в таксономично отношение като най-близък до вида Pseudomonas pseudoalcaligenes.