Section 1. Biology

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POLYSORBATES BIODEGRADATION POTENTIAL AND PLASMID STABILITY OF SOIL *PSEUDOMONAS*

Abstract. The main aim of current research was a consideration of xenobiotics biodegradation potential of more than 50 strains of 7 species and 3 subspecies of mentioned bacteria and their antibiotic resistance as interacting parts of one system of adaptation of microorganisms to changing environment. And it has not only theoretical scientific importance, for understanding of bases of *Pseudomonas* and *Stenotrophomonas* resistance plasmids stability, but also has a practical significance for solving of modern ecological problems of new multidrug resistant pathogen forming and xenobiotic biodegradation processes in soil.

Keywords: *Pseudomonas*, biodegradation, antibiotic-resistance, plasmids, lipases.

Introduction

The problem of multi-drug resistance of microbes, according to WHO reports of recent years, is one of key importance research directions for upcoming century [1]. The multiple resistance of microorganisms to antimicrobial preparations is one of

the well-known properties of *Pseudomonas*, which are being isolated from clinics [2]. But the native strains of these bacteria, which were isolated from soil and other wet surfaces of natural and artificial origin, have the wide spectrum of resistance to antibiotics of II and III generations and they are able to

stable transfer this property to other Gram-negative bacteria too. Thus it has not only medical but also ecological and archeological significance, because of very broad life areal of these microorganisms.

According to the research of recent years, *Pseudomonas* and other cave microorganisms are endangering not only the health visitors and cave researchers, but also are becoming the new object for resistance research in aspects of both resistance new types discovery and the discovery of new antibiotics [3; 4]. Due to literature data, *Pseudomonas* are highly adaptive microorganisms. They have a wide diversity of enzymes and biochemical pathways of biodegradation of various native substances and xenobiotics [5; 6]. That is why the study of relation of stable antibiotic resistance persistence and the transfer of it with xenobiotic biodegradation genes localization in bacterial genome is very significant for understanding the mechanisms of this problem.

Materials and Methods

During this research there were used strains from The National Culture Collection of Microorganisms of the MDC of "Armbiotechnology" SPC NAS RA. They were cultivated according to the standard protocols. For the resistance tests here were used 50mg/kg/

ml concentrations of 13 antibiotics of different classes and generations: penicillin/Pen, ampicillin/Amp, amoxicillin/Amx, augmentin/Amc, cefixime/Cfm, ceftriaxone/Cro, kanamycin/Kan, streptomycin/Str, gentamycin/Gen, ciprofloxacin/Cip; tetracycline/Tet; Chloramphenicol Chl) of amphenicoles, azithromycin (Azm) – of azalides of macrolides [7; 8].

Biodegradation potential was evaluated due to extracellular enzyme activity precipitation. Lipase activities tests were carried out according to standard microbiology and biochemistry protocols on solid cultural media, using substitution of carbon source to appropriate polysorbates –20,–40,–60,–65,–80,–85 with different length of fatty acids [9]. The genetical analyses was done by DNA electrophoresis, transformation and PCR. DNA isolation was done by alkaline extraction for plasmids and with benzyl-chloride method for total DNA. PCR analysis of antibiotic modification enzyme genes was done with the following primers: *aph(3')IV*, *aac(6')II*, *pCAT639*, *bla-OXA-10* [10]. The statistical verification of experiments was done by standard methods [11; 12].

Results

The results of polysorbate degradation tests are presented on (table 1).

Table 1. – Polysorbates biodegradation by *Pseudomonas*

Strain of micro-organism	Polysorbates													C+	C–
	20	40		60		65		80		85					
	G	A	G	A	G	A	G	A	G	A	G	A			
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
<i>P. geniculata</i>	9335	3+	–	3+	–	3+	–	+	–	3+	–	3+	–	5+	–
	9336	3+	–	3+	–	3+	–	+	–	3+	–	3+	–	5+	–
	9337	3+	–	3+	3+	3+	–	2+	–	3+	–	3+	3+	5+	–
	9338	2+	–	3+	–	3+	–	2+	–	3+	–	3+	–	5+	–
	9339	3+	–	2+	–	3+	–	+	–	3+	–	3+	–	5+	–
	9340	2+	–	3+	–	3+	–	2+	–	3+	–	3+	–	5+	–
	9205	3+	–	+	2+	3+	–	3+	–	3+	–	2+	–	5+	–
	9203	3+	2+	+	–	2+	+	+	+	3+	3+	3+	–	5+	–
	9212	2+	+	2+	–	2+	+	+	+	2+	–	++	+	5+	–
<i>P. aeruginosa</i>	9059	3+	–	+	–	3+	–	3+	–	3+	+	3+	3+	5+	–
	9058	3+	–	3+	–	3+	–	3+	–	3+	–	3+	–	5+	–
	9056	–	–	–	–	–	–	–	–	–	–	–	–	5+	–

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
<i>P. fluorescens</i>	9075	3+	+	3+	+	3+	–	2+	–	3+	–	3+	3+	5+	–
	9077	3+	–	3+	–	3+	–	2+	–	3+	–	3+	–	5+	–
	9252	3+	–	–	–	3+	–	+	–	3+	5+	3+	+	5+	–
	9089	3+	–	3+	–	3+	–	2+	–	3+	–	3+	–	5+	–
	9092	3+	–	3+	–	3+	–	2+	–	3+	–	3+	–	5+	–
	9095	3+	5+	2+	–	2+	–	2+	–	3+	–	3+	–	5+	–
	9096	3+	–	3+	–	3+	–	2+	–	3+	–	3+	–	5+	–

Polysorbates – 20, 40, 65, 80, 85; G – growth (after 5 days), A – activity; “+” – growth/lipase activity presence different intensity; “–” – growth/lipase activity absence; “C+” – positive control on nutrient agar media; “C–” – negative control on mineral cultural media

Table 2. – Resistance transfer in soil *Pseudomonas*

Rec	Donor	R/S of Rec	R/S of Donor	1	2	3	4	5	6	7	8	9	10	11	12	13	C2
I	pUC18 (C1)	S	Amp	–	+	–	–	–	–	–	–	–	–	–	–	–	+
II	<i>S. maltophilia</i> 9302	S	Str, Pcn, Amx Amp	+	+	+	–	–	–	–	–	–	–	–	–	–	+
III	pUC18	Cip, Cro,	Amp	–	–	–	–	–	+	–	–	–	–	–	+	–	+
IV	pUC18	Cip, S	Amp	+	–	–	–	–	–	–	–	–	–	+	–	–	+
V	VOG16	Chl, Amc, Amx, Amp Pcn, Cfm	Kan	+	+	+	+	+	+	+	+	+	–	–	–	–	+
VI	pUC18	Cfm	Amp	–	+	–	–	+	–	–	–	–	–	–	–	–	+
III	<i>S. maltophilia</i> 9285	Cip, Cfm	Kan Amc Amx Amp Cfm	–	–	–	–	+	–	–	–	–	–	–	+	–	+
VI	<i>S. maltophilia</i> 9288	Chl, Amc, Amx, Amp, Cfm,	R, Cro ^s , Tet ^s , Azm ^s , Cip ^s	–	+	+	+	+	–	+	+	–	–	–	–	–	+
IV	<i>S. maltophilia</i> 9277	Cip	Amc Amx Amp Cfm	+	+	+	+	–	–	–	–	–	–	–	+	–	+
VI	<i>S. maltophilia</i> 9304	Cfm	Cro	–	–	–	–	+	–	–	–	–	–	–	–	–	+

Antibiotics: 1 – penicillin/Pen, 2 – ampicillin/Amp, amoxicillin/Amx, 3 – augmentin/Amc, 4 – cefixime/Cfm, 5 – ceftriaxone/Cro, 6 – kanamycin/Kan, 7 – streptomycin/Str, 8 – gentamycin/Gen, 9 – ciprofloxacin/Cip, 10 – tetracycline/Tet, 11 – Chloramphenicol/Chl, 12 – azithromycin/Azm; Rec. (Recipients): I – *P. aeruginosa* 9056, II – *E. coli* DH5a, III – *S. maltophilia* 9326, IV – *P. chlororaphis*, subsp. *chlororaphis* 9329, V – *P. fluorescens* 9092, VI – *P. taetrolens* 9243; S – sensitivity, R – resistance, C1 – plasmid transmission control by *E. coli* DH5a/pUC18 plasmid, C2 – control on nutrient agar, “+” – growth, “–” – the absence of growth

Then the same procedure of transformation was repeated with other recipient strains. The same results were obtained in 6 used recipients of different species of bacteria. While the tests of stability, the divergence of results was detected. The stability of replication was detected for all the researched donors in case of *P. aeruginosa* 9056 recipients, except the *P. taetrolens* recipients which didn't demonstrate the stability after the cultivation of transformants using non selective nutrient agar media. Thus it might

be concluded that the cause of plasmid resistance in general is defined by the genome structure of *P. aeruginosa* [13]. In case of *P. taetrolens*, probably there is the system which is blocking the possibility of stable replication of donor DNA in recipient cells. The antibiotic resistance tests of all the obtained transformed strains are presented on table 5. The results of antibiotic resistance test were the same for each several donor and all the used recipients, thus in table is represented only one of 6 experiments of each series.

Figure 1. Lipase activity of different stains (A – *P. taetrolens* 9246 on polysorbate-85; B – *P. chlororaphis* subsp. *aurantiaca* 9061)

Conclusions

During the experiments, it was found out that the genes of 5 types of polysorbate degrading lipases are different. They can have both plasmid and nucleoid localization in frames of one species and one subspecies. Probably, one part of these plasmid localized lipases can play a key role for antibiotic resistance plasmid stability in some strains of *P. taetrolens*, while the stability of plasmids from *P. fluorescens*, *P. chlororaphis* and *P. aeruginosa*, is predominantly caused

by genome of recipient strains. The maximal stability was detected on both selective and non-selective cultural media in recipient cells of *P. aeruginosa*.

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