STENOTROPHOMONAS MALTOPHILIA RESISTANCE AND BIODEGRADATION POTENTIAL

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Abstract

In Current research the main goal was to study the resistance plasmids stability causes in *S. maltophilia* native strains. Using some microbiological, biochemical and genetical methods, it was noted, that the resistance to 13 mostly used in medicine, veterinary and agriculture antibiotics is stable and can be transmitted, even after the long-time cultivation without contact with any antibiotic. Then, using PCR, transformation analyses and xenobiotic tests, it was shown nucleoid and plasmid localization of studied genes. Polyphenol oxidases and caseinase genes were detected on nucleoid, while the lipases genes were indicated both on nucleoid and plasmids. It was found that biodegradation genes define the stabile replication of antibiotic resistance plasmids in a majority of *S. maltophilia studied strains.*

Keywords: *Biodegradation, Stenotrophomonas maltophilia, multi-drug resistance, plasmids.*

Introduction

S. maltophilia (former *Pseudomonas maltophilia*) are well-researched Gram-negative, aerobic, non-fermentative bacteria. This species is well-known by the versatility of metabolicc pathways, defining the huge potential of adaptation, including antimicrobial resistance, quorum sensing, etc. [1, p. 163-174]. It defines a plenty problems occurrence during the therapy of their infections [2, p. 1484-1492]. They are naturally resistant to various broad-spectrum antibiotics, due to the production of various enzymes. The pathogenesis of this opportunistic pathogen infection is an actual problem for severely immunocompromised and debilitated individuals [3, p. 57-80]. In a majority of cases in addition to native resistance this microbe has plasmids with transferable resistance [4, p. 729-748]. Thus, the

plasmid stabilityy is significant for the resistance forming and spread both in nature and clinics.

Materials Methods

The strains for this research were taken from The National Culture Collection of Microorganisms, MDC, "Armbiotechnology" SPC NAS RA. For the resistance test there were used 50mkg/ml concentrations of 13 antibiotics: β-lactams - penicillin (Pcn), ampicillin (Amp), amoxicillin (Amx), augmentin (Amc) of aminopenicillins, cefixime (Cfx), ceftriaxone (Ctx) from cephalosporins; aminoglycosides - kanamycin (Kan), streptomycin (Str), gentamycin (Gnc); fluoroquinolone – ciprofloxacin (Cip); tetracycline (Tcn); Chloramphenicol (Cam) of amphenicoles, azithromycin (Azm) - of azalide macrolides [5, p. 97-99]. The enzyme activity precipitation was done according to standard microbiology and biochemistry protocols on solid cultural media with different substrates. For caseinase activity, the milk casein destruction test was apply. For polyphenol oxidase (PPO) precipitation L-tyrosine (for *o*-diphenol oxidase or tyrosinase), α-naphthol and tannin (for *p*-diphenol oxidase or laccase) destruction tests were applied. For lipase polysorbates -20, -40, -60, -65, -80, -85 degradation was studied. The genetical analysis was done by plasmid analysis, transformation and PCR with primers*: aph(3')*IV, *aac(6')II, pCAT639*, *blaOXA-10* [6, p. 12-19].

Results

According to presented data, the majority of *S. maltophilia* studied strains are resistant to antibiotics from different classes and generations, up to multi-drug resistance. But Gnc-resistant and Stp-resistant strains of *S. maltophilia* (former *Pseudomonas maltophilia*) are absent, as well as it's noted the absence of absolutely sensitive representatives (Table 1).

Table 1. *S. maltophilia* resistance. *1 – Kan, 2 – Stp, 3 – Gen, 4 – Cam, 5 – Amc, 6 – Amx, 7 – Amp, 8 – Pcn, 9 – Cfx, 10 – Ctx, 11 – Tet, 12 – Azm, 13 – Cip, C – control on nutrient cultural media; "+" – growth, "-" – inhibition.*

strain	*Resistance to studied antibiotics*													*C*
	1	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*	*11*	*12*	*13*	
9286	-	-	-	-	+	+	+	+	-	-	-	-	-	+
9288	+	+	+	+	+	+	+	+	+	-	-	-	-	+
9289	+	+	+	+	+	+	+	+	+	+	+	+	-	+
9290	-	+	-	-	-	-	+	+	+	-	+	-	-	+
9293	-	-	-	-	-	-	-	-	+	+	-	-	-	+
9294	+	+	+	+	+	+	+	+	+	-	-	-	-	+
9298	+	+	+	-	+	+	+	+	+	+	+	+	+	+
9273	-	-	-	+	+	+	+	+	+	+	-	+	+	+
9277	-	-	-	-	+	+	+	+	+	-	-	-	-	+
9098	-	+	-	+	+	+	+	+	+	-	-	-	-	+

9285	+	-	-	-	+	+	+	+	+	-	-	-	-	+
9301	-	+	-	+	-	-	-	-	-	-	+	-	-	+
9300	-	+	-	+	-	-	+	+	+	+	-	-	-	+
9306	-	+	-	-	-	+	+	+	+	+	+	+	+	+
9304	-	+	-	+	+	+	+	+	+	-	-	-	-	+
9303	-	+	-	+	-	+	+	+	+	+	+	-	+	+
9302	-	+	-	-	-	+	+	+	-	-	-	-	-	+
9307	-	+	-	+	+	+	+	+	-	-	-	-	+	+
9305	-	-	-	-	-	+	+	+	+	-	+	-	-	+
9326	-	-	-	-	-	-	-	-	-	+	-	-	+	+
9308	-	+	-	+	+	+	+	+	-	-	+	-	-	+
9203	-	-	-	+	+	+	+	+	+	+	-	-	-	+
9310	-	-	-	+	-	+	+	+	-	-	-	-	-	+
9208	+	+	+	+	+	+	+	+	+	-	-	-	-	+

The prevailing majority of researched strains are plasmid-containing, but not all the detected plasmids are able to transfer resistance. On some of them there are detected some antibiotic modification genes (Table 2.). In some cases, antibiotic modification genes are detected as localized on bacterial chromosome of some studied strains. There are some strains with simultaneous presence of different antibiotic modifying enzymes in both types of localization.

Table 2. *S. maltophilia* different strains genetical analysis ("c+" – chromosome localized gene; "p+" – plasmid localized gene; "-" - the absence of gene/plasmid/transmission, "P" – plasmid,"++" – two plasmids, which are able to be transferred independently and to form the resistance to different antibiotics; genes: *I - aph(3')IV, II - aac(6')II, III - catB7, IV - blaOXA-10*; T – resistance transmission)

Strain	*P*	*PCR analysis*				*T*	*Strain*	*P*	*PCR analysis*				*T*
		I	*II*	*III*	*IV*				*I*	*II*	*III*	*IV*	
9286	-	-	-	-	-	-	*9289*	++	-	*p+*	*c+*	*p+*	+
9288	+	-	-	-	-	-	*9290*	-	-	-	-	-	-
9277	+	-	-	-	-	+	*9293*	-	-	-	-	-	-
9098	-	-	-	-	-	-	*9294*	+	-	-	-	-	+
9285	+	-	-	-	-	-	*9298*	+	-	-	-	-	+
9301	-	*c+*	-	-	-	-	*9273*	-	-	-	-	-	-
9300	+	-	-	-	-	+	*9302*	+	-	-	-	*p+*	+
9306	-	-	-	-	-	-	*9307*	-	-	-	-	-	-
9304	+	-	-	-	-	-	*9305*	+	-	-	-	-	+
9303	-	*c+*	-	-	-	-	*9326*	+	-	-	-	-	+
9308	-	-	-	-	-	+	*9310*	+	-	-	-	-	+
9203	+	*c+*	-	-	-	+	*9208*	-	-	-	-	-	-

Then all the strains were studied in experiments with cultivation on different mineral media with substitution of carbon source to compatible biodegradation enzyme model precipitation substrates (Table 3-5 and Fig. 1).

Table 3. Polyphenol oxidases of *S. maltophilia* of soil. ("C^+" – positive control on nutrient agarised cultural media, "C^-" – negative control on solid

mineral media, G – growth, A – Activity, "+" – growth/activity registration (in mm), "-" – the absence of growth/activity registration).

Strain	α-naphtol		L-Tyr		Tannin		C^+	C^-	Strain	α-naphtol		L-Tyr		Tannin		C^+	C^-
	G	A	G	A	G	A				G	A	G	A	G	A		
9306	2+	-	3+	10+	-	-	20+	-	*9302*	+	-	3+	5+	2+	2+	20+	-
9308	2+	-	3+	10+	-	-	20+	-	*9305*	+	-	3+	5+	-	-	20+	-
9307	-	-	3+	-	2+	-	20+	-	*9300*	2+	2+	3+	2+	-	-	20+	-
9304	2+	-	3+	15+	+	-	20+	-	*9302*	2+	2+	3+	5+	+	+	20+	-
9326	+	-	3+	10+	-	-	20+	-	*9303*	2+	-	3+	10+	-	-	20+	-

Table 4. *Stenotrophomonas maltophilia* lipase activity precipitation on solid mineral cultural media with substitution of carbon source to various polysorbates (20, 40, 60, 65, 80, 85) ("C^+" – positive control on nutrient agarised cultural media, "C^-" – negative control on mineral agarised media, G – growth, A – Activity precipitation, "+" – growth/activity precipitation registration of bacteria (in mm), "-" – the absence of growth/activity registration).

Strain	P 20		P 40		P 65		P 60		P 80		P 85		C^+	C^-
	G	A	G	A	G	A	G	A	G	A	G	A		
9298	3+	2+	2+	-	3+	-	3+	-	3+	-	3+	3+	3+	-
9300	3+	2+	3+	2+	3+	-	+	-	3+	-	3+	2+	3+	-
9301	+	-	-	-	3+	-	2+	-	3+	-	3+	-	3+	-
9302	3+	-	3+	2+	2+	-	+	-	3+	-	3+	5+	3+	-
9303	2+	-	2+	-	2+	-	2+	-	3+	-	3+	-	3+	-
9304	3+	-	2+	+	3+	+	+	+	3+	-	3+	2+	3+	-
9305	3+	2+	3+	2+	3+	-	3+	-	3+	-	3+	2+	3+	-
9306	2+	-	3+	-	3+	-	3+	-	3+	-	3+	-	3+	-
9307	3+	-	3+	-	3+	-	2+	-	3+	-	3+	-	3+	-
9308	2+	-	3+	+	3+	-	2+	+	3+	-	3+	+	3+	-
9310	2+	-	3+	-	3+	-	3+	-	3+	-	3+	-	3+	-
9326	3+	-	3+	-	3+	-	2+	-	3+	-	3+	-	3+	-
9273	2+	-	-	-	2+	-	+	-	2+	-	2+	-	3+	-
9277	2+	-	+	-	2+	-	+	-	3+	-	+	-	3+	-
9203	3+	2+	+	-	2+	+	2+	+	3+	3+	3+	-	3+	-
9288	3+	2+	2+	3+	3+	3+	3+	+	3+	3+	3+	5+	3+	-

Table 5. Caseinase of soil *S. maltophilia* ("C^+" – positive control on nutrient media, "C^-" – negative control on mineral media, G – growth, A – Activity, "+" – registration in mm, "-" – the absence of growth/activity).

Strains	Day 1		Day 2		C^+	C^-	Strains	Day 1		Day 2		C^+	C^-
	G	A	G	A				G	A	G	A		
9276	-	-	2+	-	3+	-	*9297*	-	-	3+	-	3+	-
9277	-	-	2+	10+	3+	-	*9298*	1+	2+	3+	10+	3+	-
9279	-	-	2+	-	3+	-	*9299*	1+	2+	3+	12+	3+	-
9280	2+	2+	3+	13+	3+	-	*9300*	-	-	3+	10+	3+	-
9284	-	-	1+	4+	3+	-	*9301*	3+	5+	3+	8+	3+	-
9285	-	-	3+	12+	3+	-	*9302*	2+	2+	3+	12+	3+	-
9287	-	-	2+	4+	3+	-	*9303*	-	-	3+	5+	3+	-
9288	2+	3+	3+	15+	3+	-	*9304*	-	-	3+	5+	3+	-

9289	1+	2+	3+	12+	3+	-	*9305*	-	-	3+	8+	3+	-
9290	1+	2+	3+	11+	3+	-	*9306*	1+	2+	3+	12+	3+	-
9291	1+	2+	3+	10+	3+	-	*9307*	-	-	2+	-	3+	-
9326	-	-	2+	-	3+	-	*9296*	2+	2+	3+	10+	3+	-
9293	-	-	2+	-	3+	-	*9308*	-	-	3+	12+	3+	-
9294	-	-	2+	12+	3+	-	*9310*	-	-	3+	8+	3+	-

Fig.1. Enzyme activity precipitation in soil *S. maltophilia*. (PPO: A - Tannin degradation by polyphenol oxidase of *S. maltophilia 9288*; B - L-Tyr degradation by tyrosinase of *S. maltophilia 9302*; C – L-Tyr degradation by tyrosinase of *S. maltophilia 9310, D – S. maltophilia* various strains caseinase*;* lipases*:* E - *S. maltophilia 9288* on polysorbate-85; F – *S. maltophilia 9302* on polysorbate-85, G – *S. maltophilia 9308* on polysorbate-60, H – *S. maltophilia-9298* on polysorbate-85, I – *S. maltophilia 9300* on polysorbate-40*)*

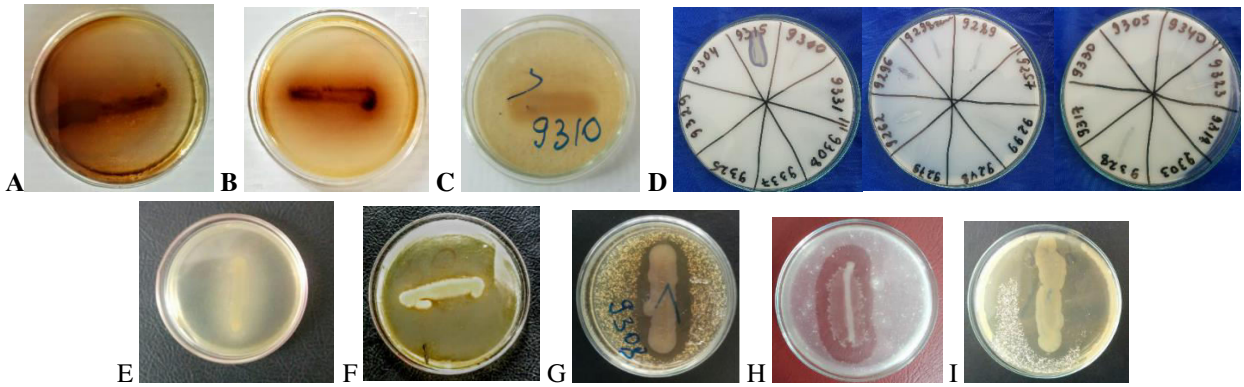

Table 6. The transformation of *P. chlororaphis 9330* recipients by *S. maltophilia* plasmids of different polysorbates biodegrading *strains*. *(*1 – the negative control with recipient non-plasmid strain transformants, 2 -7 – transformants primary selection on appropriate selective media, containing polysorbates; "C^+" – the positive control on solid nutrient cultural media, "C^- "– the negative control on mineral cultural media without carbon source; "+" – the growth of bacteria, "- "– the absence of bacterial growth.)

Donor	Polysorbates						Control	
	20	40	60	65	80	85	C^+	C^-
P. chlororaphis subsp. chlororaphis 9330	-	-	-	-	-	-	+	-
S. maltophilia 9288	+	+	+	+	+	+	+	-
S. maltophilia 9285	-	-	-	-	+	-	+	-
S. maltophilia 9302	-	+	-	-	+	-	+	-
S. maltophilia 9304	-	+	-	-	+	+	+	-
S. maltophilia 9293	-	-	-	-	-	-	+	-
S. maltophilia 9277	+	-	+	+	+	-	+	-

Table 7. Correlation between plasmid stability and resistance. (1 – Pcn, 2 – Amp, 3 – Amx, 4 – Amc, 5 – Cfx, 6 – Ctx, 7 – Kan, 8 – Gnc, 9 – Stp, 10 – Cam, 11 – Tcn, 12 – Azm, 13 – Cip, C^{+1}– the positive control on nutrient cultural media, C^{+2} – the positive control of plasmid transmission, C^- - the negative control

of recipient strain, S – sensitivity, R – resistance, "+" – the growth, "-" – the absence of growth).

Percipient strain	R/S of Donor	1	2	3	4	5	6	7	8	9	10	11	12	13	C^{+1}
P. aeruginosa 9056 (C^-)	S	-	-	-	-	-	-	-	-	-	-	-	-	-	+
P. aeruginosa 9056 + E. coli plasmid puc18 (C^{+2})	Pcn, Amp	+	+	-	-	-	-	-	-	-	-	-	-	-	+
P. aeruginosa 9056 +P. aeruginosa p5249a (C^{+3})	Amp, Amx, Amc, Kan, Pcn	+	+	+	+	+	+	+	-	-	-	-	-	+	+
S. maltophilia 9326	Pcn, Amp	-	-	-	-	-	+	-	-	-	-	-	-	-	+
P. fluorescens 9092	Kan	+	+	+	+	+	-	+	-	-	-	-	-	-	+

Then there were done the series of transformations of appropriate recipients form different species by the plasmids, which were isolated from the strains with noted biodegradation activities [7, p.1966]. Transformed strains were cultivated on various cultural media. As a result, the stability of plasmids was detected [8, p. 1073–1084]. The results of polysorbate biodegrading transformants obtaining, their stability and resistance are presented on tables 6-7. According to the collected data for one part of all the researched strains there is the direct correlation between antibiotic resistance plasmid maintains and the plasmid localization of polysorbates biodegradation genes.

Conclusion

The resistance of the researched strains of *S. maltophilia* remains stable, even after the long-time cultivation of bacteria on nutrient agar cultural media, without contact with any antibiotic. The standard tests had showed the wide spectrum of resistance to 13 antibiotics (ciprofloxacin, azithromycin, ceftriaxone, etc.) of different classes and generations as well as the possibility of transfer of resistance to other Gram-negative bacteria by plasmids.

PCR and transformation analyses of antibiotic modification enzymes genes *blaOXS-10, catB7, aac(6')II, aph(3')IV* showed the divergence in their localization both in plasmids and bacterial chromosome. Gene *catB7* is localized only in nucleoid and is not transferable. The transformation analyses showed that 3 types of polyphenol oxidases and caseinase genes were detected on bacterial chromosome, while the different polysorbates degrading lipases genes were identified both on plasmids and nucleoid. There were detected some strains with the simultaneously presenting in one cell, the different combinations of extracellular lipases encoded by both nucleoid and plasmid genes. They were differing by the substrate specificity, ensuring biodegradation of polysorbates with different length of fatty acid. The presence of polysorbate degrading lipases genes

defines the stabile replication of antibiotic resistance plasmids in about 60% of researched strains of *S. maltophilia.*

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