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The Effect of Tartaric Acid New Derivatives against The Multidrug Resistant Opportunistic Pathogenic Soil Strains of Pseudomonas Fluorescens

Bella G. Babayan[1]*, Aram R. Mikaelyan[2], Nona L. Asatryan[3], Samvel A. Bagdasaryan[4], Marina A. Melkumyan[5]

[1, 4,5] Armbiotechnology" Scientific and Production Centre (SPC), National Academy of Sciences (NAS), Republic of Armenia (RA), Republic of Armenia.

[1,2,3] National Polytechnic University of Armenia (NPUA), Republic of Armenia

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Abstract

The quantity of multidrug resistant bacteria, including phytopathogenic and pathogenic for human organism is increased caused by enlarging of antibiotic usage by mankind. And this problem is more makeable in developing countries, because of uncontrolled usage of antimicrobial preparations including the antibiotics led to new multidrug resistant bacteria selection and forming in hospitals and agricultural objects. But the initial source of resistant bacteria is soil. Thus, the search of natural, ecologically safe effective preparations against them is very important for medicine and ecology. One of the directions of searching of alternatives instead the antibiotics while the therapy of multidrug resistant infections is the usage if semisynthetic antimicrobial compounds with enlarged spectrum of properties. Tartaric acid is well-known antibacterial preservative food additive. That is why, the main aim of current research was the research of antibacterial effect of first time synthesized in our laboratory 4 derivatives of it – benzylimide, cyclohexylimide, benzyl mono amino salt and Cyclohexyl amino salt of tartaric acid on different strains of well-known by the highly adaptive metabolism - Pseudomonas fluorescens from The National Collection of Microorganisms of Microbe Depository Centre, "Armbiotechnology" Scientific & Production Center, Republic of Armenia. During the experiments it was shown that all compounds were are more effective than tartaric acid and tartrates. For the resistant representatives, which were in the minority, the absence of possibility to transfer the resistance to other Gram-negative bacteria was shown. Besides, there was showed the possibility of these compounds biodegradation by some Pseudomonas representatives.

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I. INTRODUCTION

Pseudomonas are very common well-researched Gram-negative bacteria with highly adaptive metabolism. They include opportunistic pathogens of human, animals and plant and can become a cause of various inflammatory pathologies while the patients with immune suppressed conditions, such as like postoperative patients, patients with transplanted organs, oncological diseases, AIDS, etc. In a majority of cases, the main percentage of infections are caused by *Pseudomonas aeruginosa, Pseudomonas fluorescens* and other representatives of this genus, as well as *Stenotrophomonasmaltophilia* (former *Pseudomonas maltophilia*) (Katara et al., 2012,Marcelletti and Scortichini, 2014, Deredjian, et al. 2016, Lerminiaux and Cameron, 2019, Gale et al., 2015, Wong et al., 2011Baumrin et. al, 2017). The main problem during the therapy of Pseudomonas infections is the multidrug resistance of these bacteria and the high adaptation potential of

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them to large spectrum of environmental changes. They are well-known by the abilities of various xenobiotics biodegradation (Moradali, 2017, Paras, 2015, Salam, 2016).

That is why the searching of new antibacterial alternatives against the pseudomonas different representatives carries a huge medical importance. Besides, being a very common inhabitants of wet surfaces in large diapason of temperature and pH, these bacteria can be involved in numberless quantity of consumption chains and spread their native resistance to various Gramm-negative bacteria by the mobile genetical elements in gene horizontal transfer processes. That can led to new resistant strains forming, what has not only medical but also ecological negative consequences (Adegoke et al, 2017, Demanèche et al., 2001.).

Thus, the search of a comparably ecologically safe methods against these microorganisms is a very significant problem. One of the methods for combating against the multidrug resistant microorganisms are the natural organic acids of plants and their derivatives (Babayan, 2019).

Tartaric acid is a representative of aldaric acids of plant organisms. This substance is widely represented in different plants. Tartaric acid and the salts of it (K, Na, Na/K, Ca – tartrates) are being broadly used by mankind in different scoped of actions, including food industry (food additive E-334, etc) as preservatives which are safe for human organism (Eswaranandam et. al, 2004).

The main aim of current research was to test and compare the antibacterial effect of tartaric acid well-known and 4 new synthetic derivatives of tartaric acid (fig. 1), which were elaborated in National Polytechnic University of Armenia: benzylimide, cyclohexylimide, benzyl mono amino salt and Cyclohexyl mono amino salt of Tartaric acid.

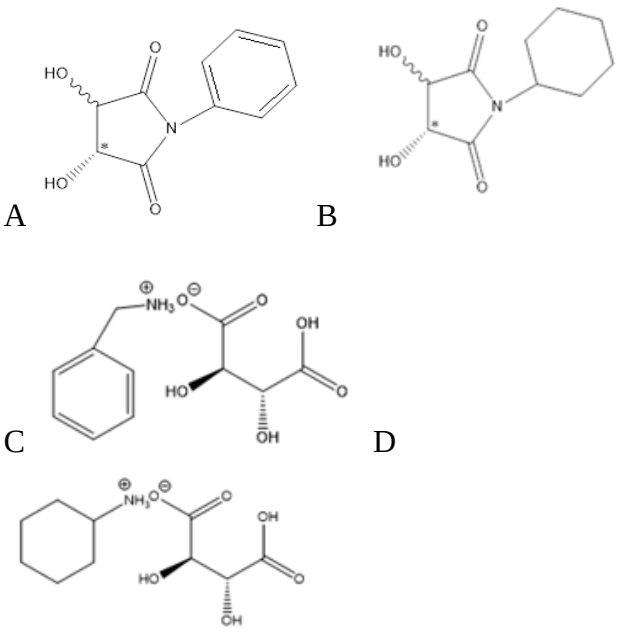

Figure 1: Tartaric acid synthetic derivatives: A – Benzylimide of tartaric acid, B -Cyclohexylimide of tartaric acid, C – Benzyl mono amino salt of tartaric acid, D – Cyclohexyl mono amino salt of tartaric acid.

II. MATERIALS AND METHODS

As the object there were used 49 strains of *Pseudomonas fluorescens* and other representatives of *Pseudomonas* from The National Microbe Collection of SPC "Armbiotechnology" NAS RA. The new derivatives of Tartaric acid were elaborated at the laboratory of New Agrochemical Creation at National Polytechnic University of Armenia (NPUA) from the natural source – the waste product of wine industry, cream of tartar (Dashchyan et al., 2014, Gharajyan et al., 2018). The antimicrobial resistance tests were done according to standard protocols (Babayan et al., 2019). DNA isolation was done by benzyl chloride and by alkaline extraction method and agarose gel electrophoresis. Transformation was done according to Mandel's method of competent cells obtaining with usage of $CaCl_2$. Biodegradation tests were done according to standard protocols, by the substitution of carbon source in cultural media by the researched compounds (Delaney et al., 2018, Hen et al., 1993,

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Lucotte and Baney, 1993, Viovy, 2000, Singh et al., 2010).

III. RESULTS AND DISCUSSION

The results of antimicrobial resistance of researched strains are represented on table 1.

During the experiments, 49 strains of Pseudomonas fluorescens were cultivated on media containing different classes of antibiotics of last generations: ceftriaxone of β-lactams, azithromycin of macrolides, ciprofloxacin of fluoroquinolones, chloramphenicol of amphenicoles and kanamycin of aminoglycosides. According to obtained data, the resistant, and especially multidrug resistant representatives are not in a a majority of researched bacteria. The resistance to kanamycin was not detected. It's very important to note that all the researched strains are demonstrating the resistance even after the long-term cultivation on nutrient agar cultural media without any contact with antibiotics. Thus, the antibiotic resistance for all the researched bacteria is the stabile property.

Then all from all the researched strains total and plasmid DNA was isolated for the comparison of their geneticalapparat. According to the obtained data, about 50 % of these strains were plasmid containing. Transformation analysis showed that not for all of them the plasmids are related with the resistance (14%).

Table 1. Antibiotic resistance of P. fluorescens soil strains (50mkg/ml) (Cro - Ceftriaxone, Kan – Kanamycin, Cip - ciprofloxacin, Chl -chloramphenicol, azithromycin, C – control on nutrient agar cultural media, "+" – growth, "-" – the absence of growth).

Strain	Cro	Kan	Cip	Chl	Azm	C	Strain	Cro	Kan	Cip	Chl	Azm	C
9120	+	-	-	-	-	+	9090	+	-	-	-	+	+
9124	+	-	-	-	-	+	9131	-	-	-	-	-	+
9098	+	-	-	-	-	+	9110	+	-	-	-	-	+
9099	-	-	-	-	-	+	9111	+	-	-	-	+	+
9134	+	-	+	-	+	+	9121	+	-	-	-	-	+
9094	-	-	-	-	-	+	9114	-	-	-	-	-	+
9140	+	-	+	+	+	+	9115	+	-	-	-	-	+
9100	-	-	-	-	-	+	9070	+	-	-	-	-	+
9205	+	-	+	-	-	+	9072	+	-	-	-	+	+
9106	-	-	-	-	-	+	9077	+	-	-	-	-	+
9108	-	-	-	-	-	+	9089	+	-	-	-	+	+
9095	+	-	-	-	+	+	9069		-	-	-	-	+
9084	+	-	+	-	-	+	9068	-	-	-	-	-	+
9123	+	-	-	-	+	+	9073	_	-	-	-	+	+
9096	+	-	-	-	-	+	9179	+	-	-	-	-	+
9185	+	-	+	-	-	+	9107	+	-	-	-	+	+
9085	+	-	-	-	+	+	9118	+	-	+	-	-	+
9081	+	-	+	-	-	+	9120	+	-	-	-	-	+
9091	-	-	-	-	-	+	9103	-	-	-	-	+	+
9092	+	-	+	-	+	+	9142	+	-	+	+	+	+
9127	-	-	-	-	-	+	9147	+	-	+	-	+	+
9152	-	-	-	-	-	+	9149	+	-	-	-	-	+
9128	-	-	-	-	-	+	9150	-	-	-	-	-	+
9132	+	-	+	-	+	+	9153	-	-	-	-	-	+
9143	-	-	-	-	-	+							

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Then all the researched strains were cultivated on media containing tartaric acid and the derivatives of it. The results are presented on table 2.

Table 2. The antibacterial effect of TA derivatives (50mkg/ml) of different representatives of Pseudomonas fluorescens (TA – tartaric acid, K –K/Na-tartrate, CI – cyclohexylimide of TA dissolved in DMSO, BI – benzylimide dissolved in DMSO, BAS – benzyl mono amino salt of TA, CAS – Cyclohexyl mono amino salt of TA; "+" – growth, "-" – the absence of growth, * - late growth after III day, ** - late growth after IV day, * late growth of singular colonies after V day, C – control on nutrient solid agar media).**

Strain	BI	CI	BAS	CAS	TA	K	C	Strain	BI	CI	BAS	CAS	TA	K	C
9120	-***	-	-**	-	+	+	+	9090	+	+*	+	+*	+	+	+
9124	-	+	-	-	+	+	+	9131	-	-	-***	-	+	+	+
9098	-	-	-***	-	+	+	+	9110	-	-	-	-	+	+	+
9099	+	-***	+**	+	+	+	+	9111	+	+	+	+	+	+	+
9134	+	+	+	+	+	+	+	9121	+	- ***	-***	+*	+	+	+
9094	+	+	-***	-	-	-	+	9114	+**	- ***	-***	-***	+*	+	+
9140	+	+	-***	-***	+	+	+	9115	-	-	-	-	+*	+	+
9100	+*	-	-***	-***	+	+	+	9070	-	-	-	-	+	+	+
9205	-	-	-	-	+	-	+	9072	-	-***	-	-***	+	+	+
9106	-	-***	-	-	+*	-	+	9077	-***	-***	-***	-***	-	-	+
9108	+	+	+	+	+	+	+	9089	-	+	+	-	-	-	+
9095	-***	-	+	+	+	+	+	9069	-	+**	+**	-	+	-	+
9084	-	-***	-***	-***	+	-	+	9068	-	-	-	-	+	-	+
9123	+	+	+	+	+	+	+	9073	+	+	+	+	+	+	+
9096	+	-***.	+	+**	+	-	+	9179	+	+	-	-	+	+	+
9185	+	+	+	+	+	+	+	9107	+	+	-	-	+	+	+
9085	+	+	-	-	+	+	+	9118	+**	-***	+**	-***	+*	+	+
9081	-	+*	-***	-	-	+	+	9120	+*	+	+***	+	+*	+	+
9091	-	+**	-***	-	+*	+	+	9103	+***	+	+***	+	-	+	+
9092	-	+*	-***	-	+*	+	+	9142	-	-	-	-	-	-	+
9127	+	+	+	-***	+	+	+	9147	+**	+**	+*	+**	+*	+	+
9152	-	+*	+*	+	+	+	+	9149	+	+	-	-	+*	+	+
9128	+*	+	+	+*	+	+	+	9150	-***	-***	-	-	+*	+	+
9132	+*	+	+*	-***	+	+	+	9153	-***	-***	-	-***	+*	+	+
Strain	BI	CI	BAS	CAS	TA	K	+	9143	-	-**	-	-***	-	+	+
							C	Strain	BI	CI	BAS	CAS	TA	K	C

According to the presented data, in a majority of cases, the effect of researched compounds is being presented by both bacteriostatic (with the prolongation of growth period) and bactericide (the absence of growth even after 7 days of cultivation) activities. Besides, in some strains it's notable the forming of singular resistant colonies after few days of cultivation. Probably it is caused by selection of resistant mutants. Probably it is related with polyphenol oxidase activity of Pseudomonas (Taranto et al, 2017). Then the resistant strains were cultivated on mineral media for biodegradation tests. The results showed that biodegradation of salts processes more intensively. Probably it is caused by their more hydrophilic properties, in opposite to hydrophobic imides. On a next stage, there were done transformation of sensitive strains of *P. aeruginosa 9056* by the plasmid DNA of these resistant strains. Thus, during the experiments there were not detected transformants, with the resistance to tartaric acid derivatives or ability to biodegradation of them on mineral media.

IV. CONCLUSION

During all the experiments with tartaric acid benzyl- and cyclohexyl- imides as well as benzyl- and cyclohexyl- mono amino salts of it on 49 native soil strains of *Pseudomonas fluorescens*, the antibacterial effect was detected for all 4 compounds. They are effective as antibacterial agents against both sensitive and multidrug resistant strains of researched Pseudomonas fluorescens. That effect in a majority of cases was stronger than in case of well-known tartaric acid derivatives usage. In some strains the effect was presented by prolongation of growth till 1-2 days.

In other part of *Pseudomonas fluorescens* representatives there were detected single colonies which were resistant to all tested compounds. Probably they were mutated by their bacterial chromosome genes. That mutations are not transmittable by plasmids in plasmid containing strains. Probably, these mutations are related with genes of polyphenol oxidases, which are presented in genome of *Pseudomonas.*

During the tests of biodegradation, cyclohexyl-derivatives showed less biodegradative properties than benzyl-derivatives. Probably it is caused by cyclohexyl radical chemical structure features and disabilities of bacterial enzymes to recognize these molecules as substrates. Soil opportunistic pathogenic strains of *Pseudomonas* can be a good models of highly adaptive pathogenic bacteria. Thus, all the tested semisynthetic substances are recommended for further research of their efficiency as antibacterial agents and ecological safeness, as potentially biodegradative compounds.

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