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Tartaric Acid Synthetic Derivatives for Multi-Drug Resistant Phytopathogen *Pseudomonas* and *Xanthomonas* Combating

B.G. Babayan
National Polytechnic University of Armenia (NPUA); "Armbiotechnology" Scientific And Production center (SPC), National academy of Sciences Republic of Armenia (NAS RA)
Yerevan, Armenia
bbg.15.04@mail.ru

M.A. Melkumyan
PhD
"Armbiotechnology" SPC, NAS RA,
Yerevan, Armenia

S.A. Bagdasaryan
"Armbiotechnology" SPC, NAS RA,
Yerevan, Armenia

A.R. Mikaelyan
PhD,
NPUA,
Yerevan, Armenia

A.M. Grigoryan
PhD
NPUA; Russian-Armenian University (RAU)
Yerevan, Armenia

Abstract— The resistance to antimicrobial preparations, according the WHO reports of recent years, is becoming the one of the most actual healthcare problems of this century. Nevertheless, the key role of antibiotics diversity increase, as well as the increase of their application scopes, the initial origin of antimicrobial resistance problem is the versatility of adaptation mechanisms potential of all microorganisms, including intraspecific gene horizontal transfer and quorum sensing. Thus, the actuality of search of new, ecologically safe and harmless for human health antimicrobial agents, among the natural and semisynthetic compounds, is being significantly increased. One of the prospective directions in these research is the derivatization of aldaric acids, isolated from plants different species, as the native antibacterial active substances, such as like: citric, acetic, tartaric, lactic,

In current research, 7 new derivatives of natural tartaric acid (TA): cyclohexylimide, benzylimide, phenylimide, benzyl mono amino salt, cyclohexyl mono amino salt, phenyl amino salt and mono ethanol amino salt of TA were tested on different strains from 6 subtypes of 3 species of phytopathogenic multi-drug resistant *Xanthomonas and Pseudomonas.* During the research it was detected the significant antimicrobial effect of studied compounds against the range of phytopathogens which

are resistant to antibiotics from different classes and generations (ciprofloxacin, chloramphenicol, ceftriaxone, azithromycin, etc.). It was detected the higher efficiency of cyclohexyl- derivatives in comparison with mono ethanol-, phenyl- and benzyl- derivatives.

Pseudomonas, *Xanthomonas*, multi-drug resistance, phytopathogenic microorganisms, complex salts of tartaric acid, imides of tartaric acid

I. Introduction

Pseudomonas and *Xanthomonas* are well-known as Gram-negative bacteria with extremely high level of adaptation various mechanisms, including the resistance to various antimicrobial agents, such as like antibiotics. Besides, they are capable to destruct different natural and toxic synthetic xenobiotics, causing their biodegradation. The diversity of these genera microorganisms is very large, but the majority of them are opportunistic pathogenic for human and animal, as well as both genera include the several percentages of phytopathogenic representatives. That is why, all the mentioned properties are becoming the main cause of the range of additional complications for the treatment of *Pseudomonas* infections in animal, human (pseudomonoses and other inflammation pathologies) and plant organism. It carries a huge importance for agriculture and medicine, as well as led to the range of ecological and healthcare problems occurrence [1, 2].

One of common phytopathogenic bacteria from *Pseudomonas* genera is *P. syringae*. It has no specific lifecycle areal, and can be isolated world around from various infected plants. As a plant pathogen, it can infect a wide range of species, and exists as over 50 different pathovars [3]. These bacteria have a wide range of genetic mechanisms for synthesis of specific enzymes and that is why it has high level of antibiotic resistance, as well as high

virulence with complex influence on plant immune system [4].

Xanthomonas is a well-researched genus of *Proteobacteria,* which includes about 40 species, the majority of which cause plant diseases, being infectants of about 400 plant species. They have many metabolic similarities with Stenotrophomonas and Pseudomonas [5, 6]. Contaminated seeds, weeds, infected plant debris are the main route of transmission. Infection starts with epiphytic stage, because *Xanthomonas* representatives the use surface polysaccharides, adhesion proteins and type IV pili to attach to the surface and can form biofilms to sustain abiotic stresses (UV, drought, etc.). These microorganisms produce xanthomonadins – the specific yellow pigments, that protect from radiation caused from natural light. Resistance to UV is mostly conferred by genes related to oxidative stress and DNA repair. Response to light is important in pathogenicity of these bacteria and regulates surface attachment and production of biofilm. One of the emphasized properties of these microorganisms is the resistance to antibiotics [7, 8]. According to World Health Organization (WHO) recent reports, multi-drug resistance and the spread of it among the various pathogens today is being extremely important. Thus, multidrug resistance is being considered as one of the research priorities of WHO for this century. It is driven by the overuse of antimicrobials in people, but also in animals, especially those used for food production, as well as in the environment. WHO is working with these sectors to implement a global action plan to tackle antimicrobial resistance by increasing awareness and knowledge, reducing infection, and encouraging prudent use of antimicrobials [9-13].

Tartaric acid is a natural aldaric acids representative, which is mostly presented in plants from algae to trees, being involved in various biosynthetic processes of their cells. This acid and the salts of it (tartrates) are well-known as antimicrobial active compounds, which are broadly used in chemical and food industry, as safe preservatives and conservation agents. One of the prospective directions of antimicrobial active compound elaboration is the derivatization of natural substances, such as like organic aldaric acid to the appropriate imides and amides [14-19]. According to the previous research, benzylimide, cyclohexylimide, cyclohexyl mono amino salt and benzyl amino salt of tartaric acid are effective against the range of opportunistic pathogenic *Pseudomonas*, *Stenotrophomonas,* as well as on 8 strains of Bacilli, isolated from cave mud. representatives. These 4 derivatives of tartaric acid had demonstrated themselves also as the biodegradable by *P. chlororaphis* group representative species (*P. chlororaphis subsp. aurantiaca, P. chlororaphis, subsp. chlororaphis, P. chlororaphis, subsp. chlororaphis, P. taetrolens,* etc.), as well as *P. fluorescens* [20, 21].

II. Materials and Methods

7 new synthetic derivatives of tartaric acid: 3 imides and 4 complex salts: cyclohexylimide, benzylimide, phenylimide, benzyl mono amino salt, cyclohexyl mono amino salt, phenyl amino salt and mono ethanol amino salt of tartaric acid, which are more hydrophilic, were synthesized by two stage simple technology from the natural cream of tartar which was elaborated in laboratory of new agrarian pesticide creation and the quality control, NPUA [22]. During the research there were used various non-pathogenic and opportunistic pathogenic strains of *Pseudomonas* and *Xanthomonas* genera from the National Culture Collection of Microorganisms Depository Center at “Armbiotechnology” Scientific and Production Center of National Academy of Sciences of Republic of Armenia [23]. The studied strains of 3 species, 5 subspecies of microbes were cultivated on different liquid and solid nutrient agarised and selective cultural media. The appropriate selective media were containing 13 types of antibiotics of different classes and generations, mainly used in medicine, veterinary and agriculture, according to standard protocols. There selective cultural media were containing 50mkg/ml compatible antibiotic. The following antibiotics of different classes and different generations were used: from β-lactamic - Pen/Penicillin, Amp/ampicillin, Amx/Amoxicillin, Amc/Augmentin, Cfx/Cefixime and Cro/Ceftriaxone; from aminoglycosides - Gen/Gentamicin, Kan/kanamycin, Str/Streptomycin; from fluoroquinolones - Cip/Ciprofloxacin; from tetracyclines - Tcn/Tetracycline; from azalides of macrolides - Azm/azithromycin; from amphenicoles - Chl/Chloramphenicol. All the used antibiotics were produced by “Astoria”. As the positive and the negative control strains there were used the following antibiotic resistant and sensitive bacteria: *E. coli DH5α*, *E. coli DH5α/pUC18*, *E. coli DH5α /VOG16*, *E. coli DH5α/pkk*, *E. coli DH5αPEC7, as well as P. aeruginosa 9056 and P. aeruginosa 5249* strains. The antimicrobial activity tests were done according to standard protocols [24, 25].

III. Results

The results of primary screening of 3 subspecies of 2 species *Pseudomonas* and *Xanthomonas* antimicrobial resistance are presented on table 1.

Table1. The resistance of phytopathogenic *Pseudomonas* and *Xanthomonas* different representatives. S – species/subspecies (A – *P. syringae, pathovar lachrymans,* B – *P. syringae, pathovar tabaci*, *C – Xanthomonas beticola), 50 mkg/ml antibiotics: Kan - kanamycin, Stp - streptomycin, Gnc - gentamicin, Cam – chloramphenicol, Amc - augmentin, Amx - amoxicillin, Amp - ampicillin, Pcn - penicillin, Cfx - cefixime, Ctx - ceftriaxone, Tcn - tetracycline, Azm –azithromycin, Cip - ciprofloxacin; CG – the positive control of growth on nutrient agarised cultural media without antibiotics; ''+'' – growth, ''-'' –inhibition, ''-*'' – single colonies after the III day of cultivation)*

Strain	*8730*	*8732*	*8734*	*8742*	*8738*	*8733*	*8731*	*8663*	*8657*	*8680*	*8681*
	A							B		C	
Kan	-	-	-*	+	+	+	-	+	+	-	-
Stp	-	-	-	+	+	+	-	+	-	-	-
Gnc	-	-*	-	+	+	-	-	+	+	-	-
Cam	-	-	-	-	+	+	+	+	-	-	-
Amc	+	-	-	-	+	+	+	-	+	-*	-
Amx	+	+	+	+	+	+	+	+	+	-*	-*
Amp	+	-	-	-	-	+	+	-	+	-	-
Pcn	+	-	+	+	+	+	-	+	+	-*	-
Cfx	+	+	+	+	+	+	-*	+	+	-*	-*
Ctx	+	+	+	+	+	+	+	+	+	-	-
Tcn	-	-	-	+	+	-	-	+	+	-	-
Azm	+	-	-	+	+	-	-	+	-	-	-
Cip	+	-	-	+	+	-	+	+	-	-	-
CG	+	+	+	+	+	+	+	+	+	+	+

As it is shown, the majority of strains are resistant to different antibiotics and demonstrate pan-drug and multi-drug resistance wide diapason. Anyhow, the sensitive representatives are observed to. Among the researched group of strains, *Xanthomonas* are less resistant then *Pseudomonas syringae*.

The results of antimicrobial activity tests are presented in tables 2 -5.

Table 2. The antimicrobial effect of Cyclohexyl mono amino salt of Tartaric Acid (CAS) and Cyclohexyl Imide of Tartaric Acid (CI) on different strains of phytopathogenic representatives of *Pseudomonas* and *Xanthomonas*. The zones of inhibition are presented in mm; *A – P. syringae path. Lachrymans, B – P. syringae path. tabaci, E - X. beticola; L – complete lysis of microbial growth zone, CG – the positive control of growth on nutrient agarised cultural media with 30mm growth zones, "+" – the absence of growth inhibition and the normal growth presence, "+/-" – less than 10% inhibition of growth; concentrations of tested compounds: I -50mkg/ml, II - 0.001M, III - 0.01M, IV - 0.05M, V - 0,1M, VI - 0,5M)*

Strain		CAS						CI					CG
		I	II	III	IV	V	VI	II	III	IV	V	VI	
8730	A	11	11	11	15	17	25	10	11	`13.6	14	15	30
8731		12	14	17	18	L	L	13	15,7	17	20	25	30
8732		12.2	20	20	20	L	L	20	21	21	24,2	25	30
8733		12	15	15	20	22	L	15	16,3	17	22	24	30
8734		L	L	L	L	L	L	L	L	L	L	L	30
8736		11	15	15.1	15.1	L	L	15	16	17.1	18.2	22	30
8738		10	10	13	15	20.5	27	11	14	14.1	14.5	15	30
8742		7	7	14	15	20	24	11	+	+	+	+	30
8653	B	+	+	+	+	+	6	L	L	L	L	L	30
8657		+/-	3.2	6	7,5	8	8,9	19.9	20.2	20.8	21.3	23.7	30
8665		4	8	9.7	10	11.2	12	9	12	13.2	14.3	15	30
8680	C	L	L	L	L	L	L	L	L	L	L	L	30
8681		L	L	L	L	L	L	L	L	L	L	L	30

Table 3. Antimicrobial effect of Benzyl Imide of Tartaric Acid (BI) and Benzyl mono amino salt of Tartaric Acid (BAS) on different strains of phytopathogenic representatives of *Pseudomonas* and *Xanthomonas*. A – *P. syringae,* B – *P. syringae path. Lachrymans*, C – *P. syringae path. tabaci*, D - *X. vesicatoria*, E - *X. beticola*; L – complete lysis of microbial growth zone, inhibition zones diameters are given in mm, CG – the positive control of growth on nutrient agarised cultural media, "+" – the absence of growth inhibition and the normal growth presence, "+/-" – 10% inhibition of growth; concentrations of tested compounds: I - 0.001M, II - 0.01M, III - 0.05M, IV - 0.1M, V - 0.5M.

Strain		BI					BAS					CG
		I	II	III	IV	V	I	II	III	IV	V	
8730	A	+/-	2.5	3	5.4	6	+/-	+/-	+/-	3	4	30
8731		+	+	+/-	+/-	+/-	+/-	+/-	+/-	+/-	2	30
8732		+/-	2.7	3	4.3	5	3	L	L	L	L	30
8733		3	3.5	6	6.9	7	2	3	3.2	4.5	10.1	30
8734		+	+	+/-	2	2.4	+/-	+/-	+/-	+/-	8	30
8736		+/-	+/-	+/-	+/-	+/-	+/-	+/-	+/-	+/-	7	30
8738		+/-	+/-	2.5	3	3.5	+/-	+/-	3	8	10	30
8742		3	5	6.4	6.5	6.9	+/-	+/-	+/-	3	11	30
8665	B	+	+/-	2	4.5	4	2	4	5.5.	7	8.1	30
8657		2	2.1	3.8	4.3	5	2	2.5	5	6	8.4	30
8843		+/-	+/-	+/-	3	5	+	+/-	+/-	4	8	30
8680	C	+/-	+/-	+/-	+/-	3.5	+	+/-	4	5	5.6	30
8681		+	+	+/-	2.8	4	+	+/-	3.8	6.2	7	30

Table 4. The antimicrobial effect of Mono amino ethanolamine complex salt of Tartaric Acid (MEAS) on different strains of phytopathogenic representatives of *Pseudomonas* and *Xanthomonas*. A – *P. syringae,* B – *P. syringae path. Lachrymans*, C – *P. syringae path. tabaci*, D - *X. vesicatoria*, E - *X. beticola*. (L – complete lysis of microbial growth zone, inhibition zones diameters are given in mm, CG – the positive control of growth on nutrient agarised cultural media, "+" – the absence of growth inhibition and the normal growth presence, "+/-" – 10% inhibition of growth; concentrations of tested compounds: I -50mkg/ml, II - 0.001M, III - 0.01M, IV - 0.05M, V - 0,1M).

Strain		MEAS					CG
		I	II	III	IV	V	
8736	A	+	+	+	+	+	30
8740		+	+	+	+	+	30
8744		+	+/-	2	6	7	30
8656		+	+	+	+	+	30
8730	B	+	+	3	4	10	30
8731		+/-	5	5	5,5	6	30
8732		+	+	+	+	6	30
8734		+	+	+/-	4.5	5	30
8733		+	+	+	+	+	30
8738		+	+	+/-	+/-	6	30
8742		+	+	+	+	+	30
8665	C	+	+	+	+	+	30
8657		+	+	+	+/-	5	30
8653		+/-	+/-	+/-		5	30
8647	D	+	+	+	+	+	30
8651		+	+	+	+	+	30
8843		+	+	+	+	+	30
8680	E	+	+	+	+	+	30
8681		+	+	+	+	+	30

Table 5. The antimicrobial effect of Phenyl Imide of Tartaric Acid (PhI) and Phenyl amino salt of Tartaric Acid (PhAS) on different strains of phytopathogenic representatives of *Pseudomonas* and *Xanthomonas*. A – *P. syringae path. Lachrymans*, B – *P. syringae path. tabaci*, C - *X. beticola*. (L – complete lysis of microbial growth zone, inhibition zones diameters are given in mm, CG – the positive control of growth on nutrient agarised cultural media, "+" – the absence of growth inhibition and the normal growth presence, "+/-" – 10% inhibition of growth)

Strain		PhI					PhAS					CG
		50	0.001	0.005	0.01	0.05	50	0.001	0.005	0.01	0.05	
8730	A	10	11	11	12	12	+/-	+/-	+/-	+/-	12	30
8731		+	+	+	+	5	+	+/-	12	15	16	30
8732		+	+	+	+	10	+	+	+/-	+/-	L	30
8733		+	+	+	+/-	+/-	10	L	L	L	L	30
8734		+	+	+	+	6	+	+	+	+/-	3.2	30
8736		+/-	+/-	3	6.3	7	+/-	+/-	+/-	8.9	10	30
8738		+	+	+	+	8	+	+	+	+	+	30
8742		+	+	+	+	+	+	+	+	+	+	30
8665	B	+	+	+	+	+	+	+/-	5.8	6	6.5	30
8657		+	+	+	+/-	5	+	L	L	L	L	30
8653		+	+	+/-	+/-	3.2	+	+	+/-	+/-	2,5	30
8680	C	+	3.1	5.5	10	L	+	+/-	4.1	8	9.2	30
8681		L	L	L	L	L	+	+/-	+/-	8	9	30

IV. Discussion

According to the data from literature, using a Cu-containing products offer some protection along with field-grade antibiotics such as oxytetracycline, which is labeled for use on some food crops in the United States [26]. Taking in consideration of tartaric acid ability to form wide range of different structure complex compounds with Cu, the new synthetic derivatives can be recommended for further research as potentially not only as primary antimicrobial agent, but also as the carrier of Cu, which can control the risks of Cu-dependent phytopathogen *Xanthomonas* plan*t i*nfection spread.

The effect of the mentioned compounds can be related with few mechanisms of resistance avoiding simultaneously. Because of structural similarity with tetracyclines and lactams, they probably can act like with the appropriate enzymes of antibiotic modification and inhibit them by binding. Their effect can be related with the changes in membrane system of antibiotic permeability. Besides, based on the chelating abilities of tartaric acid, the effect of these compounds can be related to the blocking of some ions of infection key role enzymes and signaling molecules.

V. Conclusion

As a result of experiments on different phytopathogens from *Pseudomonadaceae* and *Xanthomonadaceae,* with usage of 7 types of new synthetic derivatives of tartaric acid, it was carried out their antimicrobial activity. All the researched compounds can be considered as non-selective growth inhibitors against both sensitive and antibiotic resistant bacteria. The maximal effect was detected for cyclohexyl- derivatives in forms of complex salts. Phenyl-containing derivatives and monoethanolamine complex salt of tartaric acid are less active against the *Pseudomonas* and *Xanthomonas* phytopathogenic strains of different pathovars, than benzyl- and cyclohexyl- containing derivatives in form of imides and complex salts. In a majority of cases, the activity of salts was higher than in case of imides. Probably it is related with their higher hydrophilic properties and permeability. Thus, all the researched compounds are recommended for further mechanism of action and toxicity research as new alternatives against the multi-drug resistant bacteria.

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