Tartaric Acid Synthetic Derivatives Activity Against *Pseudomonas, Strenotrophomonas, Xanthomonas* Phytopathogens and Oppotunistic Pathogens

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Abstract

The multi-drug resistance is one of the most actual medical and ecological problem. *Stenotrophomonas*, *Xanthomonas, Pseudomonas* are common, drug resistant and highly adaptive phytopathogenic and opportunistic pathogenic microbes. Tartaric acid is natural safe antimicrobial agent. In current paper it is discussed the synthesis and antimicrobial activity of tartaric acid new derivatives, which were investigated in our laboratory. There were obtained benzyl, cyclohexyl and phenyl substituted imides as well as complex amine salts of tartaric acid. Their antimicrobial activity was tested on native soil *Pseudomonas*, *Xanthomonas* and *Stenotrophomonas* different strains from The National Microbe Collection of Scientific and Production Center (SPC) “Armbiotechnology” National Academy of Sciences of Republic of Armenia (NAS RA).

As a result of cultivation on appropriate compound containing media, as well as genetical analyses of tested microbes and docking analyses, it was found out that tartaric acid carbocyclic and aromatic imides and complex salts are effective as non-selective growth inhibitors. Complex salts of tartaric acid are biodegradable by some soil strains of *P. chlororaphis,* etc. Thus, all the elaborated compounds can be recommended for further study of their activity as an effective alternative against the multi-drug resistant microorganisms.

Introduction

Tartaric acid (TA) is the most common natural aldaric acid plants. TA and tartrates are well-known compounds, which are broadly used in chemical and food industry, as safe antibacterial agents [Lu et al. 2013; Nagata 2020; Karimi et al. 2015]. *Xanthomonas, Stenotrophomonas* and *Pseudomonas* are common inhabitants of wet surfaces of practically every where on Earth. These bacteria are involved in a huge quantity of consumption chains, as reducers and are represented by opportunistic pathogens, pathogens and non-pathogenic strains. Representatives of these genera are very close by their metabolism and wide diversity of adaptation biochemical mechanisms. They are able to biodegradation of wide spectrum of xenobiotics [Park 2016; Gomila 2017]. These bacteria include phytopathogenic (*P. syringae*), as well as pathogenic and opportunistic pathogens of human and animals (*S. maltophilia (*former *Xanthomonas* or later *Pseudomonas maltophilia), P. aeruginosa,*). Besides, they are able to synthesize various bioactive substances: plant growth stimulators, immune modulatory bioactive compounds, several pests and phytopathogenic microorganism metabolism inhibitors, etc. [Chakhtoura 2018; Choi et al. 2006]. *Xanthomonas* have many similarities to *Pseudomonas* and *Stenotrophomonas*, being a part of 2 close families *Pseudomonadaceae* and *Xanthomonadaceae* of Gram-negative bacteria. The majority of *Xanthomonas* are presented as plant pathogens, with world around spread areal. The mainly important representatives of these microorganisms are *Xanthomonas vesicatoria, Xanthomonas beticola* (former *Curtobacterium flacumfaciens pathovar beticola*) as well as phytopathogenic *Pseudomonas syringae*[Anzai et al. 2000].

The multidrug resistance of *Pseudomonas, Xanthomonas* and *Stenotrophomonas* has a huge importance because of the ability of these microorganisms to transfer this property to other various microorganisms by intraspecific gene transfer process [Adesoji et al. 2015]. In current paper there were researched the influence of synthetic derivatives of tartaric acid on growth of non-pathogenic and opportunistic pathogenic strains of soil microorganisms from genera *Stenotrophomonas* and *Pseudomonas.* The New synthetic derivatives of TA, which were synthesized by two stage technology which was elaborated in our laboratory, are presented on Figure 1 [Dashchyan et al. 2014; Peng et al. 2019].

Materials And Methods

During this research there were used non-pathogenic and opportunistic pathogenic strains of *Pseudomonas aeruginosa, P. putida, P. chlororaphis, (P. chlororaphis subsp. aurantiaca, P. chlororaphis subsp. aureofaciens, P. chlororaphis subsp. chlororaphis), P. taetrolens (which are classified in one group of P. chlororaphis and the compatible subspecies), P. geniculate, P. syringae path. syringae, P. syringae path. tabaci, P. syringae path. lachrymans, Stenotrophomonas maltophilia, Xanthomonas vesicatoria, X. beticola,* as well as some antibiotic sensitive and resistant strains of *E. coli (E. coli DH5αand E. coli DH5α*/pUC18, *E. coli DH5α* /VOG16, *E. coli DH5α*/pkk, *E. coli DH5α*PEC7) from the National Culture Collection of Microorganisms Depository Center at "Armbiotechnology" Scientific and Production Center of National Academy of Sciences of Republic of Armenia [Babayan et al. 2019a].

All the researched strains of microorganisms were cultivated on various selective cultural media with containing the tested synthetic compounds, after the tests of their antibiotic resistance to 13 antibiotics of different classes and generations. The tests were done, due to standard protocols. There selective cultural media were containing 50mcg/ml of following antibiotics: 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" [Peng et al. 2019; Babayan et al. 2019b]. All the Biodegradation tests were done according to the appropriate standards [Nemati 2016]. The statistical treatment of data was done by the standard methods. [Xuchun 20212; Buszewski et al. 2017].

Total and plasmid DNA isolation, purification and analysis as well as transformation were done due to Mandel's method of competent cells obtaining with calcium chloride usage [Delaney et al. 2018; Zhu et al. 1993; Wei Xu, et al. 2020; Green, 2019].

The synthetic derivatives of TA (Fig. 1 and 2), were obtained by the technology which was elaborated in laboratory of NPUA.

Results And Discussion

The results of all the experiments with antimicrobial effect of tartaric acid 4 new derivatives on *Stenotrophomonas maltophilia* and *Pseudomonas* different strains of different species and subspecies are represented in Tables 1–2.

Table 1
The comparison of TA derivatives antimicrobial activity on S. maltophilia **different strains (50mkg/ml).** TA – tartaric acid, K –K/Na-tartrate, Na – Na-tartrate, Cl – cyclohexylimide of TA dissolved in DMSO, BI – benzylimide dissolved in DMSO, BAS – benzylamine complex salt of TA, CAS – Cyclohexylamine complex salt of TA; "+" – growth, "-" – the absence of growth, C – control on nutrient solid agar media).

Strain	BI	Cl	BAS	CAS	TA	PhI	PhAS	EAS	K	Na	C
9303	-	-	-	-	+	-	-	+	-	-	+
9306	-	-	+	+	+	+	+	+	+	+	+
9301	-	-	+	-	+	+	+	-	+	+	+
9307	-	+	+	+	+	+	-	+	+	+	+
9310	-	-	-	-	-	-	-	-	-	-	+
9203	+	-	+	-	+	-	-	+	+	+	+

As it was shown on Table 1, the predominance of strains of *S. maltophilia* are non-sensitive to tartaric acid. The strains are more sensitive to imides then to complex salts. For this group of strains, the secondary growth was not registered. Then from all the researched strains was isolated DNA and analyzed. DNA analysis showed the presence of plasmids only in strains: *S. maltophilia 9303, 9301, 9307, 9302.*

DNA analysis of target groups of *P. putida, P. aeruginosa, P. geniculate, P. chlororaphis, P. syringae, X. vesicatoria, X. beticola* and *P. fluorescens* showed the presence of plasmids only in following strains: *9131, 9068, 9070, 9069, 9091, 9092, 9150, 9114, 9142, 9110, 9106, 9124.* On a next stage, there were done transformation of sensitive non-plasmid strains of *P. aeruginosa 9056* and *E. coli DH5α* by the plasmid DNA of these resistant strains. Both used recipients are sensitive to 13 antibiotics of 5 classes, to which were tested target groups of *Xanthomonas*, *Pseudomonas* and *Stenotrophomonas*. That is why the positive control was on antibiotic resistance plasmids of same species, and the negative control on streptomycin genes, which are encoded by bacterial chromosome genes. 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.

Table 2

Tartaric acid natural and synthetic derivatives antimicrobial effect comparison against the various strains of P. fluorescens. (50mkg/ml). N – the number of strain, TA – tartaric acid, K –K/Na- tartrate, Na – Na-tartrate, CI – cyclohexylimide of TA dissolved in DMSO, BI – benzylimide dissolved in DMSO, BAS – benzylamine complex salt of TA, CAS – Cyclohexylamine complex salt of TA; "+" – growth, "-" – the absence of growth,"+*" - late growth after III day, "+/-" - late growth after IV day, "+#" - late growth of singular colonies after 5th day, C – control on nutrient solid agar media.

Strain	BI	CI	BAS	CAS	TA	K	Na	C	Strain	BI	CI	BAS	CAS	TA	K	Na	C
9100	+*	-	+#	+#	+	+	+	+	*9070*	-	-	-	-	+	+	-	+
9205	-	-	-	-	+	-	-	+	*9072*	-	+#	-	+#	+	+	+	+
9106	-	+#	-	-	+*	-	-	+	*9077*	+#	+#	+#	+#	-	-	-	+
9108	+	+	+	+	+	+	-	+	*9089*	-	+	+	-	-	-	-	+
9095	+#	-	+	+	+	+	+	+	*9069*	-	+/-	+/-	-	+	-	-	+
9084	-	+#	+#	+#	+	-	+	+	*9068*	-	-	-	-	+	-	+	+
9123	+	+	+	+	+	+	+	+	*9073*	+	+	+	+	+	+	-	+
9096	+	+#	+	+/-	+	-	-	+	*9179*	+	+	-	-	+	+	-	+

The activity of tartaric acid synthetic derivatives against the range of phytopathogenic microorganisms is presented on table 3.

Table 3

Antimicrobial activity of tartaric acid synthetic derivatives on phytopathogenic Pseudomonas and Xanthomonas different strains. Inhibition zones are presented in mm. The cultures: a – Pseudomonas syringae, b – Xanthomonas vesicatoria; L – complete lysis of growth zone, GC – the positive control on solid nutrient agarised cultural media with 30mm maximal growth zones, "+" – normal growth and the absence of inhibition effect, "+/-" – less than 10% inhibition; the tested compounds: CAS – cyclohexylamine complex salt of tartaric acid, CI – cyclohexylimide of tartaric acid, BAS – benzylamine complex salt of tartaric acid, BI – benzylimide of tartaric acid, PhAS – Phenylamine complex salt of tartaric acid, PhI – Phenylimide of tartaric acid; the tested compounds concentrations: I- 50mcg/ml, II- 0.001M, III- 0.01M, IV- 0.05M, V- 0,1M, VI- 0,5M.

Bacterial strain		CAS						CI					GC
		I	II	III	IV	V	VI	II	III	IV	V	VI	
8736	a	15	17.2	25	25	L	L	23	23	23	23,8	L	30
8740		11	11	12	20	22	L	9.1	10	11.5	13	17	30
8744		12.1	13	15	14	18	20	14	16.2	19	20.1	22	30
8656		8	8	14.9	15.1	17	L	10.3	11,4	12	12.7	14	30
8647	b	18.2	20	19.7	20.4	22	L	20	20.8	21	21.3	22	30
8651		15	16.1	18.5	20.1	25	L	15	15,5	17,4	18,1	19	30
8843		L	L	L	L	L	L	L	L	L	L	L	30
Bacterial strain		PhI						PHAS					C
		I	II	III	IV	V	VI	I	II	III	IV	V	
8736	a	+	+	+/-	+/-	8	+	+	+	+	+	+	30
8740		+	+	+	+	+	+	+	+	+	+	+	30
8744		+	+	+/-	3.5	8	+	+	+	+	+/-	6	30
8656		+	+	+	+	+	+	+	+	+	+/-	3	30
8647	b	+	+	+	+/-	6	+	+	+	+/-	3	L	30
8651		+	+	+	+/-	5	+	+	+	+	+	+	30
8843		+	+	+	+	+	+	+	+	+	+	+	30
Bacterial strain		BAS						BI					
		I	II	III	IV	V	VI	I	II	III	IV	V	VI
8736	a	+	+	+/-	+/-	+/-	+/-	3	5	L	L	L	30
8740		+/-	5	6	6,5	7	8	5	6	6,5	7	7,2	30
8744		+	+	+/-	4	5,5	6	+	+/-	+/-	3,2	4,2	30
8656		+/-	2	3,8	5,5	6	6,5	3,9	4	4,5	5,6	L	30
8653	b	+.-	3	4,3	5,7	6,2	L	+	+	+	+	+	30
8651			+	+	+	+/-	3,4	+	+	+/-	+/-	3	30
8647			+	+	+	+/-	+/-	+	+	+/-	+/-	3,5	30

Due to data from Table 3, the maximal activity against the phytopathogenic strains of *Xanthomonas* and *Pseudomonas* was demonstrated in a majority of cases by cyclohexyl substituted derivatives of tartaric acid. Preheats, it might be defined by the affinity differences in cyclohexyl, phenyl and benzyl groups to enzymes, which are able to degradation of these compounds. The experiments of enzymes activity precipitations in various strains of Pseudomonas and Stenotrophomonas showed the high polyphenol oxidase activity in those strains cells which were resistant to tartaric acid cyclic derivatives effect [Liu at al, 2018; Babayan et al. 2020d]. According to literature data, the genes of 2 types of polyphenol oxidases in researched *Stenotrophomonas maltophilia* are encoded by their bacterial chromosome too. Then the resistant strains were cultivated on mineral media for biodegradation tests [Babayan 2020e]. The results showed that biodegradation of tartaric acid complex salts processes more intensively. Probably it is caused by their more hydrophilic properties, in opposite to hydrophobic imides.

Due to the antimicrobial activity tests, the species *P. taetrolens*, *P. chlororaphis* and *P. geniculate* were predominantly resistant to complex salts of tartaric acid, while in case of imides it was noted the inhibition of growth. Thus, for 3 subspecies of *P. chlororaphis (P. chlororaphis*, *subsp. chlororaphis, P. chlororaphis subsp. aureofaciens, P. chlororaphis, subsp. Aurantiaca)* and other resistant representatives were done biodegradation tests (table 4).

Table 4

Tartaric acid synthetic derivatives biodegradation by of P. chlororaphis 3 subspecies different strains. The bacterial strains P. chlororaphis subspecies – A – P. chlororaphis, subsp. chlororaphis, B - P. chlororaphis, subsp. aureofaciens, C – P. chlororaphis, subsp. aurantiaca; the tested compounds: CI – cyclohexylimide of tartaric acid (TA), BI – Benzylimide of TA, BAS – benzylamine complex salt of TA, CAS – Cyclohexylamine complex salt of TA; "+" - growth, "-" - absence of growth, "+*" - secondary growth after 5th day, GC^+ – the positive control on nutrient agar cultural media, GC^- - the negative control on mineral media.

Strain		CI	BI	BAS	CAS	GC^+	GC^-	Strain		CI	BI	BAS	CAS	GC^+	GC^-
A	*9189*	-	-	+	-	+	-	A	*9165*	-	-	+*	-	+	-
	9171	-	-	+	-	+	-		*9163*	-	-	-	-	+	-
	9190	-	-	+	-	+	-		*9157*	-	-	-	+	+	-
	9159	-	-	-	-	+	-		*9178*	-	-	-	_	+	-
	9177	-	-	+	+	+	-		*9172*	-	-	-	-	+	-
	9168	-	-	-	-	+	-		*9174*	-	-	-	-	+	-
	9164	-	-	-	-	+	-		*9158*	-	-	-	-	+	-
B	*9195*	-	-	+	+	+	-	C	*9066*	-	-	+	-	+	-
	9200	-	-	-	-	+	-		*9062*	-	-	+	+	+	-
	9199	-	-	-	-	+	-		*9064*	-	-	-	+	+	-

In a majority of considered strains of microorganisms, the effect of tartaric acid synthetic derivatives 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. It is correlating with well-known antibacterial activity data about the effect oa aldaric acids in general. Moreover, it was noted the forming of singular resistant colonies of some strains of *P. fluorescence*, after few days of cultivation on nutrient agar cultural media. Probably, this effect is being defined by selection of resistant mutants and is related with some enzymatic activity, for example polyphenol oxidases activity of *Pseudomonas* [Bagdasaryan et al. 2019; Janusz et al. 2020; Soong et al. 1999].

The results of bacterial growth inhibition by different derivatives of tartaric acid are represented on Fig. 3 and Fig. 4.

Both types of experiments on liquid and solid cultural media showed the maximal inhibitory activity of cyclohexyl substituted derivatives of tartaric acid. Phenyl and ethanolamine derivatives were less active against the various strains of phytopathogenic *Pseudomonas syringae, Xanthomonas vesicatoria, Xanthomonas beticola*, as well as against the animal and human opportunistic pathogenic *Stenotrophomonas maltophilia* and *Pseudomonas aeruginosa*.

Conclusion

During all the experiments with tartaric acid synthetic derivatives in forms of imides and complex salts of it on about 100 native soil strains of *Pseudomonas* and *Stenotrophomonas*, the antimicrobial effect of researched 4 compounds was noted. Benzylimide, Cyclohexylimide, as well as benzyl and cyclohexylamine complex salts of TA had demonstrated yourself as effective antibacterial agents against both sensitive and multidrug resistant representatives of both genera *Pseudomonas* and *Stenotrophomonas* isolated from soil. Antimicrobial effect of the discussed new semisynthetic compounds in a majority of cases was stronger than in case of well-known tartaric acid derivatives usage. For some strains the prolongation of growth till 1-2 days is detected. In other part of researched representatives of *Pseudomonas, Xanthomonas* and *Stenotrophomonas* there were detected single resistant colonies. For plasmid containing ones, the transformations of sensitive *Pseudomonas* representatives by the plasmids of mutants showed that the property of single colonies is not transmittable. Thus, the mutations are in nucleoid genes and cannot be transmitted to environment, by intraspecific gene horizontal transfer to other Gram-negative bacteria. Probably, these mutations are related with nucleoid localized polyphenol oxidases genes of Phytopathogenic *Xanthomonas* and *Pseudomonas*, as well as opportunistic pathogenic and *Stenotrophomonas* and *Pseudomonas.*

During the biodegradation tests, cyclohexyl- derivatives showed themselves as less biodegradative properties than the benzyl-derivatives. Perhaps, it is caused by chemical structure features of cyclohexyl radical and disabilities of appropriate bacterial enzymes to recognize these molecules as substrates, because of low affinity, absence of it or because of some allosteric regulation effects. The opportunistic pathogenic and non-pathogenic strains of *Pseudomonas* and *Stenotrophomonas*, isolated form soil can be 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.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable” in this section.

Data availability

Data used in this study were retrieved from the Clarivate Analytics PubMed Core Collection.

Competing interests

The authors declare that they have no conflict of interest.

Authors' contributions.

All authors contributed to the study conception and design. Material preparation, data collection, and analysis were performed by Baghdasaryan. A., Babayan B. The first draft of the manuscript was written by Melkumyan M., Grigoryan A. and Mikaelyan A. further extended and finalized it. All authors read and approved the final manuscript.

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Figures

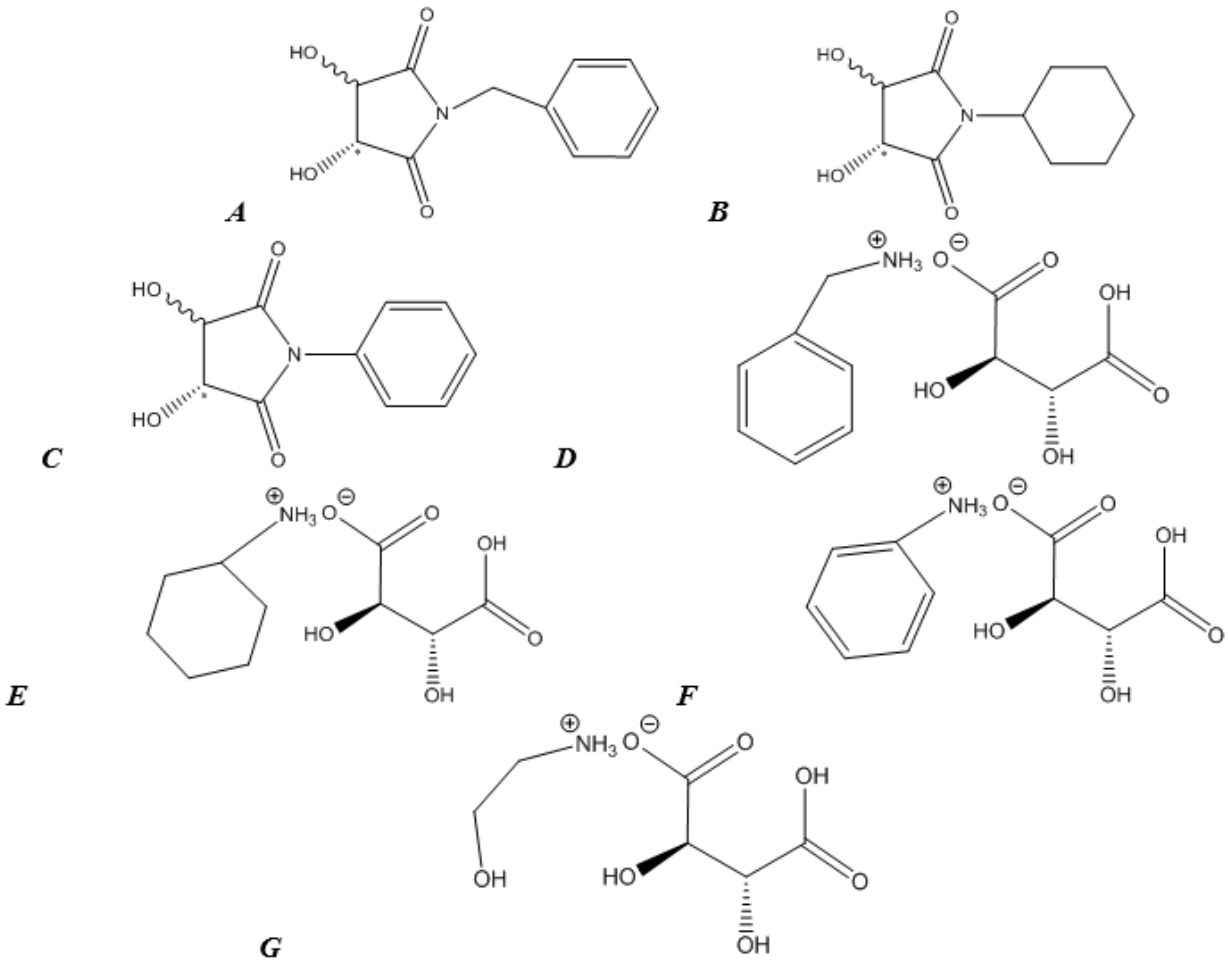

Figure 1

Synthetic derivatives of tartaric acid (TA). A – (BI) Benzylimide of TA, B – (CI) Cyclohexylimide of TA, C – (PHI) Phenylimide of TA, D – (BAS) Benzylamine complex salt of TA (previously were mentioned as Benzyl mono amino salt of TA), E – (CAS) Cyclohexylamine complex salt of TA(previously were mentioned as Cyclohexyl mono amino salt of TA), F – (PhAS) Phenylamine complex salt of TA (previously were mentioned as Phenyl mono amino salt of TA), G – (EAS) ethanolamine complex salt of TA (previously were mentioned as MEAS/monoethanolamino complex salt of TA)

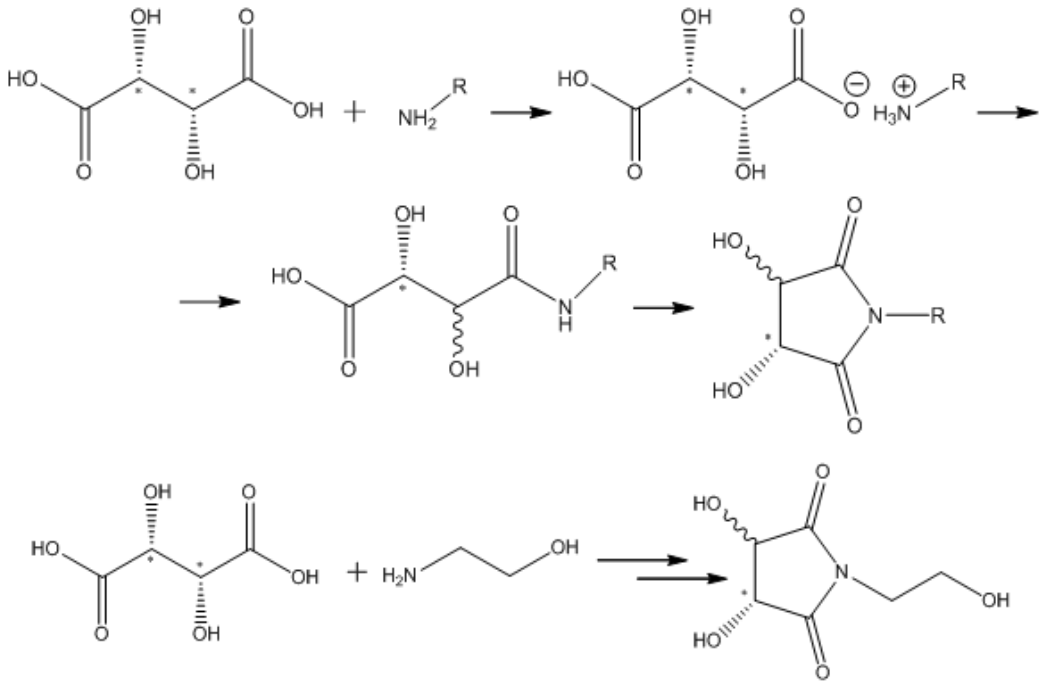

Figure 2

The principal Scheme of Tartaric acid derivatization. R = benzyl, phenyl, cyclohexyl, ethanolamine radicals in appropriate derivatives

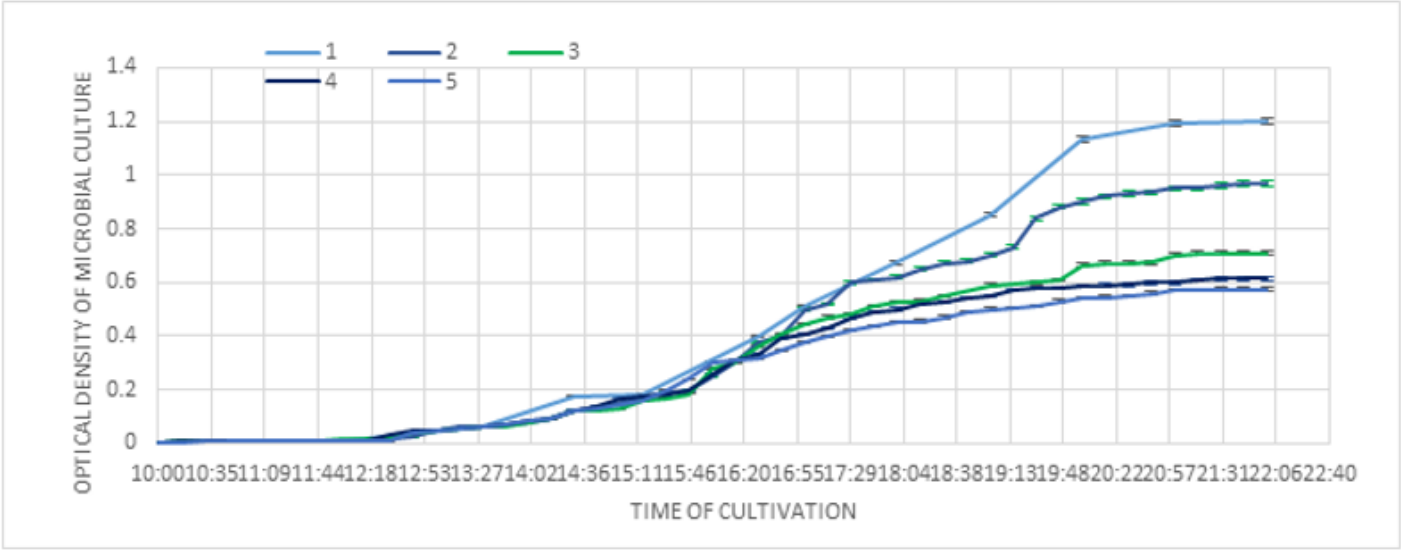

Figure 3

The effect of Tartaric Acid (TA) Synthetic Derivatives on P. aeruginosa 5249b on liquid nutrient cultural media. The culture was cultivated on liquid cultural media and then, the substances were added in 10 times less concentration then the minimal inhibitory one. 1 – positive control on cultural media, 2 – BAS (benzylamine complex salt of TA), 3 – BI (Benzylimide of TA), 4 –CAS (Cyclohexylamine complex salt of TA), 5 – CI (Cyclohexylimide of TA)

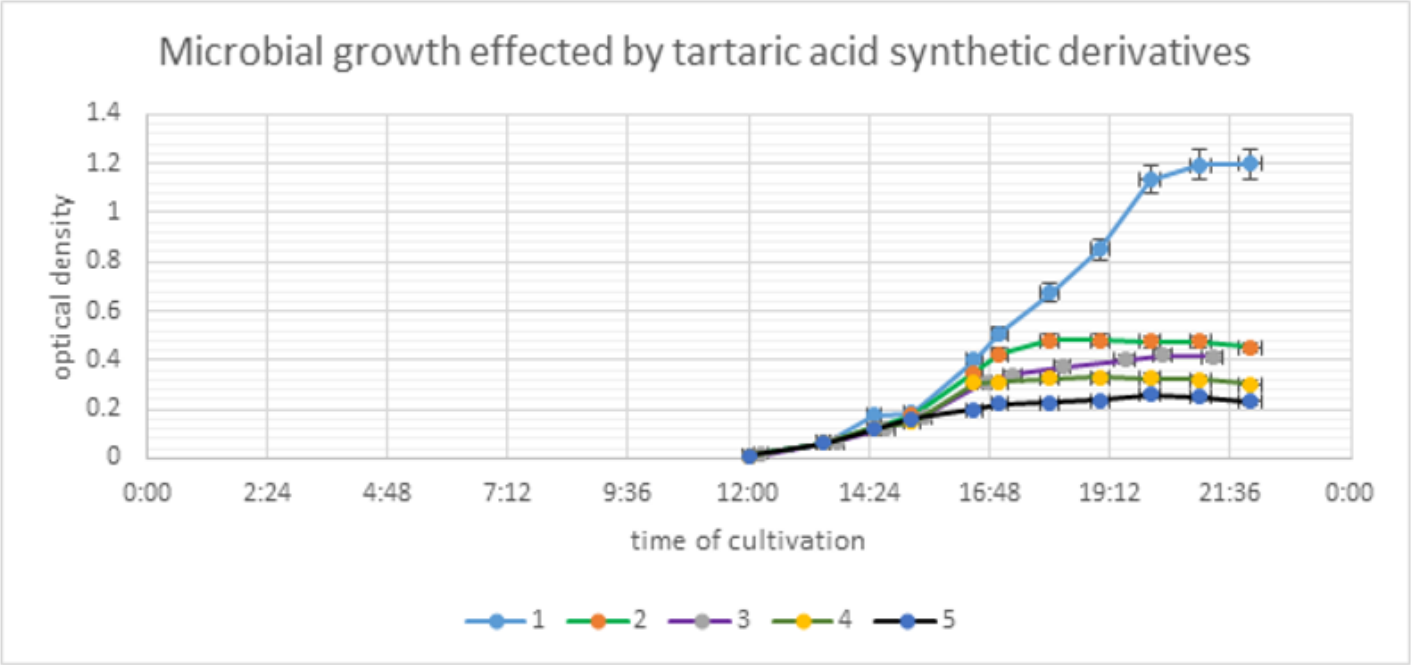

Figure 4

Phytopathogenic Pseudomonas syringae growth inhibition by tartaric acid (TA) different synthetic derivatives. The culture was cultivated on liquid cultural media and then, the substances were added in 10 times less concentration then the minimal inhibitory one. 1 – positive control on cultural media, 2 – BAS (benzylamine complex salt of TA), 3 – BI (Benzylimide of TA), 4 –CAS (Cyclohexylamine complex salt of TA), 5 – CI (Cyclohexylimide of TA)