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THE STUDY OF PROTEOLYTIC ACTIVITY OF SOME STRAINS OF PECTOBACTERIUM CAROTOVORUM REPRESENTATIVES PHYTOPATHOGENIC BACTERIA

Marina A. Melkumyan
SPC «Armbiotechnology» NAS RA
14, Gyurjyan str. 0011, Yerevan
e-mail: marmelk2109@gmail.com
ORCID iD: 0000-0003-3250-9891
Republic of Armenia

Bella G. Babayan
SPC «Armbiotechnology» NAS RA
14, Gyurjyan st. 0011, Yerevan
«Agrobiotechnology Scientific Center»,
Branch of Armenian National Agrarian University
1 Isi le Mulino str., 1101 Ejmiatsin
e-mail: arixtya12.15bbg@aol.com
ORCID: 0000-0002-3978-1209
Republic of Armenia

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Abstract

Phytopathogenic microorganisms are one of the most harmful biotic factors negatively affecting on agriculture, horticulture and forestry. Proteases of various phytopathogens including bacteria and fungi are well-known, such as like the proteases of human and animal opportunistic pathogenic bacteria. And often a proteolytic activity is related to the pathogenicity and virulence of the mentioned microbes. One of the most common pathogens of plants is *Pectobacterium carotovorum*, which is also known as *Bacillus carotovorum* or *Erwinia carotovora*. Current research is devoted to the study of extracellular proteolytic activity of *P. carotovorum* 24 strains.

Keywords: pectobacterium carotovorum, biodegradation potential, antibiotic-resistance, phytopathogenic microorganism, caseinase.

Introduction

Phytopathogenic microorganisms are presented by the large group of microbes, such as like gram-negative and gram-positive bacteria and fungi [1]. They are able to become a cause of various plant diseases. As a result of phytopathogenic microorganisms' infections bacterioses of plants are the main causes of agricultural production quality serious decrease [2,

3]. One of the common phytopathogens is *Pectobacterium carotovorum*. *P. carotovorum* phytopathogen was previously known as *Bacillus carotovorum* and then re-classified as *Erwinia carotovora* [4].

The main way of combating the pathogens in general are antimicrobial substances, such as like they are broadly used biological, complex and combined methods of plant protection. In these regards, antimicrobial resistance of phytopathogenic bacteria is one of the most important challenges for the plant protection, what has a significant impact on the quality of agricultural production [5-7]. The main components of antimicrobials preparations are antibacterial substances, such as like singular antibiotics and their combinations. But year after year, the efficiency of these substances decreases, what led to the increase of antibiotics using volumes. That is why the phenomenon of multi-drug resistance is well known and continues to become more and more dangerous problem for humanity in general [8]. In contribution to the aggravation of multi-drug resistance problem, the accessive usage of antibiotics and various pesticides led to the decrease of agricultural production quality. As a result, all of these is very negatively effects on the consumer's health [8, 9].

Thus, it is extremely important to study the factors which form the resistance stability and spread among the bacteria, for their further targeting for appropriate novel antimicrobials elaboration. One direction of it an implementation of new classes of substances for plant protection, such as like tartaric acid derivatives [10]. Another way is the usage of biopesticide, bacteriocins and the biological enemies of target phytopathogenic bacteria. But unfortunately, the main part of them is not so safe for mammalians, including human and agricultural animals. Also, one of the prospective directions of the resistance overcoming is the elaboration of key enzyme inhibitors, which are being broadly used as antiviral drugs [11-13]. In these regards, proteolytical activity is one of the useful targets, because of its relation to pathogenicity and virulence of many pathogens, including phytopathogenic bacteria, human opportunistic pathogens, etc. [14].

In current paper, proteolytic activity of some phytopathogens was discussed for further targeting for appropriate protease inhibitors elaboration and usage.

Conflict setting

During this research the bacterial strains from The National Collection of Microorganisms of Microbial Depository Center (MDC) of "Armbiotechnology" Scientific and Production Center (SPC) of National Academy of Sciences of Republic of Armenia (NAS RA) were used [15].

Extracellular proteolytic enzyme qualitative assessment was carried out milk agar with casein and gelatin appropriately. Caseinase activity was also defined by the method of photographic film [16, 17]. The resistance of studied bacteria was tested to various antibiotics of different classes and generations. There were used the mainly used compounds, which are broadly used in medicine, veterinary as well as in agriculture. The tests were carried out due to the standard protocols [18].

There were used the following antibiotics: Amp/ampicillin, Amx/Amoxicillin, Amc/Augmentin (from aminopenicillins of the class of β-lactamic antibiotics), Cfx/Cefixime,

Cro/Ceftriaxone (the I and the III generation representatives of cephalosporins from β-lactamic antibiotics), Kan/kanamycin, Stp/Streptomycin (from aminoglycoside class of antibiotics), Cip/Ciprofloxacin (from fluoroquinolone class of synthetic antibiotics), Tcn/Tetracycline (from the class of tetracycline antibiotics), Azm/Azithromycin (from azalides of the class of macrolide antibiotics), Cam/Chloramphenicol (form amphenicol class of antibiotics), produces by "Astoria" company.

The genetical analysis of the studied bacteria were carried out by the generally accepted methods of total (chromosome DNA) and plasmid DNA isolation, then the agarose gel electrophoresis (0.8%–2.5%) and transformation by the method of Mandel [19].

Digital analysis of results was performed by ImageJ software [20]. The statistical analyses were done due to standard protocols of Microsoft Excel program package.

Research Results

During the experiments which were carried out, that almost all the strains of the studied phytopathogenic bacteria are able to grow on milk agar media and destruct the casein of milk. Thus, the majority of the studied *P. carotovorum* have demonstrated the presence of extracellular caseinase activity.

The grade of its expression was different, depends to the particular strain. According to the obtained results, the maximal intensity of milk caseine degradation was noted for the following strains: *P. carotovorum 8705* and *P. carotovorum 8707.* The minimal level of extracellular caseinase activity was observed for strains *P. carotovorum 8722* and *P. carotovorum 8698.* In case of strains *P. carotovorum 8694, P. carotovorum 8692, P. carotovorum 8729, P. carotovorum 8771, P. carotovorum 8861* the extracellular caseinase activity was not observed (tab. 1).

Table 1

Extracellular caseinase of *P. carotovorum* various strains.

Strain of Bacteria	Microbial growth intensity (in mm)	Caseinase activity registration zone diameter (in mm)	Strain of Bacteria	Microbial growth intensity (in mm)	Caseinase activity registration zone diameter (in mm)
8702	1	1	*8765*	3	2
8703	2	5	*8766*	2	10
8705	2	10	*8667*	2	1
8707	2	10	*8690*	1	0
8712	3	5	*8692*	1	0
8713	1	5	*8694*	2	0
8717	1	1	*8696*	3	3
8717	1	1	*8698*	1	1
8721	1	3	*8699*	3	2
8722	2	1	*8861*	2	0
8729	0	0	*8764*	1	0
8771	1	0	*8756*	2	3

According to the results of computer analysis by the 3D reconstruction and thermogram presented on fig. 1. in strains *P. carotovorum 8698* and *P. carotovorum 8794* caseinase activity

is presented but the enzyme is not so active (Fig. 1). The previous research has demonstrated the presence of different level caseinase activity in some other phytopathogenic strains such as like *Pseudomonas syringa, Xanthomonas vesicatoria and Rhodococcus fascians* [21].

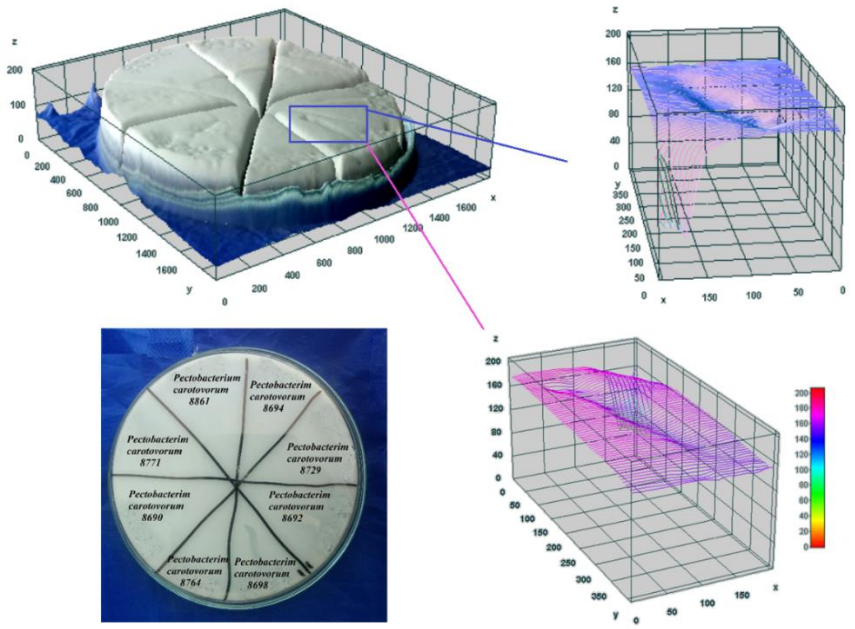

Fig. 1 *P. carotovorum* extracellular caseinase qualitative assessment on milk agar cultivation media and the digital analysis of it.

The tests of antibiotic-resistance of the studied bacteria have demonstrated the absence of strains which would be absolutely sensitive to the considered antibiotics. The majority of them have demonstrated multi-drug resistance and pan-drug resistance (tab. 2).

Table 2

Resistance (R) and sensitivity (S) of P. carotovorum different strains to antibiotics of various classes and generations

Strain	Tcn	Cip	Cam	Azm	Stp	Gnc	Kan	Cro	Cfx	Amc	Amx	Amp	Pnc
8702	S	S	S	R	S	S	S	R	R	R	R	R	R
8705	S	R	R	R	R	S	R	R	R	R	R	R	R
8717	S	R	S	R	S	S	R	R	R	R	R	R	R
8718	S	S	R	R	S	S	S	S	R	R	R	R	R
8756	R	R	S	R	R	S	R	R	R	R	R	R	R
8764	R	S	R	R	S	S	S	S	R	R	R	R	R
8765	S	S	S	S	S	S	S	S	S	R	S	R	R
8690	R	S	S	R	S	R	S	S	R	R	R	R	R
8694	R	S	S	R	S	S	R	S	R	R	R	R	R
8698	S	S	S	S	S	S	S	S	S	S	R	R	R

At the next step of research, some genetical analyses were carried out. The results of are presented on Fig. 2.

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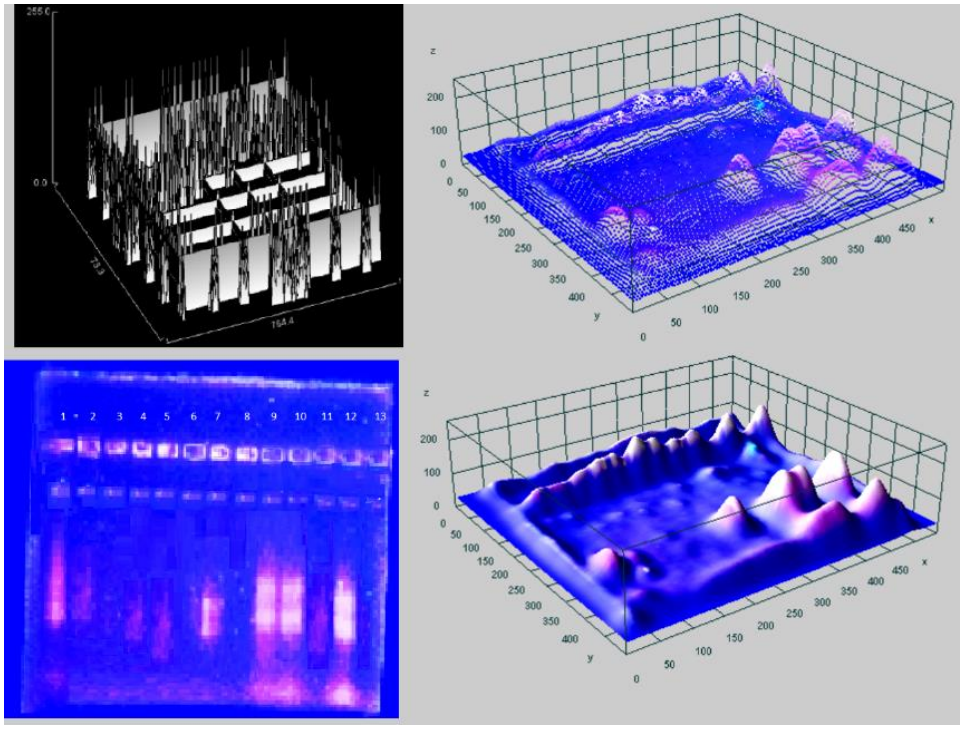

Fig. 2 DNA Electrophoresis of the studied phytopathogenic bacterial strains and the digital analysis of it

Strains: 1 – P. carotovorum 8699; 2 – P. carotovorum 8703, 3 – P. carotovorum 8758; 4 – P. carotovorum 8690; 5 – P. carotovorum 8694; 5 – P. carotovorum 8702; 6 – P. carotovorum 8705; 7 – P. carotovorum 8717; 8 – P. carotovorum 8718; 9 – P. carotovorum 8756; 10 – P. carotovorum 8764; 11 – P. carotovorum 8765; 12 – P. carotovorum 8692; 13 – P. carotovorum 8729.

The electrophoretic analyses of the isolated DNA have demonstrated the presence of plasmids in main part of the studied strains *of P. carotovorum*. During all the experiments which were done plasmid containing as well as non-plasmids strains of *P. carotovorum* were detected in both caseinase-positive and caseinase-negative strains. Also, the diapason of antibiotic-resistance of them was different too and not related to the plasmid presence in cell. According to the digital analysis of the obtained electropherograms, the molecular weight of the detected plasmids is different.

Then the transformation experiments with all the strains were carried out for the understanding of the function of the detected plasmids. Besides, the transformation experiments were carried out for the potential cryptic plasmid detection, which cannot be identified by the direct electrophoretic study duy to the small quantity of copies in cell and other factors. In experiments there were also included both antibiotic-resistant strains and the strains which demonstrated the sensitivity to them.

For that purpose, as the donor there were used the caseinase-positive strains. By the plasmid DNA, isolated from caseinase-positive strains the recipient cells of strains, which were caseinase-negative were transformed. All the results of transformations were negative. Thus, the defined plasmids are not transmitting caseinase activity. Thus, this activity is encoded by the bacterial chromosome, as a part of gens, which ensure the primary metabolism of bacteria.

Conclusion

According to the research among the 24 strains of *P. carotovorum* common plant pathogenic microorganism, the extracellular protease activity was identified. The extracellular caseinase activity was found in a majority of the studied bacteria. Caseinase activity of them is not related to the antibiotic-resistance diapason. In one part of the studied strains the plasmids with different molecular weight were identified. The experiments of transformation have demonstrated that these plasmids are not able to transfer extracellular caseinase activity. Thus, the found caseinase activity is encoded by the genes of bacterial chromosome and it can be targeted for further research of proteases inhibitors for the combating this phytopathogenic bacteria.

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ՊՐՈՏԵՈԼԻՏԻԿ ԱԿՏԻՎՈՒԹՅԱՆ ՈՒՍՈՒՄՆԱՍԻՐՈՒՄԸ «PECTOBACTERIUM CAROTOVORUM» ՖԻՏՈՊԱՏՈԳԵՆ ՄՆՐԷԻ ՈՐՈՇ ՇՏԱՄՆԵՐՈՒՄ

Մ.Ա. Մելքումյան[1], Բ.Գ. Բաբայան[1,2]
[1]ՀՀ ԳԱԱ «Հայբիոտեխնոլոգիա» ԳԱԿ
[2]«Ագրոկենսատեխնոլոգիայի գիտական կենտրոն» ՀԱՀ մ/ճ

Ֆիտոպաթոգեն միկրոօրգանիզմները ամենավնասակար կենսագործոններից են, որոնք բացասաբար են անդրադառնում գյուղատնտեսության, այգեգործության և անտառային տնտեսության վրա: Հայտնի են տարբեր ֆիտոպաթոգենների, այդ թվում՝ բակտերիաների և սնկերի, ինչպես նաև մարդկանց և կենդանիների համար

պայմանականորեն ախտածին մանրէների պրոթեզերները: Եվ հաճախ պրոտեոլիտիկ ակտիվությունը կապված է նշված մանրէների պաթոգենության և վիրուկենտության հետ: Բույսերի ամենատարածված ախտածիններից մեկը *Pectobacterium carotovorum*-ն է, որը նաև հայտնի է որպես *Bacillus carotovorum* կամ *Erwinia carotovora*: Սույն հետազոտությունը նվիրված է *P. carotovorum*-ի 24 շտամների արտաբջջային պրոտեոլիտիկ ակտիվության ուսումնասիրությանը:

Բանալի բառեր՝ Pectobacterium carotovorum, կենսաքայքայման ներուժ, հակաբիոտիկակայունություն, ֆիտոպաթոգեն մանրէ, կազեինազ:

ИЗУЧЕНИЕ ПРОТЕОЛИТИЧЕСКОЙ АКТИВНОСТИ У НЕКОТОРЫХ ШТАММОВ ФИТОПАТОГЕННЫХ БАКТЕРИЙ, ПРЕДСТАВИТЕЛЕЙ «PECTOBACTERIUM CAROTOVORUM»

М.А. Мелкумян[1], Б.Г. Бабаян[1,2]
[1]*НПЦ «Армбиотехнология» НАН РА*
[2]*«Научный центр агробиотехнологии», Филиал НАУА*

Предложена фитопатогенные микроорганизмы являются одним из самых вредоносных биотических факторов, негативно воздействующих на сельское хозяйство, садоводство и лесное хозяйство. Протеазы различных фитопатогенов, включающих бактерии и грибы, хорошо известны, как и протеазы оппортунистических патогенов человека и животных. И нередко протеолитическая активность связана с патогенностью и вирулентностью указанных микробов. Одним из наиболее распространенных патогенов растений является *Pectobacterium carotovorum*, также известная как *Bacillus carotovorum* или *Erwinia carotovora*.

Данное исследование посвящено изучению экстрацеллюлярной протеолитической активности у 24 штаммов *P. carotovorum.*

Ключевые слова: Pectobacterium carotovorum, потенциал биодеградации, антибиотик-резистентность, фитопатогенный микроорганизм, казеиназа**.**

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