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QUANTITY AND BIOLOGICAL PROPERTIES OF THE BACTERIUM *PANTOEA AGGLOMERANS* ISOLATED FROM DIFERENT GRAPE VARIETIES IN ODESA REGION

Aim. *To isolate and investigate the biological properties of Pantoea agglomerans from the internal environment of grapes and tumors selected from the vineyards of the Odessa region.* ***Methods.*** *Samples of the grapevines of Arkadia, Moldova and Odessa souvenirs varieties and tumors cut from the affected veins and were pre-sterilized, chopped into fragments, filled into a distilled water flask and stirred in a shaker at 28 °C for 3 hours. It was made 10-times dilutions and cultured on the surface of Petrie`s dishes with nutritients agar. Incubationon 28 °C, 24–48 hours. Conducted quantitative calculations, isolated pure cultures and examined their cultural, morphological, physiological and biochemical properties, determined the composition of cell lipids, and identified isolated strains. Antagonistic activity of P. agglomerans was determined by well-diffusion method.* ***Results.*** *Quantities of bacteria in inner part of grape fluctuated approximately 5,63 ± 1,3X10³CGU/см³– 2,96 ± 1,3X10⁵CGU /см³and depended from time of the year and variety of grape. Quantities of microorganisms in tumors was bigger than in non damaged vine in the same period. Weight percentage P. agglomerans in vine fluctuated from 5.7% till 68.2%, in tumors – from 9.88% till 23.08%. Strains of P. agglomerans isolated from endophytic medium of grape and tumor, characterized of similar morphological features. There were observed the ability to utilize carbohydrates, but in minnor. Pantoea strains did not consume sucrose from the tumors and 25.0% utilized raffinose in aerobic conditions, unlike strains from the intact vine. The fatty acid spectrum was represented by fatty acids containing 12 to 19 carbon atoms in the chain. Eighteen strains have been identified as P. agglomerans-GC subgroup A by fatty acid composition. For the strains examined, the dominant profiles are C16: 0, C12: 0, C14: 0, C17: 0 cyclo w7c. The isolated strains of P. agglomerans did not show antagonistic activity to the collection strains of E. carotovora, A. tumefaciens and A. vitis.* ***Conclusions.*** *The quantitative composition of the microbiota endophytic medium of the vine and the tumors was different and depended on the source of isolation, weather conditions and grape variety. The strains of P. agglomerans isolated from the vine and tumors were characterized by the same biological characteristics, with the exception of the ability to dispose of individual carbohydrates. Fatty acid composition of the investigated strains of Pantoea was represented by fatty acids with 12 to 19 carbon atoms in the chain. Antagonistic activity in the collection strains of E. carotovora, A. tumefaciens and A. vitis was not detected.*

Key words: Pantoea agglomerans, endophytic medium of grapevine and tumors, number, biological properties.

Pantoea agglomerans (Beijerinck 1888) comb. Nov [12] Earlier *Enterobacteragglomerans* (Beijerinck 1888) Ewing and Fife (1972), *Erwinia herbicola* (Löhnis 1911) Dye 1964, or *Erwinia milletiae* (Kawakami and Yoshida 1920) Magrou 1937, are gram-negative bacteria belonging to the *Enterobacteriaceae* family.

P. agglomerans, mainly, an epiphytic plant pathogen that develops on the surface of plants, or an endophyte that resides within plants [3, 19].

The widespread distribution of these bacteria in nature (they also occur in plant and animal products, in the body of animals, in water, soil, dust and air, and sometimes in humans) and their biological role in the different objects of existence are the subject of discussion and ambiguous relation to them. On the one hand, they can cause disorders in humans [18], can cause diseases of crops [8, 9], and on the other hand, they produce substances that are effective in the treatment of cancer and other diseases of humans and animals, inhibit the development of various plant pathogens, promote the growth of plants and is a potentially effective biological fertilizer and biomediator [7, 10].

Since *P. agglomerans* characterizes the versatility of biology, ecology, and the role of the environment, scientific interest in them does not subsist. In view of this, the purpose of the work was to isolate and investigate the biological properties of *Pantoea agglomerans* from the endophytic medium of grapes and tumors selected from the vineyards of the Odessa region.

Materials and methods

In this work samples (stems) from the grapes "Arcadia", "Moldova" and "Odessa souvenir" were used, as well as tumors, and cut from the affected vine grapes of these varieties.

The preparation of all samples was unified and consisted of the following stages. Samples of the grapevine and the tumors were preliminarily soaked under running water, washed with sterile distilled water, and sterilized the surface, treating 96 °C with alcohol and flaming.

After this, maintaining sterile conditions, the selected samples were chopped into fragments approximately 0.5 cm in size, 10 g of which were transferred to conical flasks of 250 cm^3 and placed on 50 cm^3 of sterile distilled water for each sample. Experimental samples were mixed in a shaker (from New Brunswick at 250 RPM) at 28 °C for 3 h. After that, a series of 10 consecutive dilutions was made. Petri dishes with nutrient agar (Merck, Germany) were cultured on the surface of 0.1 cm^3 of appropriate dilutions. The seeds were incubated at 28 °C for 24–48 hours.

It was calculated a quantitative of microorganisms that grew up, isolated pure cultures and investigated biological properties: cultural, morphological, physiological and biochemical.

For the analysis of cell lipids composition, samples were prepared with bacterial cultures which were pre-incubated at Tryptic soy agar (Merck, Germany) at a temperature of 28 ± 1 °C for 24 hours.

Three full loops of biomass were placed in the reaction vial and a concentrated NaOH solution was added. This samples was thoroughly mixed and placed on a water bath and maintained at 95–100 °C for 5 minutes. After that, the mixing was repeated and left in a water bath at 95–100 °C for 25 minutes to completely destroy the bacterial cells and purify the lipids. To the cooled suspension, a solution of acidified methanol was added and held in a water bath at 80 °C for 10 minutes to obtain the fatty acid methyl esters, which were then extracted with hexane. The extract was neutralized with 0.3 M alkaline solution and analyzed by gas chromatography [1].

The chromatographic separation of methyl esters of LC was performed on a gas chromatograph Agilent 7890 (Agilent Technologies, USA) with a capillary column ULTRA 2 and a semi-ionization detector. Samples of 2 μl were injected into the evaporator in a split mode with a coefficient of 40:1, temperature of evaporator 250 °C. The separation was carried out in the programming mode of temperature – the initial temperature 170 °C with a gradient of 5 °C / min to 270 ºC. The fatty acids content was expressed as a percentage of the total sum of peak areas.

MIDI Sherlock 4.5 software and the RSTBA6 version 6.21 fatty acid profile of the aerobic microorganisms were used to identify the strains examined for their fatty acid profile.

The antagonistic activity of the strains of *P. agglomerans* to the collection strains of *Erwinia carotovora, Agrobacterium tumefaciens* and *Agrobacterium vitis,* as well as intrinsic antagonism were determined by the hole diffusion method, measuring the growth test inhibition zones of the test strains after 24 hours of cultivation at 28 °C [2].

Statistical processing of the results of the study was carried out using the MS Excel computer program with the definition of Student's *t*-criterion. The difference was statistically significant at $P < 0.05$.

Results and discussion

The internal environment of the vine grape contains a significant number of bacteria that influence on the lasting of physiological processes in plants, promote the protection of plants against pathogens, but some, under certain conditions, can cause disease or influence on the infectious process. These bacteria include P. *agglomerans,* which can be used as an indicator of yeast and mold (mold) fungal biocontrol, pathogenic bacteria, but it can itself be an opportunistic pathogen.

Experimental studies were carried out in April-October 2017. Depending on the month of the research and the grape variety, the number of microorganisms of the internal environment of the grapes varied within the limits of 5.63 ± 1.3 ms10^3 CFU / cm^3 – 2.96 ± 1.3 ms10^5 CFU / cm^3.

The smallest number of microbiota was detected in April, the largest in September 2017, due to weather seasonal and climatic conditions.

In September and October, defects in the form of tumors were detected on grape barrels of all varieties, in the internal environment the total number of microbiota was higher in September (Fig. 1).

The obtained results indicate the sensitivity of these bacteria to weather conditions. The frequency of isolation of *P. agglomerans* was bigger in September

2017 (for sufficiently dry weather and moderate summer temperature, 28–30 °C), the smallest – in October of this year (when in the beginning of the month there was a significant decrease in temperature, 12–15 °C).

In addition to *P. agglomerans,* other microorganisms were isolated from the endophytic medium of grapes, most of which were representatives of the genera *Agrobacterium* and *Erwinia.*

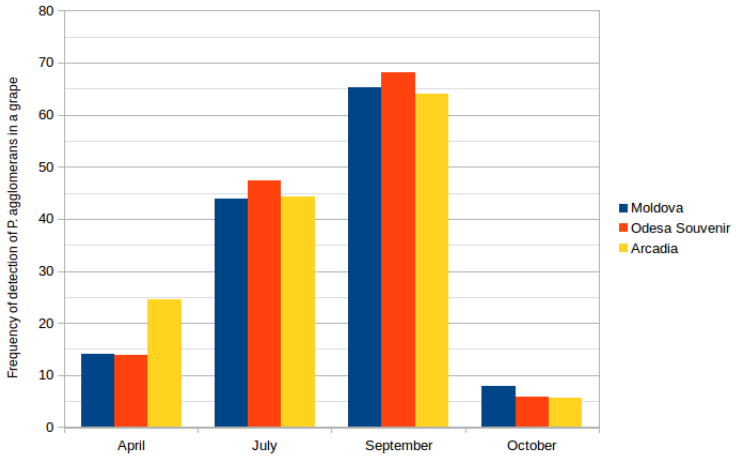

Fig. 1. Frequency of detection of bacteria *P. agglomerans* in a grape, 2017 year (data presented in percentages)

P. agglomerans were also isolated from the internal environment of the tumors. The percantage of them among the other microbiota was higher in October and amounted to slightly more than 20.0%, in September this figure was two times smaller (Figure 2).

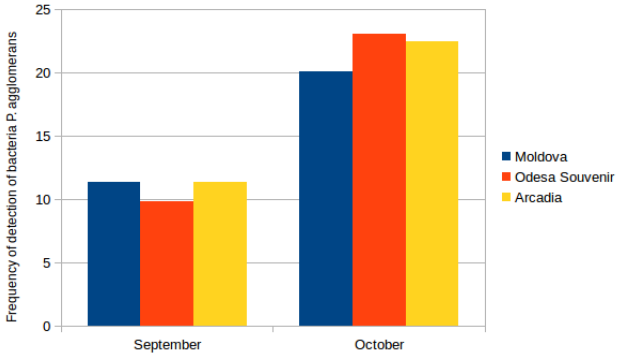

Fig. 2. Frequency of detection of bacteria *P. agglomerans* in a grape's tumor in 2017 year

Comparing the obtained data shown in Fig. 1 and 2, it should be noted that in September in the endophytic environment of the intact grapevine all varieties were dominated by *P. agglomerans,* whereas in tumors their proportion was much

smaller. In October, in the grapevine, the proportion of *P. agglomerans* decreased significantly, while in tumors, on the contrary, it increased. The taxonomic composition of the bacteria isolated from the tumors was more varied than in the grapevine. Among the isolates, bacteria of the genus *Agrobacterium, Erwinia, Xanthomonas, Achromobacter, Serratia* predominated.

Compare obtained results and data with literary sources [10, 18], it was supposed that bacteria *P. agglomerans* could be as representative in both sides, of normal microbiota of grapes and one of the potential pathogens involved in the formation of tumors.

Biological properties have shown that isolated and selected samples were coinciding with the characteristics of bacteria *P. agglomerans*. All strains of *P. agglomerans* were able to grow on nutrient agar; forming in 24 hours at 28 °C rounded, smooth, regular, carved, shiny, translucent colonies; capable for pigmentation of bright yellow color; with sizes of 0.5–3 mm.

A microscopic study has shown that isolated bacteria are gram-negative, the rod-shaped, with rounded ends. The cell sizes are defined within the range of 0.5 – 1.0 × 1 – 3 μm. Bacteria are capable to movement.Also, the optimal growth temperature was observed in the range of 25–30 °C, and it is typical for these bacteria.

All strains are oxidase-negative and catalase-positive. The ability to utilize carbohydrates was different in strains isolated from intact grapevine and from tumors (Table 1).

All tested strains were utilized only with the release of maltose, mannose, mannitol, xylose, rhamnose in aerobic and anaerobic conditions, and lactose only in anaerobic conditions. *Pantoea* strains isolated from tumors did not consume sucrose and 25.0% utilized raffinose in aerobic conditions, unlike strains from intact grapevines.

For the *Pantoea agglomerans* strains the presence of isomers of fatty acids with a carbon chain length of 12 to 19 carbon atoms were shown. Among the saturated isomers, hexadecanoic acid (C16:0) was dominant with a total content of 29.1 to 30.83% of the total sum of peak areas. The content of tetradecanoic acid (C14:0) ranged from 4.78 to 5.72%, dodecan (C12:0) – from 3.04 to 4.57%, octadecanoic acid – from 0.24 to 0.37%. The content of saturated isomers with an odd number of carbon atoms: tridecan (C13:0), heptadecan (C17:0) was within the range of no more than 0.14 and 0.34%, respectively. For all studied strains, a high content of cycloheptadecanoic acid (cyclo-C17:0) was characterized from 3.74 to 7.55% of the total sum of peak areas. For strains 1a and 4, the presence of pentadecanoic fatty acid (C15:0) in the amount less than 0.1% is shown. For these strains, the presence of both 13-methyl-pentadecane (iso) and 12-methyl-pentadecane (anteiso) isomers was characteristed. The presence of cyclo-nonadecanoic acid (cyclo-C19:0) in trace amounts for most strains has been shown.

For the fatty acid composition of all investigated strains of *Pantoea agglomerans,* the dominance of a mixture of isomers of hexadecenic acid (C16:1) was characteristic, the content of which ranged from 30.33 to 36.92%. The presence of isomers with unsaturated bonds in the 9, 10, and 11 positions of the carbon chain is shown. Dominant were 9-hexadecenic (9-C16:1) and 10-hexadecenic

Table 1

Biochemical activity of *Pantoea* strains isolated from grape and tumors

Carbons		Strains, isolated from	
		Vinegrape	Tumors
Maltose	Aerobic conditions	+	+
	Anaerobic conditions	+	+
Mannose	Aerobic conditions	+	+
	Anaerobic conditions	+	+
Mannitol	Aerobic conditions	+	+
	Anaerobic conditions	+	+
Xylose	Aerobic conditions	+	+
	Anaerobic conditions	+	+
Rhamnose	Aerobic conditions	+	+
	Anaerobic conditions	+	+
Glucose	Aerobic conditions	+	+
	Anaerobic conditions	+	+
Lactose	Aerobic conditions	-	-
	Anaerobic conditions	+	+
Sucrose	Aerobic conditions	+	-
	Anaerobic conditions	+	+
Raffinose	Aerobic conditions	-	[-]
	Anaerobic conditions	+	+

Note: "+" – positive characteristic; «-» – negative characteristic; "[-]" – positive characteristic in 25.0% strain.

(10-C16:1) isomers. Among the unsaturated isomers for all investigated strains, 11-octadecenoic fatty acid (11-C18:1) was characterized by a content of 10.43 to 14.0%. 13-Oktadecenic acid was detected in trace amounts in strains 1a, 3, 9, 15, 22x, 24, 25, 27.

For all investigated strains, the presence of hydroxylated fatty acids was characteristed. Thus, the content of 3-hydroxy-tetradecanoic acid (3-OH-C14: 0) ranged from 6.22 to 9.98% of the total sum of peak areas. The content of 2-hydroxy-dodecanoic acid was observed at a level less than 0.1% for strains 1a, 4, 15, 22x, 24, 25 and 27. For strains 35, 36, 41 and 41a, the presence of a small amount of 3-hydroxy-pentadecane acids (3-OH-C15:0) – less than 0,2% was shown.

For strains 35, 36, 41 and 41a, a small amount of 3-hydroxy-pentadecanoic acid (3-OH-C15:0) is shown to be less than 0.2%. For strains 35 and 41a, the presence of 2-hydroxy-pentadecanoic acid (2-OH-C15:0) in the amount of 0.33 and 0.4%, (Table 2), respectively, is shown.

The genus *Pantoea* was first described by Gavini [12] with the sole representatives of the species *Pantoea agglomerans*. To date, this genus has 22 validated and described species, among which *Pantoea allii, Pantoea brenneri,*

Pantoea calida, Pantoea coffeiphila, Pantoea conspicua, Pantoea intestinalis, Pantoea rodasii, Pantoea rwandensis, Pantoea theicola, Pantoea alhagi, recently posted.

Representatives of the genus *Pantoea* belong to the *Enterobacteriacea* family. One of the chemotaxonomic markers of this group is the presence of hydroxy acids in the structure of the lipopolysaccharides. The lipopolysaccharides are the main component of the external membrane of the cellular wall of gram-negative bacteria, which plays a key role in interaction of the cell with surrounding objects. Thus, in the purified lipopolysaccharides of the strain *Pantoea agglomerans* 7969 3-hydroxytradecanoic acid, dodecanova, tetradecanova, hexadecan and 2-hydroxytetradecanova were detected.

The total fatty acid composition of the typical *Pantoea agglomerans* LMG 1286T cells includes typical dodecanic, tetradecanoic, hexadecanoic, cycloheptadecanoic fatty acids in the amount of 3.8; 6; 27.1; 13.2% of the total sum of peak areas [16]. A characteristic feature is the high content of hexadecenoic and octadecenoic fatty acids – 24.2 and 11.6%, respectively. The content of 3-hydroxytradecanoic acid is 6%. Brady et al. characterized three new species of the *Pantoea* family that cause bacterial blight and dieback of eucalyptus in Colombia – *Pantoea rodasii,* Rwanda – *Pantoea rwandensis* and South Africa – *Pantoea wallisii* [5,4,6]. All isolates were characterized by a typical set of fatty acids of representatives of *Pantoea.* Strains *Pantoea rodasii, Pantoea rwandensis* had a close spectrum (Table 2). *Pantoea wallisii* had differs from the two previous slightly higher content of octadecenoic fatty acid – 18.8%, and decrease in the proportion of hexadecenoic 16.3% and tetradecanoic acid 3.5%.

Fatty acid composition of the representatives of the genus *Pantoea* has a typical set of fatty acids. It is characterized by high content of unsaturated isomers - hexa- and octadecenoic acids, saturated dodecanoic, tetradecanoic, hexadecanoic, cycloheptadecanoic fatty acids. The presence of hydroxylated isomers at least 3-hydroxydodecanoic is compulsory.

In the investigated isolates, the presence of all characteristic isomers with contents close to those described in the literature of isolates is shown (Table 3).

High similarity indices obtained when identifying investigated isolates using the automated identification system of MIDI Sherlock microorganisms allow us to conclude that they belong to the *Pantoea* family.

Also considering that the cause of some infectious diseases of the grapes is the bacteria of the genera *Erwinia* and *Agrobacterium,* and it was they who dominated in the internal environment of the vines and tumors, the ability of the isolated *P. agglomerans* strains to suppress the growth of the collection strains of *E. carotovora* (12 strains), *A. tumefaciens* (2 strains) and *A. vitis* (1 strain) was investigated. It was found that none of the investigated collection strains of phytopathogenic bacteria shown sensitivity to the metabolites of strains *P. agglomerans.*

Thus, studies have shown that the quantitative composition of the endophytic microbiota of the intact vine and tumors varied depending on the weather conditions and the grape variety, while in the tumors the number of bacteria was higher. The frequency isolation bacteria of the genus *Pantoea* was determined by the medium of selection and weather conditions and did not depend on the grape variety. The

Table 2

Fatty acid composition isolated strains from a grape and a tumors

Peak Name	**1**	**1a**	**3**	**4**	**5**	**7**	**9**	**10**	**12**	**15**	**22x**	**24**	**25**	**27**	**35**	**36**	**41**	**41a**
12:00	3.72	3.06	3.62	33	3.83	3.54	3.61	3.44	3.25	3.84	3.19	3.04	3.33	3.31	3.96	3.98	4.57	4.12
13:00	0.06	0.05	0.1	0.08	0.07	----	0.05	----	----	0.07	0.05	0.05	0.07	0.06	----	----	0.14	----
12:0 3OH	----	0.04	----	0.05	----	----	----	----	----	0.04	0.03	0.05	0.04	0.04	----	----	----	----
14:00	5.51	4.79	4.8	5.13	4.94	4.98	5.31	5.23	5.15	5.03	5.04	4.78	5.27	5.11	5.51	5.6	5.72	5.42
15:0 iso	----	*0.07*	----	*0.1*	----	----	----	----	----	----	----	----	----	----	----	----	----	----
15:0 anteiso	----	*0.06*	----	*0.11*	----	----	----	----	----	----	----	----	----	----	----	0.12	0.12	----
14:0 3OH	6.81	7.15	6.76	6.43	6.13	8.14	6.22	6.34	6.28	6.51	7.12	7.15	7.51	7.54	9.3	8.46	9.98	9.74
16:1 w7c/16:1 w6c	35.68	34.5	33.09	36.24	35.34	36.1	36.24	36.1	36.92	34.88	34.47	36.01	35.61	34.68	31.73	30.33	30.8	30.65
16:1 w5c	0.14	0.12	0.13	0.14	0.13	0.13	0.13	0.13	0.14	0.12	0.12	0.14	0.15	0.13	0.1	0.11	0.11	0.18
16:00	29.53	30.43	29.1	29.36	29.73	28.41	29.79	30.66	29.65	29.97	31.21	29.86	29.43	30.46	29.87	30.83	28.25	29.2
15:0 iso 3OH	----	----	----	----	----	----	----	----	----	----	----	----	----	----	*1.02*	*1.96*	*1.52*	*1.41*
15:0 2OH	----	----	----	----	----	----	----	----	----	----	----	----	----	----	*0.33*	----	----	*0.4*
17:0 anteiso	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	*0.19*
17:1 w8c	----	0.05	0.07	0.05	----	----	----	----	----	0.05	0.04	0.05	0.06	0.05	----	----	----	0.33
17:0 cyclo	5.25	4.8	5.88	4.54	4.3	4.57	5.32	4.67	4.42	4.6	4.89	3.74	5.33	5.17	5.97	7.11	7.55	5.64
17:00	0.19	0.24	0.48	0.31	0.33	0.26	0.21	0.17	0.18	0.3	0.2	0.26	0.27	0.25	0.26	0.34	0.4	0.43
18:0 ante/18:2 w6,9c	----	0.06	0.06	----	----	----	----	----	----	0.03	0.05	0.05	0.05	0.05	----	----	----	0.11

Table continued

18:1 w7c	12.35	13.63	14.95	13.37	14.41	13.03	12.25	12.21	13.09	13.58	12.62	14	11.91	12.16	11.59	10.64	10.43	10.83
18:1 w5c	----	0.05	0.05	----	----	----	0.05	----	----	0.04	0.04	0.04	0.05	0.05	----	----	----	----
18:00	0.21	0.3	0.29	0.25	0.29	0.3	0.24	0.24	0.21	0.23	0.25	0.24	0.21	0.24	0.26	0.37	0.24	0.34
19:0 cyclo w8c	0.09	0.1	0.15	0.1	0.1	----	0.09	0.07	0.1	0.09	0.08	0.08	0.1	0.08	0.1	0.13	0.17	0.12
Similarity Index	0.869	0.769	0.944	0.763	0.815	0.708	0.759	0.723	0.701	0.847	0.823	0.731	0.767	0.822	0.862	0.789	0.707	0.74

Table 3

The total-cellular fatty acid composition of representatives of the genus *Pantoea*

Peak Name	***P. agglomerance* LMG 1286**	***P. calida* LMG 25383**	***P. gaviviae* LMG 25382**	***P. rodasii***	***P. rwandensis***	***P. wallisii***	**Content in observing strains**
12:00	3.8	5.4	5.3	4.5	4.2	5.8	3.04-4.57
14:00	6	6.6	6.7	6.7	6.9	3.5	4.78-5.72
15:0iso	1.1	---	---	---	---	---	<0.1
14:0 3OH	11.2	6.9	7.1	13.9	14.3	15.3	6.22-9.98
16:1 w7c/16:1w6c	24.2	23.9	27.2	23.8	26.5	16.3	30.33-36.92
16:1w5c	---	---	---	---	---	---	0.1-0.18
16:00	27.1	31.8	32.1	27.4	26.1	25.6	29.1-30.38
17:0 cyclo	13.2	1.3	---	8.8	7.1	7.9	3.74-7.55
17:00	<1	<0.5	---	---	---	---	0.17-0.34

biological properties of the *P. agglomerans* strains isolated from the veins and tumors were the same except of the ability to utilize individual carbohydrates. Allocated strains are not capable to suppress the growth of collection strains of phytopathogenic bacteria.

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ЧИСЕЛЬНІСТЬ І БІОЛОГІЧНІ ВЛАСТИВОСТІ БАКТЕРІЙ *PANTOEA AGGLOMERANS*, ВИДІЛЕНИХ З РІЗНИХ СОРТІВ ВИНОГРАДУ ОДЕСЬКОЇ ОБЛАСТІ

Реферат

Мета. *Ізолювати і дослідити біологічні властивості Pantoea agglomerans із внутрішнього середовища виноградної лози і пухлин виноградної лози, відібраних з виноградників Одеської області.* ***Методи.*** *Зразки виноградної лози сортів «Аркадія», «Молдова» і «Одеський сувенір» і пухлин, зрізаних з ураженої лози цих сортів, попередньо стерилізували фламбуванням, подрібнювали на фрагменти, вносили їх у колби з дистильованою водою і перемішували в шейк ері при 28 °С протягом 3 год. Робили серію 10-ти кратних послідовних розведень і висівали на поверхню чашок Петрі з поживним агаром. Інкубували при 28 °С протягом 24–48 год. Проводили кількісний облік, виділяли чисті культури і досліджували їх культуральні, морфологічні, фізіолого-біохімічні властивості, визначали склад клітинних ліпідів та проводили ідентифікацію виділених штамів. Антагоністичну активність P. agglomerans визначали лунково-дифузійним методом.* ***Результати.*** *Чисельність бактерій у внутрішньому середовищі винограду коливалася у межах 5,63 ± 1,3×10³ КУО/см³ – 2,96 ± 1,3×10⁵ КУО/см³ і залежала від пори року і сорту винограду. Кількість мікроорганізмів у пухлинах була більшою ніж в неушкодженій лозі у той же період. Масова частка P. agglomerans у лозі коливалася від 5,7% до 68,2%, у пухлинах – від 9,88% до 23,08%. Штами P. agglomerans, виділені із ендофітного середовища винограду і пухлин, характеризувалися однаковими морфологічними, культуральними, тінкторіальними, фізіологічними властивостями. Незначні відмінності спостерігалися у здатності до утилізації вуглеводів. Штами Pantoea із пухлин не споживали цукрозу і 25,0 % утилізували раффінозу в аеробних умовах, на відміну від штамів із неушкодженої лози. Жирнокислотний спектр був представлений жирними кислотами, що містять у ланцюзі від 12 до 19 атомів вуглецю. За жирнокислотним складом вісімнадцять штамів були ідентифіковані як P. agglomerans-GC subgroup A. Для досліджуваних штамів домінантними в профілі є C16:0, C12:0, C14:0, C17:0 cyclow7c. Виділені штами P. agglomerans не проявили антагоністичної активності щодо колекційних штамів E. carotovora, A. tumefaciens і A. vitis.* ***Висновки.*** *Кількісний склад мікробіоти ендофітного середовища лози і пухлин був неоднаковим і залежав від джерела виділення, погодних умов і сорту винограду. Штами P. agglomerans, виділені із лози і пухлин, характеризувалися однаковими біологічними ознаками, за виключенням здатності до утилізації окремих*

вуглеводів. Жирнокислотний склад досліджених штамів Pantoea був представлений жирними кислотами з 12–19 атомів вуглецю у ланцюзі. Антагоністичної активності до колекційних штамів E. carotovora, A. tumefaciens і A. vitis не виявлено.

Ключові слова: Pantoea agglomerans, ендофітне середовище виноградної лози і пухлин, чисельність, біологічні властивості.

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ЧИСЛЕННОСТЬ И БИОЛОГИЧЕСКИЕ СВОЙСТВА БАКТЕРИЙ *PANTOEA AGGLOMERANS*, ВЫДЕЛЕННЫХ ИЗ РАЗНЫХ СОРТОВ ВИНОГРАДА ОДЕССКОЙ ОБЛАСТИ

Реферат

Цель. *Изолировать и исследовать биологические свойства Pantoea agglomerans из внутренней среды винограда и опухолей, отобранных из виноградников Одесской области.* ***Методы.*** *Образцы виноградной лозы сортов «Аркадия», «Молдова» и «Одесский сувенир» и опухолей, срезанных с пораженной лозы этих сортов, предварительно стерилизовали, измельчали на фрагменты, вносили в колбы с дистиллированной водой и перемешивали в шейкере при 28 °С в течение 3 ч. Делали серию 10-ти кратных последовательных разведений и высевали на поверхность чашек Петри с питательной агаром. Инкубировали при 28 °С в течение 24–48 часов. Проводили количественный учет, выделяли чистые культуры и исследовали их культуральные, морфологические, физиолого-биохимические свойства, определяли состав клеточных липидов и проводили идентификацию выделенных штаммов. Антагонистическую активность P. agglomerans определяли луночно-диффузным методом.* ***Результаты.*** *Численность бактерий во внутренней среде винограда колебалась в пределах 5,63 ± 1,3X10³ КОЕ/см³ — 2,96 ± 1,3X10⁵ КОЕ/см³ и зависела от времени года и сорта винограда. Количество микроорганизмов в опухолях была больше чем в неповрежденной лозе в тот же период. Массовая доля P. agglomerans в лозе колебалась от 5,7% до 68,2%, в опухолях – от 9,88% до 23,08%. Штаммы P. agglomerans, выделенные из эндофитной среды винограда и опухолей, характеризовались одинаковыми морфологическими, культуральными, тинкториальными, физиологическими свойствами. Незначительные различия наблюдались в способности к утилизации углеводов. Штаммы Pantoea из опухолей не разлагали сахарозу и 25,0 % утилизировали раффинозу в аэробных условиях, в отличие от штаммов с неповрежденной лозы. Жирнокислотный спектр был представлен жирными кислотами, содержащими в цепи от 12 до 19 атомов углерода. По жирнокислотному составу восемнадцать штаммов были идентифицированы как P. agglomerans-GC subgroup A. Для исследуемых штаммов доминантными в профиле являются C16:0, C12:0, C14:0, C17:0 cyclo w7c. Выделенные штаммы P. agglomerans не проявили антагонистической активности в*

отношении коллекционных штаммов E. carotovora, A. tumefaciens и A. vitis. ***Выводы.*** *Количественный состав микробиоты эндофитной среды лозы и опухолей был неодинаковым и зависел от источника выделения, погодных условий и сорта винограда. Штаммы P. agglomerans, выделенные из лозы и опухолей, характеризовались одинаковыми биологическими признаками, за исключением способности к утилизации отдельных углеводов. Жирнокислотный состав исследованных штаммов Pantoea был представлен жирными кислотами с 12–19 атомами углерода в цепи. Антагонистической активности к коллекционных штаммов E. carotovora, A. tumefaciens и A. vitis не обнаружено.*

Ключевые слова: Pantoea agglomerans, эндофитная среда виноградной лозы и опухолей, численность, биологические свойства.

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