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BACTERIA-ANTAGONISTS OF THE AGENTS OF SORYZ BACTERIAL DISEASES

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Aim. To isolate and identify bacteria with antagonist properties for biocontrol of the agents of bacterial diseases of soryz (*Sorghum oryzoidum*) and sorghum crops. **Methods.** The studies were conducted in 2021-2023. Spore-forming bacteria were isolated from the soryz samples, collected in the fields of the experimental farm of the Uman National Horticulture University (Cherkasy region, Uman). Lactic acid bacteria were isolated from soryz plants, collected in the private land plot, located between the villages of Teolyn, Vladyslavchyk, Kniazhyky in Monastyryshche community, Uman district, where Pershotravneve hamlet used to be situated. A total of 1,250 samples were analyzed. The experiment had three repeats. Spore-forming and lactic acid bacteria were isolated from the surface of soryz plants on the firm ripe stage in summer while isolating phytopathogenic bacteria. The isolates of lactic acid bacteria-antagonists were also isolated from the inner part of winter stubble stalk of soryz, collected from the tilled field. The antagonistic activity of the strains of lactic acid bacteria and spore-forming bacteria, isolated from different ecological niches, to phytopathogens of soryz and sorghum crops was determined *in vitro*. The strains of *Pseudomonas syringae,* the agents of soryz bacterial spots, were used as test-cultures: 211141a, 211141, 210341, 21034, and 210521, along with the collection strains of phytopathogens: *Pseudomonas syringae* 8299, *Pseudomonas syringae subsp. syringae* UKM B-1021, *X. oryzae* 8375, *Dickeya chrysanthemi* 8683, *Diskeya chryzanthemi* 8683. The antagonistic activity of the extracted isolates of spore-forming and lactic acid bacteria was studied using the method of radial strokes (joint cultivation of the antagonist and the strains under investigation). The bacterial isolates were deemed inactive if the growth delay zone was 0–5 mm (–), from 5 to 10 mm (+) – low activity, 11–20 mm (++) – moderate activity, over 20 mm (+++) – high activity regarding the test-cultures. To check the effect of the isolate-antagonist of phytopathogenic bacteria, artificial infecting was conducted in the field conditions. For this purpose, a diurnal culture of the antagonist was introduced into the stalk of plants in the concentration of 1×10^8 colony-forming units, and 24 h later, a culture of test-strain of the phytopathogen was administered above the previous puncture. The results were evaluated 7–14 days after the artificial infection. The experiment had three repeats. The isolates of bacteria which demonstrated their antagonistic properties regarding the phytopathogenic bacteria were identified by their morphological properties, Gram staining, catalase test, profile of carbohydrate fermentation and mass-spectrometry (MALDI-TOF – Matrix Assisted Laser Desorption/Ionization) using VITEK MS mass-spectrometer. **Results.** Thirty-eight spore-forming bacterial isolates were extracted from soryz; among these, 21030, 21095, 21040, ASV1, ASV3, B4 demonstrated their antagonistic activity towards the investigated phytopathogenic bacteria. Isolate 21040 showed high antagonistic activity to most test-strains of *P. syringae* from soryz (the zone of negative culture – 23–30 mm) and lower activity regarding the collection cultures. Isolates B4 and AVS3 demonstrated their selective activity regarding the investigated phytopathogens. Twenty isolates of lactic acid bacteria were extracted. Higher antagonistic activity was noted for the isolates of lactic acid bacteria 8/1 and F1 to the strains of *P. syringae,* isolated from soryz and collection cultures. The highest antagonistic activity of isolate 8/1 was noted regarding test-strains of *P. syringae* 210521 and *X. oryzae* 8375 (the zone of negative culture – 40–35 mm). In the field conditions, the treatment of sorghum plants with F1 affected the pathological process that developed due to the impact of the phytopathogenic bacteria *P. syringae*, which led to the reduction in disease symptoms. The taxonomic position of the isolates of bacteria, which seem to be promising for the control of disease agents, was determined. In terms of morphology of cells and colonies, the biochemical profile, and mass-spectrometry MALDI-TOF, the spore-forming isolates 21040 and B4 were identified as *Bacillus subtilis*, and ASV3 – as *Bacillus vallismortis.* The identified isolates of lactic acid bacteria were *Lactobacillus pentosus* F1

and *Lactobacillus sakei* 8/1. **Conclusions.** In addition to phytopathogenic bacteria, from soryz plants we isolated the strains of spore-forming bacteria *Bacillus subtilis* 21040, B4, *Bacillus vallismortis* AVS3 and such lactic acid bacteria as *Lactiplantibacillus pentosus* and *Lactobacillus sakei* 8/1 (*Latilactobacillus sakei* 8/1), promising for the elaboration of methods for the biocontrol of the agents of bacterial diseases.

Key words: soryz (*Sorghum oryzoidum*), phytopathogenic bacteria, soryz bacterial spots, spore-forming bacteria, bacilla, lactic acid bacteria, collection cultures, antagonistic activity, biocontrol, mass-spectrometry.

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INTRODUCTION

Agriculture is an important branch of economy. The diseases of crops, caused by bacterial pathogens, result in serious limitations to agricultural production and lead to considerable annual losses on the global scale (Sundin et al, 2016). Traditional agriculture uses chemical fertilizers and pesticides to enhance performance and to control disease agents and pests. However, the increase in their application impacts the ecological equilibrium and safety of food products and is the main factor of environmental pollution (Raman et al, 2022). Therefore, as an alternative to chemical preparations, researchers pay great attention to the elaboration of ecologic methods of protection against crop disease agents, using microbial preparations, based on different species of microorganisms and their metabolites (Bergsma et al, 2022).

Among bacteria-antagonists of plant disease agents, special attention is paid to the spore-forming bacteria of genus *Bacillus. These bacteria* are characterized with high viability in the environment, they are tolerant to anthropogenic effects and are technological both in production and application. The bacteria of *Bacillus* group are microorganisms, inhabiting a number of various environments. They are well-known producers of a wide spectrum of antagonistic compounds of different structure and cover from 5 to 8 % of the total genome, designated for the biosynthesis of secondary metabolites. The strains of *Bacillu*s genus demonstrate their ability to conduct biocontrol mainly via the inhibition of the growth of plant pathogens and triggering of systemic resistance in plants and competing with plant pathogens for ecologic niches (Fira et al, 2018).

The foundation of biopreparations for the biocontrol of disease agents consists of the representatives of rhizosphere microorganisms of genus *Bacillus*, which demonstrate high antagonistic activity regarding the phytopathogenic microorganisms due to the synthesis of exometabolites of different nature – antibiotics, bacterial toxins, volatile organic substances, biosurfactants (Compant et al, 2005), phytohormones and vitamins (Araújo et al, 2005; Chen et al, 2010), which have a positive impact on the growth and development of plants and protect them from phytopathogenic microorganisms.

The *Bacillus* bacteria colonize the rhizosphere of plants, and the root exometabolites, released by plants, serve as nutrients for bacteria (Saharan et al, 2011).

Several researchers demonstrated high antagonistic activity of *Bacillus* bacteria to the agents of fungal and bacterial diseases of plants (Lapa et al, 2005; Drahovoz et al, 2014; Hrabova et al, 2015). It was demonstrated that *Bacillus* bacteria inhibit the development of the infection, caused by *Clavibacter michiganensis* subsp. *michiganensis* on tomatoes. The presowing treatment of the seeds with the suspensions of *Bacillus subtilis* IMV B-7023 and *Bacillus pumilus* 3 enhanced the resistance of plants to bacterial cancer, which could have occurred due to the synthesis of biologically active substances with antimicrobial properties by these bacteria. A considerable stimulating effect on the growth and development of tomatoes was ensured by the strain of *B. subtilis* IMV B-7023, which is a component of bacterial preparations for plant production (Roi et al, 2012).

The antagonistic activity is also notable for saprotrophic strains of *Bacillus,* isolated from the surface of green leaves of root artichoke. It was determined that the strain of *Bacillus subtilis* UzNU-18 demonstrated high antagonistic activity to phytopathogenic fungi and phytopathogenic bacteria (Davranov et al, 2019).

A relevant role in the study of antagonist microbes is also attributed to lactic acid bacteria. The distribution of lactic acid bacteria (LAB) in soil is minimal and not dominant, but they are considered to be promising candidates for agriculture, promote plant health, and soil fertility. Thus, they are closely considered for the purposes of sustainable agriculture. LAB metabolites promote the growth of plants and stimulate the growth of shoots and roots. As fertilizers, they may promote biodecomposition, increase the content of organic substances in soil and produce metabolites of organic acids

and bacteriocins. *Lactobacillus* spp. may be remarkable potential agents of biocontrol, since they produce biologically active substances (organic acids, bacteriocins, phenyl-lactic acid, cyclic dipeptides, fatty acids) with antimicrobial properties. In addition, it was found that *Lactobacillus* spp. are capable of detoxication of micotoxins (Degrassi et al, 2020; Baffoni et al, 2015).

According to scientific literature, plants interact with many groups of lactic acid microorganisms, including *Enterococcus, Lactobacillus, Lactococcus*; the most notable among them being the coccal forms of *Streptococcus lactis*, *S. cremori*s, *S. durans*, *S. faecalis*. As for rod-like forms, the most common are *Lactobacillus plantarum* and *L. brevis,* which are found in the rhizosphere, on the surface, fruit, and flowers of plants (Mundt et al, 1968; Rodríguez R et al, 2019).

Wide spreading on fruit (mango), vegetables (tomatoes, squash, potatoes, eggplants), grains (rice, sorghum) is noted for *Lactobacillus plantarum*, which is found on the surface of plants and fruit (Daranas N et al, 2019).

The groups of LAB on plants may also manifest themselves as growth stimulators (Merlich et al, 2016) and antagonists of phytopathogenic microorganisms (Roi et al, 2012; Steglinska et al, 2022).

The studies of some scientists (Visser et al, 1986; Merlich et al, 2016) showed the antagonistic activity of the representative of LAB, *Lactobacillus plantarum*, isolated from plant surfaces, towards the test strains of phytopathogenic bacteria of genera *Xanthomonas*, *Pectobacterium, Pseudomonas* and *Rhizobium* (Visser et al, 1986; Merlich et al, 2016). Some authors remarked on the high antagonistic activity of *L. plantarum* in the MRS medium to both potentially pathogenic and phytopathogenic microorganisms. The investigated strains of *L. plantarum* produce complexes of metabolites with antagonistic activity. It was shown that lactic acid and hydrogen peroxide are the main factors of the antagonistic effect of most *L. plantarum* strains, investigated in this work (Harmasheva et al, 2015). There is also a discussion on the role of LAB in maintaining the health of soil and enhancing the performance of crops (Raman et al, 2022; Lamont et al, 2017). The authors found that LAB can improve the soil structure, increase the availability of nutrients, and inhibit the growth of pathogenic fungi. They also determined that bacteria can promote the growth of plants (Abhyankar et al, 2022).

MATERIALS AND METHODS

The studies were conducted in 2021–2023. Spore-forming bacteria were isolated from the soryz samples, collected in the fields of the experimental farm of the Uman National Horticulture University (Cherkasy region, Uman). Lactic acid bacteria were isolated from soryz plants, collected in the private land plot, located between the villages of Teolyn, Vladyslavchyk, Kniazhyky in Monastyryshche community, Uman district, where Pershotravneve hamlet used to be situated. A total of 1,250 samples were analyzed. The experiment had three repeats.

The spore-forming bacteria were isolated from the surface of soryz plants (*Sorghum oryzium*) – a leaf, a stalk, seeds in the firm ripe stage in summer, during the release of phytopathogenic strains, causing bacterial infections (Patyka et al, 2017). To isolate potentially active antagonists of phytopathogenic bacteria, which can be distributed by insects in soryz fields, we took pollen samples from soryz and isolated lactic acid bacteria from them by the method described in (Belhadj H et al, 2014).

To isolate lactic acid bacteria from soryz, the samples were taken from two plots of different fields, one – with organic fertilizer (cow dung), and the other – without any fertilizers. The isolates of lactic acid bacteria-antagonists were also extracted from the winter stubble stalk of soryz, collected from the tilled field. The inner part of stalk pulp was taken. The pulp pieces were extracted from the upper and lower parts, close to the root. The pulp was pestled in the mortar with some sterile water, the homogenates were incubated at 30°C for 24–72 h. After the incubation, the homogenates were applied to the slides with Lactobakagar. The dishes were incubated at 30 °C for 24–72 h in microaerophilic conditions. The colonies, notable for LAB, were isolated and sown to separate specific isolates.

The antagonistic activity of the strains of LAB and spore-forming bacteria, isolated from different ecological niches, to phytopathogens of soryz and sorghum crops was determined *in vitro*. The following bacterial cultures, the agents of soryz bacterial spots, were used as test-cultures: 211141a, 211141, 210341, 21034 and 210521, along with the collection strains of phytopathogens: *Pseudomonas syringae* 8299, *Pseudomonas syringae* subsp. *syringae* UKM B-1021, *X. oryzae* 8375, *Dickeya chrysanthemi* 8683.

The antagonistic activity was studied using the method of radial strokes (joint cultivation of the antagonist and the strains under investigation) by the well-known method (Patyka et al, 2017).

The strains of *Lactobacillus* and *Bacillus* were deemed inactive if the growth delay zone was 0–5

mm (–), from 5 to 10 mm (+) – low activity, 11–20 m (++) – moderate activity, over 20 mm (+++) – high activity regarding the test cultures (Patyka et al, 2017).

To check the impact of the antagonists of LAB on sorghum crops in the field conditions, the culture of LAB F1, cultivated for 48 h/37 °C on solid culture medium MRS in facultative anaerobic bacteria, was diluted in sterile running water to the concentration of 1×10^8 colony-forming units (CFU) and administered into the stalk of grain sorghum on the stage of panicle earing. The experiment was repeated three times.

The antagonism of LAB to sorghum plants was checked using the modified method of (Daranas, 2019). For the study purposes, we selected the strains of test culture of phytopathogenic bacteria, to which the antagonist demonstrated different inhibiting effects. A diurnal culture of the antagonist was administered into the plant stalk in the concentration of 1×10^8 CFU, and 24 h later, a culture of the phytopathogen strain was introduced above the previous injection site. The results were evaluated 7–14 days after the artificial infection.

The isolates of bacteria which demonstrated their antagonistic properties regarding the phytopathogenic bacteria were identified by their morphological properties, Gram staining, catalase test, and profile of carbohydrate fermentation (Bucka-Kolendo et al., 2021). The taxonomic position of the extracted bacterial isolates was confirmed by mass-spectrometry (MALDI-TOF – Matrix Assisted Laser Desorption/Ionization) using VITEK MS mass-spectrometer (Mellmann et al, 2008; Kačániová et al, 2019; Tsuchida et al, 2020; Bucka-Kolendo et al, 2021). For this purpose, *a calibration curve* of the strain of *Escherichia coli* ATCC 8739, cultivated for 24 h, was prepared. A thin layer of cells of this strain was applied to the target calibration well and 1 µl MATRIX was added to each target calibration well. Then pure cultures of the investigated bacteria were prepared for 18–24 h, and the colony of 1 µl was applied to the target well. The introduced culture was immediately administered 1 µl MATRIX into the center of the target well. The target well was kept until it went completely dry and MATRIX crystals were formed on it. The slide was placed into the device which was then switched on using the corresponding software till the process completion. The obtained results are reflected in the form of a system on the chart, a row of lines – a spectrum, which corresponds to different molecules that separated from the sample. The identification of microorganisms is based on obtaining a total mass-spectrum of proteins in the range of 1,000–10,000 Da and the bioinformational comparison of the obtained spectrum against the database of reference spectra. If the spectrum of the investigated sample coincides with one of the spectra in the database, the system identifies the sample. If the mass-spectrum of the investigated bacterial strain coincides with one of the spectra in the database of reference bacterial species, the system identifies it. The range of the probability for accurate identification in percentage is from 60 to 99 (Tsuchida et al, 2020).

RESULTS

To control the agents of bacteriosis of soryz and sorghum crops, we investigated the bacteria-antagonists, isolated from the surface of soryz plants in summer and from winter stubble of soryz plants, and determined the antagonistic activity of spore-forming strains and lactic acid bacteria.

We found that isolates 21030, 21095, 21040, ASV1, ASV3, B4, isolated from soryz, which were similar to bacteria of genus *Bacillus* in their morphology, demonstrated antagonistic activity to the investigated phytopathogenic bacteria from soryz and collection strains (**Table 1, Fig. 1**).

Among spore-forming isolates, 21040 demonstrated high antagonistic activity to most test strains from soryz, for instance, to *P. syringae* 210521 (zone of negative culture – 30 mm), *P. syringae* 211141a (23 mm), *P. syringae* 21034 (25 mm), *Dickeya chrysanthemi* 8683 (20 mm), *X. oryzae* 8375 (18 mm). However, poor antagonistic effect was noted regarding strains *P. syringae* 8299 (3 mm), *P. syringae* 211141 (4 mm).

Isolate 21030 was moderately active (zone of negative culture – 13–18 mm) towards the strains of *P. syringae* 211141, 211141a, 210521, *X. oryzae* 8375.

Isolates 21040, 21095, ASV3, ASV1, B4 had poor activity regarding *P. syringae* 211141 and no activity regarding *P. syringae* subsp. *syringae* UKM B-1021 and *P. syringae* 8299.

Isolate B4 manifested a high level of antagonism to *P. syringae* 21034 (30 mm) and *P. syringae* 210521 (21 mm).

High antagonistic activity was manifested by the isolates of lactic acid bacteria, isolated from the residues of soryz plants which were either on the soil surface or in soil at the depth of 1–5 cm. The prevailing part of plant residues were parts of roots with the stalk of 15–20 cm (**Fig. 2, *b*, *c***), the inner part of stem pulp was wet (**Fig. 1, *a***). The pulp pieces were extracted from

Table 1. The antagonistic activity of spore-forming isolates towards the agents of bacterial diseases

Agent	Strain	Negative culture zones (in mm) at the effect of spore-forming isolates					
		21030	21040	21095	ASV1	ASV3	B4
Bacteria strains, isolated from soryz							
P. syringae	210521	15	30	20	13	16	21
P. syringae	211141a	14	23	13	11	10	18
P. syringae	211141	13	4	5	6	7	5
P. syringae	210341	10	18	10	10	0	0
P. syringae	21034	0	25	23 BA	0	23 BA	30
Collection strains of bacteria							
Dickeya chrysanthemi	8683	20	20	12	12	5	10
X. oryzae	8375	18	18	12	16	0	12
P. syringae subsp. *syringae*	UKM B-1021	3	0	0	0	0	0
syringae	8299	0	3	5	0	23 BA	0

Note: "BA" – bacteriostatic action.

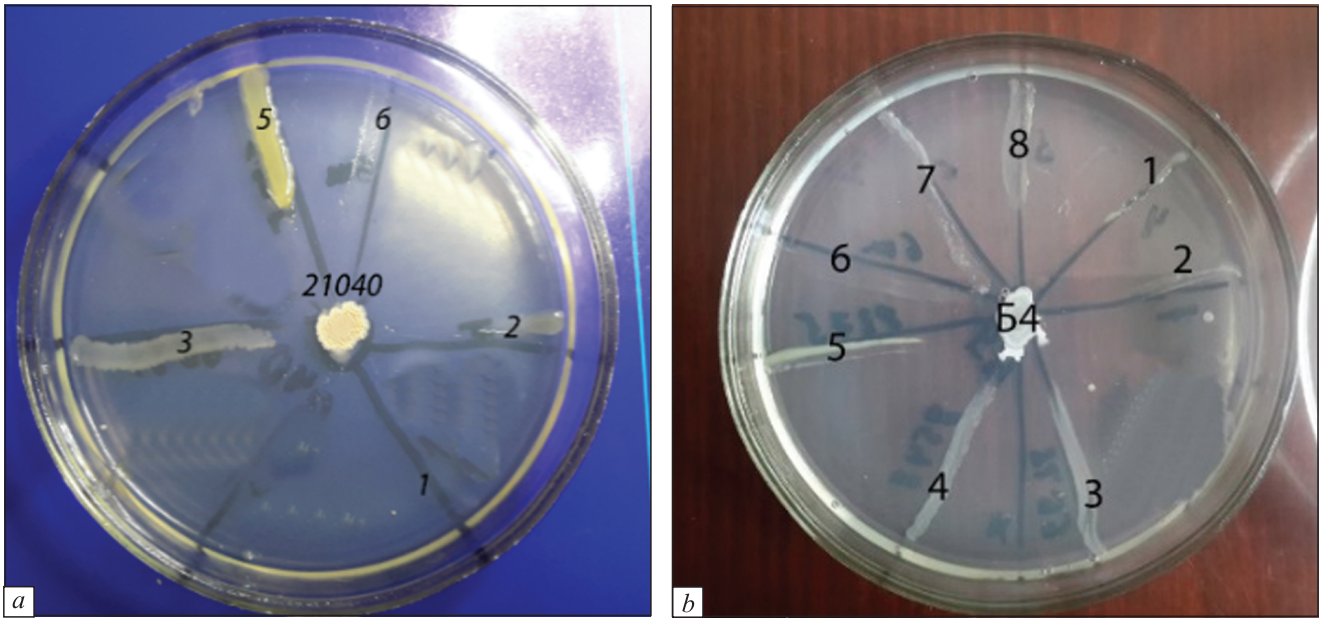


Fig. 1. The antagonistic effect of spore-forming bacteria: *a* – *Bacillus subtilis* 21040, *b* – *B. subtilis* B4 regarding phytopathogenic bacteria*:* 1 – *P. syringae* 211141a, 2 – *P. syringae* 210521, 3 – *P. syringae* 8299, 4 – *P. syringae* 8548, 5 – *X. oryzae* 8375, 6 – *Diskeya chryzanthemi* 8683, 7 – *P. syringae* 210341, 8 – *P. syringae* 211141

the upper and lower parts of the stalk closer to the root, after cutting off the upper part of the stalk (Fig. 2, *b***)**.

Higher antagonistic activity was manifested by the extracted isolates of lactic acid bacteria 8/1 and F1 (**Table 2**).

The highest antagonistic activity of isolate 8/1 was noted regarding test strains of *P. syringae* 210521 and *X. oryzae* 8375 (zone of negative culture – 40–35 mm). The isolate 8/1 demonstrated high antagonistic activity regarding strains *P. syringae* 211141a (30 mm), 211141 (20 mm), 210341 (30 mm), and was moderately active regarding *Dickeya chrysanthemi* 8683 (11 mm). High antagonistic activity was registered regarding the collection strains of *X. oryzae* 8375 and *P. syringae* subsp. *syringae* UKM B-1021.

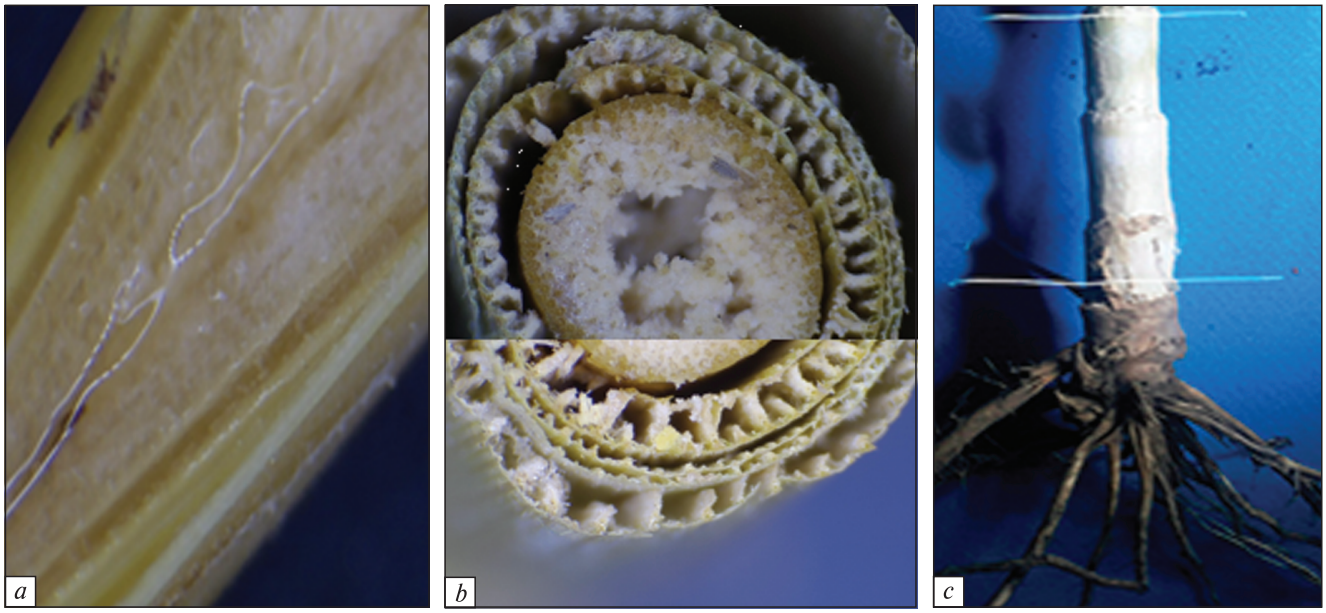


Fig. 2. A stalk, from which the antagonists were isolated in winter: *a* – the inner part, containing moisture, *b* – a cross-cut of the stalk; *c* – a part of the stalk, from which samples were extracted

Isolate F1 demonstrated high antagonistic activity regarding four strains from soryz: *P. syringae* 211141a (zone of negative culture – 40 mm), *P. syringae* 210521 (32 mm), *P. syringae* 211141 (34 mm), *P. syringae* 21034 (20 mm), and the collection strain of *P. syringae* subsp. *syringae* UKM B-1021 (54 mm), moderate activity was observed regarding *P. syringae* 210341 (15 mm), *Diskeya chrysanthemi* 8683 (16 mm).

We found the effect of isolate F1 on the pathological process, caused by the strains of phytopathogenic bacteria *P. syringae* (**Fig. 3**). The results of artificial inoculation are a decrease in or the absence of infection symptoms.

However, the treatment of grain sorghum plants with the isolate F1 did not affect the pathological process in the stalk of a plant, which gets developed under the effect of phytopathogenic bacterium *X. oryzae* 8375.

The results of the treatment of sorghum plants correlate with the data, obtained while investigating the antagonism of LAB regarding the test cultures of phytopathogenic bacteria *in vivo.*

Since the extracted isolates of bacteria-antagonists are perspective for the elaboration of biopreparations for the control of phytopathogenic bacteria, we conducted their identification.

By their morphology, the isolates taken from the surface of soryz plants, which demonstrate antibacte-rial activity towards phytopathogenic bacteria, are si-milar to bacteria of genus *Bacillus*. These are large, branched, irregular in form, white colonies; the cells are gram-positive, rod-like, and spore-forming (**Fig. 4**).

The biochemical profile of the extracted isolates with the antagonistic activity to the phytopathogenic bacteria was evaluated on the microbiological analyzer VITEK® 2 Compact (**Table 3**).

Table 2. The antagonistic effect of lactic acid bacteria regarding the agents of bacterioses

Bacteria, strains	Zones of negative culture of phytopathogens at the effect of the isolates of lactic acid bacteria, in mm	
	8/1	F1
P. syringae 210521	40	32
P. syringae 211141a	30	40
P. syringae 211141	20	34
P. syringae 210341	30	15
P. syringae 21034	10	20
Dickeya chrysanthemi 8683	11	16
X. oryzae 8375	35	15
P. syringae subsp. *syringae UKM* B-1021	33	54
P. syringae 8299	5	14

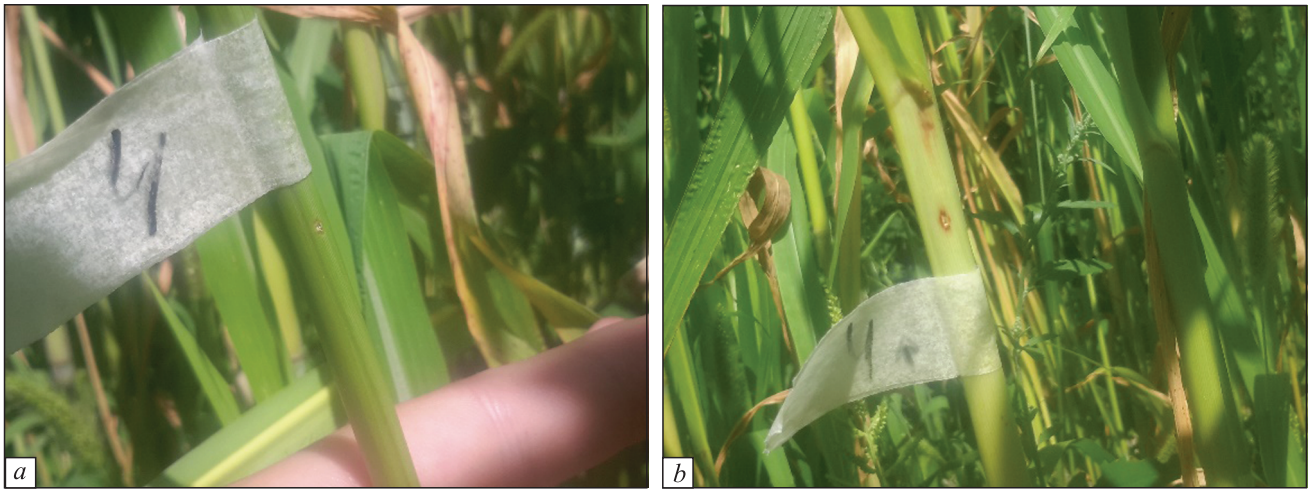
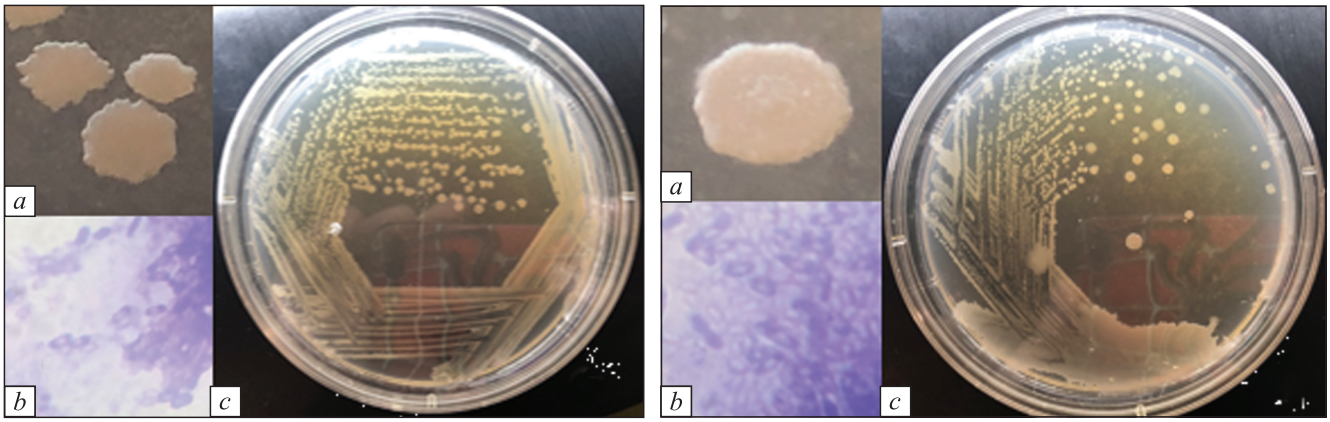


Fig. 3. The results of artificial infecting of grain sorghum with the strain of *P. syringae* 211141a*: a* – a plant, previously treated with the culture of antagonist F1, *b* – a control plant without any treatment



21040 B4

Fig. 4. A photograph of the colonies and cells of spore-forming microorganisms: *a* – colonies of spore-forming bacteria, *b* – spores and cells, *c* – colonies

By the obtained properties, the spore-forming isolates B4, 21040, and ASV3 had a biochemical profile, typical for bacteria *Bacillus subtilis*.

According to the results of MALDI–TOF, the mass-spectra of isolates B4 and 21040 were identical and corresponded to *Bacillus subtilis* (**Fig. 5, Table 4**).

Bacillus is a large genus of gram-positive bacteria, widespread in soil and other media. It is notable for good resistance and ability to form highly stable spores which can survive in severe conditions, including extreme temperatures, dry conditions, etc. (Tu et al, 2021). *Bacillus subtilis* bacteria inhibit the reproduction of phytopathogenic bacteria by the products of its activity and stimulate the immunity of plants to diseases, which decreases the possibility of recurrent infections (Araújo et al, 2005; Chen et al, 2010).

Isolate ASV3 had a biochemical profile, typical for *B. vallismortis*. In scientific literature, *B. vallismortis* is described as an antagonist with antifungal activity (Degrassi et al, 2020).

Thus, the extracted spore-forming isolates with high antagonistic activity regarding the phytopathogens of sorghum crops were identified as *Bacillus subtilis* 21040*, Bacillus subtilis* B4 and *Bacillus vallismortis* ASV3.

The isolates of bacteria F1 and 8/1, extracted from the remains of soryz stalk in winter, which manifested antagonistic properties to phytopathogenic bacteria, had a rod-like form (**Fig. 5**). By their morphological and biochemical properties, they were similar to bacteria of genus *Lactobacillus* (**Table 5)**.

Isolate F1 was extracted from the soryz stalk, collected from the fields in which organic fertilizers

Table 3. The biochemical identification of spore-forming isolates-antagonists using the VITEK/BCL card

Isolate B4. Bacillus subtilis Sample ID 1362070215556220. Identification accuracy 95 %.

1	BXYL	+	3	LysA	–	4	AspA	–	5	LeuA	+	7	PheA	+	8	ProA	–
9	BGAL	–	10	PyrA	+	11	AGAL	+	12	AlaA	–	13	TyrA	+	14	BNAG	–
15	APPA	–	18	CDEX	–	19	dGAL	–	21	GLYG	(+)	22	INO	+	24	MdG	+
25	ELLM	–	26	MdX	–	27	AMAN	–	29	MTE	–	30	GlyA	+	31	dMAN	–
32	dMNE	+	34	dMLZ	–	36	NAG	–	37	PLE	+	39	lRHA	–	41	BGLU	+
43	BMAN	+	44	PHC	–	45	PVATE	+	46	AGLU	+	47	dTAG	–	48	dTRE	+
50	INU	–	53	dGLU	+	54	dRIB	+	56	PSCNa	–	58	NaCl 6.5%	+	59	KAN	–
60	OLD	–	61	ESC	+	62	TTZ	–	63	POLYB_R	_						

Notes: the results in brackets demonstrate weak reactions, BXY – beta-xylosidase; LysA – lysine arylamidase; AspA – aspartate arylamidase; LeuA – leucine arylamidase; PheA – phenylalanine arylamidase; ProA – proline arylamidase; BGAL – beta-galactosidase; PyrA – pyrrolidonyl arylamidase; AGAL – alpha-galactosidase; AlaA – alanine arylamidase; TyrA – tyrosine arylamidase; BNAG – beta–N–acetyl glucosaminidase; APPA – alanine, phenyl, proline arylamidase; DEX – cyclodextrine; dGAL – D–galactosidase; GLYG – glycogen; INO – myoinositol; MdG – methyl–A–D–glucopyranoside; ELLM – Ellman; MdX – methyl-D-xylosidase; AMAN – alpha mannosidase; MTE – maltotriose; GlyA – glycine arylamidase; dMAN – D-mannitol; dMNE – D-mannose; dMLZ – D-melezitose; NAG – N-acetyl-D-glucosamine; PLE – palatinose; lRHA – L-rhamnose; BGLU – beta-glucosidase; BMAN – beta-mannosidase; PHC – phosphoryl choline; PVATE – pyruvate; AGLU – alpha-glycosidase; dTAG – D-tagatose; dTRE – D–trigalase; INU – inuline; dGLU – D-glucose; dRIB – D-ribose; PSCNa – putrescine; KAN – kanamycin-resistant; NaCl – sodium chloride; OLD – oleandomycin-resistant; ESC – esculin; TTZ – tetrazolium red; POLYB_R – polymyxin-B-resistant.

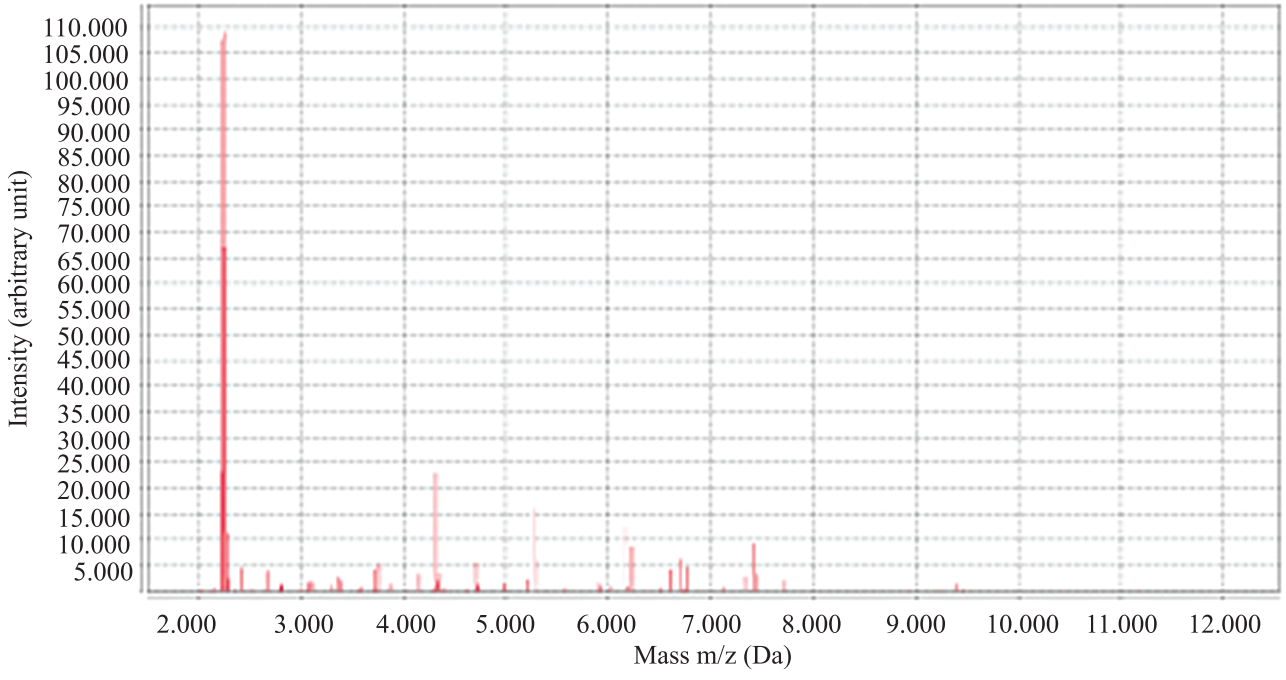


Fig. 5. The mass-spectrum of isolate B4

had been administered in two previous seasons. Isolate 8/1 was extracted from the plot with soryz plants without previous administration of organic fertilizers.

According to the results of these studies, isolate F1 had a biochemical profile and mass-spectrum, typical for *Lactobacillus pentosus (Lactiplantibacillus pentosus*), and isolate 8/1 – *Lactobacillus sakei (Latilacto-*

Table 4. The accuracy of identification of bacterial isolates using Vitek MS-DS

Isolate	Name	Identification accuracy, %
B4	*Bacillus subtilis*	95
21040	*Bacillus subtilis*	95
ASV3	*Bacillus vallismortis*	95

Table 5. Morphological and biochemical characteristics of lactic acid bacteria

Tests	Characteristics of isolates	
	8/1	F1
Gram staining	+	+
Form of cells	Short rods	Rods
Catalase	+	+
Utilization:		
saccharose	+	+
xylose	+	+
fructose	+	+
rhamnose	+	+
sorbose	+	+
maltose	+	+
arabinose	+	+
lactose	–	–
glucose	+	+
mannose	+	+

Note. "+" – a positive result; "–" a negative result.

bacillus sakei) *(***Fig. 7, Table 6**). The probability (accuracy) of identification was 99.9 %.

According to the scientific literature, the antagonistic activity was noted for the representative of lactic acid bacteria *Lactobacillus plantarum* from plant surfaces to the test-strains of phytopathogens *Xanthomonas campestris, Erwinia carotovora* (Merlich et al, 2016).

The studies on DNA-DNA hybridization demonstrated that *Lactobacillus plantarum*, *Lactobacillus pentosus* and *Lactobacillus paraplantarum* form a closely related group, known as "a group of *Lactobacillus plantarum*" of gene Rec Lb. *plantarum* and Lb. *pentosus* Hind II *Lactobacillus pentosus* (Torriani S et al., 2001). Fatty acids of *L. pentosus* demonstrate their antifungal activity regarding fungal pathogens (Lipinska, 2014; Raman et al, 2022).

According to the results of our study, isolate 8/1 had a biochemical profile, typical for *Lactobacillus sakei*. The probability (accuracy) of identification was 99 %.

Lactobacillus sakei (Latilactobacillus sakei) is a gram-positive rod-like anaerobic bacterium. The scientific literature states that this species decreases the number of viable microorganisms of *Enterobacteriaceae* and *Pseudomonas* sp. families, and in case of long-term storing, *L. sakei* in the medium with pathogens manifests a more inhibiting effect (Zhang, 2018).

The most frequently mentioned tomato plants were *L. plantarum, L. fermenti*, *L. brevis,* whereas *L. casei, L. viridescens, L. cellobiosus, L. salivarius* and *L. buchneri* were obtained from a small number of samples. Wide yet sporadic distribution of lactic acid bacteria in small amounts demonstrates that these organisms do not usually flourish on plant surfaces (Mundt et al, 1968).

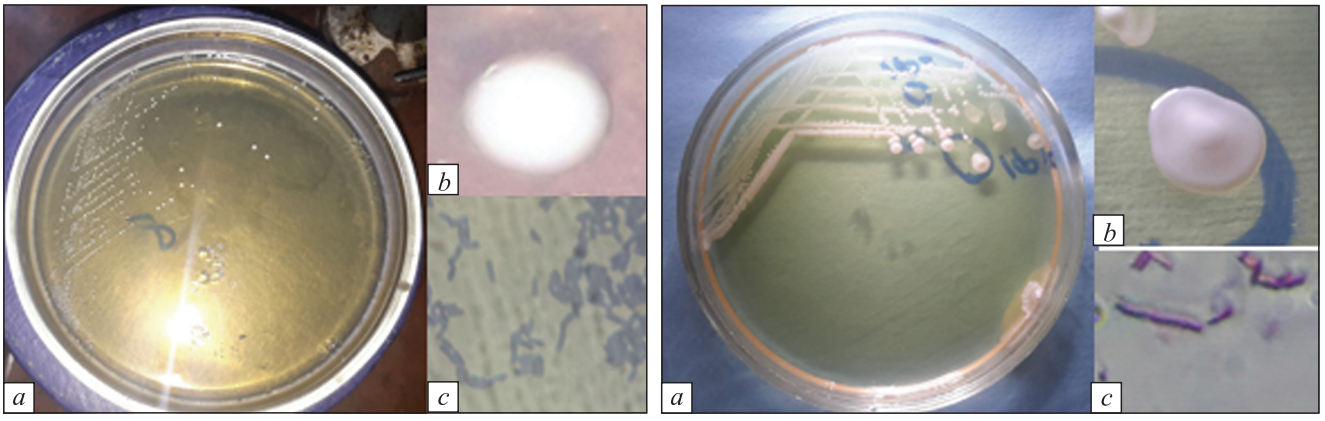


Fig. 6. Lactic acid bacteria: *a* – general view, *b* – colonies; *c* – microphotograph

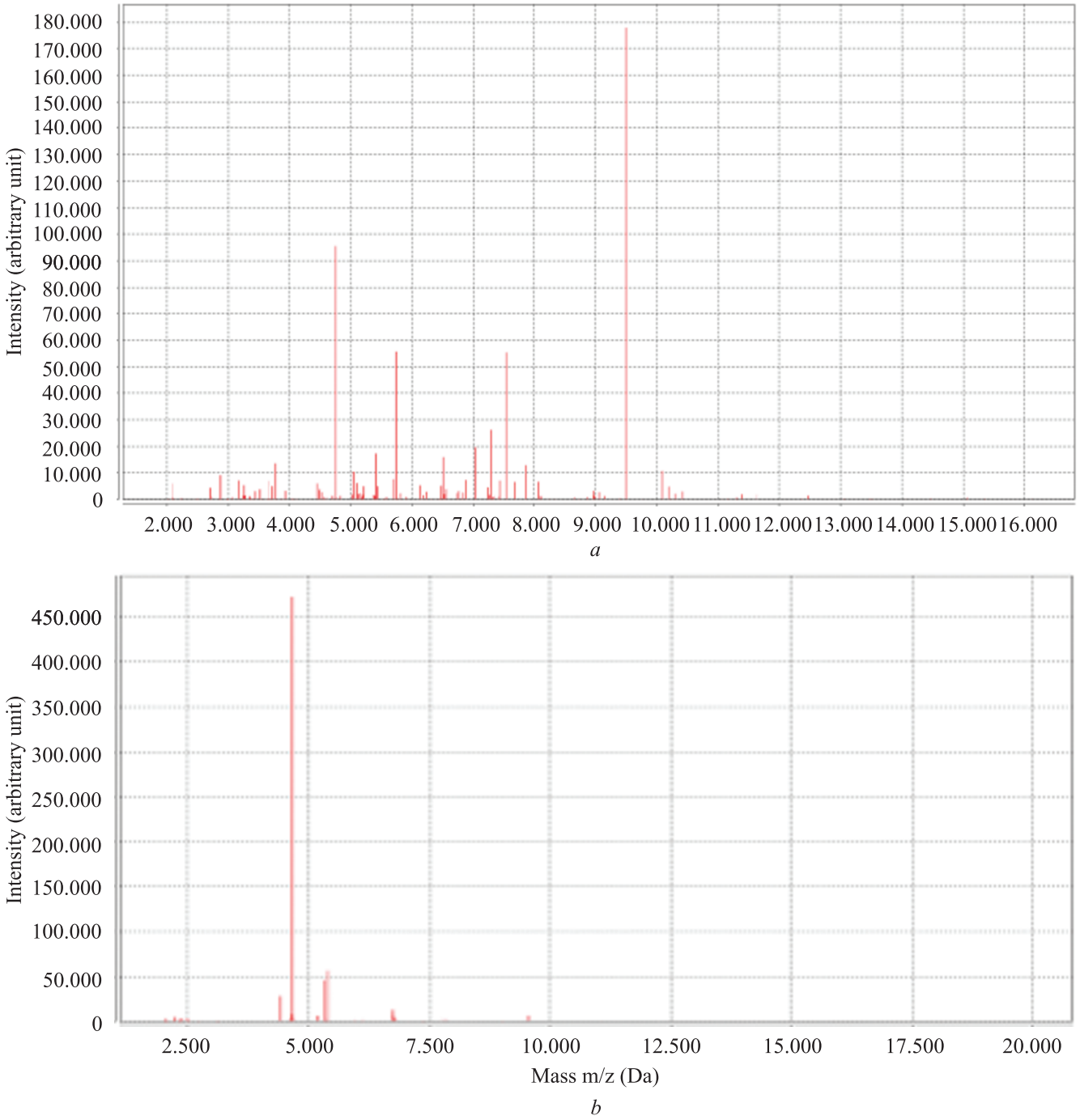


Fig. 7. Representative mass-spectra: *a* – isolate F1, *b* – isolate 8/1

In 2020, based on the molecular and genetic studies, the taxonomic position of *Lactobacillus pentosus* (*ex* Fred et al, 1921) Zanoni et al. 1987 was changed into *Lactiplantibacillus pentosus* (Zanoni et al, 1987; *ex* Fred et al, 1921) Zheng et al. 2020, and *Lactobacillus sakei* corrig. Katagiri et al. 1934 into *Latilactobacillus sakei* (Katagiri et al, 1934) Zheng et al. 2020 (Zheng et al, 2020).

Table 6. The accuracy of identification of bacterial isolates using Vitek MS-DS

Isolate	Name	Identification accuracy, %
F1	*Lactobacillus pentosus*	99.9
8/1	*Lactobacillus sakei*	99.9

DISCUSSION

Bacterial diseases are known to cause considerable damage to agriculture, decreasing the quality and quantity of the cultivated products. The same is true for the agents of bacterial diseases of soryz (*Sorghum oryzoidum*). Therefore, in our studies, we used the strains of the agent of soryz bacterial spots and sorghum crops as test-culture in the search for microbes-antagonists.

Unreasonable doses of mineral fertilizers, the use of chemical means to control plant diseases and the failure to comply with the technology of their application bring harm to the environment (Raman et al, 2022). Thus, scientists search for ecological methods of protection from the agents of crop diseases, which could be found in microbes-antagonists for the elaboration of biopreparations on their basis (Dragovoz et al, 2014; Hrabova et al, 2015; Davranov et al, 2019; Bergsma et al, 2022).

In the scientific literature, the main attention is paid to spore-forming and LAB with antagonistic properties regarding the agents of bacterial diseases of fungal and bacterial nature (Lapa et al, 2005; Merlich et al, 2016; Steglinska et al, 2022). Therefore, while isolating the agents of soryz bacterial diseases, we paid attention to the colonies of spore-forming and lactic acid bacteria, selected for the studies. We found that some spore-forming and lactic acid isolates demonstrated their antagonistic activity both to the strains of *Pseudomonas syringae*, isolated from soryz, and to the collection culture of genera *Pseudomonas, Xanthomonas, Dickeya.* The spore-forming isolates of bacteria (21095, 21030, 21040, B4, AVS3) and lactic acid bacteria (F1, 8/1) were heterogeneous by their antagonistic effect regarding the strains of phytopathogens under our investigation. The highest antibacterial activity was found in the isolates 21040, B4, AVS3, F1, 8/1. The investigated isolates of bacteria-antagonists demonstrated higher antibacterial activity regarding strains *P. syringae,* isolated from soryz bacterial spots than to the collection strains of *P. syringae* 8299, *P. syringae* subsp. *syringae* UKM B-1021, *X. oryza e*8375, *D. chrysanthemi* 8683. Similar results were described by other researchers who registered the antagonistic activity of strains of *Bacillus subtilis* IMV B-7023 and *Bacillus pumilus* 3 to phytopathogenic bacteria (Roi et al, 2012). These strains differed in their antagonistic effect regarding *Clavibacter michiganensis* subsp. *michiganensisi* and demonstrated a higher stimulating impact on the growth and development of tomatoes during the treatment of seeds with the suspension of *B. subtilis* IMV B-7023 (Roi et al, 2012). The strains of LAB *L. plantarum* were heterogeneous by their spectra of antagonistic activity and differed in terms of potentially pathogenic and phytopathogenic microorganisms (Harmasheva et al, 2015).

Although the rhizosphere bacteria of genus *Bacillus,* which have antagonistic properties regarding the phytopathogenic microorganisms, are considered to be the main component of biopreparations for the protection of plants from disease agents (Compant et al, 2005; Araújo et al, 2005), we isolated the spore-forming bacteria from the surface of plants. Some authors also described that saprophytic strains of genus *Bacillus,* isolated from the surface of plants of root artichoke demonstrated high antagonistic activity to phytopathogenic fungi (*Fusarium oxysporum, Fusarium culmorum, Fusarium solani, Rhizoctonia solani, Phytophtora capsici, Alteranria alternata*) and phytopathogenic bacteria (*Pseudomonas syringae, Erwinia carotovora, Xanthomonas beticola*) (Davranov et al, 2019).

The scientific literature describes the antagonistic activity of the representative of LAB, *Lactobacillus plantarum*, isolated from plant surfaces regarding test-strains of *Xanthomonas campestris*, *Pectobacterium carotovora, Pseudomonas syringae* and *Rhizobium radiobacter* (Visser et al, 1986; Merlich et al, 2016). The level of antagonism of *L. plantarum* regarding phytopathogens differed depending on the strain of lactobacilla, its origin and the strain of phytopathogen (Merlich et al, 2016).

We found that in the field conditions, the isolate of LAB F1, which had high antibacterial activity regarding the phytopathogenic bacteria, impacted the pathological process that developed at the effect of phytopathogenic bacteria *P. syringae*, which led to the reduction in infection symptoms or even their complete absence.

The perspective bacteria with high antagonistic properties (isolates 21040, B4, AVS3, F1, 8/1), found by us, were identified based on morphological, cultural, and biochemical properties and mass-spectrometry MALDI-TOF.

According to the studied biological properties, the spore-forming isolates B4, 21040, and ASV3 had a biochemical profile, typical for bacteria *Bacillus subtilis*.

The mass spectrometry MALDI-TOF demonstrated that isolates B4 and 21040 were identical and corresponded to the species of *Bacillus subtilis.* Isolate ASV3 had a biochemical profile, typical for *B. vallismortis*, which is most closely related to *Bacillus sub-*

tilis. According to the data of Roberts et al, (1996), *B. vallismortis* can be distinguished from *B. subtilis* only by its differences in the composition of fatty acids in the entire cell.

In the scientific literature, *B. vallismortis* is described as an antagonist with antifungal activity, which may demonstrate its potential as an alternative resource for the biocontrol of plant diseases, but the antagonistic activity of this species regarding phytopathogenic bacteria has not been studied sufficiently (Degrassi et al, 2020).

In terms of biological properties, the isolate of LAB F1 had a biochemical profile and mass-spectrum, typical for *Lactobacillus pentosus (Lactiplantibacillus pentosus*), and isolate 8/1 – for *Lactobacillus sakei (Latilactobacillus sakei*).

L. casei was often isolated from the surface of tomato plants (Mundt et al, 1968). We isolated *L. pentosus* bacteria with the antagonistic properties regarding the agents of bacterial diseases, which, according to the data of some authors, was insufficiently studied as an antagonist of phytopathogenic bacteria (Lipinska, 2014; Raman et al, 2022).

Since the late 2000s, the technology MALDI-TOF has been used in medicine for rapid identification of bacterial species (Mellmann et al, 2008), and the obtained results are diagnostically relevant. The advantages of this technology are convenience, rapidness, and accuracy in comparison with traditional biochemical methods (Tsuchida et al, 2020). MALDI-TOFMS analysis is a method, widely used for the identification of microbial species, since it ensures rapid obtaining of reliable results (Kačániová et al, 2019). The researchers demonstrated that thirteen out of fifteen strains of *Pseudomonas*, identified using MALDI-TOFMS analysis, were additionally confirmed by the sequencing of gene 16S rDNA (Kačániová et al, 2019). It was shown that MALDI-TOFMS is more accurate for the identification of some bacterial species than partial sequencing of gene 16S rRNA (Mellmann et al, 2008).

CONCLUSIONS

1,250 samples of soryz were analysed. Thirty-eight spore-forming bacterial isolates were extracted; among these, 21030, 21095, 21040, ASV1, ASV3, B4 demonstrated their antagonistic activity towards the investigated phytopathogenic bacteria, including the agent of soryz bacterial spot *Pseudomonas syringae* and collection bacteria of genera *Pseudomonas, Xanthomonas, Dickeya*.

Twenty isolates of LAB were extracted. We determined high antagonistic activity of the isolates of LAB F1 and 8/1, extracted from the remains of soryz plants which were either on the surface or in soil, at the depth of 1–5 cm. The prevailing majority of the remains were parts of roots with the stalk of 15–20 cm. It was determined that the isolates of LAB demonstrated higher antibacterial activity to the investigated test-cultures of phytopathogenic bacteria than spore-forming isolates. The antagonistic activity of isolate F1 was confirmed in the field conditions. Under artificial infection, it reduced the symptoms of the pathological process, caused by the phytopathogenic bacteria *P. syringae.*

Based on the investigated biological properties, the isolates of spore-forming bacteria, extracted from soryz plants, were identified as *Bacillus subtilis* 21040, *B. subtilis* B4, *Bacillus vallismortis* AVS3. The isolates of LAB were identified as *Lactiplantibacillus pentosus and Lactobacillus sakei* 8/1 (*Latilactobacillus sakei).* The isolated bacteria-antagonists are recommended for the biocontrol of the agents of bacterial diseases.

To identify the antagonists of genera *Lactobacillus* and *Bacillus,* we used MALDI-TOF mass-spectrometry which ensures the identification of microorganisms in one day with considerably high accuracy. The versatility of this method is much more accurate than traditional biochemical methods.

Adherence to ethical principles. All the experimental results, presented in this article, were obtained without the use of any animals.

Conflict of interests. The authors declare the absence of any conflicts of interests.

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Бактерії-антагоністи збудників бактеріальних хвороб соризу

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Мета. Виділити та ідентифікувати бактерії з антагоністичними властивостями для біоконтролю збудників бактеріальних хвороб соризу (*Sorghum oryzoidum*) і соргових культур. **Методи.** Дослідження проводили

впродовж 2021–2023 років. Споротворні бактерії виділяли із зразків рослин соризу, зібраних на полях дослідного господарства Уманського національного університету садівництва (Черкаська обл. м. Умань). Молочнокислі бактерії виділені із рослин соризу, відібраних на приватній земельній ділянці, розташованій між населеними пунктами Теолин, Владиславчик, Княжики у Монастирищенській громаді Уманського району у розташуванні колишнього хутора Першотравневе. Всього проаналізовано 1250 зразків. Повторність досліду триразова. Спороутворюючі та молочнокислі бактерії виділяли із поверхні рослин соризу у фазі твердого зерна в літній період під час виділення фітопатогенних бактерій. Ізоляти молочнокислих бактерій-антагоністів також виділяли із внутрішньої частини стебла зимової стерні соризу, відібраної із зораного поля. Визначення антагоністичної активності штамів молочнокислих бактерій та споротворних бактерій, виділених із різних екологічних ніш, до фітопатогенів соризу та соргових культур проводили *in vitro.* Як тест-культури використовували штами *Pseudomonas syringae*, які є збудниками бактеріальної плямистості соризу: 211141a, 211141, 210341, 21034 та 210521 та колекційні штами фітопатогенів: *Pseudomonas syringae* 8299, *Pseudomonas syringae subsp. syringae* УКМ В-1021, *X. Oryzae* 8375, *Dickeya chrysanthemi* 8683, *Diskeya chryzanthemy* 8683. Дослідження антагоністичної активності виділених ізолятів спороутворюючих і молочнокислих бактерій проводили за методом радіальних штрихів (сумісного культивування антагоніста та досліджуваних штамів). Ізоляти бактерій вважалися неактивними при утворенні зони затримки росту 0–5 мм (–), від 5 до 10 мм (+) – низька активність, 11–20 мм (++) – середня активність, більше 20 мм (+++) – високоактивні щодо тест-культур. Для перевірки дії ізолята-антагоніста на фітопатогенні бактерії в польових умовах проводили штучне зараження. Для цього однодобову культуру антагоніста вводили у стебло рослин у концентрації 1×10^8 колонієутворюючих одиниць, а через 24 год вище від попереднього місця уколу вводили культуру тест штаму фітопатогена. Результати оцінювали через 7–14 діб після штучного інфікування. Повторність досліду триразова. Ізоляти бактерій, які виявляли антагоністичні властивості до фітопатогенних бактерій, ідентифікували на основі їх морфологічних характеристик, забарвлення за Грамом, каталазного тесту, профілю вуглеводної ферментації та за допомогою мас-спектрометрії (MALDI-TOF – Matrix Assisted Laser Desorption/Ionization) на мас-спектрометрі VITEK MS. **Результати.** Із соризу виділено 38 спороутворюючих ізолятів бактерій, з яких ізоляти 21030, 21095, 21040, ASV1, ASV3, B4 виявляли антагоністичну активність до досліджених фітопатогенних бактерій. Ізолят 21040 проявив високу антагоністичну активність до більшості тест-штамів *P.syringae* із соризу (зона відсутності росту 23–30 мм) і меншу активність до колекційних культур. Ізоляти Б4 iAVS3 виявляли вибіркову активність до досліджених фітопатогенів. Виділено 20 ізолятів молочнокислих бактерій. Вищу антагоністичну активність відмічено стосовно ізолятів молочнокислих бактерій 8/1 та F1 до штамів *P. syringae,* ізольованих із соризу та колекційних культур. Найвищу антагоністичну активність ізоляту 8/1 відмічено стосовно тест-штамів *P. syringae* 210521 та *X. oryzae* 8375 (зона відсутності росту 40–35 мм). У польових умовах обробка рослин соргових культур ізолятом F1 впливала на патологічний процес, який розвивається за дії фітопатогенної бактерії *P. syringae*, що призводить до зниження симптомів захворювання. Встановлено таксономічне положення ізолятів бактерій, які перспективні для контролю збудників хвороб. За морфологією клітин і колоній, біохімічного профілю, мас-спектрометрії MALDI-TOF спороутворюючі ізоляти 21040 і Б4 були ідентифіковані як *Bacillus subtilis*, aASV3 як *Bacillus vallismortis.* Ізоляти молочнокислих бактерій ідентифіковані як *Lactobacillus pentosus* F1 та *Lactobacillus sakei* 8/1. **Висновки.** З рослин соризу крім фітопатогенних бактерій нами ізольовані штами споротворних бактерій *Bacillus subtilis* 21040, Б4, *Bacillus vallismortis* AVS3та молочнокислих бактерій (*Lactiplantibacillus pentosus*)та *Lactobacillus sakei* 8/1 (*Latilactobacillus sakei* 8/1, які перспективні для розробки методів біоконтролю збудників бактеріальних хвороб.

Ключові слова: Сориз (*Sorghumoryzoidum*), фітопатогенні бактерії, бактеріальна плямистість соризу, споротвірні бактерії, бацили, молочнокислі бактерії, колекційні культури, антагоністична активність, біоконтроль, мас-спектрометрія.

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