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BIOLOGICAL PROPERTIES OF THE AGENT OF SORYZ BACTERIAL SPOT IN UKRAINE

Soryz is a new promising agricultural crop. Sorghum leaf spots are one of the most common and harmful diseases of these crops. Improving the technology of growing agricultural crops requires the development of methods of controlling their pathogens, based on data on their distribution and properties. There is no information on the taxonomic status and properties of the causative agents of soryz bacterial spot in Ukraine. The ***aim*** *of the work was to identify the causative agent of bacterial spots of a new sorghum crop — soryz in Ukraine and to study its biological properties.* ***Methods****. Identification of the causative agent of soryz bacterial spots was carried out in the Cherkasy and Kyiv regions of Ukraine in 2019-2023. Isolation of the pathogen and study of its morphological-cultural, physiological-biochemical properties were carried out by classical microbiological methods using the NEFERMtest24 (MikroLaTEST®, ErbaLachema, Czech Republic) and API 20NE (Biomerieux, France) test systems. Electron microscopy, chromatographic separation, and identification of fatty acids were conducted at the Center for Collective Research of the Zabolotny Institute of Microbiology and Virology of the National Academy of Sciences of Ukraine (IMV). The antigenic properties of isolated bacteria were investigated by the agglutination reaction with antisera to five serological groups of Pseudomonas syringae strains. Identification of the obtained bacteria was carried out on the basis of their phenotypic properties and the results of MALDI-TOF mass spectrometry on a VITEK MS mass spectrometer.* ***Results.*** *Affecting by spotting was noted in 2—27% of soryz plants, and the development of symptoms of damage was 1—4 points. Bacteria with sorghum disease symptoms were isolated, from which ten virulent isolates were studied in detail. According to the phenotypic properties, 9 isolates that were similar to the characteristics of the typical strain of P. syringae UCM B-1027^T, were identified as P. syringae van Hall 1902. One isolate belongs to phytopathogenic bacteria of the genus Pseudomonas according to its main properties, but its taxonomic status within the genus needs to be clarified. In the cellular lipids strains isolated from affected soryz plants, as well as in the type strain of P. syringae UCM B-1027^T, such fatty acids as dodecanoic, tetradecanoic, hexadecanoic, octadecanoic, cis-9-hexadecenoic, cis-11-octadecenoic, cis-9,10-methylene hexadecanoic, cis-9,10-methylene octadecanoic acids were identified. In the cellular lipids of strains from soryz, fatty acids with an even number of carbon*

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atoms predominate, the total content of which is more than 60% of all detected fatty acids. Isolates from soryz also contain 3-hydroxydecanoic, 2-hydroxydodecanoic, and 3-hydroxydodecanoic fatty acids, the presence of which confirms the belonging of the isolated bacteria to the species P. syringae. According to the antigenic properties, nine strains of P. syringae isolated from soryz are homogeneous and belong to serogroup I. One strain of Pseudomonas sp. does not belong to any of the studied serogroups that parasitize grain crops. Belonging to the causative agent of soryz bacterial spots to the species P. syringae was confirmed by the MALDI-TOF mass spectrometry method. ***Conclusions****. Therefore, on the base of our results, it has been established that the main causative agent of soryz bacterial spots in Ukraine is P. syringae. According to antigenic properties, the population of the causative agent of soryz bacterial spots is homogeneous, which makes it possible to develop serological rapid tests for the detection of the causative agent. By comparing the biological properties of collection strains from sorghum, a species of Pseudomonas holci, which does not exist in modern taxonomy, with the properties of strains from soryz, the typical strain of P. syringae UCM B-1027^T, we established that P. holci bacteria isolated in the Department of Phytopathogenic Bacteria of the IMV in 1968-1971 belong to the species P. syringae.*

Keywords*: Pseudomonas syringae, soryz, bacterial spot, virulence, serological properties, fatty acids, mass spectrometry.*

Sorghum (*Sorghum bicolor* L.) is one of the oldest agricultural crops in the world which has a high food, energy, and fodder value [1] and is the fifth cereal crop in the world after wheat, rice, corn, and barley. According to the United Nations Agricultural Organization, in 2022, about 74.5 million tons of sorghum was produced in the world. Sorghum attracts special attention as a promising crop to ensure food security in the face of climate change and anthropogenic degradation of agricultural land [2—4].

Ukrainian breeders have developed a new variety of sorghum called soryz (*Sorghum orysoidum*) [5]. The new crop inherited the properties of sorghum (drought and heat resistance, salinity tolerance, undemanding to soil conditions, etc.) and has good taste, high vitreousness, hardness of the endosperm, and the ability to extrusion.

In all cultivation regions, sorghum crops are affected by leaf spots, which leads to significant crop losses [6—8]. The agents of leaf diseases of sorghum crops can be both micromycetes and bacteria [6]. Among the agents of bacterial diseases of sorghum, the following species are known: *Burkholderia andropogonis*, *Xanthomonas campestris* pv. *holcicola*, and *Pseudomonas syringae* [6, 9, 10]. The development of methods for controlling the agents of any agricultural crop requires the availability of data on the spread of phytopathogens in the region of cultivation. Despite the stable area of sorghum cultivation in Ukraine (about 50.000 hectares) [11], data on the spread of the causal agents of bacterial diseases are practically absent. On the territory of Ukraine, employees of the Department of Phytopathogenic Bacteria of the IMV of the NAS of Ukraine have identified an agent of bacterial leaf spot of sorghum *Pseudomonas holci* [12]. However, this species is absent in the modern classification of bacteria. In connection with climate change and anthropogenic factors, it is possible to predict the expansion of ranges and the increase in the harmfulness of phytopathogenic bacteria, therefore, attention should be focused on the spread of these bacteria on sorghum crops.

Therefore, the **aim** of the work was to identify the causative agent of bacterial spot of the new sorghum crop — soryz in Ukraine and to study its biological properties.

Materials and methods. To establish the prevalence of bacterial spot and identify its causative agent, sorghum crops were surveyed in the farms of the Uman district, Cherkasy region, namely the experimental farm of the Uman National University of Horticulture, private farms of the Mankivska and Zhashkivska hromadas, and experimental crops of Kyiv region. Surveys of sorghum crops to determine the severity of bacterial infection were conducted using the diagonal method in 20 points, selecting 5 plants/point. The development of symptoms of infection in each plant was assessed on a scale of 0 to 5 (0 —

undamaged, 5 — severely damaged plant). The assessment was carried out for the stem, leaves, and panicle using the following scale: from 1 to 10% of the stem and leaves turn red (1 point), 11—29% of the seeds have red dots (2 points), 20—30% of the seeds with red dots and infection of the outer stem and leaves (3 points), 50—79% of the leaf and 50—70% of the stem are affected by spots (4 points), unripe seeds, 80—90% of the leaf and 70% of the stem with oozing spots (5 points).

To isolate from the agent, plant samples with typical symptoms of bacterial leaf spot were collected: dark green spots, which later turn a lighter color with different shades. Most of them are concentrated in the upper part of the leaf, small in size, round in shape, and increase over time.

To isolate the causative agent, plant samples with typical signs of bacterial spot damage were selected. Isolation of bacteria was carried out by plating the pieces of plants, pounded with 0.1 mL of sterile tap water, on potato agar. For the bacteria isolation, parts of plants were selected on the verge of healthy and damaged tissues [13].

The morphology and structure of bacterial colonies were studied after 72 h growing on potato agar in Petri dishes [14], first observing with the naked eye or a magnifying glass, and then with a digital microscope *Yizhan Digital Microscope* at 120X magnification. Cell morphology and motility were determined in Gram-stained and «crushed drop» preparations respectively under a Sigeta MB-201 microscope using a one-day bacterial culture grown in nutrient broth (NB) [13, 14]. For the initial identification of the isolates from soryz, the LOPAT test was used [14].

A sample of bacteria for electron microscopy was grown in a liquid culture on an NB medium. The morphological features of bacterial cells were studied using transmission electron microscopy (TEM) with a JEM-1400 electron microscope (Jeol, Japan) at the Center for Collective Use of the Zabolotny Institute of Microbiology and Virology of the NAS of Ukraine (IMV).

To study the virulent properties of the isolated strains, artificial inoculation of soryz plants (*Sorghum orysoidum* (L.) Moench, variety Titan, in the field and other sorghum in the greenhouse and on field plots: sugar sorghum (*Sorghum saccharatum* (L.) Moench.), variety Silosne 42, common sorghum, grain sorghum, grain (*Sorghum bicolor* subsp. *bicolor*), variety Svey, sudan grass (*Sorghum bicolor* subsp. *drummondii*), variety Dniprovs'ka 54) was carried out. Artificial infection of sorghum plants was carried out by injecting a drop of bacterial suspension with a density of 1×10^7 CFU/mL. The development of visible signs of damage indicated virulent properties. The results of artificial infection were analyzed on a 5-point scale after 7—14 days, evaluated by the size of the necroses [15].

The ability of *P. syringae* to cause the hypersensitive reaction on the tobacco variety Samsun was determined by the leaf infiltration method [14]. A suspension of a one-day bacterial culture with a concentration of 1×10^7 CFU/mL was injected under the epidermis of the leaves using a syringe. Sterile tap water was used as a negative control. The presence of necrosis was observed after 24 h.

The biochemical properties of bacterial isolates were studied by their ability to assimilate individual carbohydrates as the only source of carbon nutrition using classical microbiological methods [13]. The NEFERMtest24 test system (MikroLaTEST®, ErbaLachema, Czech Republic) and API 20NE (Biomerieux, France) test system were used to study the physiological and biochemical properties of bacterial isolates. The results were analyzed according to the tables provided by the manufacturer. Enzymatic and oxidative glucose metabolism (OF-test) was determined using a microplate (OFtest, Erba Lachema).

For comparative studies, strains of phytopathogenic bacteria from the Ukrainian Collection of Microorganisms of the IMV [16] and the collection of the Department of Phytopathogenic Bacteria of the IMV were used: *P. syringae* UCM B-1027^T, *P. holci* 8300, 8299, 8301; *P. syringae* pv. *atrofaciens* UCM B-1011^T.

The oxidase activity was determined by the method described by N. Kovach using a 1% solution of N, N-dimethyl-n-phenylenediamine sulfate. A positive oxidase reaction was detected by the color change of the bacterial mass to dark red in 5—10 sec [13].

The pectolytic activity was determined by inoculating a one-day bacterial culture on peeled and sliced potatoes incubated under sterile conditions in a humid chamber in a Petri dish [13].

In order to identify the isolated bacteria, the determination of the fatty acid composition of total cellular lipids was carried out. For this purpose, fatty acid methyl ethers were obtained during methanolysis of whole bacterial cells in a 5% solution of sulfuric acid in methanol [13]. Chromatographic separation and identification of fatty acids was carried out at the Collective Use Center of the IMV. The separation of the fatty acid methyl esters was carried out on the chromatographic mass spectrometric system Agilent 6890N/5973 inert. Peaks were identified by comparing their retention times with the retention times of standard Bacterial Acid Methyl Ester Mix, as well as using an integrated database of NIST 02 mass spectra. The content of individual fatty acids was determined as the percentage of the total peak area.

Antigenic properties of bacterial strains were studied by agglutination reactions [17]. Antisera to *P. syringae* strains of nine serological groups [18]: *P. syringae* UCM B-1027^T, *P. syringae* pv. *atrofaciens* UCM B-1013 — serogroup I; *P. syringae* pv. *atrofaciens* K1025 — serogroup II; *P. syringae* pv. *atrofaciens* UCM B-1011^T — serogroup IV; *P. syringae* pv. *atrofaciens* 948 — serogroup V; *P. syringae* pv. *atrofaciens* UCM B-1115, and *P. syringae* 8299 — serogroup VI were used in the studies.

The phytopathogenic bacteria isolated from soryz were identified by comparing their properties with the characteristics of bacteria listed in the Bacterial Identifier [19] and the collection strains of bacteria.

Confirmation of the taxonomic position of the isolated phytopathogens was carried out using Mass Spectrometry MALDI-TOF on a VITEK MS mass spectrometer. A calibration curve of the *Escherichia coli* strain ATCC 8739 grown for 24 h was prepared. A thin layer of cells from this strain was plated onto a calibration target well, and 1 μL of MATRIX was added to each calibration target well. The time was allowed until the material on the target wells dried completely and yellow crystals formed.

First, pure cultures of the studied bacteria were prepared for 18—24 h, and a colony in a volume of 1 μL was applied to the target well.1 μL of MATRIX was immediately added to the applied culture in the center of the target well. The target wells were allowed to dry completely and MATRIX crystals — to form. The slide was placed in the instrument, and the instrument was run using the software until the process was completed.

Results. During the examination of soryz plants, the presence of 2 to 27**%** of plants affected by spotting with the development of symptoms of 1—4 points was noted in all farms' products. The largest number of plants with signs of leaf spot was observed in the farms of Kyiv region. In some areas, up to 40**%** of plants were affected by spotting.

The main symptoms of spotting on plant leaves were rounded grey spots, red streaks, red dry spots with red-brown borders, and red elongated spots of various shapes: round or amorphous, elongated with a dried black or greenish center (Fig. 1).

In areas where a significant number of plants were affected by spotting, a decrease in crop and the formation of fewer grains per panicle were also observed.

With a significant development of symptoms of spotting, the damage also spread to the stem of the plants: reddening in the lower part of the stems, closer to the root, and brown elongated continuous spots from 0.5 to 3 cm, which spread up the stem (Fig. 1).

Bacterial isolates obtained from soryz plants affected by leaf spot were diverse in colony color (gray transparent, gray-white, yellow, orange),

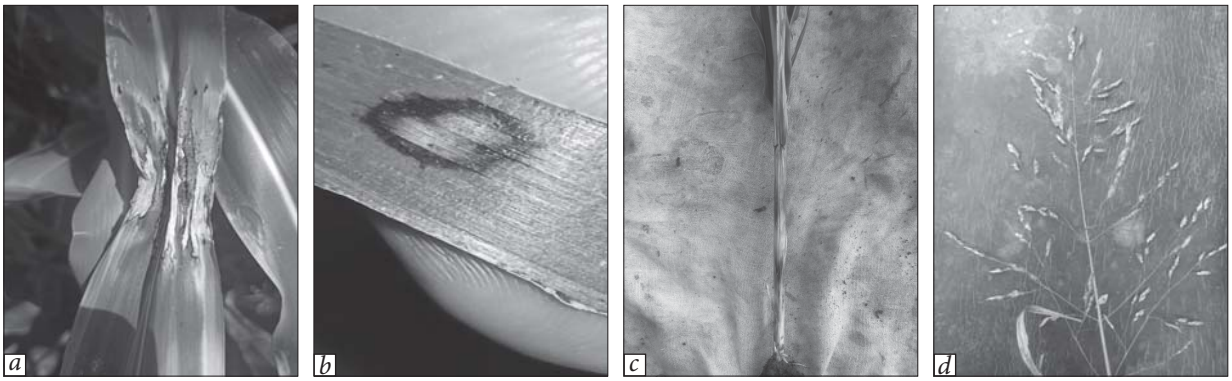

Fig 1. Natural lesions of soryz plants: *a, b* — leaf; *c* — stem; *d* — panicle of plants with a high level of damage

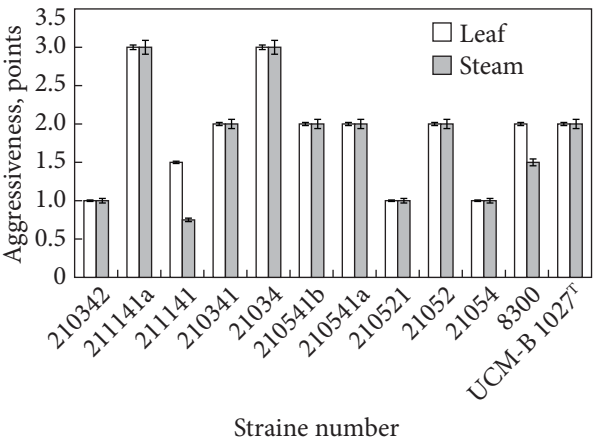

Fig. 2. The average aggressiveness of obtained bacterial isolates on soryz plants (variety Tytan): 210342, 211141a, 211141, 210341, 21034, 210541b, 210541a, 210521, 21052, and 21054 — isolates from soryz, 8300 — *Pseudomonas holci*, UCM B-1027^T — *Pseudomonas syringae*

Table 1. **Characteristics of bacteria isolated from soryz by LOPAT test**

Bacteria studied	Biochemical tests*				
	L	O	P	A	T
Bacteria isolated from soryz	+	—	—	—	+
P. syringae UCM B-1027^T	+	—	—	—	+
P. holci 8300	+	—	—	—	+

*L— levane formation; O — presence of oxidase; P— tissue maceration; A —arginine dehydrolase; T — hypersensitivity reaction

round, flat or slightly convex in shape, dense, semi-transparent or opaque, and slimy with a wavy or even edge. In artificial inoculation in the field and greenhouse conditions, the main part (60%) of the isolates was found to be virulent to the soryz variety Tytan.

It is necessary to note that isolates that formed gray semi-transparent flat with wavy edges of colonies were virulent to soryz. Among them, we selected 10 most typical isolates in terms of morphological features but different in aggressiveness for further study of biological properties and identification (Fig. 2).

Bacterial cultures isolated from affected soryz plants are virulent not only for soryz of the Tytan variety but also for sugar sorghum (*Sorghum saccharatum* (L.) Moench.), Silosne 42 variety, common sorghum, grain sorghum (*Sorghum bicolor* subsp. *bicolor*), Sway variety, and Sudan grass (*Sorghum bicolor* subsp. *drummondii*), variety Dniprovska 54 [15].

The morphological characteristics of the selected ten virulent isolates from soryz are similar to those of the genus *Pseudomonas* [19]. For the initial identification of these isolates, the LOPAT test was used to determine the affiliation of phytopathogenic bacteria to the *P. syringae* species. It was found that all isolates were oxidase-negative, produced levane on sucrose medium, did not produce pectate lyase and argenine dehydrolase, and caused a hypersensitivity reaction on tobac-

co leaves, which is typical for phytopathogenic bacteria of the *Pseudomonas* genus (Table 1).

These results indicate that the bacteria isolated from soryz belong to the phytopathogenic bacteria of the *P. syringae* species.

Electron and light microscopy methods revealed that the studied bacteria were Gram-negative, motile, and rod-shaped (Table 2). The size of the cells was 0.5—0.8 × 1.0—1.7 μm.

The isolates differed in their ability to use individual carbohydrates as the only source of carbon nutrition (use of sucrose, sorbitol, and inositol) and the presence of gelatinase (Table 2), but most of them were similar to the characteristics of P. *holci* strain 8300 and the typical *P. syringae* strain UCM B-1027^T. Only isolate 21052 was different, as it did not use mannitol, arabinose, and galactose like the other isolates from soryz and collection strains.

To obtain more characteristics of the bacterial isolates from soryz, the NEFERMtest 24 test system was used (Table 3).

It was found that all isolates of bacteria from soryz had negative results for the tests on urease, arginin, ornithin, lysin, acetamid, N-acetil-β-D-glucosamidase, simmons citrate. Isolate 21052 differed from the other isolates by not utilizing xylosa, galaktose, saccharose, inositol, esculine and not forming β-glukosidase.

Therefore, based on the conducted research, the similarity of nine isolates of bacteria (210342, 211141a, 211141, 210341, 21034, 210541b, 210541a, 210521, 21054) with collection strains from sorghum *P. holci* 8300 and 8299 and the type strain *P. syringae* UCM B-1027^T was established. The isolates were identified as *P. syringae* van Hall 1902. Isolate 21052 belongs to the genus *Pseudomonas*, but its taxonomic status within the genus needs to be clarified.

In the fatty acid composition of the cellular lipids of strains isolated from affected soryz plants, as well as in the type strain of *P. syringae* UCM B-1027^T and the strain *P. holci* 8300, dodecanoic, tetradecanoic, hexadecanoic, octade-

Table 2. **Physiological and biochemical properties of bacteria isolated from soryz plants**

Tests	Isolates from soryz					*P. holci* 8300	*P. syringae* UCM B-1027^T
	210342, 21034, 210541в 210541a	211141a 210341	210521 21054	211141	21052		
Gram staining	—	—	—	—	—	—	—
Cell shape	r	r	r	r	r	r	r
Mobility	+	+	+	+		+	+
Spore formation	—	—	—	—	—	—	—
Fluorescent pigment	+	+	+	+	+	+	+
Production of indole and hydrogen sulfide	—	—	—	—	—	—	—
Gelatinase	—	+	—	—	—	+	+
Nitrate reduction	—	—	—	—	—	—	—
Carbohydrate utilization:							
Glucose, anaerobically	—	—	—	—	—	—	—
Glucose, fructose, glycerol	+	+	+	+	+	+	+
Mannitol, arabinose, galactose	+	+	+	+	—	+	+
Lactose, rhamnose, maltose, dulcitol, inulin, salicin	—	—	—	—	—	—	—
Sorbitol, inositol	+	+	—	+	+	+	+
Sucrose	+	+	—	+	—	+	+

canoic, cis-9-hexadecenoic, cis-11-octadecenoic, cis-9,10-methylenehexadecanoic, cis-9,10-methyleneoctadecanoic fatty acids were identified (Table 4). In the cellular lipids of the isolates from soryz, fatty acids with an even number of carbon atoms predominated, namely hexadecanoic, cis-9-hexadecenoic, and cis-11-octadecenoic acids. Their total content was more than 60% of all detected fatty acids. Isolates from soryz also contained 3-hydroxydecanoic, 2-hydroxydodecanoic, and 3-hydroxydodecanoic fatty acids, the presence of which confirms the belonging of the isolated bacteria to the species *P. syringae*.

To determine the antigenic affinity of *P. syringae* strains isolated from soryz, agglutination reactions were performed with antisera to strains of bacteria that occur on sorghum and cereal crops [17, 18]. It was found that the strains of bacteria from soryz showed serological affinity to representatives of four *P. syringae* serogroups (I, II, IV, VI). However, the titers of the agglutination reaction depended on the serogroup strain to which it was obtained. None of the ten studied strains of bacteria reacted in high titer (12800—25600) with antiserum to the strain *P. syringae* UCM B-1027^T (Table 3) and belonged to serogroup I. With anti-

Table 3. **Properties of bacteria isolated from soryz according to the results of NEFERMtest 24 (MikroLaTEST, ErbaLachema)**

Test	Isolates from soryz				*P. holci* 8299	*P. syringae* UCM B—1027^T
	211141	210541a 210342 21034	21052	210341		
Urease	—	—	—	—	—	—
Arginin	—	—	—	—	—	—
Ornithin	—	—	—	—	—	—
Lysin	—	—	—	—	—	—
Acetamid	—	—	—	—	—	—
β — Glukosidase	+	+	—	+	+	+
N—acetil—β—D—glucosamidase	—	—	—	—	—	—
Simmons citrate	—	—	—	—	—	+weak
Lactose	—	—	—	—	—	—
Mannitol	+	+	—	+	+	+
Trehalose	—	—	—	—	—	—
Xylosa	+	+	—	+	+	+
Arabinose	+	+	+	+	+	+
α — Galaktosidase	—	—	—	—	—	—
β — Galaktosidase	—	—	—	—	—	—
Malonate	—	—	—	—	—	—
Galaktose	+	+	—	+	+	+
Maltose	—	—	—	—	—	—
Cellobiose	—	—	+	+	—	—
Saccharose	+	+	—	+	+	+
Inositol	+	+	—	+	+	+
γ — Glutamyltransferase	+	+	+	+	+	—
Phosphatase	—	+	—	+	+weak	+
Esculine	+	+	—	+	+	+

sera to strains of serogroups II and IV, all cultures from soryz reacted in a low titer (100—800). All strains from soryz reacted in the same low titer (1600) with antiserum to strain 8299 (serogroup VI). Strain 21052 differed: it reacted in a low titer with antisera to *P. syringae* serogroups I, II, and VI and did not react at all with antiserum to the strain of serogroup IV.

Therefore, the obtained results indicate the serological affinity of the *P. syringae* strains from soryz with the strains of *P. holci* from sorghum and other crops.

To confirm the affiliation of the isolated strains of bacteria from soryz to the species *P. syringae*, matrix-assisted laser desorption/ionization (MALDI-TOF) was used. Identification of microorganisms was based on obtaining a general mass spectrum of proteins in the range of 1000—10000 Dalton and bioinformatics comparison of the obtained spectrum with the database of reference spectra of known species of bacteria.

MALDI-TOF MS confirmed that the strains of *P. syringae*, the causative agents of soryz bacterial spots, and the collection strain *P. holci* 8300 isolated from sorghum plants are identical (Fig. 3).

So, on the basis of the conducted research, we have established that the main causative agent of soryz bacterial spots is *P. syringae* species.

Discussion. Soryz (*Sorghum orysoidum*) is a new promising agricultural crop, in fact, it is a new variety of cereal sorghum [5]. The new crop has inherited the properties of sorghum (drought and heat resistance, salt tolerance, tolerance to soil conditions, etc.) and has good taste, high vitreousity, endosperm hardness, and extrusion ability [5]. Researchers from the Department of Phytopathogenic Bacteria of the IMV studied bacterial diseases of sorghum and found that sorghum is affected by the bacterium *P. holci* [12], which is not currently included in the modern bacterial taxonomy. Therefore, the virulent bacteria isolated from soryz were studied in comparison with the properties of strains isolated from sorghum and collection cultures.

Table 4. **Fatty acid composition of total cellular lipids of *P. syringae* strains isolated from soryz**

Fatty acids	Amount of fatty acids (% of total peak area), strains									The average value
	P. syringae	*P. holci*	Isolated from soryz							
	UCM B-1027^T	8300	21034	210341	210342	210541в	210541а	211141	211141а	
C10:0 3-OH	2.75±0.13	0.54±0.02	0.56±0.02	0.55±0.02	0.55±0.02	0.55±0.02	0.56±0.02	0.37±0.01	0.42±0.02	0.51
C12:0	6.25±0.31	9.03±0.45	8.78±0.44	10.18±0.49	10.27±0.50	10.50±0.52	8.79±0.44	8.7±0.43	5.72±0.28	9.00
C12:0 2-OH	2.15±0.11	0.72±0.03	0.75±0.03	0.90±0.04	0.72±0.03	0.88±0.03	0.94±0.04	0.39±0.01	0.42±0.02	0.71
C12:0 3-OH	1.25±0.06	0.21±0.01	0.15±0.01	0.2±0.01	0.2±0.01	0.15±0.01	0.2±0.01	0.15±0.01	0.15±0.01	0.17
C14:0	0.25±0.01	0.72±0.03	0.56±0.02	0.72±0.03	0.54±0.02	0.53±0.02	0.56±0.02	0.38±0.01	0.22±0.01	0.50
C16:1	31.75±1.58	24.09±1.20	23.71±1.18	24.15±1.20	23.22±1.17	23.78±1.18	24.15±1.20	25.70±1.28	27.92±1.39	24.66
C16:0	34.25±1.71	24.25±1.20	24.85±1.23	24.30±1.21	23.96±1.19	23.78±1.18	24.91±1.24	25.70±1.28	27.92±1.39	25.06
C17: 0 cyclo	0.5±0.02	2.72±0.13	2.62±0.13	2.18±0.10	2.70±0.13	1.96±0.09	1.87±0.09	1.94±0.09	0.85±0.04	2.02
C17:0	0	0.72±0.03	0.56±0.02	0.56±0.02	0.72±0.03	0.54±0.02	0.75±0.03	0.39±0.01	0.21±0.01	0.53
C18:1	18.25±0.91	22.80±1.13	23.30±1.16	23.05±1.15	23.25±1.16	23.40±1.17	23.60±1.18	24.50±1.22	27.50±1.37	24.08
C18:0	2.5±0.12	10.80±0.53	10.05±0.50	9.40±0.47	10.81±0.54	10.89±0.54	10.49±0.52	9.65±0.48	7.40±0.37	9.81
C19:0 cyclo	0.1±0.01	3.40±0.17	4.11±0.20	3.81±0.19	3.06±0.15	3.04±0.15	3.18±0.15	2.13±0.10	1.27±0.06	2.94

According to the results of the studies, it was found that soryz in Ukraine is affected by bacterial spotting, with symptoms similar to those described by researchers in Ukraine on sorghum [2]. The most characteristic lesions were recorded on the leaves in the form of rounded or elongated grey spots with red or brownish-red borders, red strokes, and red dry elongated spots with red-brown borders.

On the stem, reddening was more often detected, starting from the lower part of the stem, closer to the root, and brown elongated continuous spots. Such a variety of symptoms is typical for the sorghum disease, which, in addition to the name «bacterial spot», has synonyms: red bacteriosis and red bacterial blight. The percentage of affected plants on soryz ranged from 2 to 40%, depending on the year and location of the study. This variation in the natural damage to sorghum crops has been explained by some researchers. Bacterial spot incidence is increased by cold weather, rain, and high humidity [20]. Gaudet and Kokko [10] detected *P. syringae* on 8 to 67% of the sorghum seeds from different lots and reported P. *syringae* to be the cause of poor sorghum seedling emergence in Alberta, Canada. They found large numbers of bacteria inside the seed coat, distal to the coleorhiza, and on the exterior of the seed in the region of the embryo, after 3 days of germination.

Ten isolates of bacteria isolated from soryz, whose colonies were round, grey, translucent, smooth, with wavy edges, compacted, and with a raised center, were virulent to soryz of the Ti-

Table 5. **Results of agglutination reactions of *P. syringae* strains from soryz with antiserum to strains of different serological groups**

Antigens of strains	Titers of agglutination reaction with antiserum to strains of *P. syringae*, serogroups				
	UCM B-1027^T	8300	K1025	PDDCC 4394	8299
	I serogroup	I serogroup	II serogroup	IV serogroup	VI serogroup
Collection					
K1025*	200	400	12800-25600	1600	12800
UCM B-1027^T	25600	3200	100	400	n/i
8299**	100	100	6400	12800	25600
8300**	12800	6400—12800	200	800	1600
8301**	200	400	1600	400	12800
From soryz					
21054	12800	6400	100	400	3200
211141a	25600	6400	800	200	1600
211141	25600	6400—12800	800	400	1600
210521	6400—12800	800	400	400	1600
210541в	12800	6400	800	800	1600
210541a	12800	6400—12800	800	200	1600
21052	800	800	100	0	1600
210342	12800	12800	200	100	1600
210341	12800	6400	200	200	1600
21034	12800	6400—12800	800	400	1600

Collection strains: «*» — *P. syringae* pv. *atrofaciens*, «**» — *P. holci* strains from sorghum

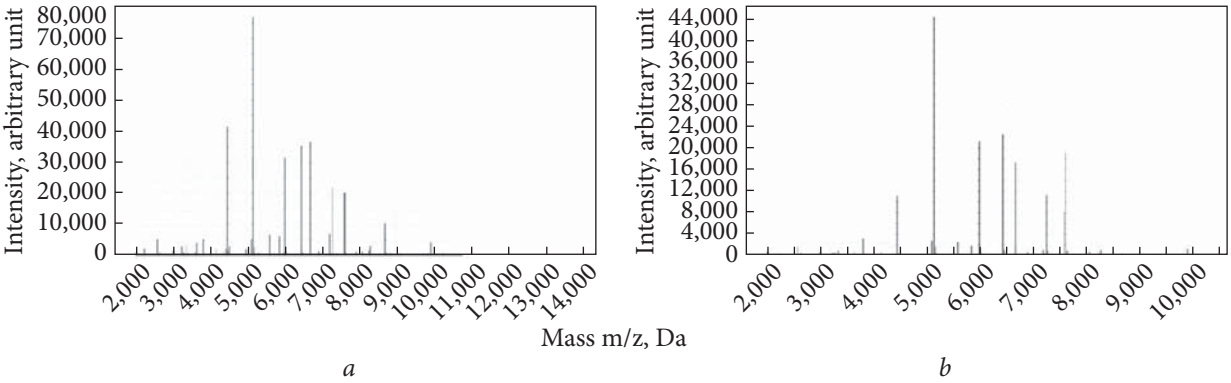

Fig. 3. Mass spectra of strains: *a* — 211141a from soryz; *b* — collection strain *P. holci* 8300 from sorghum.

tan variety. According to the morphology of the colonies, virulent isolates were most similar to phytopathogenic bacteria of the genus *Pseudomonas* [19]. These isolates were heterogeneous in aggressiveness (from one to three points). In addition to soryz, they infected sorghum varieties (sugar sorghum and common sorghum), Sudanese grass, and segetal vegetation under artificial inoculation [15].

The isolates from soryz did not differ by the LOPAT test from the typical strain of *P. syringae* UCM B-1027^T and the collection strain from sorghum *P. holci* 8300. The isolated bacteria were identified by comparing their properties with those of the typical *P. syringae* UCM B-1027^T strain and the Bacterial Identifier [19]. According to morphological and biochemical properties, nine bacterial isolates (210342, 211141a, 211141, 210341, 21034, 210541b, 210541a, 210521, and 21054) were identified as *P. syringae* van Hall, as their properties were found to be similar to the typical strain of *P. syringae* UCM B-1027^T. We found that some characteristics (the use of sucrose, sorbitol, and inositol, the presence of gelatinase) of the isolates from soryz differed, but most of them were similar to the typical *P. syringae* strain UCM B-1027^T. These minor differences do not go beyond the biological properties of the *P. syringae* group, for which heterogeneity in the use of some carbon sources has been noted by other researchers [21]. *Pseudomonas syringae*, the causative agent of bacterial spot, is a polyphage and one of the most common phytopathogens that cause bacterial diseases of various crops [9].

Isolate 21052, which differed in some properties from the characteristics of the typical strain of *P. syringae* UCM B-1027^T, was identified as *Pseudomonas* sp.

An important diagnostic test for bacterial identification is the determination of the fatty acid composition of total cellular lipids. As known, the most important value for the taxonomy of bacteria of the genus *Pseudomonas* is given to the oxy-substituted fatty acids [22]. In all strains isolated from soryz, 3-hydroxydecanoic, 2-hydroxydodecanoic, and 3-hydroxydodecanoic fatty acids were identified in amounts less than 5%. The results we obtained on the fatty acid composition of the cellular lipids of *P. syringae* are in agreement with the literature data. According to D.E. Stead [22], saturated, unsaturated, and oxy acids are found in the cells of phytopathogenic bacteria of the genus *Pseudomonas*. On the basis of the detected hydroxy acids (2-hydroxy, 3-hydroxy, and iso-branched-3-hydroxy acids), called core, phytopathogenic bacteria of the genus *Pseudomonas* were divided into six groups. Pathovars of *P. syringae* belong to group 1, subgroup 1a, all members of which contain 10:0 3-OH, 12:0 2-OH, and 12:0 3-OH hydroxy acids in amounts less than 5—6% [22].

In terms of antigenic properties, nine strains of *P. syringae* isolated from soryz are homogeneous and serologically related to serogroup I. The strain *Pseudomonas* sp. 21052 does not belong to any of the studied serogroups, which may indicate that it belongs to a new serological group. *P. syringae* strains of serogroup I are common on many crops: sorghum, Sudanese grass, lupine, rye, oats, cabbage, segetal vegetation, and fruit trees [17, 18]. It should be noted that the strains of the sorghum bacterial spots are divided into four serogroups — I, II, IV, VI, while the strains from soryz belong to only one — serogroup I. This can be explained by the fact that soryz is a newly bred crop and pathogens have not yet adapted to it. On the other hand, there is evidence of some restriction of *P. syringae* strains of some serogroups to the host plant and the geographical location of isolation of the pathogen strains. The agent of soryz bacterial spotting was confirmed by MALDI-TOF mass spectrometry to be *P. syringae*. Since the late 2000s, MALDI-TOF technology has been used in medicine for the rapid identification of bacterial species [23]. The results obtained using MALDI-TOF are diagnostically significant. MALDI-TOF MS analysis is a method that has been used extensively for the identification of microbial species as it provides an opportunity to obtain reliable results quickly [24]. The researchers showed that thirteen of the fifteen strains of *Pseudomonas* identified by MALDI-TOF MS analysis were further confirmed by 16S rDNA gene sequencing. This study revealed the high discriminatory power of mass spectra and 16S rDNA gene sequencing technology for the identification of *Pseudomonas* species associated with freshwater fish [24]. MALDI-TOF MS has been shown to be more accurate than partial 16S rRNA gene sequencing for the identification of some bacterial species (species of members of the *B. cepacia* complex) [23].

Conclusions. The results of our study showed that the main pathogen of soryz bacterial spot in Ukraine is the species *Pseudomonas syringae*. The population of the pathogen is homogeneous in terms of antigenic properties, which makes it possible to develop serological rapid tests for the detection of the pathogen in the future. The obtained results complement the scheme of serogrouping of phytopathogenic bacteria of the *P. syringae* group [18] with strains of the agent of soryz bacterial spot that belong to serogroup I. As for the collection strains from sorghum of the species *P. holci*, absent in modern taxonomy, comparing their biological properties with the properties of strains from soryz, the typical strain of *P. syringae* UCM B-1027^T, and the properties of *P. syringae* [19], we can state that the causative agent of sorghum bacterial spotting isolated in the Department of Phytopathogenic Bacteria of the IMV of the NAS of Ukraine in 1968—1971, is actually a species of *Pseudomonas syringae*.

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БІОЛОГІЧНІ ВЛАСТИВОСТІ ЗБУДНИКА БАКТЕРІАЛЬНОЇ ПЛЯМИСТОСТІ СОРИЗУ В УКРАЇНІ

Сориз є новою перспективною сільськогосподарською культурою. Плямистості листя соргових є одними з найпоширеніших і шкідливих хвороб цих культур. Удосконалення технології вирощування сільськогосподарських культур вимагає розроблення методів контролю їхніх збудників, що базуються на даних щодо їхнього поширення та властивостей. Відомості стосовно таксономічного статусу та властивостей збудників бактеріальних плямистостей соризу в Україні відсутні. **Мета**. Метою роботи було виявити збудника бактеріальної плямистості нової соргової культури — соризу в Україні та вивчити його біологічні властивості. **Методи.** Виявлення збудника бактеріальної плямистості соризу здійснювали у Черкаській та Київській областях України в 2019—2023 роках. Ізолювання збудника, вивчення його морфолого-культуральних, фізіолого-біохімічних властивостей проведено класичними мікробіологічними методами та з використанням тест-систем NEFERMtest24 (MikroLaTEST®, ErbaLachema, Czech Republic) і API 20NE (Biomerieux, France). Електронну мікроскопію, хроматографічне розділення та ідентифікацію жирних кислот виконано у Центрі колективного користування Інституту мікробіології і вірусології НАН України. Антигенні властивості ізольованих бактерій вивчені за реакцією аглютинації з антисироватками до штамів *P. syringae* п'яти серологічних груп. Ідентифікація виділених бактерій проведена на основі їхніх фенотипових властивостей та з використанням Малді-тоф мас-спектрометрії на мас—спектрометрі VITEK MS. **Результати**. Ураженість плямистістю відмічали у 2—27% рослин соризу за розвитком симптомів ураження 1—4 бали. З характерних для соргових симптомів захворювання виділені бактерії, з яких детально досліджено десять вірулентних ізолятів. З них за фенотиповими властивостями 9 ізолятів, подібними до характеристик типового штаму *Pseudomonas syringae* УКМ В-1027^T, були ідентифіковані як *P. syringae* van Hall 1902. Один ізолят за основними властивостями належить до фітопатогенних бактерій роду *Pseudomonas*, але його таксономічний статус в межах роду потребує уточнення. В жирнокислотному складі клітинних ліпідів, ізольованих з уражених рослин соризу штамів**,** як і у типового штаму *P. syringae* УКМ В-1027^T та штаму *P. holci* 8300, ідентифіковано додеканову, тетрадеканову, гексадеканову, октадеканову, *cis*-9-гексадеценову, *cis*-11-октадеценову, *cis*-9,10-метиленгексадеканову, *cis*-9,10-метиленоктадеканову жирні кислоти. У клітинних ліпідах бактерій переважали жирні кислоти з парним числом вуглецевих атомів, сумарний вміст яких становив понад 60% від усіх виявлених жирних кислот. Ізольовані із соризу штами також містять 3-гідроксидеканову, 2-гідроксидодеканову і 3-гідроксидодеканову жирні кислоти, наявність яких підтверджує належність виділених із соризу бактерій до виду *P. syringae.* За антигенними властивостями дев'ять штамів *P. syringae*, ізольовані з соризу, однорідні і належать до серогрупи I. Один штам *Pseudomonas* sp. не належить до жодної з досліджених серогруп, що паразитують на зернових культурах. Належність цього збудника бактеріальної плямистості соризу до виду *P. syringae* було підтверджено методом мас-спектроскопії MALDI-TOF. **Висновки.** Отже, на основі наших результатів встановлено, що основним збудником бактеріальної плямистості соризу в Україні є *P. syringae.* За антигенними властивостями популяція збудника бактеріальної плямистості соризу є однорідною, що робить можливим розроблення серологічних швидких тестів для його виявлення. Порівнюючи біологічні властивості неіснуючого в сучасній систематиці колекційних штамів із сорго виду *P. holci* з властивостями штамів із соризу, типового штаму *P. syringae* УКМ В-1027^T, ми встановили, що бактерії *P. holci,* виділені у відділі фітопатогенних бактерій у 1968—1971 рр., належать до виду *P. syringae*.

Ключові слова: *Pseudomonas syringae, сориз, бактеріальна плямистість, вірулентність, серологічні властивості, жирні кислоти, мас-спектрометрія.*