BACTERIAL MICROFLORA OF SUGAR BEET SEEDS

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The paper shows the results of bacterial microflora of sugar beet seeds study, with the analysis of morphological, culture-biochemical and virulent characteristics of isolated bacteria. The presence of pathogenic bacteria was observed on seeds pretreated with fungicide. The antagonistic activity of bacterial microflora of sugar beet seeds in relation to known bacterial diseases agents of this culture was investigated.

Keywords: sugar beet, seeds, bacterial microflora, antagonism, artificial infection.

Crop seeds is a substrate for various microorganisms: micromycetes, bacteria, mycoplasma and viruses [10]. Seeds microbiota is divided into the several groups. Epiphytic microbiota – microorganisms that colonize the seed surface and consume plant cells waste products. Under the normal conditions, these organisms do not invade the internal tissues and do not harm the plant [4]. Under the favorable conditions, epiphytes proliferate on the surface of plants and can cause a biological barrier that prevents parasites penetration into the plant tissue. Endophytic bacteria colonize internal tissues, but do not cause disease symptoms and do not have adverse effects on plants [11]. However, the composition of epiphyte and endophyte microbiota can include microorganisms that may be able not only to penetrate into the inner parts of plants and develop there, but also to cause diseases of seeds and plants [4].

Use of pesticides is required nowadays as the basis of plant protection. However, the use of compounds toxic to the certain types of pathogens, can cause the appearance of the resistant forms of pathogens and development of pathogenic organisms of other taxonomic groups. It was shown [3] that on the surface of sugar beet seeds treated with highly toxic insecticides Carbofuran and Gaucho, fungicide Tachygaren and moderately toxic fungicide TMTD, contained even more bacterial cells than on non treated seeds (100 – 160 thousand and 30 – 40 thousand, respectively).

Therefore, the aim of our work was to analyze the composition of biological properties and microbiota composition of sugar beet seeds, intact and treated with pesticides.

Materials and methods. Seeds of sugar beet hybrids Olexandria and Umansky CS-97, selected on the breeding station of the Institute of Bioenergy Crops and Sugar Beet of NAAS of Ukraine were used in the studies.

Hybrid Olexandria – single seed triploid on sterile base, with high yield potential and sugar content. It was entered into the Registry of Plant Varieties of Ukraine in 1997 [7]. The studied seeds of Olexandria hybrid were treated with fungicide Maxim XL 035 FS.

Umansky CS-97 – is single seed diploid MS hybrid oriented on high sugar content yield. It is resistant to premature seeding and tolerant to swift moth and Cercospora spot. It was entered into the Registry of Plant Varieties of Ukraine in 2003 [7]. The intact seeds of Umansky CS-97 hybrid were used.

Germinating energy and germination of studied hybrids were determined according to DSTU [5].

For bacteria isolation the seeds were rinsed for 15 minutes with running and sterile water and grounded in a mortar with 2 ml of saline solution. Then the small amount of received suspension was plated on potato agar (PA) with thick strokes from one edge of the Petri dish to another and cultivated at a 28 °C temperature.

For comparative studies the collection strains of sugar beet bacterial diseases were used: *Pseudomonas wieriingae* (Elliot) Savulesku 1947 strains 7923, 7921; *Pseudomonas syringae* pv. *aptata* (Brown & Jamieson 1913) Yong, Dye & Wilkie 1978 strains 8544, 8545; *Hanthomonas ahonopodis* Starr & Garces 1950 strains 6, 10, 22, 7325, 8715, *Rhizobium vitis* (Smith & Townsend 1907) Conn 1942 strains 9052, 9054, 8628, stored in the collection of cultures of pathogenic bacteria in the Zabolotny Institute of Microbiology and Virology NAS of Ukraine

Cultural, physiological, biochemical and morphological properties of the isolated bacteria were determined using the classical methods [1, 9]. The formation of pigments was determined visually under ultraviolet light for bacteria cultivated on King B. medium. Bacteria were identified with the Bergey identification guide [6].

To determine the presence of pectinase enzymes the ability of isolates to macerate potato pieces was studied. Selected isolates were also tested for their ability to induce hypersensitivity reactions and antagonistic activity against the bacterial diseases strains of sugar beet [1].

Pathogenic characteristics of isolates was determined by artificial infection of sugar beet (hybrids Baccara, Olzhych) and beans (Mavka variety). For this the suspension of bacteria (at concentration of 10^9 cells / ml of sterile tap water) was applied to the surface of plant leaves, followed by their injury with needle [1]. Experiments repetition – 5–7 – fold.

Results and discussion. The observation survey of sugar beet crops in Uladovo-Lyulenetskiy experimental breeding station (Vinnytsia region) conducted in 2012 had showed that during the growing season plants has symptoms of bacterial leaf spot, bacterial gummosis and root canker. To determine the presence of pathogenic bacteria on seeds and influence of seed protectants on microbiota composition the bacteriological analysis of the samples was performed.

Seeds of both hybrids, taken for analysis, was visually healthy and were characterized by the parameters given in Table 1. Seedlings from seed samples were also healthy, without visible signs of damage.

18 different types of bacterial isolates were isolated from seeds samples, 12 of them – from hybrid Umansky CS-97 and 6 – from hybrid Olexandria (Table 2). The larger number of bacteria isolated from the untreated seeds of hybrid Umansky CS-97 indicate the greater diversity of its microbiota.

TABLE 1. Germinating rate and laboratory germination of sugar beet seeds

Hybrid	Germinating energy (day 4), %	Laboratory germination (day 10), %
Umansky CS-97	68	93
Olexandria	70	90

TABLE 2. Results of bacteriological analysis of sugar beet seeds

Samples		Number of bacterial isolates		
Hybrid	Treatment with chemicals	Total	With colonies	
			Crème/ orange color	Grey/white color
Umansky CS-97	Without treatment	12	11	1
Olexandria	Maxim XL 035 FS	6	3	3

In total, the single isolate with grayish white colony and 11 isolates with colonies of yellow color (from cream to orange) from untreated seeds of hybrid Umansky CS-97 were selected. Three isolates of yellow and 3 isolates of grayish-white colored colonies were selected from the seeds of hybrid Olexandria. The color intensity of the grayish- white yellow isolates had depended a lot on the batch of potato agar. All selected isolates were analyzed by their morphological and biochemical properties (Table 3).

All identified isolates were Gram-negative, oxidase negative, and unable to form spores. 61% of them were mobile. 83% of isolated bacteria had bacilli shape of the cells, while 17% – small cocci. 44% of selected bacteria were facultative anaerobes (B-27, B-28, B-29, B-31, B-35, B-46, B-47, B-39). The presence of fluorescent pigment was observed in isolates B-30, B-36 and B-40.

As a carbon source isolates B-27, B-28, B-29, B-30, B-31, B-33, B-47, B-36, B-38, B-39, B-40 in different extent can utilize rhamnose, sucrose, xylose, maltose, arabinose, mannitol and sorbitol. Isolates B-41, B-45 and B -37 were able to utilize only some of the sugars: B-41 and B-45 – arabinose, B-37 – xylose, maltose and arabinose.

Seventeen percent of isolates were identified as the members of the *Pseudomonas* genus (B-30, B-36, B-40) – aerobic mobile bacilli, capable to produce a fluorescent pigment and cannot form spores.

Isolates B-27, B-28, B-35, B-46 and B-39 were identified as *Pantoea agglomerans* species.

Isolates B-41 and B-45 were related to the methylotrophic bacteria. These bacteria constantly inhabit the phyllosphere of agricultural, medicinal, ornamental and wild plants [8].

The remaining eight isolates had represented saprophyte microflora, which cannot be determined in our tests.

TABLE 3. Morphological and cultural-biochemical properties of bacteria isolated from the sugar beet seeds.

Tests	Bacteria, isolated from sugar beet seeds																	
	Umansky CS-97												Olexandria					
	B-27	B-28	B-29	B-30	B-31	B-32	B-33	B-34	B-35	B-41	B-45	B-47	B-36	B-37	B-38	B-39	B-40	B-46
Gram staining	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Mobility	+	+	+	+	–	–	–	–	+	+	+	–	–	+	–	+	+	+
Shape of cell	B	B	C	B	B	B	B	B	B	C	B	B	B	B	C	B	B	B
Spores	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Fluorescent pigment	–	–	–	+	–	–	–	–	–	–	–	–	+	–	–	–	+	–
Oxidase	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Carbon source:																		
Glucose (anaerobic)	K	K	K	–	K	–	–	–	K	–	–	K	–	–	–	K	–	K
Glucose (aerobic)	K	K	K	K	K	K	–	–	K	–	–	K	K	K	–	K	K	K
Rhamnosa	K	K	K	K	K	K	K±	–	n/d	–	–	K	K±	–	K±	K	K	n/d
Sucrose	K	K	K	K	K	K	K	–	n/d	–	–	K	K±	–	K±	K	K±	n/d
Xylose	K	K	K	K	K	K	K	–	n/d	–	–	K	K	K	K	K	K	n/d
Maltose	K	K	K	K	K	K	K	–	n/d	–	–	K	K	K±	K	K	K	n/d
Arabinose	K	K	K	K	K	K	K	–	n/d	K±	K±	K	K	K	K	K	K	n/d
Mannitol	K	K	K	K	K	K	K	–	n/d	–	–	K	K	–	K±	K	K±	n/d
Sorbitol	K	K	K	K	K	K	K	–	n/d	–	–	K	K	–	K±	–	K±	n/d
Colony color	yellow	yellow	gray	yellow	yellow	yellow	yellow	yellow	yellow	pink	pink	yellow	gray	gray	gray	yellow	crème	yellow

Notes: +/- – presence or absence of the sign; K – acid production (change of medium color); K± – less intensive acid production; ± – partial presence of sign;

B – bacilli; C – cocci; n/d – not determined

Virulence and antagonistic properties of the selected isolates are depicted in Table 4.

Anticipating the presence of pectinase bacteria among the selected isolates that cause soft rot, the ability of isolates to macerate potato pieces was studied. It was established that none of isolated isolates were able to cause maceration of potato, thus indicating the absence of rot pathogens of sugar beet among them (Table 4).

TABLE 4. Virulent and antagonistic properties of bacteria isolated from sugar beet seeds

Isolates	Test				
	Hypersensitive reaction	Potato maceration	Antagonism	Artificial infection	
				Sugar beet	Beans
B-27	n/d	—	—	—	—
B-28	n/d	—	—	—	—
B-29	n/d	—	—	—	—
B-30	+	—	—	+	+
B-31	n/d	—	—	—	—
B-32	n/d	—	+	—	—
B-33	n/d	—	—	—	—
B-34	n/d	—	—	—	—
B-35	n/d	—	—	—	—
B-41	n/d	—	—	—	—
B-45	n/d	—	—	—	—
B-47	n/d	—	—	—	—
B-36	—	—	—	—	+
B-37	n/d	—	—	—	—
B-38	n/d	—	—	—	—
B-39	n/d	—	—	—	—
B-40	+	—	+	+	+
B-46	n/d	—	—	—	—

Notes: +/- — presence or absence of the sign; +* — antagonism to *Xanthomonas axonopodis* 6; +** — antagonisms *Pseudomonas syringae* pv. *aptata* 8545; n/d — not determined

The ability of isolates to induce hypersensitivity reactions was examined in bacteria identified as *Pseudomonas* sp. Isolates B-30 and B-40 initiated the formation of brown necrosis on leaves of tobacco. The ability to induce hypersensitivity reactions is associated with virulent bacterial properties. Isolate B-36 was not able to induce the hypersensitivity reactions and obviously represents the saprophytes microbiota.

At artificial infection of sugar beet plants, it was revealed that isolate B-30 causes appearance of the yellow stripes on the leaves and weak deformation of veins in the injection site. Isolate B-40 had caused leaves spotting — aqueous during first 3

– 4 days, and later increasing in size and darken. The remaining isolates had not reveal any pathogenic properties at artificial infection of sugar beet plants. Artificially infected bean plants with isolates B-30, B-40 and B-36 had yellow stripes on the leaves.

Thus, pathogenic for sugar beet bacteria were found on intact seeds of hybrid Umansky CS-97, and on the seeds of Olexandria hybrid treated with fungicide Maxim XL 035. That had confirmed our previous investigations which have shown that Maxim fungicide has no effect on phytopathogenic bacteria – pathogens of sugar beet [2].

The antagonistic activity of bacteria isolated from the seeds to known pathogens of sugar beet was tested. The weak antagonistic activity was determined in isolate B-40, in relation to *Hanthomonas ahonopodis* 6 (halo of growth inhibition – 18 mm), and isolate B-32, in relation to *Pseudomonas syringae* pv. *aptata* 8545 (halo of growth inhibition – 30 mm). The remaining isolates had no antagonistic activity.

It was established that microbiota of untreated seeds of sugar beet is more diverse than on seeds, treated with fungicide. However, sugar beet pathogenic bacteria (isolates B-30 and B-40) were isolated from the both samples. This indicates that the fungicidal disinfectant Maxim XL 035 FS used for pre-sowing seeds treatment was unable to protect sugar beet seeds from bacterial pathogens.