UDC 632.3:579.84

Bacterial biome of sugar beet with black root of sugar beet

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The purpose. To isolate and identify bacteria in structure of biome of plants of sugar beet, contaminated with black root of sugar beet. **Methods.** Field — for sampling plants with disease symptoms; laboratory (microbiological and phytopathological) — for allocation of bacteria in pure culture, determination of their disease-inciting power; biochemical — for determination of properties of the isolated bacteria. **Results.** Bacteriological analysis of samples of plants of sugar beet with typical symptoms of black root of sugar beet is carried out. **Conclusions.** Except for the basic causal organisms of black root of sugar beet, in structure of biome of plants of sugar beet, contaminated with black root of sugar beet they fixed presence of bacteria of stem Bacillus and pectolytic-active bacteria of Pectobacterium carotovorum which are capable to initiate putrefaction or reinforce pathological process caused by other microorganisms.

Key words: *sugar beet, black root of sugar beet, contamination, biome, bacteria, properties, identification.*

https://doi.org/10.31073/agrovisnyk201805-04

Introduction. Sugar beet due to its national economic significance occupies the leading position among technical crops in Ukraine. The classical technology of their cultivation involves agrotechnical, biological and chemical methods for limiting the disease development, as well as weeds and pests distribution [7]. However, unsatisfactory level of plants protection from harmful organisms, insufficient fertilization and other irregularities of cultivation technology are the main factors of root crops yield reducing [9].

Widespread disease of sugar beet in all sugar beet sowing areas is black leg [5, 6, 8, 9]. In recent years, significant development of black leg disease has been observed in Vinnytsya (30,5%), Chernihiv (16%), Cherkasy (15,6%), Lviv (15,0%), Kyiv and Sumy (14%) regions [4].

The first signs of affection appear on the seedlings before the germination on the soil surface. The disease develops in the phase of the beginning of plant growth («fork-shape»), the first and the second pairs of these leaves. At the beginning of the pathological process on the mesocotyl or rootlet appears a transparent and then a brownish stain or strip. Later it is browning and subsequently blackening, afterward is spreading onto the underground part of the seedling. Affected areas begin to rot and interceptions are formed very often [5]. The symptoms and disease progression vary depending on the phase of plant development, types of pathogens, the agro-climatic zone of cultivation and weather conditions [8, 10].

The main factor of black leg disease is the adverse environmental conditions that decrease the plant's resistance to microorganisms and affection of weakened seedlings occurs. Many types of soil micromycetes could cause a pathological process in the initial stages of sugar beet plants growth, however, the most often plants are affected by representatives of the following genera *Aphanomyces, Phytium, Fusarium, Phoma, Rhyzoctonia* [6, 8].

Saprophytic and pathogenic bacteria can populate into weakened and damaged tissues of seedlings, initiate or intensify the infectious process. For the selection of effective means of protecting agricultural crops from disease, it is important to consider the specific composition of pathogens that cause damage to plants with various symptoms and consequences for plants.

The purpose of research – Isolation and identification bacteria in the biome of sugar beet plants affected by black leg disease.

Materials and methods. The research materials were sugar beet sprouts with marked symptoms of black leg disease, selected on experimental plots in the Uladovo-Lyulinetska experimental breeding station. They characterized by blackening of the underground part of the seedling with interceptions of dark tissues, located at the bottom of mesocotyl.

For the isolation of bacteria from the washed plants pieces which cover a part of the affected and healthy tissue were cut out, then pounded in a mortar with 0,5 ml water and used for bacteria growing on the surface of potato agar (PA). Bacteria were grown at the temperature of 28°C during 5 days.

Morphological, cultural and biochemical characteristics of allocated isolates were determined using classical methods [3, 12]. The pigmentation was determined visually under ultraviolet light for bacteria cultivation on King B medium and meat-peptone agar. The ability to consume certain carbohydrates as a single source of carbon nutrition investigated on the medium of Omelyanskyi sample, which contained a bromothymol blue indicator. As a source of carbon (0,5%) used: fructose, lactose, arabinose, sucrose, raffinose, galactose, rhamnose, maltose, dulcitol, inositol, sorbitol, and inulin. The type of glucose assimilation (O-F test) was determined by sowing on the Omelyanskyi medium with glucose and an indicator in two test tubes. After sowing, one of the tubes was filled with vaseline oil in a layer of 1 cm. The presence of bacterial growth was determined by changing the color of the medium.

The ability of bacterial isolates to macerate pieces of potatoes, beets and carrot were studied in order to determine the pectinolytic enzyme presence. The ability to induce the hypersensitivity reaction was determined by introducing a suspension of bacterial cells under the epidermis of tobacco leaves by the Klement's method. Suspension of bacteria (in concentration of $1x10^7$ CFU/ml of sterile tap water) was used [12]. Identification was carried out in accordance with Determinant of Burge [11].

Results and analysis. In the result of bacteriological analysis of investigated samples of sugar beet sprouts with typical symptoms of black leg disease, different morphological types of bacterial isolates were isolated: 65% of which had colonies of gray color, 15% – white and 20% – light-yellow color. The growth of isolated bacteria in potato agar colonies differed among themselves according to the level of transparency, they had a smooth or wrinkled surface and equal or palmate edges.

The control check of pathogenic properties carried out through artificial contamination of sugar beet plants of hybrids of domestic and foreign breeding (Ukrainian ChS-72 and Crocodile). It is found that none of the isolates of bacteria isolated from black leg damaged plants of sugar beet for artificial contamination of plants of both hybrids does not cause the visible symptoms of affection. In addition, no one isolate caused an over-sensitivity reaction in tobacco leaves.

For the determination of pectolytic activity of bacteria isolates, selected from plants damaged by black leg, the presence of isolates that cause maceration of potatoes pieces was determined. In order to study the ability to macerate tissues of other cultures, the bacterial mass was supported on the pieces of table beet and carrots. After 24-hours stay in Petri dishes in a thermostat at a temperature of 27 °C, the ability of 5 isolates of bacteria to soften the tissues of table beet and carrot pieces was confirmed. The softening of the pieces was intense and the formation of exudates on their surface observed (Fig. 1).

Fig. 1 Tissue maceration of potato pieces, table beet and carrot caused by isolates of bacteria, selected from sugar beet plants affected by black leg disease

From the literature it is well known that harmfulness of black leg affection leads to the death of affected seedlings, which is often caused because of resowing [8]. However, most phytopathologists consider the main reason of black leg disease is micromycetes affection. At the same time, the affection by pectolytic bacteria can also cause the loss of the sowings. The precise definition of the etiology of the black leg pathogen is a key for the successful implementation of measures to limit this disease.

All isolates which characterized by pectolytic activity were selected for the further identification.

On the surface of the KA medium, three pectolytically active isolates (K-3, K-9 and K-16) formed smooth, shiny with equal edges, semitranslucent gray colonies. Isolates K-3, K-9 and K-16 are gram-negative, oxidase-negative, mobile sticks with fermentative type of glucose adsorption and well-defined pectolytic activity that is why they were previously identified as representatives of the *Pectobacterium* genus.

For more definite identification of isolated bacteria the spectrum of carbohydrates fermentation and a number of other properties determined and obtained results compared with research results of other investigations and materials of the determinant (Table 1).

Table 1. Characteristics of pectolytically active isolates of bacteria selected from sugar beet plants that are affected by black leg disease

Factors	Isolates from sugar beet	*Pectobacterium carotovorum* [11]	*Pectobacterium carotovorum* subsp. *carotovorum* [2]
Gram staining	–	–	–
Motility	+	+	+
Cell shape	R	R	R
Sporulation	–	–	–
Fluorescent pigment	–	–	–
Oxidase	–	–	–
Pectinolitic activity	+	+	+
Reaction of hypersensitivity	–	–	u/r
Use of carbon sources: Glucose (anaerobic)	A	A	A
Glucose (aerobic)	A	A	A
Fructose, Lactose, Sucrose,	A	A	A

Raffinose			
Galactose, Rhamnose	A	A	A
Maltose	A	X	–
Dulcitol	A±	–	–
Mannitol, Inositol	A	A	A
Sorbitol	A	X	–
Arabinose	A	–	A
Inulin	A±	X	u/r
Reduction of nitrates	+	+	+
Indole production	–	–	–
H_2S production	–	–	–

Note: «+» - presence of property, «–» - absence of property, «A» – formation of acid (change in the color of the medium); «X» – strains variability, «R» - rods, u/r – the result is undefined.

Thus, according to the following features as Gram stain, mobility, cell shape, absence of spore formation, ability to cause rotting of plant tissues, absence of indole and hydrogen sulfide formation, nitrate reduction ability and use of glucose, fructose, lactose, maltose, raffinose, galaxies, dulcitol, mannitol, sorbitol, raffinose and inositol, selected from the sugar beet plants affected by black leg disease, bacteria isolates of K-3, K-9 and K-16 are identical to the genus *Pectobacterium carotovorum* Thompson et al. 1984.

The presence of pectolytic activity also characterized the isolates K-14 and K-19, which formed on KA medium wrinkled, non-transparent colonies of light cream color with a diameter of 2-3 mm. By their biological properties, they are gram-positive, oxidaze-negative mobile spore-forming sticks that don't reduce nitrates, don't form hydrogen sulfide and indole and absorb arabinose. On the basis of a comprehensive assessment, K-14 and K-19 isolates are related to the *Bacillus* genus.

It is known that sugar beet plants affection by black leg could cause the development of a number of rottenness both during vegetation period and during storage of sugar beet [10]. Stimulators of mouldering may be isolated bacteria with strongly pronounced pectolytic activity, which caused the maceration of beet, potatoes and carrots tissues.

It is known that the consequences of disease are manifested in various deformations of root crops and the reduction of their mass [10]. Thus, in case of a weak development of the disease (25%), the mass of root crops is reduced by almost 20%, and with strong (75%) – by 43% and more, simultaneously decreases the sugar output from 26 to 45% [8]. This indicates the need to protect sugar beet plants from pathogens that cause lesions including black leg disease, also the growth of isolated and identified bacteria *P. carotovorum* should be limit. In the scientific literature there is an evidence that pathogenic *P. carotovorum* 8982 bacteria under laboratory testing conditions are not susceptible to the recommended by the manufacturer conditions and ten times increased doses of such preparations as (active substances): 25 g/l fludioxonil, 100 g/l penconazole, 250 g/l dipheconazole, 700 g/kg thiophanate methyl, 640 g/kg mancozeb + 40 g/kg meylexyl and 500 g/kg benomyl [1]. The issues of the indicated species sensitivity of bacteria to chemical plant protection products should be studied as well. Such chemical plant protection products are used for the purification of sugar beet seeds in order to protect plants at initial growth periods and development from a set of harmful organisms in the soil and which are capable to cause seedlings damage by plant diseases.

Conclusions

As a result of conducted research of biome composition of sugar beet plants affected by black leg disease, based on the determination of morphological, physiological and biochemical properties, identified the bacteria of the genus *Bacillus* and pectolyticaly active bacteria *Pectobacterium*

carotovorum that are capable to cause rotting of plants or settle into affected tissue by other pathogens

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