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The Species Composition of Microorganisms of the *Secale cereal* L., 1753 †

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Abstract. Rye (*Secale cereal* L., 1753) of the «Marusenka» variety is one of the most stable and high-yielding zoned objects. A comprehensive study of the species composition of microorganisms was conducted using standard microbiological techniques. 120 samples of stems, leaves and soil collected during such phenological phases of plant development as tillering, stalking, grain filling and ripeness were examined. We isolated 23 strains of bacteria that were assigned to 6 genera (*Bacillus*, *Erwinia*, *Staphylococcus*, *Kurthia*, *Microbacterium* and *Psychrobacillus*) and 15 species, of which 2 are gram-negative rods, 4 are gram-positive rods, 16 are gram-positive spore rods and 1 is gram-positive cocci. Based on the data obtained, it can be concluded that in various phenological phases, the greatest contamination of the rye plants of the «Marusenka» variety is observed by such bacterial species as: *Bacillus halodurans*, *Erwinia carotovora* and *Psychrobacillus psychrodurans* (with an occurrence index from 70 to 90%). 9 strains of fungi were also isolated, which were assigned to 4 genera (*Alternaria*, *Fusarium*, *Ramichloridium*, *Rhizopus*) and 4 species. At various phenological phases, only one species of Alternaria alternata prevails with an occurrence index from 70 to 100%.

Keywords: associate microorganisms; rye

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1. Introduction

The Rye is the most important grain crop from which bread is baked. Rye grain contains up to 18% protein, a large number of vitamins (mainly B group), is used for the production of cereals, pasta. Straw has a great nutritional value, it is used as a building material, for making paper, bedding for animals, etc. Infection of viral, fungal and bacterial pathogens is one of the most important causes of loss of crop yields. Therefore, the importance of constant monitoring of the phytocenoses of the culture is increasing, which formed the basis of this work, the purpose of which was to study the associative microorganisms of rye plants (*Secale cereale* L., 1753) of the «Marusenka» variety, collected in the fields of the Federal State Budget Scientific Institution «FANC South-East» (Saratov, Russia) [1,2].

2. Methods

The work was carried out on the basis of the Chair of Microbiology and Plant Physiology of SSU named after N. G. Chernyshevsky. The study of the main microbiological indicators (species composition, population size, occurrence index) of strains isolated from the surface, internal environment and rhizosphere of plants was carried out. The morphological and cultural characteristics of the isolates, and their biochemical properties, such as the ability to use various sugars (glucose, sucrose, arabinose, xylose, lactose,

maltose, sorbitol, mannitol), the ability to hydrolyze casein, gelatin, starch, and ammonia production were studied, hydrogen sulfide and acetoin, nitrate reduction and nitrogen fixation. The enzymatic activity of isolated microorganisms was studied, in particular, lipolytic activity (medium with Tween-80 and various oils—sesame, olive and sunflower), cellulolytic and pectolytic activity. And also, the presence of catalase and oxidase activity in the strains was determined. When studying resistance to abiotic factors, the ability of strains to grow at various temperatures (+10 and +43 °C), pH (5, 9, 11) and NaCl concentrations in the medium (2, 5, 7, 10, 15%) [3–7].

3. Results

During the study, 27 strains of microorganisms were isolated from 120 samples of stems, leaves and soil, which were assigned to 10 genera (*Bacillus*, *Erwinia*, *Fusarium*, *Kurthia*, *Microbacterium*, *Psychrobacillus*, *Staphylococcus*, *Alternaria*, *Ramichloridium*, *Rhizopus*).

Indices of occurrence (IO) and quantitative indicators of microorganisms (CFU/cm²) isolated from the surface of plants are presented in Table 1.

Table 1. Indices of occurrence (IO) and quantitative indicators of increase (lg CFU/cm²) isolated from the surface of plants.

Types of Bacteria	Tillering		Developing Stem		Grain Swelling		Ripeness	
	lg CFU/cm²	IO	lg CFU/cm²	IO	lg CFU/cm²	IO	lg CFU/cm²	IO
Bacillus atrophaeus	0.1–1	90	–	–	–	–	–	–
Bacillus halodurans	0.2–5	90	–	–	–	–	–	–
Bacillus megaterium	0.1–1	80	–	–	–	–	–	–
Erwinia carotovora	–	–	–	–	0.5–1	60	–	–
Erwinia uredovora	–	–	–	–	–	–	0.2	100
Kurthia zopfii	0–1	70	–	–	–	–	–	–
Staphylococcus xylosus	–	–	0.2–1	40	–	–	–	–
Alternaria altenata	–	–	0.1–1	70	0.1–1	100	0.1	100
Fusarium caeruleum	–	–	–	–	–	–	0.2	100
Ramichloridium mackenziei	–	–	0.5–1	70	–	–	–	–

It has been established that in the phenological phase «tillering» the highest contamination of the surface of rye plants of the «Marusenka» variety with such bacterial species as *Bacillus atrophaeus*, *Bacillus halodurans*, *Bacillus megaterium* and *Kurthia zopfii* (with an occurrence index of 70 to 90%) is observed. In the phenological phase «tillering» on the surface of plants of winter rye variety «Marusenka» there are such species as: *Bacillus atrophaeus* (0.1–1 CFU/cm²), *Bacillus halodurans* (0.2–5 CFU/cm²), *Bacillus megaterium* (0.1–1 CFU/cm²), *Kurthia zopfii* (0.1–1 CFU/cm²). At the phenological stage «developing stem» a number of species are observed: *Alternaria alternata* (0.1–1 CFU/cm²), *Ramichloridium mackenziei* (0.5–1 CFU/cm²), *Staphylococcus xylosus* (0.2–1 CFU/cm²). In the phenophase «grain swelling» microbial species were found: *Alternaria alternata* (0.1–1 CFU/cm²) and *Erwinia carotovora* (0.5–1 CFU/cm²). At the stage of «ripeness» of plants, the following species were found: *Alternaria alternata* (0.1 CFU/cm²), *Erwinia uredovora* (0.2 CFU/cm²) and *Fusarium caeruleum* (0.2 CFU/cm²) with an occurrence index of 100%. One species isolated by us (*Alternaria alternata*) was found at several stages of plant development studied. When studying the internal environment of rye plants of the «Marusenka» variety (Table 2), 5

species of bacteria were isolated, assigned to 3 genera (*Bacillus*, *Erwinia*, *Psychrobacillus*) and 2 species of fungi, assigned to 2 genera (*Alternaria* and *Ramichloridium*).

Table 2. Indices of occurrence (IO) and quantitative indicators of microorganisms (lg CFU/g) isolated from the internal environment of plants.

Types of Bacteria	Tillering		Developing Stem		Grain Swelling		Ripeness	
	lg CFU/g	IO	lg CFU/g	IO	lg CFU/g	IO	lg CFU/g	IO
Bacillus badius	0.2–5	90	–	–	–	–	–	–
Bacillus halodurans	–	–	0.7	70	–	–	2.5	80
Erwinia carotovora	–	–	–	–	3.3	80	–	–
Erwinia tracheiphila	–	–	–	–	–	–	2.7	80
Psychrobacillus psychrodurans	–	–	–	–	3.3	80	–	–
Alternaria alternata	–	–	2.0	20	–	–	–	–
Ramichloridium mackenziei	–	–	3.5	30	–	–	–	–

From the internal environment of plants at the «tillering» stage, one species was isolated *Bacillus badius* (0.2–5 CFU/g) with an occurrence index of 90%. *Alternaria alternata*, *Bacillus halodurans* and *Ramichloridium mackenziei* were isolated at the stalking stage. *Bacillus halodurans* predominates (0.7 CFU/g, IO 70%).

At the grain filling stage, *Erwinia carotovora* (3.3 CFU/g, occurrence index 80%) and *Psychrobacillus psychrodurans* (3,3 CFU/g, occurrence index 80%) were found.

At the stage of ripeness of plants in the internal environment of rye, the species *Bacillus halodurans* (2.5 CFU/g, occurrence index 80%) and *Erwinia tracheiphila* (2.7 CFU/g, occurrence index 80%) were found. The species *Bacillus halodurans* was isolated twice (developing stem and ripeness stages).

The rest of the species isolated from the endosphere of the rye variety «Marusenka» did not repeat during the four stages studied. In the study of the root soil, 9 species of bacteria were identified, assigned to 4 genera (*Bacillus*, *Erwinia*, *Microbacterium* and *Psychrobacillus*), where the *Bacillus* genus is represented by the largest number of species; and 1 fungal species, *Rhizopus* genus (Table 3).

Table 3. Indices of occurrence (IO) and quantitative indicators of microorganisms (CFU/g) isolated from the rhizosphere of plants.

Types of Bacteria	Tillering		Developing Stem		Grain Swelling		Ripeness	
	lg CFU/g	IO	lg CFU/g	IO	lg CFU/g	IO	lg CFU/g	IO
Bacillus algicola	11	10	–	–	–	–	–	–
Bacillus benzoevorans	11	60	–	–	–	–	–	–
Bacillus funiculus	–	–	8.0	70	–	–	–	–
Bacillus halodurans	–	–	8.7	80	–	–	–	–
Bacillus nealsonii	9.6	10	–	–	–	–	–	–
Erwinia carotovora	–	–	–	–	8.7	50	–	–
Erwinia tracheiphila	–	–	–	–	–	–	7.5	60
Microbacterium lacticum	8.0	40	–	–	–	–	–	–
Psychrobacillus psychrodurans	–	–	8.1	80	7.9	70	–	–

Rhizopus stolonifer	6.0	90	–	–	–	–	6.0	80

The highest contamination was observed during the tillering period, and is represented, for the most part, by microorganisms of the genus *Bacillus*, as well as representatives of the genera *Microbacterium* and *Psychrobacillus*. At the stage of stalking, microorganism species were isolated: *Bacillus funiculus*, *Bacillus halodurans*, *Psychrobacillus psychrodurans*. At the grain filling stage, *Erwinia carotovora* species were found in the amount of 8.7 CFU/g and with an occurrence index of 50% and *Psychrobacillus psychrodurans* in the amount of 7.9 CFU/g and with an occurrence index of 70%. In the phenophase of «ripeness», representatives of the genus *Erwinia* and *Rhizopus* were isolated from the rhizosphere of rye plants of the «Marusen'ka» variety. The predominant species is *Rhizopus stolonifer* (6.0 CFU/g with an occurrence index of 80%).

4. Discussion

The study of biological properties showed that the isolated strains have catalase activity to a greater extent (83%), many (72%) are capable of reducing nitrates and (44%) are capable of utilizing citrate. The isolated strains form gaseous products from peptone weakly, only 11% are capable of releasing hydrogen sulfide—H_2S, ammonia (NH_3) was not detected during the experiment. An insignificant part of the isolates hydrolyzes gelatin (28%) and starch (44%), have proteolytic properties. Species of microorganisms *Bacillus funiculus*, *Bacillus badius*, *Bacillus halodurans* have good lipolytic activity. They decompose media with the addition of polysorbate (tween-80), sesame, olive and sunflower oils, and also have a pronounced amylolytic and pectolytic ability. *Erwinia tracheiphila* has a high amylolytic activity. *Bacillus funiculus* and *Bacillus badius* also used media supplemented with starch and pectin.

The ability of microorganisms to form acid from various carbohydrates was revealed. The saccharolytic properties of the isolated strains are weakly expressed. The obtained microorganisms are able to use xylose (61%), a small part of the isolated cultures is able to use lactose and sorbitol. Basically, the resulting bacteria can make good use of mannitol, sucrose and glucose as a source of nutrition and energy.

Studying the resistance of strains to various environmental conditions, they were cultivated at critical temperatures (10 °C and 43 °C), various pH of the medium (5, 9, 10), salt concentrations (NaCl) in the medium from 2 to 15%, and as well as growth under anaerobic conditions. The microorganisms isolated during the study are represented by obligate aerobes (72%) and facultative anaerobes (28%). Isolates, for the most part, do not withstand low temperatures, only 17% are able to give colonies at 10 °C, when the cultivation temperature rises to 43 °C, they become unviable. A pH range of 5 to 10 is acceptable for growing cultures on dense nutrient media. A low concentration of NaCl in the nutrient medium (2–7%) is optimal for cultivating the isolated strains. With an increase in concentration to 15%, growth inhibition is observed (no dominant halophilic bacteria were found). When establishing community indices, between different comparison groups (according to the stages of plant development–tillering, developing stem, grain swelling, ripeness) of the species composition of bacteria isolated from the winter rye variety «Marusenka», the simultaneous presence of associated microorganisms was noted in the selected categories. On the surface of cereals, in the comparison group «Developing stem-Grain swelling» of grain, the index of generality was 10% of the species, in the comparison group «Developing stem-Ripness»—10%. In the internal environment of plants, the community index, in the comparison group «Developing stem-Ripness», is 14%. Bacteria isolated from the rhizosphere of cereals have a community index of 10% in the comparison group «Developing stem-Grain swelling». Isolated species were not repeated in other comparison groups. Phytopathogenic microorganisms of the species *Alternaria alternata*, *Fusarium caeruleum* and *Rhizopus stolonifer* were isolated from shoots, rhizosphere and internal environment [8].

Institutional Review Board Statement:

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Data Availability Statement:

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