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The Species Composition of Microorganisms of the *Triticum aestivum* L., 1753 †

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Abstract: Wheat (*Triticum aestivum* L., 1753) of the «Kalach-60» 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 18 strains of microorganisms that were assigned to 6 genera of fungi and bacterium (*Aphanocladium*, *Alternaria*, *Fusarium*, *Bacillus*, *Listeria*, *Staphyloccocus*,) and 15 species, of which 1 are gram-positive rods, 9 are gram-positive spore rods, 1 is gram-positive cocci and 3 species of fungi. Based on the data obtained, it can be concluded that in various phenological phases, the greatest contamination of the wheat plants of the « Kalach-60» variety is observed by such bacterial species as: *Bacillus drentensis*, *B. horikoshii*, *B. psychrodurans*, *B. halodurans*, *B. okuhidensis*, *B. oleronius* (with an occurrence index from 70 to 100%). Fungi *Aphanocladium album*, *Alternaria altenata*, *Fusarium oxysporum* prevails at various phenological phases with an occurrence index from 70 to 100%.

Keywords: associate microorganisms; wheat

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1. Introduction

Wheat is one of the most important cereal crops, which plays a decisive role in our lives and occupies a special position among other cultivated cereals in Russia [1]. The winter form of this plant is especially significant, since it can exceed the spring form by 2–3 times in yield. Unfortunately, the defeat of viral, fungal and bacterial pathogens is one of the most important reasons for the loss of crop yields. Therefore, the importance of constant monitoring of crop phytocenoses is increasing, which served as the basis for this study, the purpose of which was to study the associative microorganisms of wheat plants (*Triticum aestivum* L., 1753) of the «Kalach 60» variety, collected in the fields of the Federal State Budget Scientific Institution «FANC South-East» (Saratov, Russia) [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. We have studied the main microbiological indicators (species composition, population size, occurrence index) of strains isolated from the surface, internal environment and rhizosphere of plants [3–5].

During the study, analyzed the morphological and cultural characteristics of microorganisms, 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, produce ammonia, hydrogen sulfide and acetoin, reduce nitrates and fix nitrogen. The enzymatic activity of the isolates was studied, in particular, catalase, oxidase, lipolytic, cellulolytic and pectolytic activities. Different degrees of resistance of strains to abiotic environmental factors were revealed, such as the ability to grow at high and low temperatures (+10 and +43 °C), pH (5, 9, 11) and NaCl concentrations in the medium (2%, 5%, 7%, 10% and 15%) [6–8].

3. Results

During the study, 18 strains of microorganisms were isolated from 120 samples of stems, leaves and soil, which were assigned to 6 genera (*Bacillus*, *Listeria*, *Staphyloccocus*, *Aphanocladium*, *Alternaria*, *Fusarium*).

It can be seen in the Table 1, that during the tillering period, on the surface of plants of the «Kalach 60» wheat variety, a microorganism of the species *Bacillus psychrodurans* with IO = 100% was detected, in an amount of 0–1 lg CFU/cm². Species *Bacillus drentensis* with IO = 70% and −1–0 lg CFU/cm², *Aphanocladium album* with IO = 100%, in the amount of −0.3–0 lg CFU/cm² are present at the stalking stage. According to the results of the study, microorganisms of the species *Bacillus horikoshii* with a 90% IO content were identified, in an amount of 0–1.7 lg CFU/cm².

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
Alternaria altenata	–	–	–	–	–	–	0–1.7	100
Aphanocladium album	–	–	−0.3–0	100	–	–	–	–
Bacillus drentensis	–	–	−1–0	70	–	–	0–1	100
Bacillus horikoshii	–	–	–	–	0–1.7	90	–	–
Bacillus psychrodurans	0–1	100	–	–	–	–	–	–
Fusarium oxysporum	–	–	–	–	–	–	0–1	100
Listeria innocua	–	–	–	–	–	–	0–1	100

In the phase of milky ripeness, microorganisms of the species *Bacillus drentensis* were isolated with a IO of 100%, in an amount of 0–1 lg CFU/cm², *Listeria innocua* with a IO of 100%, in an amount of 0–1 lg CFU/cm², *Alternaria altenata* with a IO of 100%, in the amount of 0–1.7 lg CFU/cm² and *Fusarium oxysporum* with 100% IO, in the amount of 0–1 lg CFU/cm².

According to the results shown in Table 2, in the internal environment of the plant during the tillering period, there are microorganisms of the *species Bacillus halodurans* in the amount of 4–4.7 lg CFU/g, with a IO = 100% and *Bacillus okuhidensis* with a IO of 100%, in an amount of 4–4.7 lg CFU/g.

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
Alternaria alternata	–	–	–	–	0–1	90	–	–
Aphanocladium album	–	–	2.7–3	70	–	–	–	–
Bacillus halodurans	4–4.7	100	–	–	–	–	–	–
Bacillus okuhidensis	4–4.7	100	–	–	–	–	–	–
Bacillus oleronius	–	–	3–4.7	100	–	–	–	–
Fusarium oxysporum	–	–	–	–	3–4.7	100	–	–

In the stalking phase, there are other types of microorganisms from the genus *Bacillus oleronius* whose occurrence index = 100%, in the amount of 3–4.7 lg CFU/g and *Archanocladium album* with 100% IO, in the amount of 2.7–3 lg CFU/g.

At the stage of grain filling, microorganisms of the species *Alternaria alternata* were found in the amount of 0–1 lg CFU/g, the occurrence index = 90%, and *Fusarium oxysporum* with a IO of 100%, in the amount of 3–4.7 lg CFU/g.

According to the data given in Table 3, it can be seen that the highest seeding of winter wheat plants of the «Kalach 60» variety was during the period of tillering, stalking and grain filling, and was represented by microorganisms of the species *Bacillus benzoevorans*, with an occurrence index = 50%, in the amount of 10–11 lg CFU/g, *Bacillus horikoshii* with 50% IO, in the amount of 8–9 lg CFU/g, *Bacillus oleronius* with 70% IO, in the amount of 8–9.7 lg CFU/g and *Staphyloccocus xylosus* with 90% IO, in the amount of 8–9 lg CFU/g.

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 benzoevorans	10–11	50	–	–	–	–	–	–
Bacillus horikoshii	8–9	50	–	–	–	–	–	–
Bacillus oleronius	–	–	8–9.7	70	–	–	–	–
Staphyloccocus xylosus	–	–	–	–	8–9	90	–	–

4. Discussion

Were used standard tests to identify one or another activity of the strains [3]. Isolates, to a greater extent, have the ability to reduce nitrates, almost no one is able to form ammonia and hydrogen sulfide from peptone.

The ability of microorganisms to form acid from various carbohydrates was revealed. It was found that most strains use glucose (78%), sucrose (64%) and arabinose (64%).

Species of microorganisms *Bacillus drentensis*, *Bacillus oleronius*, *Listeria innocua* have pronounced lipolytic properties. They actively gave colonies and clearing around the growth zone on media with the addition of polysorbate (tween-80), as well as with the addition of sesame, olive and sunflower oils in the medium. Species *Staphyloccocus xylosus* actively exhibited lipolytic properties in the medium with the addition of polysorbate and insignificantly in the medium with the addition of sunflower oil; in other cases, no active growth was observed on such media. The *Bacillus drentensis* species showed marked enzymatic activity: it actively grew and used media supplemented with pectin, starch, and cellulose.

In terms of resistance to various environmental conditions, the isolated strains, for the most part, cannot withstand high and low temperatures; at pH 9 in the medium and 10, maximum growth is visible. With an increase in the concentration of NaCl in the medium from 2% to 15%, growth inhibition is observed.

When calculating the community indices, between different groups of comparison (by stages of plant development—tillering, developing stem, grain swelling, ripeness) of the species composition of bacteria isolated from winter wheat variety «Kalach-60», the simultaneous presence of associated microorganisms was identified in the selected categories. Species of bacteria of the exosphere of cereals, in the comparison group «Developing stem-Ripness» have a commonality index of 10%. Isolated species in other comparison groups were not repeated, as well as throughout all the phenological stages we studied—not a single species that we isolated was found twice.

During the study, we have isolated phytopathogenic microorganisms of the species *Alternaria alternata*, *Fusarium oxysporum* and *Aphanocladium album*, the identification of strains 1, 2 and 3 continues.

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