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DETECTION OF BACTERIOSIS PATHOGENS SIGNIFICANT FOR GRAIN EXPORT AND A COMPLEX OF ASSOCIATED MICROORGANISMS IN GRAIN CROPS (ON THE EXAMPLE OF TIMIRYAZEVSKAYA FIELD EXPERIMENTAL STATION

O.Yu. SLOVAREVA[1] ✉, M. MUVINGI[2], A.B. IAREMKO[1], V.N. IGONIN[3], V.S. RUBETS[3]

[1]*All-Russian Plant Quarantine Center,* 32, ul. Pogranichnaya, Bykovo, Ramenskiy District, Moscow Province, 140150 Russia, e-mail slovareva.olga@gmail.com (✉ corresponding author), an_ya94@mail.ru;
[2]*Peoples' Friendship University of Russia,* 6, ul. Miklukho-Maklaya, Moscow, 117198 Russia, e-mail mufaromuvingi@gmail.com;
[3]*Russian State Agrarian University — Timiryazev Moscow Agricultural Academy*, 49, ul. Timiryazevskaya, Moscow, 127550 Russia, e-mail: valentina.rubets50@gmail.com, selection@rgau-msha.com
ORCID:
Slovareva O.Yu. orcid.org/0000-0001-6022-5955
Muvingi M. orcid.org/0000-0001-7700-1296
Iaremko A.B. orcid.org/0000-0003-3295-8080
Igonin V.N. orcid.org/0000-0001-8218-4285
Rubets V.S. orcid.org/0000-0003-1233-8837
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Abstract

According to official statistics, about 130 million tons of cereals are produced annually in Russia. In the Unified List of quarantine objects of the Eurasian Economic Union is the causative agent of wheat yellow mucous bacteriosis *Rathayibacter tritici*. This species is subject to detection during import and, if the importer requires, during export of wheat. Due to the need for regulation, there is a diagnostic method for *Rathayibacter tritici* in quarantine phytosanitary laboratories. For other pathogens of bacteriosis in grain crops, such as *Rathayibacter rathayi*, *Pseudomonas fuscovaginae*, *Pseudomonas cichorii*, *Pseudomonas fluorescens*, *Pseudomonas syringae, Acidovorax avenae,* E*rwinia rhapontici*, *Xanthomonas translucens*, *Clavibacter tessellarius*, etc., there are no diagnostic methods, due to which no detections have been recorded in the practice of diagnostic phytosanitary laboratories. The listed types are regulated by importing countries that purchase more than half of all grain products intended for export in Russia. Bacterioses pose a serious threat to grain production, and the possible damage they cause to the crop is estimated at 10-40 %. The bacteria can cause disease outbreaks or be latent in plants depending on environmental conditions and almost never cause symptoms on grain. In this regard, it is possible to detect causative agents of bacteriosis only in the laboratory using the method of inoculation on nutrient media, which often takes a week or more. Reliable identification of each type of bacteria is possible only with the use of molecular methods. It is required to develop PCR tests that allow the identification of target bacteria directly in samples without using the cultural method, which will significantly simplify and speed up the procedure for confirming the compliance of the state of Russian grain batches with the requirements of importers. The development of molecular methods for diagnosing causative agents of bacterioses in grain crops is possible only after studying their species composition in plants and grain, while the diversity of living bacteria in vegetative plants is significantly higher than in grain. Information on the species composition of bacteria on grain crops will make it possible, using genomic analysis, to detect species-specific PCR targets and develop diagnostic PCR tests for the rapid identification of bacterial species that are especially dangerous and important for grain export. Previously, a large-scale study of the bacterial composition in grain crops was not carried out, and therefore, there is no list of bacteria that can be found together in one sample. There is also no complete list of all bacteria that can be found in cereals. At the same time, for bioinformatic prediction of a species-specific PCR target, it is necessary to know all the species that can be found in the analyzed sample, from which the target species should be distinguished. The composition of the bacterial microbiota may differ depending on the crop and variety, so the maximum diversity of different crops and varieties will provide more complete information. Humid and moderately warm summer conditions in the Central region are ideal for the development of bacteriosis. In connection with the foregoing, sampling was carried out on the territory of the Timiryazevskaya field experimental station (Moscow), where hybridization, selection and variety testing of several hundred varieties of

grain crops are carried out annually. The work is devoted to the detection and identification of bacteria in samples of grain crops of the Timiryazevskaya field experimental station (Moscow). The objects of the study were bacterial isolates from grain samples in 2020. Bacteria were identified by sequencing the amplicons obtained by PCR with primer pairs PSF/PSR, SyD1/SyD2, and 8UA/519B and comparing the resulting sequences using the BLAST service with sequences posted in GenBank (https://blast.ncbi.nlm. nih.gov). As a result, 55 samples of grain crops were collected, 171 bacterial isolates were isolated and identified, including 34 isolates identified to species. Bacterial diversity is represented by 14 species. Among them, there are phytopathogens *Pantoea ananatis*, *Clavibacter michiganensis*, *Rhodococcus fascians*, *Pseudomonas trivialis*, *Pseudomonas viridiflava* and *Pseudomonas syringae*. The highest frequency of occurrence, 70.9 %, was noted in species belonging to the genus *Pseudomonas*. Representatives of the genera *Frigoribacterium* (36.4 %), *Clavibacter* (16.4 %), *Arthrobacter* (12.7 %) and *Rhodococcus* (10.9%) also have a high frequency of occurrence. The results of the study can be used in the development of fast and reliable methods for diagnosing especially dangerous and important bacterial species for grain export. In addition, during the study, bacteria were isolated that belong to certain genera, but do not belong to any of the known species, which makes them promising for further study to describe new species in the microbiota of grain crops.

Keywords: diagnostics of phytopathogens, grain crops, bacterioses, PCR, sequencing

According to information provided by the Federal State Statistics Service (https://rosstat.gov.ru/), winter and spring grain crops (wheat, rye, barley, triticale, and oats) are grown annually in Russia on an area of more than 41 million hectares, and the gross harvest of products is about 130 million tons. According to the Customs Statistics of Foreign Trade of the Russian Federation (http://stat.customs.ru/), the Russian Federation annually exports more than 39.5 million tons of grain (analysis for the period from 2019 to 2021).

Plant diseases caused by bacterial pathogens significantly limit crop production and cause significant annual losses globally [1-3]. Bacterioses of cereals pose a serious economic threat, since, according to various estimates, they can reduce yields by 10-40% depending on environmental conditions and the stage of plant ontogenesis in which the infection occurred [4, 5]. The problem requires systemic control of the spread of bacterial infections [1, 6]. In accordance with the Decision of the Council of the Eurasian Economic Commission No. 157 dated November 30, 2016 (as amended by the decisions of the Council of the Eurasian Economic Commission No. 31 dated 03.29.2019, No. 74 dated 08.08.2019, No. 54 dated 05.18.2021, No. 98 dated 05.10.2021, and No. 109 dated 07.15.2022), only the causative agent of wheat yellow mucous bacteriosis *Rathayibacter tritici*, is regulated on grain crops, namely on regulated products under HS codes 1001 and 1008600000 (https://www.alta.ru/tamdoc/16sr0157/). The specified species is subject to identification during import and, subject to the requirements of the importer, during export of wheat. Due to the need for regulation, a diagnostic technique for *Rathayibacter tritici* has been developed and used in quarantine phytosanitary laboratories in the Russian Federation. In quarantine phytosanitary laboratories, there are no methods to identify other dangerous plant pathogenic bacteria, e.g., the causative agents of bacteriosis of grain crops *Rathayibacter rathayi*, *Pseudomonas fuscovaginae*, *Pseudomonas cichorii*, *Pseudomonas fluorescens*, *Pseudomonas syringae*, *Acidovorax avenae*, *Erwinia rhapontici*, *Xanthomonas translucens* and *Clavibacter tessellarius*. According to the Federal Service for Veterinary and Phytosanitary Surveillance (https://fsvps.gov.ru/ru) and the European and Mediterranean Plant Protection Organization (https://gd.eppo.int/), the listed species are regulated in grain products by the phytosanitary requirements of a number of countries, including those importing Russian grain. One or more of these bacteria species is regulated in Egypt, Jordan, Turkey, Morocco, Tunisia, Nigeria, Pakistan, Cameroon, Taiwan, Serbia, South Africa, Brazil, Israel, Colombia and Mexico, the countries that, according to External Customs Statistics trade of the Russian Federation, purchase in Russia more than half of all grain products intended for export.

Bacteria that colonize plant vascular tissue [7], cannot be controlled under field conditions. Often, infected plants produce grain which is a source of infection [3, 4, 8]. Plant pathogenic bacteria can survive for a long time in plants and seeds without showing symptoms [9, 10]. Among the causative agents of bacteriosis of grain crops, *Erwinia rhapontici* can cause characteristic symptoms (pink pigmentation) on seeds [11, 12]. For most bacterioses of grain crops, characteristic symptoms are various stripes, streaks and constrictions on the leaves, burns, yellowing, watery spots or necrosis, depending on the stage of the disease. It is noted that the ears, including seeds and glumes, are usually asymptomatic, but can still harbor infection and be a source of infection [13]. The most effective way to prevent the spread of seed-borne diseases is early laboratory diagnosis [14]. Thereof, both fundamental and practical research require reliable, unified and highly sensitive methods for identification of pathogens to differentiate these species despite the diversity of plant microbiota. For example, this is important in studies of plant-pathogen interaction, acquisition of plant resistance, the plant-pathogen system ecology [15, 16], as well as in breeding varieties for disease resistance, biotechnologies of infection-free cell and tissues cultures, phytosanitary monitoring, export and import quarantine control [16-18]

The fastest and most reliable approach to diagnosing plant pathogens is the use of species-specific PCR tests [19]. To predict the PCR target using bioinformatics methods and validate the resulting primers, information about the species composition of the microbiota of the object is required. It is important to have the most complete collections of both target bacterial isolates and possible accompanying microbiota from which the test must differentiate the target regulated species. However, systematic and large-scale screening of grain crops for the presence of bacterial phytopathogens significant for export has not yet been carried out in the Russian Federation.

The climatic conditions of Moscow are characterized by high humidity [20] and can promote the growth of bacteria inside and on the surface of the plant, which increases the likelihood of their detection. In this work, in samples of grain crop varieties collected from testing sites and hybridization plots at the Moscow field experimental station of the Timiryazev Russian State Agrarian University, along with bacteria exhibiting economically useful and neutral properties, we have identified for the first time the plant pathogens *Pantoea ananatis*, *Clavibacter michiganensis*, *Rhodococcus fascians*, *Pseudomonas trivialis*, *Pseudomonas viridiflava* and *Pseudomonas syringae*.

Our goal was to collect and identify bacterial isolates in samples collected at the Timiryazevskaya field experimental station to form a collection of pathogenic and non-pathogenic microbiota of grain crops.

Materials am methods. Samples (one sample for one variety) of wheat, triticale and rye plants were collected on May 13, 2020 at the variety testing sites and hybridization plots (the field experimental station of the Timiryazev Russian State Agrarian University, 2020-2021). The sample of winter crops consisted of 5-15 plant stems cut at the first internode; the sample of spring crops consisted of 15 seedlings. If symptoms were present, both symptomatic plants and healthy vegetative material were selected.

Individual analytical samples were prepared as previously described [21]. Collected plant material stored at 4 °C in the dark was used within 1 week after collection. Plant tissue (5-10 g) crushed using sterilized scissors was added with 20 ml of phosphate-buffered saline (per 1 liter of distilled water, 2.9 g $Na_2HPO_4 \cdot 12H_2O$, 0.2 g $KH_2PO_4 \cdot 2H_2O$, 8 g NaCl and 0.2 g KCl; pH 7.0-7.2) and left on the shaker for 1 hour at 200 rpm. Then the liquid part was passed through filters with a pore size of 3-5 μm and centrifuged for 10 minutes

at 10,000 g and 4 °C. The supernatant was removed, and the pellet was suspended in 1 ml of phosphate-buffered saline.

Bacteria were isolated on CRL medium [21] by plating 20 μl analytical aliquot onto three Petri dishes according to the Drigalski method. After 5-7 days, individual colonies were subcultured onto CRL medium using a sterile bacteriological loop. The entire morphotypical diversity of colonies grown on the plates was collected. Small fragment of individual colony from each pure culture taken with a sterile bacteriological loop was suspended in 200 μl of distilled water.

Suspensions were used for DNA extraction using a commercial Proba-GS kit (ZAO AgroDiagnostica, Russia) in accordance with the manufacturer's instructions.

All DNA samples were tested in duplicate by classical PCR. Amplification (a T100 thermal cycler, Bio-Rad, USA) was performed using oligonucleotides synthesized at ZAO Evrogen (Russia) and ready-made mixtures for PCR 5× Mas^{DD}TaqMIX-2025 (ZAO Dialat, Russia). The first test was performed with primers PSF/PSR (PSF: 5′-AGCCGTAGGGGAACCTGCGG-3′, PSR: 5′-TGACTGCCAAGGCATCCACC-3′) [22]. Several copies of the 610 bp sequence amplified with the indicated primers are located in tRNA in bacteria of the genus *Pseudomonas*. The PCR mixture for one reaction was 16 μl water, 5 μl 5× Mas^{DD}TaqMIX-2025, 1 μl each primer at a concentration of 10 μmol and 2 μl DNA. Amplification program was 95 °C for 10 min; 25 cycles of 95 °C for 20 s, 64 °C for 15 s, 72 °C for 15 s; 72 °C for 2 min. The PCR product was detected after electrophoretic separation in a 1.5% agarose gel using a gel documentation system (Bio-Rad, USA). Representatives of the genus *Pseudomonas* were found among all isolates. DNAs of cultures from which a 610-bp PCR product was obtained were tested with primers SyD1/SyD2 (SyD1: 5′-CAGCGGCGTTGCGTCCATTGC-3′′, SyD2: 5′-TGCCGCCGACGATGTAGACCAGC-3′) [22]. The primers identify *Pseudomonas syringae* and amplify a 1040 bp product. The PCR mixture per reaction was 17.4 μl water, 5 μl 5× MasDDTaqMIX-2025, 0.3 μl each primer at a concentration of 10 pmol and 2 μl DNA. Amplification program was 95 °C for 10 min; then 25 cycles of 95 °°C for 20 s, 64 °C for 15 s, 72 °C for 45 s; 72 °C for 7 minutes. The PCR product was detected in a 1.5% agarose gel horizontal electrophoresis. If a 1040 bp product was present, the amplicon remaining in the tube was purified using the GeneJET PCR Purification Kit (Thermo Fisher Scientific, USA) and used for Sanger sequencing with Big Dye Kit, BigDye®XTerminator™ Purification Kit (Thermo Fisher Scientific, USA) on an AB-3500 genetic analyzer (Applied Biosystems, USA) according to an adapted method [23]. In the absence of 1040 bp amplicons, sequencing of the PCR product was performed with primers PSF/PSR. For DNA samples for which no PCR products were obtained with primers SyD1/SyD2 or PSF/PSR, PCR was performed with primers 8UA/519B (8UA: 5′-AGAGTTTGATCMTGGCTCAG-3′, 519B: 5′-GTATTACCGCGGCKGCTG-3′) for the 16-23S rRNA region [24]. The PCR mixture for one reaction was 14 μl water, 5 μl 5× Mas^{DD}TaqMIX-2025, 2 μl each primer at a concentration of 10 μmol and 2 μl DNA. Amplification protocol was 96 °C for 10 min; 35 cycles of 95 °C for 15 s, 55 °C for 30 s, 72 °C for 30 s; 72 °C for 10 min. The PCR product was detected by a 1.5% agarose gel horizontal electrophoresis. Amplicon residues not used for electrophoresis were subjected to purification and sequencing as described above.

Sequencing results were processed using the BioEdit program (https://bioedit.software.informer.com/). The deciphered nucleotide sequences were compared using the BLAST service with sequences deposited in GenBank (https://blast.ncbi.nlm.nih.gov). The identification result was considered to be

the organism with the maximum similarity (Max score), automatically calculated by the BLAST service based on the calculation of the Query coverage and Percent identity indicators. If several such organisms were found in a taxon, the oldest taxon was considered the result of identification.

For each identified species and genus, the frequency of occurrence (A) was calculated using the formula [25]: $A = B/C \times 100\%$, where B is the number of samples on which a bacterium with a certain species was found, C is the total number of analyzed samples. When calculating the frequency of occurrence of bacterial genera, both isolates identified to species and isolates identified only to genus were accounted.

Results. The period of plant sampling for winter grain crops was during the booting stage, and for spring grain crops during the seedling phase. There were no symptoms of bacterial diseases on the plants during the sampling period of winter grain crops. Chlorosis occurred on spring rye seedlings. A total of 55 samples of grain crops were selected (Table 1).

1. Collected samples of grain crops (Timiryazevskaya field experimental station, Russian State Agrarian University — Timiryazev Moscow Agricultural Academ, Moscow, 2020)

Crop	Variety
Secale cereale L.	Snezhana, Verasen, Unnamed
× *Triticosecale* Wittm. & A.Camus	Alexander, Victor, Nemchinovsky 56, Valentin 90, Timiryazevskaya 150
Triticum turgidum L.	Donskoy Yantar, Terra
Triticum durum Desf.	Pobeda 70
Triticum dicoccum Schrank	Untitled
Triticum sphaerococcum Percival	Eremeevna
× *Triticosecale* (Wittm. & A. Camus) *sphaerococcum*	Titus
Triticum aestivum L.	Zhiva, Alekseevich, Urup, Morozko, Timiryazevskaya Yubileynaya, Moskovskaya 56, Turquoise, Timiryazevka 150, Count, Vassa, Moskovskaya 39, Doublet, Cavalier, Scarlet Dawn, Nemchinovskaya 24, Legend, Avesta, Inna, Stan, Ascetic, Velena, Vanya, Artel, Nemchinovskaya 85, Videya, Don Lyra, Sineva, Moskovskaya 40, Don 107, Steppe, Governor of the Don, Rostovchanka, Vekha, Nemchinovskaya 57, Augusta, Soberbash, Anka, Gurt, Antonina, Nemchinovskaya 17, Bezostaya 100

N o t e. The sample of winter crops consisted of 5-15 plant stems cut at the first internode; the sample of spring crops consisted of 15 seedlings. One sample was taken from one variety.

Among the collected samples, 14 are rye *Secale cereale* L., triticale × *Triticosecale* Wittm. & A. Camus, × *Triticosecale* (Wittm. & A. Camus) *sphaerococcum*, turgid wheat *Triticum turgidum* L., hard wheat *Triticum durum* Desf. and spherical wheat *Triticum sphaerococcum* Percival, 41 samples are common wheat *Triticum aestivum* L. (see Table 1).

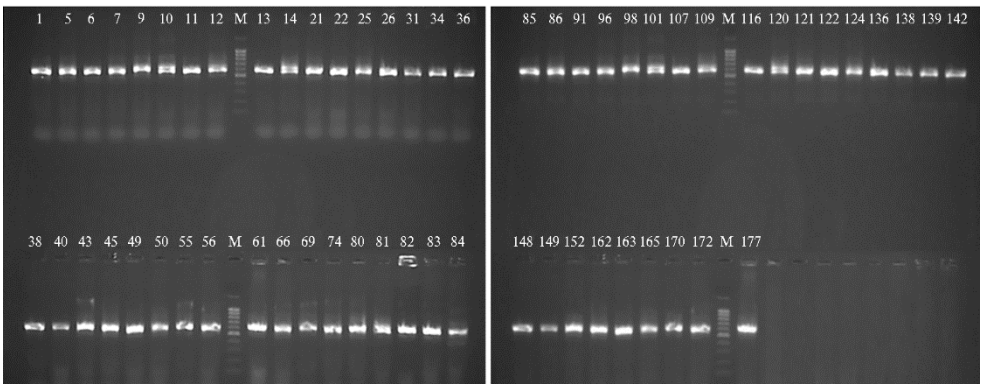

Fig. 1. PCR products with primers PSF/PSR (610 bp) for DNA samples of bacterial isolates from cereal varieties: 1 — Snezhana; 5 — Alive; 6, 7 — Alekseevich; 9-12 — Morozko; 13, 14 — Timiryazevskaya Yubileinaya; 21 — Moskovskaya 56; 22 — Turquoise; 25, 26 — Timiryazevka 150; 31 —

Alexander; 34, 36, 38 — Donskoy amber; 40 — Victor; 43 — *Triticum dicoccum* Schrank (no name); 45 — Nemchinovsky 56; 49 — Eremeevna; 50 — Titus; 55, 56 — Moskovskaya 39; 61 — Doublet; 66-69 — Cavalier; 74 — Scarlet dawn; 80 — Nemchinovskaya 24; 81, 82 — Victory 70; 83, 84 — Legend; 85, 86 — Avesta; 91, 96, 98 — Verasen; 101 — Inna; 107 — Terra; 109 — Timiryazevskaya 150; 116 — Stan; 120-122 — Ascetic; 124 — Velena; 136 — Videa; 138, 139 — Don lyre; 142 — Sineva; 148 — Don 107; 149 — Steppe; 152 — Rostovchanka; 162 — Soberbash; 163, 165 — Anka; 170 — Antonina; 172 — Nemchinovskaya 17; 177 — *Secale cereale* L. (no name); M — DNA length marker 100+ bp DNA ladder (100-1000 bp (ZAO Evrogen, Russia) (Timiryazevskaya field experimental station, Russian State Agrarian University — Timiryazev Moscow Agricultural Academy, Moscow, 2020).

A total of 171 bacterial isolates derived from the collected samples. PCR with primers PSF/PSR revealed a 610 bp amplicon in 60 tested DNA samples of bacterial cultures (Fig. 1).

PCR with primers SyD1/SyD2 generated a 1040 bp amplicon for eight bacterial culture DNA samples tested (Fig. 2).

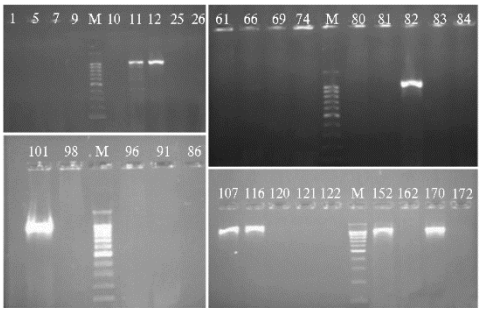

Fig. 2. PCR products with primers SyD1/SyD2 (1040 bp) obtained for DNA samples of bacterial isolates from cereal varieties: 1 — Snezhana; 5 — Zhiva; 7 — Alekseevich; 9-12 — Morozko; 25, 26 — Timiryazevka 150; 61 — Doublet; 66, 69 — Cavalier; 74 — Scarlet dawn; 80 — Nemchinovskaya 24; 81, 82 — Victory 70; 83, 84 — Legend; 86 — Avesta; 91, 96, 98 — Verasen; 101 — Inna; 107 — Terra; 116 — Stan; 120-122 — Ascetic; 152 — Rostovchanka; 162 — Soberbash; 170 — Antonina; 172 — Nemchinovskaya 17; M — DNA length marker 100+ bp DNA ladder (100-1000 bp (Evrogen, Russia) (Timiryazevskaya field experimental station, Russian State Agrarian University — Timiryazev Moscow Agricultural Academy, Moscow, 2020).

For the remaining 103 DNA samples from bacterial cultures, 500 bp amplicons were obtained in PCR with primers 8UA/519B (Fig. 3).

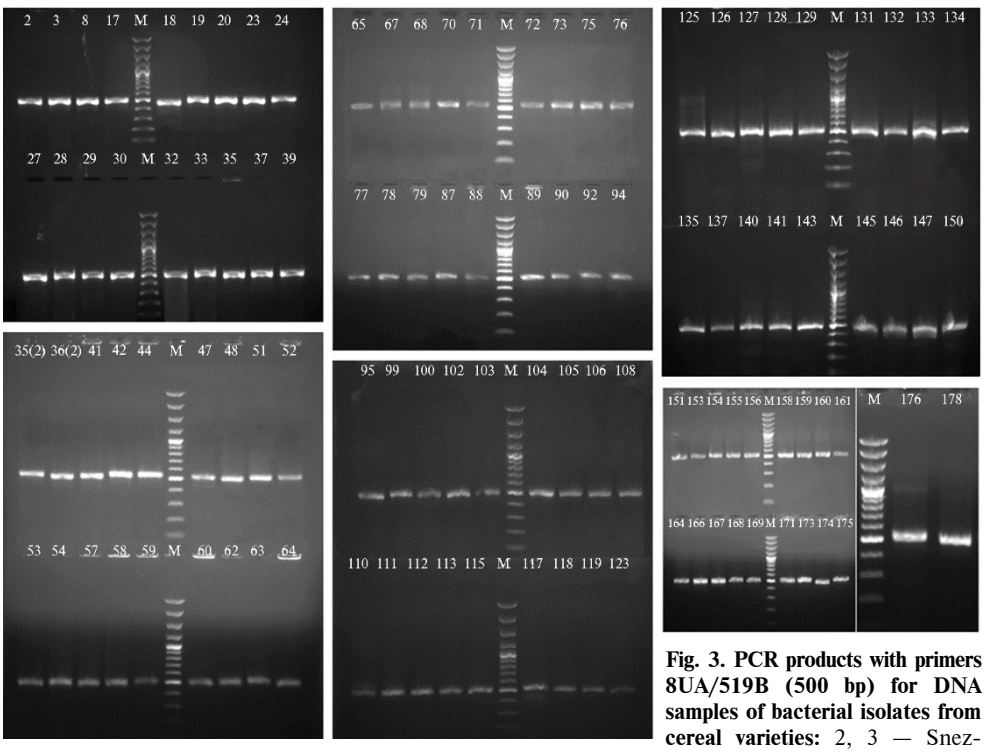

Fig. 3. PCR products with primers 8UA/519B (500 bp) for DNA samples of bacterial isolates from cereal varieties: 2, 3 — Snezhana; 8 — Urup; 17-20 — Timiryazevskaya Yubileinaya; 23-24 — Turquoise; 27 — Timiryazevka 150; 28-30 — Count; 32, 33, 35, 37 — Donskoy amber; 39 — Victor; 35(2), 36(2) — Donskoy amber; 41-42 — Vassa; 44 — Nemchinovsky

56; 47-48 — Eremeevna; 51-52 — Titus; 53-54, 57-58 — Moskovskaya 39; 59-60 — Valentin; 62-65 — Doublet; 67-68 — Cavalier; 70-73, 75-76 — Scarlet dawn; 77-79 — Nemchinovskaya 24; 87-90 — Avesta; 92, 94 — Verasen; 95, 97, 99 — Verasen; 100, 102-103 — Inna; 104-106, 108 — Terra; 110-111 — Timiryazevskaya 150; 112-114, 117 — Stan; 118-119 - Ascetic; 123 — Velena; 125 — Va-nya; 126-129, 131-132 — Artel; 133-134 — Nemchinovskaya 85; 135 — Videa; 137 — Don lyre; 140-141 — Sineva; 143, 145-146 — Moskovskaya 40; 147 — Don 107; 150 — Governor of Don; 151 — Rostovchanka; 153 — Milestone; 154-155 — Nemchinovskaya 57; 156, 158-159 — Augusta; 160-161 — Soberbash; 164 — Anka; 166-169 — Edge; 171 — Nemchinovskaya 17; 173-175 — Bezostaya 100; 176, 178 — spring rye Secale cereale L. (no name); M — molecular weight marker GeneRuler 100 bp Plus (100-1000 bp) (Thermo Fisher Scientific, USA) (Timiryazevskaya field experimental station, Russian State Agrarian University — Timiryazev Moscow Agricultural Academy, Moscow, 2020).

2. Alignment of nucleotide sequences from Sanger sequencing for the collected bacterial isolates (BLAST service, https://blast.ncbi.nlm.nih.gov; Timiryazevskaya field experimental station, Russian State Agrarian University — Timiryazev Moscow Agricultural Academy, Moscow, 2020)

1	2	3	4	5	6	7
			Secale cereale L.			
Snezhana	1	SF/PSR	*Pseudomonas* sp.	990	100 %	99.09 %
Snezhana	2	8UA/519B	*Rhodococcus* sp.	353	75.0 %	86.73 %
			Rhodococcus fascians	350	89.0 %	83.62 %
Snezhana	3	8UA/519B	*Rhodococcus* sp.	619	91.0 %	91.23 %
Verasen	97	8UA/519B	*Arthrobacter* sp.	787	100 %	97.22 %
Verasen	98	PSF/PSR	*Pseudomonas* sp.	959	100 %	98.53 %
			Pseudomonas graminis	948	100 %	98.17 %
Verasen	99	8UA/519B	*Staphylococcus pasteuri*, *Staphylococcus* sp., *Staphylococcus warneri*	909	100 %	99.80 %
			Triticum aestivum L.			
Zhiva	5	PSF/PSR	*Pseudomonas trivialis*	902	96.0 %	96.26 %
Alexeyevich	6	PSF/PSR	*Pseudomonas* sp.	959	100 %	98.53 %
			Pseudomonas graminis	948	100 %	98.17 %
Alexeyevich	7	PSF/PSR	*Pseudomonas poae*	1075	99.0 %	99.49 %
Urup	8	8UA/519B	*Erwinia papayae*, *Erwinia* sp., *Erwinia billingiae*	551	99.0 %	98.71 %
Morozko	9	PSF/PSR	*Pseudomonas viridiflava*	712	99 %	88.17 %
Morozko	10	PSF/PSR	*Pseudomonas syringae*, *Pseudomonas syringae* pv. *aptata*	575	100 %	99.68 %
Morozko	11	PSF/PSR	*Pseudomonas syringae* pv. *atrofaciens*, *Pseudomonas syringae*	1596	100 %	97.18 %
Morozko	12	PSF/PSR	*Pseudomonas syringae* pv. *atrofaciens*, *Pseudomonas syringae*	1596	100 %	97.18 %

Note. 1 — variety from which the isolate was isolated, 2 — isolate number, 3 — pair of primers (for more details, see the Materials and methods section), 4 — microorganism with maximum similarity, 5 — maximum score, 6 — query coverage (query coverage), 7 — percent identity. The table is presented in full on the http://www,agrobiology.ru.

Purification, sequencing, and processing using the BioEdit program allowed us to obtain nucleotide sequences for each of isolates and align these sequences in the BLAST service (https://blast.ncbi.nlm.nih.gov). Examples of the results obtained are shown in Table 2 (see in full on the website http://www.agrobiology.ru).

Some species of identified bacteria were found only in one sample of grain crops (Table 3). The Gram-positive bacterium *Rathayibacter festucae* isolated from a sample of triticale cv. Timiryazevskaya 150, was originally identified in 2002 from a leaf gall caused by the nematode *Anguina graminis* on red fescue [26]. The genus *Rathayibacter* includes 6 species, among which *Rathayibacter tritici* and *Rathayibacter rathayi* are pathogenic for grain crops and are regulated by phytosanitary requirements of a number of countries (https://fsvps.gov.ru/ru, https://gd.eppo.int/).

The species *Pseudoclavibacter helvolus* identified in the winter rye sample (see Table 3) is a gram-positive bacterium that does not have phytopathogenic properties [27]. The gram-negative bacterium Paucimonas lemoignei, isolated from turgid wheat variety Donskoy Yantar (see Table 3), is not characterized as a phytopathogen [28].

3. Identification of bacteria in samples of grain crops (except for *Triticum aestivum* L.) (Timiryazevskaya field experimental station, Russian State Agrarian University — Timiryazev Moscow Agricultural Academy, Moscow, 2020)

Crop	Variety	Identified as
Secale cereale L.	Snezhana	*Pseudomonas* sp., *Rhodococcus* sp.
	Verasen	*Pseudomonas trivialis*, *Micrococcus* sp., *Staphylococcus* sp., *Pseudomonas* sp., *Arthrobacter* sp.
	Unnamed	Actinomycetales bacterium, *Pseudomonas* sp., *Pseudoclavibacter helvolus*
× *Triticosecale* Wittm. & A.Camus	Alexander	*Pseudomonas* sp.
	Victor	*Frigoribacterium* sp., *Pseudomonas* sp.
	Nemchinovsky 56	*Dyadobacter* sp., *Pseudomonas* sp.
	Valentin 90	*Clavibacter michiganensis*, *Frigoribacterium faeni*
	Timiryazevskaya 150	*Pseudomonas* sp., *Frigoribacterium* sp., *Rathayibacter festucae*
Triticum turgidum L.	Donskoy amber	Uncultured bacterium, *Frigoribacterium faeni*, *Paucimonas lemoignei*, Uncultured Enterobacteriaceae bacterium, Uncultured soil bacterium, *Pantoea ananatis*, *Frigoribacterium* sp., *Salinibacterium* sp., *Pseudomonas* sp.
	Terra	*Arthrobacter* sp., *Rhodococcus* sp., *Clavibacter michiganensis*, *Pseudomonas syringae*, *Frigoribacterium* sp.
Triticum durum Desf.	Pobeda 70	*Pseudomonas viridiflava*, *Pseudomonas syringae*
Triticum dicoccum Schrank	Untitled	*Pseudomonas* sp.
Triticum sphaerococcum Percival	Eremeevna	*Frigoribacterium* sp., *Sanguibacter* sp., *Pseudomonas* sp.
× *Triticosecale* (Wittm. & A. Camus) *sphaerococcum*	Titus	*Pseudomonas* sp., Bacterium, Uncultured bacterium

N o t e. The sample of winter crops consisted of 5-15 plant stems cut at the first internode; the sample of spring crops consisted of 15 seedlings. One sample was taken from one variety.

The species *Pantoea ananatis* identified in a sample of turgid wheat variety Donskoy Yantar (see Table 3) is a gram-negative bacterium that is the causative agent of various plant bacterioses [29] and, according to the Federal Service for Veterinary and Phytosanitary Surveillance (https://fsvps.gov .ru/ru), is regulated by the Colombian quarantine list. *Pantoea ananatis* was reported to promote active metabolism in plants [30].

In single samples of 41 winter soft wheat specimens (Table 4), we found both phytopathogenic and economically useful bacteria.

The gram-positive bacterium *Arthrobacter chlorophenolicus* isolated from wheat variety Sineva (see Table 4) is economically useful and increases the drought resistance of plants [31]. The gram-negative soil bacterium *Pseudomonas chlororaphis* from wheat variety Asket (see Table 4) is used as a bioagent against plant diseases [32].

Bacteria were also isolated the presence of which was noted in several samples of cereal crops (see Tables 3, 4). Species of gram-positive bacteria *Clavibacter michiganensis* and *Rhodococcus fascians* and gram-negative *Pseudomonas trivialis*, *Pseudomonas viridiflava* and *Pseudomonas syringae* (including the pathovar *Pseudomonas syringae* pv. *syringae*) have been identified, for which phytopathogenic properties have been described [4, 33-37]. In addition, the gram-positive bacteria *Frigoribacterium faeni*, usually isolated from soil, plant phyllosphere and other sources, for which economically valuable or pathogenic properties have not been noted, have been isolated and identified [38]. Gram-negative bacteria *Pseudomonas graminis* and *Pseudomonas poae* have also been isolated (see Tables 3, 4) which are usually found in soil or plants and are used to control plant diseases [39, 40].

4. Identification of bacteria in samples of winter soft wheat *Triticum aestivum* L. (Timiryazevskaya field experimental station, Russian State Agrarian University — Timiryazev Moscow Agricultural Academy, Moscow, 2020)

Variety	Identified as
Alive	*Pseudomonas trivialis*
Alexeyevich	*Pseudomonas* sp., *Pseudomonas poae*
Urup	*Erwinia* sp.
Morozko	*Pseudomonas viridiflava*, *Pseudomonas syringae*
Timiryazevskaya Yubileinaya	*Pseudomonas* sp., *Frigoribacterium* sp., Uncultured bacterium, *Clavibacter* sp., *Kineococcus* sp.
Moskovskaya 56	*Pseudomonas* sp.
Turquoise	*Pseudomonas* sp., *Pantoea* sp., Uncultured bacterium
Timiryazevka 150	*Pseudomonas* sp., *Pseudomonas poae*, Uncultured bacterium
Graph	*Curtobacterium* sp., *Arthrobacter* sp., *Streptomyces* sp.
Vassa	*Frigoribacterium* sp.
Moskovskaya 39	*Frigoribacterium* sp., *Clavibacter* sp., *Pseudomonas trivialis*, *Pseudomonas* sp.
Doublet	*Frigoribacterium* sp., Bacterium, *Curtobacterium* sp.
Cavalier	*Pseudomonas* sp., Uncultured bacterium, Bacterium, *Pseudomonas graminis*
Scarlet dawn	*Oerskovia* sp., *Cellulomonas* sp., *Frigoribacterium* sp., *Microbacterium* sp., *Pseudomonas* sp., Bacterium
Nemchinovskaya 24	Microbacteriaceae bacterium, *Frondihabitans* sp., *Curtobacterium* sp., *Pseudomonas* sp.
Legend	*Pseudomonas graminis*, *Pseudomonas poae*
Avesta	*Pseudomonas poae*, *Clavibacter* sp., *Frigoribacterium* sp., *Sphingomonas* sp.
Inna	*Clavibacter michiganensis*, *Pseudomonas syringae,* Uncultured bacterium, *Microbacterium* sp.
Mill	*Rhodococcus fascians*, *Arthrobacter* sp., *Phycicoccus* sp., *Pseudomonas syringae* pv. *syringae*, *Frigoribacterium* sp.
Ascetic	*Frigoribacterium* sp., *Bacillus* sp., *Pseudomonas viridiflava*, *Pseudomonas chlororaphis*, *Pseudomonas* sp.
Velena	*Rhodococcus* sp., *Pseudomonas viridiflava*
Vania	*Plantibacter* sp.
Artel	Uncultured bacterium, Unidentified microorganism, *Frigoribacterium* sp., *Curtobacterium* sp., Rhizosphere soil bacterium, *Sphingomonas* sp.
Nemchinovskaya 85	*Clavibacter* sp., *Frigoribacterium* sp.
Videya	Bacterium, *Pseudomonas* sp.
Don lyre	*Plantibacter* sp., *Pseudomonas* sp., *Pseudomonas syringae* pv. *syringae*
Sineva	*Frigoribacterium* sp., *Arthrobacter chlorophenolicus*, *Pseudomonas* sp.
Moskovskaya 40	*Clavibacter* sp., *Arthrobacter* sp.
Don 107	*Frigoribacterium* sp., *Pseudomonas* sp.
Steppe	*Pseudomonas* sp.
Governor of Don	*Frigoribacterium* sp.
Rostovite	Bacterium, *Pseudomonas* sp.
Milestone	*Erwinia* sp.
Nemchinovskaya 57	*Rhodococcus fascians*, Uncultured bacterium
Augusta	*Bacillus* sp., Uncultured bacterium
Soberbash	*Clavibacter* sp., *Pseudomonas* sp., Bacterium
Anka	*Pseudomonas trivialis*, Bacterium, *Pseudomonas* sp.
Gurt	*Pseudomonas* sp., *Agreria* sp., *Frondihabitans* sp., *Sphingomonas* sp.
Antonina	*Pseudomonas syringae*
Nemchinovskaya 17	*Rhodococcus* sp.
Bezostaya 100	*Athrobacter* sp., *Micrococcus* sp., *Frigoribacterium* sp.

N o t e. The sample of winter crops consisted of 5-15 plant stems cut at the first internode; the sample of spring crops consisted of 15 seedlings. One sample was taken from one variety.

These data revealed that the frequency of occurrence of bacteria of the genera *Pseudomonas*, *Frigoribacterium*, *Clavibacter*, *Arthrobacter* and *Rhodococcus* on grain crops of the Timiryazev field experimental station was more than 10% (Table 5). The diversity of pseudomonads, the most common bacteria in the studied samples with 70.9% frequency of occurrence, is represented by six species (see Table 5). Of these species the *Pseudomonas chlororaphis*, *Pseudomonas graminis* and *Pseudomonas poae*, according to the sprcial literature, have properties beneficial to plants, and three species, the *Pseudomonas syringae*, *Pseudomonas trivialis* and *Pseudomonas viridiflava* are plant pathogens.

Bacteria of the genus *Frigoribacterium*, the second most abundant group (see Table 5), are common members of the plant microbiota, promoting plant growth and adaptation [41]. Common representatives of soil and plant microbiota also include the bacteria *Arthrobacter* sp., which we found in samples of grain

crops with an occurrence frequency of 12.7%.

5. Frequency of occurrence of bacterial species and genera in grain crop samples (Timiryazevskaya field experimental station, Russian State Agrarian University — Timiryazev Moscow Agricultural Academy, Moscow, 2020)

Genus	Frequency, %	Species	Frequency, %
Agreria sp.	1.8	–	
Arthrobacter sp.	12.7	*Arthrobacter chlorophenolicus*	1.8
Bacillus sp.	5.5	–	
Cellulomonas sp.	1.8	–	
Clavibacter sp.	16.4	*Clavibacter michiganensis*	5.5
Curtobacterium sp.	7.3	–	
Dyadobacter sp.	1.8	–	
Erwinia sp.	3.6	–	
Frigoribacterium sp.	36.4	*Frigoribacterium faeni*	3.6
Frondihabitans sp.	3.6	–	
Kineococcus sp.	1.8	–	
Microbacterium sp.	3.6	–	
Micrococcus sp.	3.6	–	
Oerskovia sp.	1.8	–	
Pantoea sp.	3.6	*Pantoea ananatis*	1.8
Paucimonas sp.	1.8	*Paucimonas lemoignei*	1.8
Phycicoccus sp.	1.8	–	
Plantibacter sp.	3.6	–	
Pseudoclavibacter sp.	1.8	*Pseudoclavibacter helvolus*	1.8
Pseudomonas sp.	70.9	*Pseudomonas chlororaphis*	1.8
		Pseudomonas graminis	3.6
		Pseudomonas poae	7.3
		Pseudomonas syringae	12.7
		Pseudomonas trivialis	7.3
		Pseudomonas viridiflava	7.3
Rathayibacter sp.	1.8	*Rathayibacter festucae*	1.8
Rhodococcus sp.	10.9	*Rhodococcus fascians*	3.6
Salinibacterium sp.	1.8	–	
Sanguibacter sp.	1.8	–	
Sphingomonas sp.	5.5	–	
Staphylococcus sp.	1.8	–	
Streptomyces sp.	1.8	–	

N o t e. Dashes mean that species within the genus have not been identified.

We found a prevalence of the pathogenic species *Clavibacter michiganensis* at a frequency of 5.5%. It is most likely that the detected bacteria belong to the subspecies *Clavibacter michiganensis* subsp. *tessellarius* (*Clavibacter tessellarius* sp. nov.) which is the causative agent of bacterial mosaic in wheat, since *Clavibacter michiganensis* subspecies are highly specific to the host plant, and it is the tessellarius subspecies that infects wheat [42].

Among the bacteria *Rhodococcus* sp., the frequency of which was 10.9%, only *Rhodococcus fascians* are reported to cause plant diseases [34].

The frequency of occurrence of other identified bacterial genera and species was less than 10%.

Thus, we studied the microbiota of local grain crops (Timiryazevskaya field experimental station, 2020) and revealed field isolates that can enter a unified collection of grain bacteria in order to create species-specific primers that will be a key part of the developed regulatory documents on the detection and identification of quarantine and export-significant pathogens of bacteriosis of grain crops. The specified regulatory documents which are of very great demand will be used by phytosanitary laboratories for phytosanitary control. Gien the regional characteristics of soil-climatic and agrotechnical conditions and the biodiversity of isolates [43-45], we believe that standard strains from foreign collections of microorganisms (if available) are less suitable for these purposes. Let us note that previously no large-scale study of the bacterial composition in grain crops has been carried out in Russia, and therefore there is no information about the species composition of bacteria that can be found together in one sample. There is also no complete list of bacteria that can be found in grain crops in

Russia.

We conducted a study of the composition of the microbiota on crops of economically important crops using classical microbiology methods to isolate bacteria from samples and molecular genetic methods to identify isolated isolates. The data obtained can add to the knowledge of bacteria living in plants and will be useful for developing a general understanding of the microbiome of target crops in the field.

Bacteria were isolated from seedlings of spring crops (rye) and from green plants of winter crops in the stage of emerging into the tube, which could affect the number of some types of bacteria in plants compared to others and, indirectly, the composition of the resulting bacterial associations [4, 5, 13]. Strains were isolated on Petri dishes based on the diversity of morphotypes, which is quite subjective and in any case does not allow detection of uncultivable microorganisms [45]. However, this does not contradict the tasks that we set for ourselves when carrying out the work. i.e., to isolate cultures for a collection of bacterial phytopathogens and their accompanying microbiota that are phytosanitary important for grain export. We do not extend the obtained data on the composition and frequency of occurrence of bacteria to other grain crops, even in the same agroclimatic zone.

It is important to note that we identified pathogenic, neutral and beneficial species in the microbiota of grain plants. More knowledge about pathogens can improve the phytosanitary assessment of cereal crops, while candidate bacteria can be found among beneficial species to develop new drugs for the biological control of phytopathogens. In addition, we identified bacteria that belong to certain genera, but do not belong to any of the known species, which makes them promising for further study and the possibility of describing new species of the grain crop microbiota.

So, at testing sites and hybridization plots of the Timiryazevskaya field experimental station, we collected 55 samples of grain crops of which 171 bacterial isolates were purified and 37 isolates were identified to species using molecular genetic methods. The identified bacterial diversity is represented by 14 species. Among them, phytopathogens include *Pantoea ananatis*, Cl*a*vibacter *michiganensis*, *Rhodococcus fascians*, *Pseudomonas trivialis*, *Pseudomonas viridiflava* and *Pseudomonas syringae*. *Pantoea ananatis* is listed and watched by the North American Plant Protection Organization. *Rhodococcus fascians* is regulated as a quarantine organism in Argentina, Brazil, Chile, Mexico, etc. *Pseudomonas viridiflava* is a quarantine organism for Mexico and is regulated as a non-quarantine organism in Switzerland and the UK. *Pseudomonas syringae* (and, in particular, *Pseudomonas syringae* pv. *syringae* which we discovered on grain crops), is a quarantine organism for such importers of Russian grain as Taiwan, Mexico, Colombia and Jordan, and is also regulated by the phytosanitary requirements of Egypt and Zimbabwe and Great Britain in grain products as a non-quarantine species (https://fsvps.gov.ru/ru, https://gd.eppo.int/). Bacteria with economically useful properties were also isolated and identified, these are *Arthrobacter chlorophenolicus*, *Pseudomonas chlororaphi*s, *Pseudomonas graminis* and *Pseudomonas poae*. Other identified species, the *Rathayibacter festucae*, *Pseudoclavibacter helvolus*, *Paucimonas lemoignei* and *Frigoribacterium faeni*, according to published data, do not have pronounced harmful or beneficial properties. The highest frequency of occurrence (70.9%) was characteristic of the genus *Pseudomonas* species. Representatives of the genera *Frigoribacterium* (36.4%), *Clavibacter* (16.4%), *Arthrobacter* (12.7%) and *Rhodococcus* (10.9%) also have a high frequency of occurrence. The experimental data on the bacteria species composition we obtained in grain crops can be used to identify the spread

of bacterioses on the territory of the Russian Federation and to bioinformaticsally analyze bacterial genomes in the search for species-specific genetic markers of quarantine objects.

REFERENCES

1. Singh J., Chhabra B., Raza A., Yang S.H., Sandhu K.S. Important wheat diseases in the US and their management in the 21st century. *Frontiers in Plant Science*, 2023, 13: 1010191 (doi: 10.3389/fpls.2022.1010191).
2. Krawczyk K., Wielkopolan B., Obr palska-St plowska A. *Pantoea ananatis*, a new bacterial pathogen affecting wheat plants (*Triticum* L.) in Poland. *Pathogens*, 2020, 9(12): 1079 (doi: 10.3390/pathogens9121079).
3. Sapkota S., Mergoum M., Liu Z. The translucens group of *Xanthomonas translucens*: complicated and important pathogens causing bacterial leaf streak on cereals. *Molecular Plant Pathology*, 2020, 21(3): 291-302 (doi: 10.1111/mpp.12909).
4. Tambong J. Bacterial pathogens of wheat: symptoms, distribution, identification, and taxonomy. In: *Wheat*. IntechOpen, 2022 (doi: 10.5772/intechopen.102855).
5. Butsenko L., Pasichnyk L., Kolomiiets Y., Kalinichenko A., Suszanowicz D., Sporek M., Patyka V. Characteristic of *Pseudomonas syringae* pv. *atrofaciens* isolated from weeds of wheat field. *Applied Sciences*, 2021, 11(1): 286 (doi: 10.3390/app11010286).
6. Sundin G.W., Castiblanco L.F., Yuan X., Zeng Q., Yang C.H. Bacterial disease management: challenges, experience, innovation and future prospects: challenges in bacterial molecular plant pathology. *Molecular Plant Pathology*, 2016, 17(9): 1506-1518 (doi: 10.1111/mpp.12436).
7. Wichmann F., Vorhölter F., Hersemann L., Widmer F., Blom J., Niehaus K., Reinhard S., Conradin C., Kölliker R. The noncanonical type III secretion system of *Xanthomonas translucens* pv. *graminis* is essential for forage grass infection. *Molecular Plant Pathology*, 2013, 14(6): 576-588 (doi: 10.1111/mpp.12030).
8. Xie G.L., Zhang G.Q., Liu H., Lou M.M., Tian W.X., Li B., Zhou X.P., Zhu B., Jin G.L. Genome sequence of the rice-pathogenic bacterium *Acidovorax avenae* subsp. *avenae* RS-1. *Journal of Bacteriology*, 2011, 193(18): 5013-5014. (doi: 10.1128/JB.05594-11).
9. Nandi M., Macdonald J., Liu P., Weselowski B., Yuan Z.C. *Clavibacter michiganensis* ssp. *michiganensis*: bacterial canker of tomato, molecular interactions and disease management. *Molecular Plant Pathology*, 2018, 19(8): 2036-2050 (doi: 10.1111/mpp.12678).
10. Coutinho T.A., Wingfield M.J. *Ralstonia solanacearum* and *R. pseudosolanacearum* on *Eucalyptus*: opportunists or primary pathogens? *Frontiers in Plant Science*, 2017, 8: 761 (doi: 10.3389/fpls.2017.00761).
11. McMullen M.P., Stack R.W., Miller J.D., Bromel M.C., Youngs V.L. *Erwinia rhapontici*, a bacterium causing pink wheat kernels. In: *Proceedings of the North Dakota Academy of Sciences*. Grand Forks, ND, 1984, 38: 78.
12. Wise K.A., Zhao Y.F., Bradley C.A. First report of pink seed of pea caused by *Erwinia rhapontici* in North Dakota. *Plant Disease*, 2008, 92(2): 315 (doi: 10.1094/PDIS-92-2-0315A).
13. Asaad S., Sands D.C., Mohan S.K. Chapter 4: Detection of *Pseudomonas syringae* pv. *syringae* in wheat seeds. In: *Detection of plant-pathogenic bacteria in seed and other planting material, Second Edition*. AIP Publications, 2017: 21-26 (doi: 10.1094/9780890545416.004).
14. Boykin L.M., Sseruwagi P., Alicai T., Ateka E., Mohammed I.U., Stanton J.L., Kayuki C., Mark D., Fute T., Erasto J., Bachwenkizi H., Muga B., Mumo N., Mwangi J., Abidrabo P., Okao-Okuja G., Omuut G., Akol J., Apio H.B., Osingada F., Kehoe M.A., Eccles D., Savill A., Lamb S., Kinene T., Rawle C.B., Muralidhar A., Mayall K., Tairo F., Ndunguru J. Tree lab: portable genomics for early detection of plant viruses and pests in Sub-Saharan Africa. *Genes (Basel)*, 2019, 10(9): 632 (doi: 10.3390/genes10090632).
15. Gorshkov V.Yu. *Bakteriozy rasteniy: molekulyarnye osnovy formirovaniya rastitel'no-mikrobnykh patosistem* [Plant bacterioses: molecular basis for the formation of plant-microbial pathosystems]. Kazan', 2017 (in Russ.).
16. Nair S., Manimekalai R. Phytoplasma diseases of plants: molecular diagnostics and way forward. *World Journal of Microbiology and Biotechnology*, 2021, 37(6): 102 (doi: 10.1007/s11274-021-03061-y).
17. Ignat'eva I.M., Slovareva O.Yu. *Tezisy dokladov 22-y Vserossiyskoy konferentsii molodykh uchenykh «Biotekhnologiya v rastenievodstve, zhivotnovodstve i sel'skokhozyaystvennoy mikrobiologii» (Moskva, 07-09 noyabrya 2022 goda)* [Abstracts of the 22nd All-Russian Conference of young scientists "Biotechnology in crop production, animal husbandry and agricultural microbiology" (Moscow, November 07-09, 2022)]. Moscow, 2022: 140 (in Russ.).
18. Ignatyeva I.M., Karimova E.V., Prikhodko S.I. Diagnostics of the bacterial blight pathogen of bean *Xanthomonas axonopodis* pv. *phaseoli* in plant and seed material of grain legumes using molecular genetics methods. *AIP Conference Proceedings*, 2021, 2388: 030014 (doi: 10.1063/5.0068504).

19. Islam M.R., Hossain M.R., Kim H.T., Jesse D.M.I., Abuyusuf M., Jong H.J., Park J.I., Nou I.S. Development of molecular markers for detection of *Acidovorax citrulli* strains causing bacterial fruit blotch disease in melon. *International Journal of Molecular Sciences*, 2019, 20(11): 2715 (doi: 10.3390/ijms20112715).
20. Korneykova M.V., Soshina A.S., Gavrichkova O.V. *Mikologiya i fitopatologiya*, 2021, 55(4): 256-270 (doi: 10.31857/S0026364821040085) (in Russ.).
21. Slovareva O.Y. Detection and identification of pathogens of bacterial diseases of wheat and barley in the Russian Federation. *Microbiology Independent Research Journal (MIR Journal)*, 2020, 7(1): 13-23 (doi: 10.18527/2500-2236-2020-7-1-13-23).
22. Kazempour M.N, Kheyrgoo M., Pedramfar H., Rahimian H. Isolation and identification of bacterial glum blotch and leaf blight on wheat (*Triticum aestivum* L.) in Iran. *African Journal of Biotechnology*, 2010, 9(20): 2860-2865.
23. Belkin D.L., Bondarenko G.N., Yaremko A.B., Uvarova D.A. *Karantin rasteniy. Nauka i praktika*, 2019, 28(2): 31-34 (in Russ.).
24. Honda-Takinami R., Hata J., Matsuoka K., Hoshi S., Koguchi T., Sato Y., Akaihata H., Kataoka M., Ogawa S., Nishiyama K., Suzutani T., Kojima Y. Association between the presence of bacteria in prostate tissue and histopathology in biopsies from men not complaining of lower urinary tract symptoms. *Fukushima Journal of Medical Science*, 2022, 68(3): 161-167 (doi: 10.5387/fms.2022-34).
25. Minaeva L.P., Samokhvalova L.V., Zavriev S.K., Stakheev A.A. First detection of fungus *Fusarium coffeatum* in the territory of the Russian Federation. *Sel'skokhozyaistvennaya biologiya* [*Agricultural Biology*], 2022, 57(1): 131-140 (doi: 10.15389/agrobiology.2022.1.131eng).
26. Dorofeeva L.V., Evtushenko L.I., Krausova V.I., Karpov A.V., Subbotin S.A., Tiedje J.M. *Rathayibacter caricis* sp. nov. and *Rathayibacter festucae* sp. nov., isolated from the phyllosphere of *Carex* sp. and the leaf gall induced by the nematode *Anguina graminis* on *Festuca rubra* L., respectively. *International Journal of Systematic and Evolutionary Microbiology*, 2002, 52(6): 1917-1923 (doi: 10.1099/00207713-52-6-1917).
27. Raghupathi P.K., Herschend J., Røder H.L., Sørensen S.J., Burmшlle M. Draft genome assembly of two *Pseudoclavibacter helvolus* strains, G8 and W3, isolated from slaughterhouse environments. *Genome Announcements*, 2016, 4(2): e00077-16 (doi: 10.1128/genomeA.00077-16).
28. Jendrossek D. Transfer of [*Pseudomonas*] *lemoignei*, a gram-negative rod with restricted catabolic capacity, to *Paucimonas* gen. nov. with one species, *Paucimonas lemoignei* comb. nov. *International Journal of Systematic and Evolutionary Microbiology*, 2001, 51(3): 905-908 (doi: 10.1099/00207713-51-3-905).
29. Weller-Stuart T., De Maayer P., Coutinho T. *Pantoea ananatis*: genomic insights into a versatile pathogen. *Molecular Plant Pathology*, 2017, 18(9): 1191-1198 (doi: 10.1111/mpp.12517).
30. Lu L., Chang M., Han X., Wang Q., Wang J., Yang H., Guan Q., Dai S. Beneficial effects of endophytic *Pantoea ananatis* with ability to promote rice growth under saline stress. *Journal of Applied Microbiology*, 2021, 131(4): 1919-1931 (doi: 10.1111/jam.15082).
31. Miranda-Ríos J.A., Ramírez-Trujillo J.A., Nova-Franco B., Lozano-Aguirre Beltrán L.F., Iturriaga G., Suárez-Rodríguez R. Draft genome sequence of *Arthrobacter chlorophenolicus* strain Mor30.16, isolated from the bean rhizosphere. *Genome Announcements*, 2015, 3(3): e00360-15 (doi: 10.1128/genomeA.00360-15).
32. Liu F., Yang S., Xu F., Zhang Z., Lu Y., Zhang J., Wang G. Characteristics of biological control and mechanisms of *Pseudomonas chlororaphis* zm-1 against peanut stem rot. *BMC Microbiology*, 2022, 22(1): 9 (doi: 10.1186/s12866-021-02420-x).
33. Hwang I.S., Oh E.J., Song E., Park I.W., Lee Y., Sohn K.H., Choi D., Oh C.S. An apoplastic effector Pat-1Cm of the gram-positive bacterium *Clavibacter michiganensis* acts as both a pathogenicity factor and an immunity elicitor in plants. *Frontiers in Plant Science*, 2022, 13: 888290 (doi: 10.3389/fpls.2022.888290).
34. Park J.M., Koo J., Kang S.W., Jo S.H., Park J.M. Detection of *Rhodococcus fascians*, the causative agent of lily fasciation in South Korea. *Pathogens*, 2021, 10(2): 241 (doi: 10.3390/pathogens10020241).
35. Sawada H., Fujikawa T., Tsuji M., Satou M. *Pseudomonas allii* sp. nov., a pathogen causing soft rot of onion in Japan. *International Journal of Systematic and Evolutionary Microbiology*, 2021, 71(1). (doi: 10.1099/ijsem.0.004582).
36. Lipps S.M., Samac D.A. *Pseudomonas viridiflava*: An internal outsider of the *Pseudomonas syringae* species complex. *Molecular Plant Pathology*, 2022, 23(1): 3-15 (doi: 10.1111/mpp.13133).
37. Xin X.F., Kvitko B., He S.Y. *Pseudomonas syringae*: what it takes to be a pathogen. *Nature Reviews Microbiology*, 2018, 16(5): 316-328. (10.1038/nrmicro.2018.17).
38. Zhuo Y., Jin C.Z., Jin F.J., Li T., Kang D.H., Oh H.M., Lee H.G., Jin L. *Lacisediminihabitans profunda* gen. nov., sp. nov., a member of the family Microbacteriaceae isolated from freshwater sediment. *Antonie Van Leeuwenhoek*, 2020, 113(3): 365-375 (doi: 10.1007/s10482-019-01347-8).
39. Crovadore J., Calmin G., Chablais R., Cochard B., Schulz T., Lefort F. Whole-genome sequence of *Pseudomonas graminis* strain UASWS1507, a potential biological control agent and biofertilizer isolated in Switzerland. *Genome Announcement*, 2016, 4(5): e01096-16 (doi: 10.1128/genomeA.01096-16).

40. Sun H., Kong L., Du H., Chai Z., Gao J., Cao Q. Benefits of *Pseudomonas poae* s61 on *Astragalus mongholicus* growth and bioactive compound accumulation under drought stress. *Journal of Plant Interactions*, 2019, 14(1): 205-212 (doi: 10.1080/17429145.2019.1611958).
41. Shen S.Y., Fulthorpe R. Seasonal variation of bacterial endophytes in urban trees. *Frontiers in Microbiology*, 2015, 6: 427 (doi: 10.3389/fmicb.2015.00427).
42. Li X., Tambong J., Yuan K.X., Chen W., Xu H., Lйvesque C.A., De Boer S.H. Re-classification of *Clavibacter michiganensis* subspecies on the basis of whole-genome and multi-locus sequence analyses. *International Journal of Systematic and Evolutionary Microbiology*, 2018, 68(1): 234-240 (doi: 10.1099/ijsem.0.002492).
43. Sennikov V.A., Larin L.G., Rossinskaya T.M., Belolyubtsev A.I., Korovina L.N. *Izvestiya Timiryazevskoy sel'skokhozyaystvennoy akademii*, 2005, 1: 141-146 (in Russ.).
44. Belolyubtsev A.I., Asaulyak I.F. *Izvestiya Timiryazevskoy sel'skokhozyaystvennoy akademii*, 2013, 4: 66-84 (in Russ.).
45. Dumova V.A., Pershina E.V., Merzlyakova Ya.V., Kruglov Yu.V., Andronov E.E. The main trends in dynamics of soil microbiom during a long-term field experiment as indicated by high throughput sequencing the 16S-rRNA gene libraries. *Sel'skokhozyaistvennaya biologiya* [*Agricultural Biology*], 2013, 5: 85-92 (10.15389/agrobiology.2013.5.85rus) (in Russ.).