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Разработка и оптимизация методов выявления и идентификации бактериозов, значимых для экспорта и импорта Российской зернопродукции

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Диссертация на соискание учёной
степеникандидата
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INTRODUCTION

Relevance of the topic. Many countries, such as China, Egypt, Indonesia, the United States of America, Cuba, Canada, Zambia, and Algeria, have phytosanitary regulations for the importation of grains that are considering plant pathogenic bacteria as Plant Quarantine organisms. As a result, the National quarantine lists are developed. These lists serve as an important measure to prevent the spread of important bacterial pathogens to other countries.

Information on the National Plant Quarantine Organisms lists is available in such websites as the International Plant Protection Convention (IPPC) under the United Nations' (UN) Food and Agriculture Organisation (FAO) which highlights shows 227 entries of phytosanitary requirements of countries like Zambia, Alegria, China, and Canada. The European and Mediterranean Plant Protection Organization (EPPO), which has more than 40 member countries including Russia, maintains such a list. This helps with the diagnosis of bacteria during import and export. As of now, some species of bacteria with phytosanitary significance in cereals have been barely classified, and some, like *Rathayibacter tritici*, are known as yel bacterial ear rot of wheat, gumming disease of wheat, yellow slime of wheat, or mucus bacteriosis.

Rathayibacter tritici (Carlson & Vidaver) Zgurskaya, Evtushenko, Akimov & Kalakoutskii (EPPO code CORBTR) is a gram-positive pathogenic bacteria associated to seed galls caused by plant pathogenic nematode *Anguina tritici*. In infected plants the seed grains either fail to form or become puny. [1]. As shown in the figure 1.1 crops infected with the bacteria have deformed ears and within the ears yellow bacterial mucilage is formed.

Figure 1.1 Cereal crops infected with *Rathayibacter tritici*. (Photograph is made by CIMMYT)

Rathayibacter tritici is a plant quarantine organism in Tunisia, Kazakhstan, Uzbekistan, Georgia, Moldova, The Eurasian Economic Union (EAEU), and USA. Currently it is widely distributed in Australia, Zambia, Egypt and China. In the Russian Federation, *Rathayibacter tritici* is under EAEU regulation.

Gram-negative gamma-proteobacteria *Pseudomonas fuscovaginae* (ex Tanii et al.) Miyajima, Tanii & Akita (EPPO code PSDMFU) causes brown sheeth rot as shown in figure 1.2 in cereal grain crops [2]. Currently in Egypt it is an absent quarantine organism. This phytopathogen is widely spread in countries like Tanzania, China Iran Agentina and also has been detected in Russia [3].

Figure 1.2 Cereal crops infected with *Pseudomonas fuscovaginae*. (Photograph is made by Etienne Duveiller)

Pseudomonas syringae has numerous pathovars that cause different diseases in grain plants [4]. *P. syringae* pv. *syringae* van Hall (EPPO Code: PSDMSY) has been seen to cause die back or leaf blight [5]. Reduction in seed yield, growth of plant organs, plant height and as a result plant yield are noted when plants are affected by the presence of this pathogen [4]. Synonimic to PSDMSY *P. syringae* pv. *japonica Loehnis* & Hansen has been noted to caused striations on the nodes of plants and dull blackish discolored areas on the wheat plants [5]. Whilst *Pseudomonas syringae* pv. *atrofaciens* (EPPO Code: PSDMAT) has been noted to cause basal glume blotch on wheat and triticale [6].

In oats *Pseudomonas syringae* pv. *striafaciens* caused stripe blight [6]. Whilst *Pseudomonas syringae* pv. *coronafaciens* (Elliott) Young, Dye & Wilkie has been noted to be the main cause of halo blight [7, 8]. These pathogens lead to the eventual withering of plants from the tips and yield losses [6].

Pseudomonas cannabina pv. alisalensis (*syn. P. syringae* pv. *alisalensis*) has been noted to cause leaf spot in oats [9]. Spreading has been attributed to aphids and also rain splash and leaf contact meaning that even machines can transport the pathogens from one place to another. Survival is prominent of plant and seed debris thus warranting the need to clear any and all plant and seed debris after each season [6]. *P. syringae* pv. *syringae* has been noted to cause basal kernel blight of barley [10]. *Pseudomonas syringae* pv. *striafaciens* causes bacterial stripe of barley [11]. Some pathogens are carried across fields, countries, and even continents, so the interest is in knowing exactly how they are transported or transported by *Pseudomonas syringae* pv. *atrophaciens* was noted in Iran only in 2005–2006 [5]. *Pseudomonas syringae* infected wheat plant is shown in Figure 1.3 below. Losses of up to 50% have been noted internationally as a result of infestations of *Pseudomonas syringae* is a quarantine object in Mexico [4].

Figure 1.3 Cereal crops infected with *Pseudomonas syringae*. (Photograph is made by Plantvillage)

Xanthomonas translucens (ex Jones et al.) Vauterin et al. (EPPO Code: XANTTL) has several pathovars that cause diseases in grain plants. They include *X. translucens* pv. *cerealis* (brown stripes in cereal grains), *X. translucens* pv. *translucens* (purple-black discoloration of garin seeds and black husk), *X. translucens* pv. *graminis* (initial wilting of grains and the eventual necrosis), and *X. translucens* pv. *undulosa* (black and brown stripes at the top of the scales or over the entire surface of the scales). Symptoms are shown in Fig 1.4.

Figure 1.4 Cereal crops infected with *Xanthomonas translucens*. (Photograph is provided by Ebrahim Osdaghi)

This pathogen causes bacterial leaf streak in cereal grain crops and has a quarantine status pathogen in Mexico (*X. translucens pv. cerealis*), Brazil and Israil (*X. translucens* pv. *graminis*), Tunisia, Egypt, Morocco, Chile, Jordan, Türkiye, EPPO, and Inter-African Phytosanitary Council (IAPSC) (*X. translucens* pv. *translucens*).

These phytopathogens have been noted to cause losses in yield under favourable weather conditions (hot and wet climate).

The degree of development of the topic. At the beginning of the study, there were no approved for Russian Federation guidelines for the detection and identification of

pathogens of bacteriosis of cereal crops – *P. fuscovaginae*, *P. syringae* (pathovars *syringae*, *atrofaciens*, *coronafaciens* and *lapsa*) and *X. translucens* (pathovars *translucens*, *undulosa*, *graminis*, *cerealis* and *secalis*). Existing guidelines for the detection and identification of *R. tritici* do not allow for reliable and rapid diagnosis of the causative agent of yellow mucosal bacteriosis. Scientific articles contain a description of molecular genetic diagnostic tests that require mandatory approbation and applicability assessment. In laboratories on the territory of the Russian Federation, the identification of bacterial species significant for grain export is carried out by isolation on media and assessment of the morphology of colonies, but without molecular analysis, these methods are unreliable even with the successful isolation of bacteria with morphologically similar colonies. Information in the literature on the distribution of the studied bacteria in the territory of the Russian Federation is based mainly on visual examinations and methods of classical microbiology. that are not sufficient for reliable identification.

The objects of the study

The causative agents of bacterial diseases in grain crops: *Rathayibacter tritici, Pseudomonas fuscovaginae, Pseudomonas syringae* and, *Xanthomonas translucens.*

Objective and specific tasks of the study: development and optimization of methods for the detection and identification of bacterial pathogens significant to the import and export of agricultural products by Russia.

To achieve this goal the following tasks had to be completed:

1. Collection of plant samples in different regions of the Russian Federation and examination for the presence of DNA of *Rathayibacter tritici*, *Pseudomonas fuscovaginae, Pseudomonas syringae* and *Xanthomonas translucens.*

2. Development of PCR -based diagnostics for the identification of *Xanthomonas translucens.*

3. Determination of the optimal nutrient medium for isolation *of Rathayibacter tritici.*

4. Optimization of seed sample preparation for simultaneous detection and identification *of Rathayibacter tritici, Pseudomonas fuscovaginae, P. syringae* pv. *coronafaciens* and *X. translucens* pv. *translucens*.

5. Characterization of bacterial microbiota in the plant samples from Moscow, the Stavropol region and the Republic of Crimea.

The scientific novelty of the work:

1. Plant samples of cereal grains from several regions of the Russian Federation were examined for bacterial infection by molecular methods; presence of *X. translucens* and *P. syringae* was confirmed.
2. Based on bioinformatic analysis of the genomes of genus *Xanthomonas* we identified nucleotide sequences unique to *X. translucens,* and developed new primers for PCR assay of this species.
3. Improved methods for cereal grains sample preparation and identification of pathogens have reduced assay time to 6 hours.
4. Bacterial microbiota associated with cereal grain crops in phytocenoses in Moscow, Stavropol region, and the Republic of Crimea was assayed by microbiologic and PCR methods.

Theoretical and practical significance. The dissertation is devoted to the study of pathogens of bacterial plant diseases that are significant for the export and import of grain crops, and the development of methods for their diagnosis. The Russian Federation exports more than 40 million tons of grain annually, and most of the total production goes to countries that regulate certain types of bacteria on grain. These countries also include the largest importers of Russian grain, Egypt and Türkiye. To ensure the phytosanitary requirements of grain importers, methodological recommendations are required for the detection and identification of regulated phytopathogenic bacteria. These bacteria include *Pseudomonas fuscovaginae, P. syringae* (pathovars *syringae, atrofaciens, coronafaciens* and *lapsa*), *Xanthomonas translucens (*pathovars *translucens*, *undulosa*, *graminis, cerealis and secalis*) and *Rathayibacter tritici*. At the start of the study, there were no diagnostic protocols or guidelines for *P. fuscovaginae, P. syringae* and *Xanthomonas translucens*, and methodological recommendations for *R. tritici,* a quarantine object for the EAEU countries and other countries, did not contain molecular genetic research methods.

The available information on bacterial species important for grain import, including nomenclature, biology, distribution data, phytosanitary status, affected crops and diagnostic methods, was collected and summarized. Sample preparation processes, methods of isolation of cultures and PCR tests were tested and optimized. The applicability of some PCR tests was assessed. The results of the research were used in the development of methodological recommendations of the FSBI "VNIIKR" for the detection and identification of pathogens bacteriosis of grain crops, which have now been put into operation and recommended for use by testing laboratories in the territory of the Russian Federation.

Basic provisions for defense:

1. Developing PCR tests for the identification of *Xanthomonas translucens*.
2. Identification of *Rathayibacter tritici*, *Pseudomonas fuscovaginae*, *Pseudomonas syringae*, *Pseudomonas fuscovaginae* and *Xanthomonas translucens* from the bacteria cultivated in Moscow, the Republic of Crimea and Stavropol region.

3. Identification of cultivated bacteria in Moscow, the Republic of Crimea and Stavropol region.
4. The protocols of seed sample preparation for the detection and identification of *Rathayibacter tritici*, *Pseudomonas fuscovaginae*, *P. syringae*, and *Xanthomonas translucens* were optimized.

Approbation of the work. The results of the research were presented at 3 international scientific conferences: the 20th All-Russian Conference of Young Scientists, dedicated to the memory of Academician of the Russian Academy of Agricultural Sciences Georgy Sergeevich Muromtsev, Moscow, October 27–29, 2020; the International Scientific Conference "Plant Protection in the Context of the Transition to Precision Farming", Institute of Plant Protection, Priluki (Republic of Belarus), July 27–29, 2021; the 11th International Scientific and Practical Conference "Biological Defense plants are the basis for stabilizing Agroecosystems", Krasnodar, September 12–16, 2022.

Contribution by the author personally. The applicant took part in establishing the study's goals and objectives, collected and analyzed the data, processed and evaluated the information, and generated publications as a co-author.

Structure and volume of thesis. An introduction, three chapters, conclusions, and a bibliography make up the dissertation work. On 193 pages, the information is presented along with 31 tables, 54 figures, and diagrams. There are 142 sources in the list of references.

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CHAPTER 1
LITERATURE REVIEW

1.1 General information on the causative agents of bacterial diseases of grain crops

This section provides brief characteristics of the causative agents of bacterial diseases of cereals of interest to phytosanitary services due to their presence in the quarantine list of importing countries. The most detailed are the causative agents of bacterial diseases of cereals belonging to the genera *Triticum* L. (wheat), *Secale cereale* L. (rye), *Avena* L. (oats) and *Hordeum* L. (barley) of the Poaceae family.

Quarantined products are represented by the entries indicated in Table 1.1

Table 1.1 Names of positions and TN VED codes of the EAEU of the studied products (https://classifikators.ru/tnved).

HS Code	Name of the position
1001	Wheat and meslin
1002	Rye
1003	Barley
1004	Oats

When analyzing information sources, a list of harmful organisms (bacteria) regulated by importing countries for Russian grain products was compiled (Table 1.2).

Table 1.2 Causative agents of bacteriosis of cereals and their phytosanitary status for importing countries

No p/n	Name of the pest organism (bacteria)	Country	Phytosanitary status	Source
1	*Pseudomonas fuscovaginae*	Egypt	Absent quarantine organism (A1)	[3]

		Nigeria, Sri Lanka	Unspecified status	Based on the request of the State
2	*Pseudomonas syringae* pv. *atrofaciens*	Egypt, Brazil	Absent quarantine organism (A1)	[3]
		Mexico	Quarantine organism	[3]
		Syrian Arab Republic, Pakistan	Unspecified status	Based on the request of the State
3	*Pseudomonas syringae* pv. *coronafaciens*	Mexico	Quarantine organism	[3]
		Egypt	Absent quarantine organism (A1)	[3]
		East Africa, South Africa	Limited-prevalent quarantine organism	[3]
4	*Pseudomonas syringae* pv. *syringae*	Mexico	Quarantine organism	[3]
		Egypt	Regulated non-quarantined organism	[3]
		Jordan	Limited-prevalent quarantine organism	[3]
		Pakistan	Unspecified status	Based on the request of the State
5	*Xanthomonas translucens* pv. *cerealis*	Mexico	Quarantine organism	[3]
6	*Xanthomonas*	South	Absent quarantine	[3]

	translucens pv. *graminis*	Africa, Brazil	organism (A1)	
		Israel	Quarantine organism	[3]
		Egypt	Included in phytosanitary requirements	[1]
7	*Xanthomonas translucens* pv. *translucens*	Egypt, Chile	Absent quarantine organism (A1)	[3]
		Jordan, Turkey	Limited-prevalent quarantine organism	[3]
		Morocco, Tunisia	Quarantine organism	[3]
		Nigeria	Prohibited for import	[1]
		Syrian Arab Republic, Pakistan, Israel, South Africa	Unspecified status	Based on the request of the State
8	*Xanthomonas translucens* pv. *undulosa*	Nigeria	Quarantine organism	[3]
		South Africa	Unspecified status	Based on the request of the State
9	*Rathayibacter tritici*	Tunisia, USA	Quarantine organism	[3]
		Pakistan, Bangladesh	Unspecified status	Based on the request of the State

Nine excisting bacteriosis of grain crops that have phytosanitary value for quarantine products are going to be studied.

Thus, nine exciters of grain crop bacteriosis have phytosanitary value for quarantine products.

1.1.1 *Rathayibacter tritici*

The Systematic statement Kingdom: Bacteria, Subkingdom: Posibacteria, Type: Actinobacteria, Subclass: Actinobacteridae, Order: Actinomycetales, Family: Microbacteriaceae, Genus: *Rathayibacter*, Species: *Rathayibacter tritici* [17]. **The computer code on EPPO is** CORBTR. **Scientific name:** *Rathayibacter tritici* [Carlson and Vidaver, 1982 Zgurskaya et al., 1993 [17]. **The list of synonyms.** *Clavibacter tritici*; *Corynebacterium michiganense* pv. *tritici*; *Corynebacterium tritici*; *Phytomonas tritici*; *Pseudomonas tritici* [3]. **Common names:** bacterial ear rot of wheat, gumming disease of wheat, yellow slime of wheat (English), bactériose des épis du blé (French), bacteriosis de las espigas del trigo (Spanish) [3]. **Geographical distribution.** Distributed in Africa (Egypt, Ethiopia, Morocco, Zambia), Asia (Afghanistan, China, India, Iran, Iraq, Pakistan), Europe (Cyprus), Oceania (Australia) as shown in figure 1.5 [18]. Of these countries *R. tritici* is a quarantine pathogen in China and Australia to date.

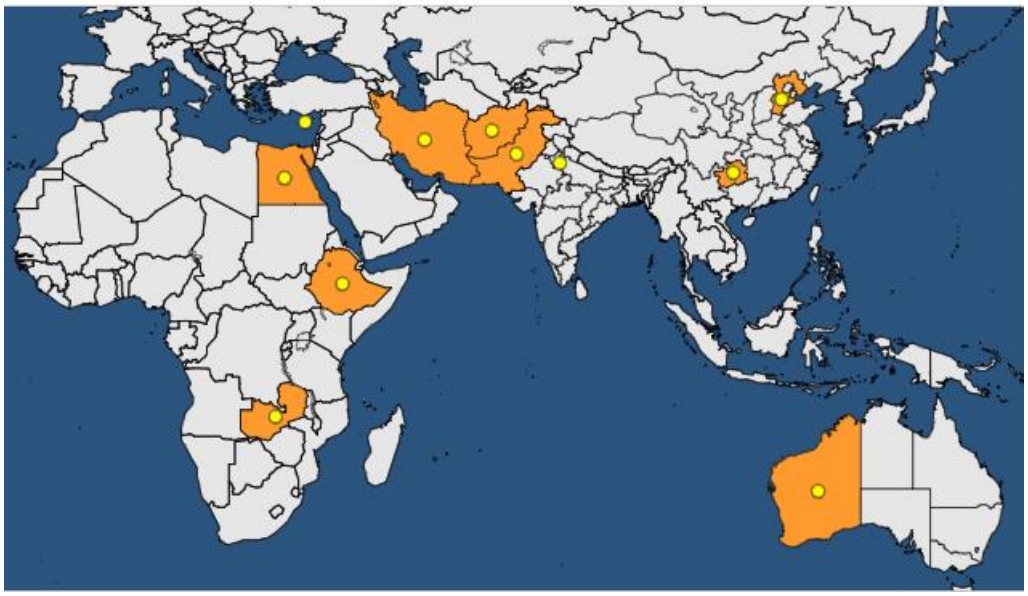

Figure 1.5 Geographical distribution of *R. tritici* N.B the areas marked represent the areas in which the bacterium is present [3]

Description of morphology. Gram-positive, non-motile and rod-shaped bacterium. On the medium it forms colonies of yellow color, round, regular shape, up to 3 mm in diameter [19].

Information about the affected plants. It infects cereal grain crops

including barley and wheat [3;19]. *R. tritici* is known to cause a wide range of diseases in cereal grain crops, including spike blight also known as tundu disease and gummosis [19]. **Migration and distribution methods.** It is transmitted by mainly a nematode vector known as *Anguina tritici* [20]. This nematode can survive in larvae form in the soils for years. Infection occurs with the help of a nematode larvae, which remain viable in the soil for five to seven years, and in dry grain - more than twenty. At the same time, the causative agent of the disease can persist in nematode galls for up to two years. The disease spreads on grain crops in the form of foci [19]. *Rathayibacter tritici* is a soil bacterium which thrives in wheat seeds, and also in the soil for more than two years and seven years, respectively [20]. **Symptoms.** On the sprouts along the leaf margin and between the veins, longitudinal white or yellowish discoloration is observed, over time, exudate appears on them, and they turn yellow. Severely affected leaves dry up and die. Weakly affected plants lag behind in development and form dwarf spikelets. In the next stages of development, white, less often yellowish narrow longitudinal stripes are formed, folding, twisting and licking of leaves are observed, stems and ears. The spike, together with the wrapping leaf, turns yellow, curves, forming a shapeless, seedless ugly mass that is covered with viscous yellowish mucus. *R. tritici* is known to cause a wide range of diseases in cereal crops, including spike blight also known as tundu disease and gummosis [19]. Once it affects plants it is known to cause seed galls in the grain plants ears and as a result spike blight referred to as yellow ear rot, Tundu disease or yellow slime rot [21]. *Anguina tritici* is a nematode known as a vector of this bacterial phytopathogen. *R. tritici* is a fervent phytopathogen that has been noted to be causing significant yield losses internationally in countries like Iran, China, Australia, Pakistan and Cyprus [22; 23]. **Harmfulness and economic significance.** 30 – 70% yield losses were observed in [14].

1.1.2 *Xanthomonas translucens*

The Systematic statement Kingdom: Bacteria, Subkingdom: Negibacteria, Type: Proteobacteria, Class: Gammaproteobacteria, Order: Xanthomonadales, Family: Xanthomonadaceae, Genus: Xanthomonas, Species: *Xanthomonas*

translucens [17]. Bacteria belonging to this species are gram-negative. It affects plants belonging to the species Poaceae. Some pathovars have the capability to infect and can be isolated from pistachio trees and also ornamental asparagus trees. *X. translucens* strains affect both broad and narrow host ranges. Mostly have a narrow host range causing bacterial leaf streak (BLS) also known as cereal leaf streak (CLS) diseases [24; 25]. This bacterial species has numerous pathovars distinguished by their biochemical and molecular and ranges of hosts. As a result, they are placed into two major groups that is graminis and translucens. The graminis group affects plants in the forage grass species. This includes the following pathovars arrhenatheri, graminis, poae, phlei and pistaciae. *X. translucens* causes bacterial wilt. The translucens group affects plants in the cereal crop species whose pathovars include undulosa, translucens, cerealis, hordei, and secalis. *X. translucens* in this group causes leaf streak [26]. There are several Xanthomonas species, and each has a particular pathophysiology and host target. Because xanthomonads have rather narrow crop preferences, each species is unique to a single host [27]. These bacteria are known to affect cereal grain crops, *Xanthomonas translucens* pv. *hordei* affects barley, *Xanthomonas translucens* pv. *undulosa* affects wheat, barley, and rye, *Xanthomonas translucens* pv. *secalis* affects rye, *Xanthomonas translucens* pv. *hordei-avenae* affects barley and oat, and *Xanthomonas translucens* pv. *cerealis* affects wheat, barley, rye, and oat [26; 28].

Symptoms of BLS disease start off as water-soaked streaks (Figure 1b), develop and become translucent necrotic lesions on leaves and spikes (Figure 1c). At times yellow bacteria exudates oozes can be spotted on the leaf surface of infected plants see figure 1a. In severe cases the entire leaf surface is affected. Dark purple streaks known as black chaff develop on glumes see figure 1.6 [29].

Figure 1.6 signs and symptoms of BLS on leaf surfaces and spikes that is 1a) yellow bacteria exudates oozes b) water-soaked streaks c) translucent necrotic lesions d) black chaff Photo [26].

Individual passenger goods are also included in the lists of importing countries. Among them: *X. translucens* pv. *cerealis, X. translucens* pv. *translucens, X. translucens* pv. *graminis and X. translucens* pv. *undulosa.*

Xanthomonas translucens* pv. *cerealis

The computer code on EPPO: XANTCE. **Scientific name:** *Xanthomonas translucens* pv. *cerealis* [28; 3]. **Common name:** bacterial streak of grasses [3]. **A list of synonyms.** *Xanthomonas campestris pv. cerealis* (Hagborg) Dye; *Xanthomonas translucens* f. sp. *cereal* [3; 28]. **Geographical distribution**: Africa (Ethiopia, Kenya, Madagascar, Morocco, South Africa, Tanzania, Tunisia, Zambia), Asia (Azerbaijan, China, Georgia, India, Iran, Israel, Japan, Kazakhstan, Malaysia, Pakistan, Syria, Turkey, Yemen), Europe (Romania, Russia, Ukraine), North America (Canada, Mexico, USA), Oceania (Australia), South America (Argentina, Bolivia, Brazil, Paraguay, Peru, Uruguay) [18]. **Quarantine status**: Mexico has classified the phytopathogen as a high-risk quarantine pathogen. As a result, *X. translucens pv. cerealis* is under zero tolerance and under strict quarantine control [3]. **Environmental Requirements:** The bacteria thrive in areas that have humid and rainy springs, in some cases areas where the main system of irrrigation is sprinker. Morphology the colonies are saprophytic and yellow. **Information about the affected plants.** It affects cereals such as wheat, rye, barley

and oats. **Migration** It is seed bourne as such *X. translucens* pv. *cerealis* is transmitted by seeds. Leaves and sterms have also been noted to carry the pathogen. **Symptoms.** It is believed that the bacterium affects rye to a greater extent, but on other cereals symptoms also appear. Watery dark green spots appear on the leaves, which gradually increase, darken and turn into brown stripes. The surface of the stripes in conditions of high humidity is usually covered with milky white drops of bacterial exudate, and in dry weather a thin transparent film or yellowish dry granules form on them [30]. **Harmfulness and economic significance:** Economic significance depends mainly on the climate of the environment, in areas that have humid, warm springs it can cause losses [26]. This bacterium strain is seed bourne thus making infected seeds a great source of the bacteria acting as a highly efficient inoculum, and this may be detrimental to yields [31]. This bacterium strain is of moderate to low importance in other areas whose climates are not conjusive [18]. Losses of up to 40% have been noted and has been seen to vary depending on the prevailing climate, the amount of inoculum, the susceptibility of the host [32; 26]. **Method of diagnosis:** Primers designed by Langlois et al. (2017) were not pathovar specific to date the most efficient method of identification is pathogenicity test on creal grain plants (also wild grasses) and small grain cereals and the use of molecular phylogenetics [25].

Xanthomonas translucens* pv. *translucens

The computer code on EPPO is XANTTR. **Scientific name:** *Xanthomonas translucens* pv. *translucens* [3]. **Common name:** bacterial leaf streak of barley, bacterial leaf streak of wheat, black chaff of cereals [3]. **A list of synonyms.** *Pseudomonas translucens*; X. *campestris* pv. *hordei*; *X. campestris* pv. *translucens*; *X. translucens*; *X. translucens pv. hordei* [28; 22; 3]. **Geographical distribution.** Africa (Ethiopia, Kenya, Madagascar, Morocco, Tanzania, Tunisia, Zambia, South Africa), Asia (Azerbaijan, China, Georgia, India, Iran, Israel, Japan, Kazakhstan, Malaysia, Pakistan, Syria, Turkey, Yemen), Europe (Romania, Russia, Ukraine), North America (Canada, Mexico, USA), Oceania (Australia), South America (Argentina, Bolivia, Brazil, Paraguay, Peru, Uruguay) as shown in figure 1.7 [18].

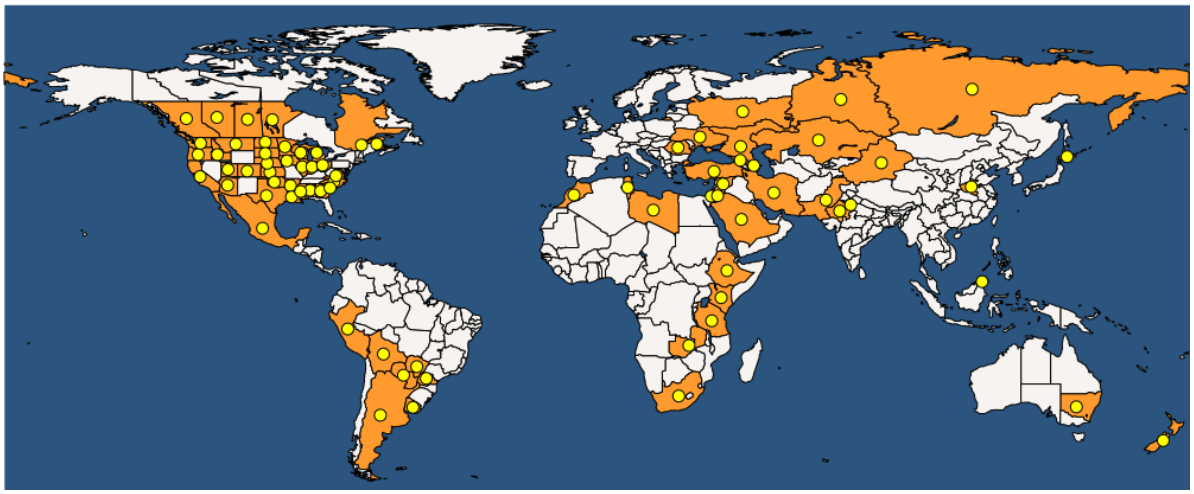

Figure 1.7 Geographical distribution of *Xanthomonas translucens* pv. *translucens* N.B the areas marked represent the areas in which the bacterium is present [3]

Qurantine status: The bacterium is qualified as an A2 qurantine phytopathogen on IAPSC (Inter-African Phytosanitary Council) and eppo. In Asia it is considered a regulated quarantine agent. **Enviromental Requirements:** It has been seen to thrive in areas with high humidity that is high precipitation. **Morphology:** colonies are known to be light yellow, with smooth and convex margins and mucoid. The bacteria are gram-negative, with one polar flagellum and also [34]. **Information about the affected plants.** The main host plant is barley. It affects rye and wheat, as well as grasses: meadow timothy, bromus and creeping wheatgrass. **Migration and distribution methods.** The pathogen can be widely spread by seeds through trade between countries [35]. In the field, bacteria are transmitted by droplet-liquid moisture. In addition, aphids caught in sticky exudates can carry the bacterium and transmit it to wheat and barley, thereby contributing to the spread over long distances [36]. **Symptoms.** On the leaves, narrow, water-soaked stripes appear, yellowish in barley and triticale, necrotic in the center with edges of rusty color in wheat. Bacterial mucus is released and dries to a thin layer similar to scales, which can exfoliate. On young plants, symptoms are practically not manifested. On grains, symptoms of "black husk" with a purple-black discoloration of the surface appear. Symptoms appear 10 – 14 days after inoculation [35]. **Harmfulness and economic significance.** Direct crop losses were estimated to be between 10% or less and 40%, depending on the authors (EPPO. Data Sheets on Quarantine Pests. *X. translucens* pv. *translucens*). **Methods of diagnosis:** The

injection technique where the bacteria are injected into susceptible host plants. Another method that was developed included the use of polyclonal antiserum in the ELSA procedure which was able to detect 5000 c.f.u./ml of the phytopathogen. Primers were developed that can identified a wide range of *Xanthomonas* pathovars [35].

Xanthomonas translucens* pv. *graminis

The computer code on EPPO is XANTGR. **Scientific name:** *Xanthomonas translucens* pv. *graminis* [3]. **Common names:** bacterial wilt of grasses [3]. **A list of synonyms.** *Xanthomonas campestris* pv. *graminis* [22]; *Xanthomonas graminis* [37]. **Geographical distribution.** Europe (Belgium, France, Germany, Netherlands, Norway, Poland, Switzerland, United Kingdom) as shown in figure 1.8 [18].

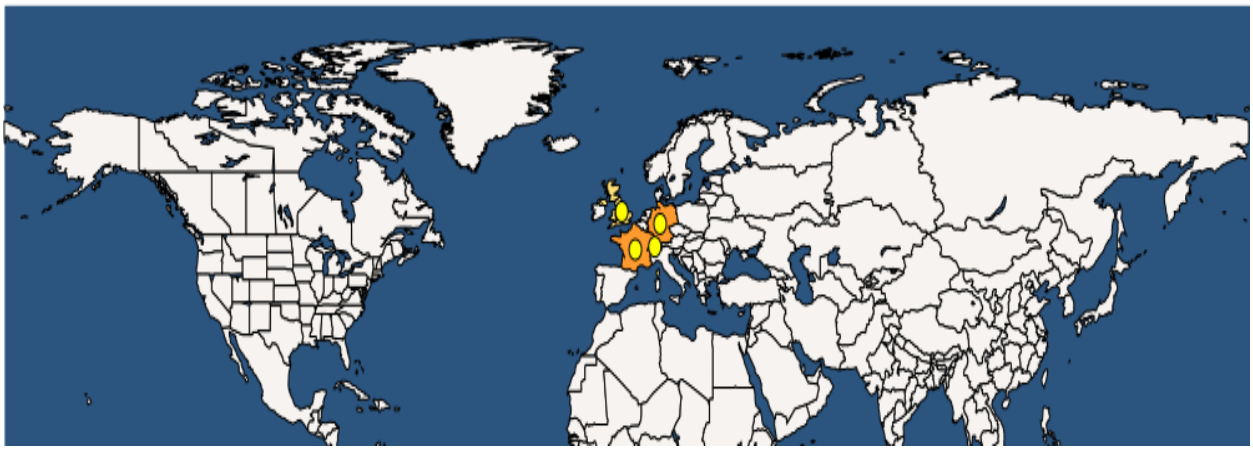

Figure 1.8 Geographical distribution of *Xanthomonas translucens* pv. *graminis* N.B the areas marked represent the areas in which the bacterium is present [3]

Quarantine status. It is noted as an A2 qurantine pathogen. **Environmetal requirements.** Known to thrive in warm humid conditions. **Morphology.** Bacteria known to be non-spore forming rod shaped with a single polar flagella and gram negative. **Information about the infected plants.** It affects cereals, including wheat, rye, barley and wheat. of the wild species, it can affect the hedgehog. **Migration and distribution methods.** The pathogen enters the plant through injuries and initially colonizes the protoxyl lacuna, from where it migrates to the vascular tissue [38]. Transmitted by seeds [18]. Symptoms The leaves of infected plants tend to wilt causing bacterial wilt. Yellow stripes are a common feature on tillers of plants and and times on the leaf blades. Upon inculation the pathogen is so

severe that highly susceptible plants fall prey and die within three weeks. Deforemed inflorescences and reduced regrowth are common site in resistant or less susceptible plants [37; 39]. **Harmfulness and economic significance.** Inoculation by a pathogen leads to wilting of leaves and necrosis of the entire plant. Selection of resistant varieties based on recurrent phenotypic selection has led to the emergence of varieties with improved partial resistance to bacterial wilt, but completely resistant varieties are absent. The disease can lead to significant losses in the yield of cereal plants depending on the susceptibility of the host [38]. **Method of Diagnosis.** Pathogenecity tests have been used for diagnosis where plants are inculated with the bacteria and the symproms were observed after 28days [40]. Genome sequencing after the amplification of the bacteria is another method of diagnosis [41]. Non-graminis publicly available data used in genome comparison, a method in which non-graminis strains were annotated *de novo*. This analysis was done using EDGAR 2.0 [40]. Predicted singletons and virulence-contributing gene clusters were characteristed and identified using a method called singleton and gene cluster analysis. This was done by combined approach of signal peptide prediction by SignalP 4.0 [42], BLASTP analysis [43] and conserved domain search [44]. Mortility assay is another method that has been used for the diagnosis of this bacterium. In this method the bacterial colonies are transferred on to nutrient medium and photographed 10 days after incubation [40].

Xanthomonas translucens* pv. *undulosa

The computer code of the EPPO is XANTTU. **Scientific name:** *Xanthomonas translucens* pv. *undulosa* [3]. **Common names** bacterial leaf streak of wheat, black chaff of wheat, brown streak of wheat the causative agent of black bacteriosis of wheat. [3]. **A list of synonyms.** *Phytomonas translucens* var. *undulosa* Stapp; *X. campestris* pv. *undulosa*; *X. translucens* f. sp. *undulosa* [3; 22]. **Geographical distribution.** Phytopathogen is distributed in Africa (Ethiopia, Kenya, South Africa, Tanzania), Asia (China, Georgia, India, Israel, Kazakhstan, Malaysia, Pakistan), Europe (Belgium, France, Russia, Sweden, Ukraine), North America (Canada, Mexico, USA), Oceania (Australia) and South America (Brazil)

as shown in figure 1.9 [18].

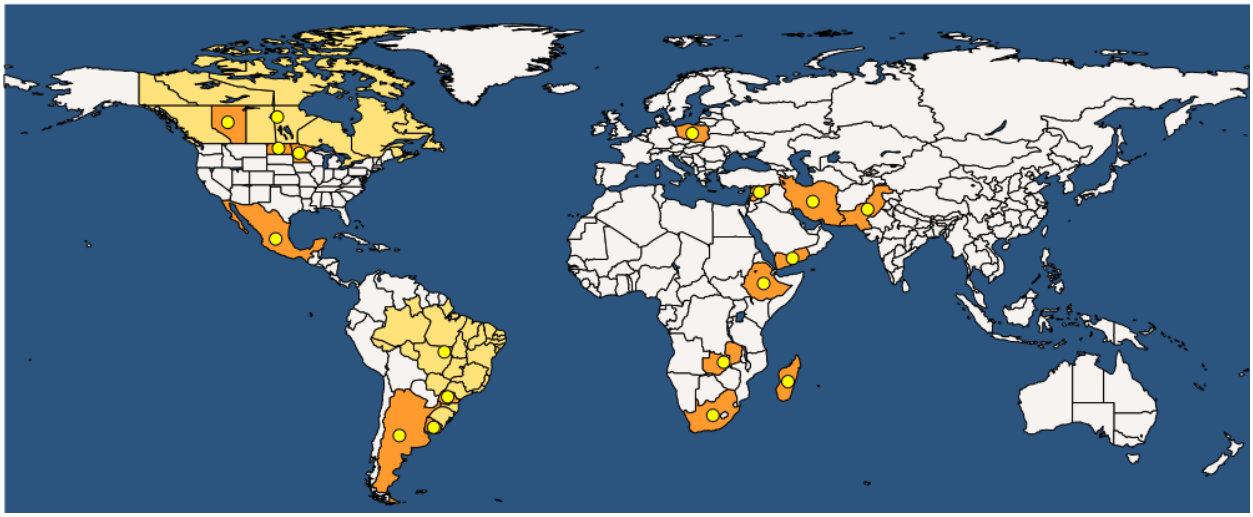

Figure 1.9 Geographical distribution of *Xanthomonas translucens* pv. *undulosa* N.B the areas marked represent the areas in which the bacterium is present [3]

Environmental requirements. The pathogen that causes BLS in wheat, *X. translucens* pv. *undulosa*, can overwinter in weed grasses. **Information about the affected plants.** The pathogen affects oats, wheat and barley [45]. **Symptoms.** It affects all organs of wheat plants in the growing season, with the formation of transparent stripes that eventually become yellow and brown, and liquid light green spots that increase in size, become chlorotic, dark yellow, brown with a black border. On the stem at the base of the leaves, black-brown spots or brown longitudinal stripes form that can spread throughout the stem. It affects the spikelets, leading to the appearance of black and brown spots or stripes at the top of the scales or over the entire surface of the scales (Figure 1.10).

Figure 1.10 – Symptoms of *Xanthomonas translucens* pv. *undulosa*. Photo: cimmyt/5071612063

With a strong lesion, the ear decreases, deforms, curves, and an unfulfilled grain develops. Under natural conditions, in wet weather, exudate appears on the grain in the form of mucus of whitish or yellowish color, which in the process of drying turns into granules or a gray mass. Harm is manifested in a decrease in the total number and length of ears, the number of grains in the ear. The development of the disease is facilitated by high relative humidity 70 -80% and a temperature of 25-30°C [30]. **Harmfulness and economic significance.** It leads to a decrease in the mass of 1,000 grains by 60– 62%. The grain yield decreases by 15 –90%, depending on the variety, geographical location and climatic conditions [30]. It brings about 30 –40% of crop losses worldwide [45]. **Method of diagnosis.** A limited number of housekeeping genes have been subjected to multilocus sequence typing (MLST) and multilocus sequence analysis (MLSA) in order to evaluate genetic diversity among closely related strains, predict pathovar designation, and differentiate closely related bacterial species [46; 47; 48]. Studies of bacterial pathogens that were not possible with the traditional morphological techniques can now be conducted thanks to the highly reproducible molecular analyses and the ability to compare bacterial strains across studies with the help of sequence data [48; 49]. Prior MLSA investigations have identified pathovars of *X. translucens* and distinguished between distinct *Xanthomonas* species using four housekeeping genes (rpoD, dnaK, fyuA, and gyrB) [48; 49]. By definition, planta assays are required to confirm pathovar identification, but MLSA is helpful in predicting pathovar designation [22].

1.1.3 *Pseudomonas fuscovaginae*

The Systematic position Kingdom: Bacteria, Subkingdom: Negibacteria, Type: Proteobacteria, Class: Gammaproteobacteria, Order: Pseudomonadales, Family: Pseudomonadaceae, Genus: *Pseudomonas*, Species: *Pseudomonas fuscovaginae* [17]**. The computer code of the EPPO is** PSDMFU. **Scientific title:** *Pseudomonas fuscovaginae* [17; 50]. **Common name:** sheath brown rot (English)

cereal shell leaf brown rot [2; 3]. **Geographical distribution.** Distributed in Asia (China, Indonesia, Iran, Japan, Malaysia, Philippines, Nepal, South Korea), Europe (Russia, Serbia), Oceania (Australia), South America (Brazil, Bolivia, Argentina, Colombia, Ecuador, Peru, Uruguay), North America (Costa Rica, Cuba, Dominican Republic, El Salvador, Guatemala, Jamaica, Mexico, Nicaragua, Panama, Trinidad and Tobago), Africa (Burundi, Congo, Rouen, Tanzania and Madagascar) [18]. **Quarantine status** Egypt has catogerised *Pseudomonas fuscovaginae* as an A1 pest. A1 pests are those that are not found in a particular nation, even though they may be in that nation's close neighbours [3]. **Environmental requirements Description of morphology.** *P. fuscovaginae* belong to the *P. fluorescens* group. This bacterium is a gram-negative fluorescent bacterium [51]. Phytopathogen was first identified and described as the causative agent of rice bacteriosis in Japan in 1976 [52]. **Information about the affected plants.** It mainly affects wheat, but *P. fuscovaginae* is now considered a plant pathogen for other cereals, including corn, sorghum and wheat [52]. **Migration Symptoms.** Young plants darken and die if infected [53]. Adult rice plants develop brown-black, water-soaked spots on the adaxial side of the leaf sheath of the flag leaf [54]. The spike is poorly formed, the grain becomes discolored or may not be tied at all [52]. **Harmfulness and economic significance.** Infected seedlings have been known to die within one to two weeks [55]. **Current methods of dianosis.** Pathogenicity tests have been used for the diagnosis of this bacterium [55].

1.1.4 *Pseudomonas syringae*

The Systematic statement Kingdom: Bacteria, Type: Proteobacteria, Class: Gammaproteobacteria, Order: Pseudomonadales, Family: Pseudomonadaceae, Genus: *Pseudomonas*, Species: *Pseudomonas syringae* [17]. **Scientific name:** *Pseudomonas syringae* [17]. Pathovars of this bacterium have been listed in the top 10 list of plant pathogenic bacteria in microbiology [27]. According to a number of sources, this species has no subordinate taxa [17; 56]. Wet, chilly weather is generally favourable for *P. syringae* caused diseases; ideal disease temperatures

range from 12 to 25 °C (54 to 77 °F), though this might vary depending on the pathovar [57].

P. syringae is also able to withstand temperatures that are somewhat below freezing. The severity of infection increases in these below-freezing temperatures [58]. These bacteria can overwinter on plant tissues that appear healthy, but they can also do so on sick plant tissues, such as areas with gummosis and, in certain situations, necrosis (sap oozing from tree wounds). Rain or irrigation water in the spring will carry the bacteria onto leaves and blooms, where it will proliferate and endure the summer [58] *P. syringae* will proliferate and spread during this part of its life cycle, known as the epiphyte phase, but it won't produce an illness. The disease will begin as soon as it enters the plant through the stomata on a leaf or as necrotic areas on leaves or woody tissue [59]. After that, the pathogen will take advantage of the intercellular space and spread, producing cankers and leaf spots. *P. syringae* is also able to withstand temperatures that are somewhat below freezing. The intensity of the illness in trees such as sour cherry, apricot, and peach is heightened by these below-freezing conditions [58]. Other sources mention various pathovars of this species, including those significant for cereals [3; 18]. It is evident that *P. syringae* continues to produce commercially significant plant diseases and has had a significant influence on the scientific comprehension of microbial pathogenicity [27]. Individual passenger goods are also included in the lists of importing countries. Among them: *P. syringae* pv. *atrofaciens, P. syringae* pv. *coronafaciens*, *P. syringae* pv. *syringae* [59].

Pseudomonas syringae* pv. *atrofaciens

The computer code on EPPO: PSDMAT. **Scientific name:** *Pseudomonas syringae* pv. *atrofaciens* [3; 22; 63]. **Common name:** basal glume rot of wheat [3]. **Synonym:** *Pseudomonas syringae* pv. *atrofaciens*. **Geographical distribution.** The bacterium was found in Africa (Morocco, South Africa, Zimbabwe), Asia (Iran), Europe (Bulgaria, Czech Republic, Russia, Germany, Italy, Romania, Ukraine), North America (Canada, USA), Oceania (Australia, New Zealand) [18]. **Quarantine status** Egypt and Brazil have catogerised *Pseudomonas syringae* pv. *atrofaciens* as an A1 pest. A1 pests are those that are not found in a particular

nation, even though they may be in that nation's close neighbours. In Mexico *Pseudomonas syringae* pv. *atrofaciens* has been categorized as a Quarantine Phyotpathogen [3]. **Information about the affected plants.** Under natural conditions, in addition to wheat, phytopathogen causes lesions of rye, barley and oats [30]. It can also affect tomato and tobacco [18]. **Migration** Rain splash spreads *P. syringae* pv. *atrofaciens*, which is seed-borne, across plants. [57]. **Symptoms.** A characteristic feature is the decay of the lower part of the sterm, as well as the tillering node. The spotting of various parts of plants, wilting of individual leaves or the whole plant [30]. Transparent liquid, oily, brown, whitish or yellow elongated spots with a brown or red-brown border form on the leaves. On leaves, a clear, liquid border that is typically greasy and brownish-whitish appears [60]. On the leaves themselves, there are instances where long, yellow dots with a brown or reddish-brown border appear. Occasionally, dark strokes emerge that, when combined, give the lower portion of the stem a shade of black. Necrotic brown patches form on tissue, which weakens and causes individual leaves or the entire plant to wilt, occur during the decay of the tillering node on the lower portion of the stem and the leaves [57]. The wrapping leaf may occasionally develop dry rot, while the upper leaves wrinkle and turn yellow-brown. A severe lesion results in a persistent browning of numerous scales, deformity of the ear's stems and rod. When there is a severe lesion, numerous scales continuously turn brown, and the ear's stems and rod become distorted [60]. An unfilled brown grain with a darker embryo form in these areas. The bacterium is known for causing basal glume rot of wheat [27]. **Harmfulness and economic significance.** The lesion is up to 15%, but this figure increases in years susceptible to the development of the pathogen, and can reach 30-80%. With a strong degree of development of the disease, from 10 to 80% of the ears are affected. Basal bacteriosis worsens the commercial qualities of cereals, reduces the mass of grains, germination energy and germination of seeds [60]. The greatest development of the disease of cereals is noted in years with elevated temperatures in spring and summer, with a large amount of precipitation and high humidity during spikes [30]. **Method of diagnosis.** This bacterium has been identified based on the MLSA scheme [60].

Pseudomonas syringae* pv. *coronafaciens

The computer code on EPPO: PSDMCO. **Scientific name:** *P. syringae* pv. *coronafaciens* [3; 22; 49]. **Common names:** bacterial blight of oat, chocolate spot of corn, halo blight of oat (English), taches aréolées de l'avoine (French), bakterielle Blattdürre: Hafer, bakterielle Blattfleckenkrankheit: Hafer, Fleckenbakteriose: Hafer (German), manchas areoladas de la avena (Spanish) [3]. **Geographical distribution.** Africa (Ethiopia, Kenya, Zimbabwe, Morocco), Asia (Japan, South Korea, Uzbekistan), Europe (Denmark, Germany, Ireland, Poland, Romania, Russia, Serbia, United Kingdom), North America (Canada, Mexico, United States), South America (Argentina, Chile, Brazil), Oceania (Australia, New Zealand) [18]. **Information about the affected plants.** The main host plants are rye and oats. The pathogen can also affect other economically valuable crops of the Poaceae family [18]. **Symptoms.** Several types of lesions develop on rye leaves. The first type is observed at the border of healthy tissue and frost-affected leaf tips. The lesions are round or elliptical in shape, light or dark brown in color. The second type is linear, spots of light or dark brown color, spread 5–10 cm along the leaf, capturing the middle vein. Brown necrotic tissue is bordered by a narrow strip of yellow wilted tissue. Typical lesions are formed when the temperature rises up to 15-22°C [49]. First, brown spots appear along the edge of the leaf or along the vein with oval chlorous zones around a diameter of 15 mm. After a few days, the color of the spots turns pale, lightens, and the tissue in the center becomes indented and necrotic. Almost always, the pathogen causes intensive lightening of the leaf plate tissue. The central zone is bordered by a light yellow-green halo 2–3 mm wide. All the tissue of new lesions necroticizes and becomes light brown, and old lesions have a dark brown color. The affected areas merge and often cover the entire leaf. Both the wrapping leaf and the ear of rye are also damaged. If the lesion develops during the flowering period, no seeds are formed [30].

Pseudomonas syringae* pv. *syringae

The computer code on EPPO is PSDMSY. **Scientific name:** *Pseudomonas*

syringae pv. *syringae* [3]. **Common names:** bacterial canker of lilac, bacterial canker of pear, bacterial canker of stone fruits, bacterial canker of trees, bacterial leaf spot of sorghum, bacterial stem blight of alfalfa, black pit of citrus, blast of citrus, brown spot of bean, chocolate spot of maize (English) [3]. **Synonyms:** *Phytomonas vignae*; *Pseudomonas holci*; *Pseudomonas japonica*; *Pseudomonas medicaginis*; *Pseudomonas striafaciens* var. *japonica*; *Pseudomonas syringae* pv. *japonica, Pseudomonas vignae* [3; 22; 61]. **Geographical distribution.** The pathogen is common in Africa (Algeria, Egypt, Ethiopia, Kenya, Lesotho, Libya, Malawi, Morocco, Nigeria, South Africa, Tanzania, Tunisia, Uganda, Zimbabwe), Asia (Afghanistan, Azerbaijan, Bangladesh, China, Georgia, India, Iran, Iraq, Israel, Japan, Kazakhstan, Kyrgyzstan, Lebanon, North Korea, Pakistan, South Korea, Sri Lanka, Thailand, Turkey, Uzbekistan, Vietnam), Europe (Austria, Belarus, Belgium, Bosnia and Herzegovina, Bulgaria, Cyprus, Czech Republic, Serbia, Denmark, France, Germany, Greece, Hungary, Ireland, Latvia, Lithuania, Moldova, Montenegro, Netherlands, North Macedonia, Norway, Poland, Portugal, Romania, Russia, Serbia, Slovenia, Spain, Sweden, Switzerland, Ukraine, United Kingdom), North America (Barbados, Canada, El Salvador, Guatemala, Honduras, Mexico, Panama, Puerto Rico, USA), Oceania (Australia, New Zealand), South America (Argentina, Brazil, Chile, Uruguay, Venezuela) [18].

Environmental Requirements This phytopathogen is known to thrive in warm and moist conditions with little radiation exposure [64]. Although some of the strains within this pathovar are sensitive to environmental conditions *P. syringae* pv. *syringae* (*Pss*) strain B728a has been seen to thrive in stressful conditions as sunlight and dryness that are hostile to bacterial growth [62]. **Information about the affected plants.** It can affect a wide range of plants of different species and families; of the cereals, these are wheat, rye, barley and oats [18]. Up to 40 plant species can be infected by *Pss* [63]. **Symptoms.** Symptoms are more pronounced on barley plants in the form of spots on the leaves. Over time, the spots spread along the vein, become light brown, and then dark brown. The spike is most affected. At the heart of the husk, watery brownish lesions form that spread to the spikelets, which leads to their loss. The critical period of grain damage

continues from the stage of milk ripeness to waxy and is 65-74%, respectively. The optimal temperature for inoculation and development of bacteriosis from 28 to 30°C [30]. Comparative Genomics has been used to disgnosize the pathogen in which comparative analysis of *Pss* strains (Pss B728a and Pst DC3000) to find the homologous genes between *Pseudomonas* spp [64].

1.2. Information on extracted crops and the regions from which sampleas were collected

Climatic conditions are essential when selection of crops to cultivate, they also affect crop productivity, composition and yield flactuations. Majority of the Russian Federation is so cold that the only crops exceptional for cultivation are either early-maturing or hardy crops. Frost occurrence within the Russian Federation provide great variability of conditions and crops that can florish and grow within the region mainly in terms of the first and last occurrence of frost (White, 1987).

The length of the growing season is limited by frost and range of crops to be cultivated tends to be restricted by the lack of days over 20°C. Essentialy the period in which areas are free from killing frost is the growing season. In the Black Earth region, famous for its rich soils the growing season is only 130 to 160 days long. The growing season in the major agricultural region the North Caucasus and Volga basin, south of the Russian Federation is characterized by a growing period of 165 to 200 days. In the central regions of the Russian Federation, the growing season only lasts between 110 and 130 days. Further to the north, the growing season could be shorter than 110 days (Arkhangelsk oblast). In the steppe regions of Western Siberia, the growing season decreases to between 115 and 130 days (Khomyakov et al., 2001). The growing season everywhere in the Russian Federation is considerably shorter than in Western Europe (260 to 300 days) .

During the growing season winter rye flourishes in cold and dry enviroments thus making it an ideal crop in the central and northern parts of the Russian Federation. The root system of rye aids in penetration of the gray podzolic soils mainly found in the forest zones. Due to the nature of the root system that tends to be hard compacted soils are penetrated more easily and as a result deep ploughing becomes a lesser requirement. This cereal crop is well known for competing better with weeds (White, 1987).

In regions of low moisture and early frost barley thrives as it can withstand these conditions. Well-drained loam soils are most ideal for barley although it

produces good yield in clay soils. As a result of these peculiarities this cereal crop has a wide geographical distribution in the Russian Federtation. Barley is grown in the Arid warmer regions and the cooler northern regions of the country. The area occupied by barley is constantly increasing due the growth in demand for the crop both for animal and human consumption.

Oats are mainly produced in forest zones as they can easily withstand the acid regime of poor podzolic soils. This cereal crop tends to be more vulnerable to weather conditions especially moisture and heat and as a result are easily impacted by drought (Kruchkov and Rakovskaya, 1990).

In the Russian Federtation, the most important food crop under cultivation is wheat, which is planted on more than 50 percent of the cereal-crop area since the 1960s and 1970s. This cereal crop is very susceptible to soil characteristics like acidity and cool weather. Due to these factors geographical distribution of the crop is limited to the steppe and wooded steppe zones. In the Ukraine, the Northern Caucasus and the Black Earth regions, the main crop under cultivation was wheat as their regional conditions for crop overwintering are most favorable (Kruchkov and Rakovskaya, 1990).

Winter wheat production was limited by late but hot summers, dry autumns, and frequently a light snow cover in a severe winter which happen to be the climatic characteristics of the south of Western Siberia and northern Kazakhstan. As a result, these areas produce spring wheat (Kruchkov and Rakovskaya, 1990).

1.3. Information on current diagnosis tests available

It is established that the regulatory document on detection and identification is available only for *Rathayibacter tritici* (Table 1.2). It should be noted that this method does not describe the PCR tests. The lack of the possibility of PCR identification of the target bacterium by this method reduces its relevance. For *Pseudomonas syringae, Xanthomonas translucens*, *Pseudomonas fuscovaginae*, and *Rathayibacter tritici*, a description of real-time PCR (PCR-RT) is found in the literature (Table 1.2). PCR-RT is the most preferred diagnostic test for routine

diagnostics due to minimal time and labor costs, so tests based on PCR-RT are subject to assessment of applicability first [65].

At least one PCR test was found for each target bacterium (Table 1.2). Most PCR tests, according to the authors, are species-specific, which suggests that there is no need for subsequent sequencing. However, in the process of evaluating the applicability of PCR in testing a large number of closely related bacteria, results may be obtained that may impose restrictions on the use of tests. For example, when testing PSF/ PSR primers to identify the species *P. syringae* [5], PCR products were obtained for all bacteria of the genus *Pseudomonas* [66]. Each PCR test listed in Table 2.1 is subject to testing.

No species-specific PCR tests were found for pathogens of the species *X. translucens*. There is a source that has published primers for PCR and subsequent sequencing to determine the pathway [48]. The sequencing results, due to the use of the NCBI database for analyzing the obtained sequences, can be used for scientific purposes, but their use for diagnostics by an accredited laboratory in the analysis of a sample is not applicable due to the low degree of confidence. Thus, the development of species-specific PCR tests for *X. translucens* is required.

Recommendations for isolation on culture media were found for each target bacterium (Table 1.3). Among the proposed media options, it is necessary to experimentally identify the most optimal one.

Table 1.3. Diagnostic tests for grain crop bacterial pests.

Bacteria	**Presence/absence of a diagnostic technique**	**Isolation**	**PCR**
P. fuscovaginae	Absent	KB, NA [50]	qPCR *Pseudomonas fuscovaginae* by OOO «Sintol», Russia; 6 primer pairs called Pf8 [50]
P. syringae	Absent	KB [67], SPTPsjA [68]	SyD1/ SyD2 [5]; PsyF/ PsyR [67]; rpoD-F/ rpoD-R, gyrB-F/ gyrB-R, cts-F/ cts-R, gapA230F/ gapA942R [69]

Bacteria	**Presence/absence of a diagnostic technique**	**Isolation**	**PCR**
Rathayibacter tritici	Present	YGM [70], NBY, NA, 523M [71]	qPCR BTRITF2/ BTRITR2, BTRITP1; qPCR BTRITF1/ BTRITR1, BTRITP1 [71]; 5 pairs of primersRt-xF/ Rt-xR [72]
X. translucens	Absent	WB, NBY [48], YDC, KM-1, WB Boric Acid-cephalexin, XTS [23]	PCR for housekeeping genes proD, dnaK, fyuA и gyrB [48]

NA – Nutritive agar; YDC – Yeast Extract-Dextrose-Calcium Carbonate; SNR medium – Sorbitol Neutral Red medium; KB – King B medium; SPTPsjA – serine-potassium tellurite-based Psj-selective agar; PPGA – potato-peptone-glucose agar; NBY – nutrient broth with yeast extract.

Surveys were conducted on an area of more than 1 000 ha of wheat, barley, triticale, oats and rye crops in Moscow, the Republic of Crimea and Stravrapol region (Table 1.3). Bacteria colonies were isolated, some of which were identified, while others are in the process of determining their species.

CHAPTER 2
MATERIALS AND METHODS

2.1 Materials

The research materials were strains of bacteria from both foreign and domestic collections shown in appendix 3 Table 3.1 (appendix 3). Foreign collections were from Germany and France whereas domestic were from the bacterial collection of the Federal State Budgetary Institution "VNIIKR", bacterial isolates, plant and seed material of grain crops. When running diagnostic PCR tests, optimizing sample preparation for research and optimizing methods for isolating cultures, strains No.0337 from the collection of bacteria of the Federal State Budgetary Institution (FGBU) "VNIIKR" *Xanthomonas translucens* pv. *translucens* was used (source – German Collection of Microorganisms and Cell Cultures, Leibniz Association, Germany DSMZ 18974). *Rathayibacter tritici* strains from the collection of bacteria of the FGBU “VNIIKR” No.0378 (source – Egypt CFBP (Collection for plant associated Bacteria) since 1952 its identification number is 1385). Strain No.0335 *P. fuscovaginae* (source – German Collection of Microorganisms and Cell Cultures, Leibniz Association, Germany DSMZ 7231-0205-001) and strains No.0222 *Pseudomonas syringae* pv. *syringae* (source – United Kingdom National Collection of Plant Pathogenic Bacteria 1089/1087) and No.0440 *P. syringae* pv. *coronofaciens* (source – France CIRM-CFBP, since 1958 its identification number is 13852216), also from the FGBU “VNIIKR” collection.

The origin of the bacterial strains used and a description of the material for each experiment is given in its description.

Air-dry wheat grain free from the bacterial objects of the study (*R. tritici, P. fuscovaginae, P. syringae* and *X. translucens*) was also used in the study.

Plant material from various regions of the Russian Federation were collected. A total of 181 cereal plant samples were collected during the study. From Moscow region, 55 cereal plant samples were collected as follows: wheat 47 samples, triticale 5 samples, rye 3 samples, from the Republic of Crimea 60 cereal plant samples as folows: wheat 29 samples, barley 22 samples, oats 7 samples, cereal-

bean mixture 2 samples and from Stavropol region 66 cereal plant samples as follows: wheat 47 samples, barley 17 samples, triticale 1 sample and cereal mixture 1 sample.

During the study bacterial isolates were collected from the cereal plant samples. A sum of 434 bacterial isolates were selected. From Moscow region 168 bacterial colonies were selected, the Republic of Crimea 102 colonies and Stavropol region 164 bacterial colonies were selected.

For the search for targets in the designing of *Xanthomonas translucens* primers maximum available sample of genomic assemblies of *X. translucens* used are presented in table 2.1.

Table 2.1 Genomic assemblies of *Xanthomonas translucens* strains from NCBI RefSeq used to search for targets

No p/n	**Genomic assemblies used**	**No p/n**	**Genomic assemblies used**
1	GCF_000313775.1	6	GCF_001269865.1
2	GCF_000331775.1	7	GCF_001282765.1
3	GCF_000334075.1	8	GCF_001282805.1
4	GCF_000807145.1	9	GCF_001282885.1
5	GCF_001021935.1	10	GCF_001455815.1

The maximum available sample of genomic assemblies of *X. translucens*, presented in Table 2.1, was used to search for genome regions identical in all assemblies of the target bacterium.

In the designing of *X. translucens* primers, during the search for the specified targets, 161 genomic assemblies of 25 other (non-target) species of bacteria of the genus *Xanthomonas* that do not belong to species *X. translucens* (Table 2.2) were used.

Table 2.2 Species of bacteria of the genus *Xanthomonas* and the number of their genomic assemblies from NCBI RefSeq used to search for targets

No p/n	View	Number of assemblies	No p/n	View	Number of assemblies
1	*Xanthomonas albineans*	10	14	*Xanthomonas hyacinthi*	2
2	*Xanthomonas anoxopodis*	10	15	*Xanthomonas maltophilia*	10
3	*Xanthomonas arboricola*	10	16	*Xanthomonas melonis*	3
4	*Xanthomonas bromi*	2	17	*Xanthomonas oryzae*	10
5	*Xanthomonas campestris*	10	18	*Xanthomonas phaseoli*	10
6	*Xanthomonas cassavae*	1	19	*Xanthomonas pisi*	2
7	*Xanthomonas citri*	10	20	*Xanthomonas populi*	3
8	*Xanthomonas codiaei*	1	21	*Xanthomonas prunicola*	3
9	*Xanthomonas cucurbitae*	2	22	*Xanthomonas sacchari*	7
10	*Xanthomonas dyei*	3	23	*Xanthomonas theicola*	2
11	*Xanthomonas euvesicatoria*	10	24	*Xanthomonas vasicola*	10
12	*Xanthomonas fragariae*	10	25	*Xanthomonas vesicatoria*	10
13	*Xanthomonas hortorum*	10			

2.2 Methods

During the research, the researcher was guided by fire safety rules, electrical safety rules, rules for the use of pressure vessels and rules for working with chemical reagents approved in the laboratories of the FGBU “VNIIKR”. At every stage of the study, personal protective equipment that is laboratory coat, and gloves were used, and when working with chemical reagents.

Some tests required additional goggles and a respirator which were used. In order to prevent contamination, 70% ethyl alcohol (C2H5OH) and aqueous solutions of chlorine-containing agents, such as “Belizna” (RF) and “Desitabs”, “NPH COMPANY” (RF) were used.

2.2.1 Cereal plant sampling in different regions of the Russian Federation.

Sampling was carried out during the vegetative period. This depended on the region, sampling period and sowing time at which the phases could be: early leaf,

tillering, stem elongation, heading and ripening. When the plants were at early leaf or tillering phase the sample collected consisted of 5-15 whole plants, and when the plants were at stem elongation, heading or ripening phases the sample consisted of 5-15 stems cut at the first internode including the ear, stem and leaves.

A visual inspection of the crop was carried out for the presence of symptoms in the form of spotting, strokes and stripes, as well as signs of wilting and deformation of the leaves. Plants with symptoms were collected and healthy plants were collected when no symptoms were noted on plants. When collecting the material, the following reagents, consumables and inventory were used; non-powdered nitrile gloves, chlorine, medical scalpel, scissors, inspector's bag, bags with a latch and paper filters.

When sampling from hybridization plots and variety testing plots, one sample was taken from one variety. When sampling from production fields, one sample was taken from one field. Sampling fields were located randomly along the route of movement. In cases where the varieties were known they were recorded. Diluted chlorine was used to clean and disinfect the gloves and instruments before each plant sample collection. The collected samples were then placed in a bag with paper filters, sealed and marked. The coordinates from which each sample was collected were recorded. These samples were then transported to the laboratory and stored at 2–8 °C awaiting sample preparation for less than a week which was done within this time frame.

2.2.2 Sample preparartion

2.2.2.1 Plant sample preparation.

Plant sample preparation was carried out within 1 week after sample collection. Prior to sample preparation, the samples were stored at 4°C in the dark. Measures to prevent contamination were applied throughout the sample preparation process. Samples were removed from the package and visually inspected to detect bacterial disease symptoms of cereal crops with symptoms like chlorotic spots, chlorotic streaks, wilting and twisting. When symptoms were noted plant tissue was taken at the junction of the affected and healthy areas. In the case of healthy

samples, that is those that were symptomless upon visual inspection plant tissue was also taken.

An analytical sample (suspensions of the microbiota) was prepared from each of the plant tissue samples in the laboratory. About 5g of plant tissues cut with sterilized scissors, 30ml of phosphate-buffered saline (PBS Appendix 2 table 3) was added to the laboratory specimen. The sample was then shaken vigorously on a Unimax 2010 rotary shaker (Heidolph, Germany) at 200rpm for 45-60 min. The extract was separated from impurities of plant tissues by gravity filtration using the fine filters “Blue Ribbon”, poresize 3-5µm. The extracts were centrifuged for 10min at 4°C (10,000 g, Allegra X-30R, Beckman Coulter, Denmark). The supernatant was removed, and the precipitate was re-suspended in 1ml of PBS and this resulting suspension was the the analytical sample used in this research. Of the analytical sample: 200µl was used for DNA extraction, 200µl was transferred into separate tubes and used for bacterial isolation and to the 600µl two drops of glycerine were added and stored for future use.

2.2.2.2 Optimization of seed sample preparation.

In the optimization of seed sample preparation, air-dry grains of wheat infected with bacterial suspensions were the objects of the study. For guaranteed sample uniformity the grains were poured into a container and stirred. An electronic scale (AJH-4200CE, Vibra, Japan), was used to ensure that every laboratory grain sample weighed 25±0.2g. These were then transferred into homogenization bags to which 54ml of PBS (Appendix 2 table 3) was added [eppo]. Using a special tripod, the bags were placed on an orbital shaker (Unimax 2010, Heidolph, Germany) at 100rpm for 2 hours.

Prepared bacterial suspensions were used for the infection of the laboratory grain samples. 6ml of one of the dilutions was added-2, 3, 4 or 5 was added to the homogenization bags that contained the laboratory grain samples. One of the homogenization bags was left uninfected as the negative control. The bags were placed in the homoginzer (Bag Mixer 400SW, Interscience, France) with the position of the blades closest to the homogenizer door and at speed 4 for 5 mins the

bags were homogenized. After the homogenization bags were placed on a shaker at 100rpm for 15min, so that the flour settled as a mesure to reduce inhibition. The liquid part of the samples was then poured into centrifugal tubes with a volume of 50ml and centrifuged at 5minutes, 1200g, 4°C (Allegra X-30R, Beckman Coulter, Denmark) so as to easily separate the flour from the desired supernatant. The supernatant was then moved into clean centrifuge tubes and centrifuged again for 10minutes at 10 000g, 4°C. Centrifugation was done twice so as to have a lesser amount of flour and as such prevent PCR inhibition. After the second centrifugation the supernatant was then removed and 1 ml of PBS (Appendix 2 table 2) was added to the sediment, shaken on a vortex and the resulting analytical sample was transferred to a microtube.

200µl of each analytical sample in double repetition, as well as 200 µl of 3, 4, 5 and 6 dilutions of bacterial suspensions in PBS (Appendix 2 table 2) were used for DNA extraction via the sorption method (Proba-GS, AgroDiagnostics, Russia).

Conventional and real-time PCR were run using species-specific primers for the diagnostics of bacteria in the extracted DNA and the results were interpreted.

2.2.3 Cultural and morphological methods

2.2.3.1 Isolation of bacterial cultures.

For the isolation of bacterial cultures analytical samples extracted during plant sample preparation were used. The regions from which the plant samples were taken, the date of plating, the nutrient media, the µl plated and dates of plating are indicated in table 2.3.

Table 2.3– Plating conditions for isolation of bacteria from cereal samples collected in different regions.

Sample collection region	Plating date	Nutrient media	µl of the analytical sample plated	T, °C	Date of colony selection	Transferred nutrient media
Moscow	14–15	CRL	20 µl by	25	18–22	CRL

Sample collection region	Plating date	Nutrient media	µl of the analytical sample plated	T, °C	Date of colony selection	Transferred nutrient media
region	May 2020		stretching into 3 petri-dishes		May 2020	
Republic of Crimea	08–10 June 2021	YDC	50 µl by stretching into 2 petri-dishes	25	14–17 June 2021	YDC
Stavropol region	30 May–03 June 2022	R2A	10–20 µl by stretching into 4 petri-dishes	25	09–21 June 2022	R2A

The composition of the nutrient media used in the experiment is shown in table 1.1., Appendix 1.

The analytical samples were plated onto nutrient media in Petri-dishes using the Drigalsky spatula. In order to determine the best results for bacterial isolation different isolation methods were applied and compared.

For samples from Moscow region 20 µl were stretching on 3 Petri-dishes, for samples from Crimea 50 µl were stretching on 2 Petri dishes and for samples from Stavropol region 10–20 µl were stretching on 4 Petri-dishes.

Plating results for the analytical samples from these regions are shown in the table 2.3, was done on different universal nutrient media so as to determine the best. R2A [73], YDC [23] and CRL nutrient media [65] (Appendix 1 table 1.1) were the nutrient medias used. Nutrient medias were selected based on their potential for maximum bacterial diversity growth, according to literature.

After plating, the Petri dishes were tightly wrapped with parafilm and kept at 25°C in an incubator (MIR-254, Panasonic Healthcare Co., Ltd., Japan) for several days, as indicated at the table 2.3. After incubation, colonies of all morphotypes were selected and their characteristics were recodered (table 2.3). Selection of colonies varied in time frame amongst the nutrient medias.

For Moscow samples, colony selection was done 4 days after plating. For Crimea, the colony selection was done 6 days after plating and this took 3 days. Stavropol region's analytical samples were incubated for 10 days thereafter colony selection was done for 12 days. Due to the different compositions in the nutrient media the incubation and the time of colony selection differed with R2A providing a longer time for colony selection.

Colonies of various morphotypes were collected and re-plated on Petri dishes with the same nutrient media as used in initial plating see table 2.3. This was done using a bacteriological loop in order to obtain an isolated pure bacterial culture. Separate colonies were then selected using a bacteriological loop and placed into microtubes with 200µl PBS (Appendix 2 table 2.2) and also placed in microtubes and stored at -80°C for future use.

2.2.3.2 Determination of the optimal nutrient medium for *Rathayibacter tritici*.

Plating was on two different nutrient media YPGA and NBY that are recommended for *Rathayibacter tritici* according to literature data analysis [19].

In the determination of the optimal nutrient medium for *Rathayibacter tritici*, a single 7-day bacterial colony of pure *Rathayibacter tritici* (0378 VNIIKR collection strain) (Appendix 3 table 3.1) was added to 1ml PBS (Appendix 2 table 2.1). After the Eppendorf tube was centrifuged and vortexed. A series of consecutive dilutions was done.

After this, 50µl of dilutions 4 and 5 were then plated using the spread plate technique [75**Error! Reference source not found.**] onto the two different nutrient media, YPGA [76] and NBY [19] nutrient media (Appendix 1 table 1.1). Each dilution was plated in 10 petri-dishes making the total 20 petri-dishes per media. The petri-dishes were sealed with parafilm and incubated for 5 days at 25^{o}C in the MIR-254, Panasonic Healthcare Co., Ltd., Japan incubator.

2.2.3.3 Preparation of bateria suspensions and determination of bacteria concentration.

In the optimization of seed sample preparation, cultures were plated on nutrient media from literature: King B [77], L.B [77] and YPGA [76] (Appendix 1 table 1.1). The nutrient media were selected based on the analysis of literature, on the biological characteristics of the studied bacteria and this was based on the genera to which the target bacteria (Appendix 3 table 3.1) belonged.

Pure bacterial cultures were used for bacterial suspension preparation. For the preparation of bacterial suspensions, the 7-day pure live cultures of *X. translucens* pv. *translucens* (0337 VNIIKR collection strain), *R. tritici* (0378 VNIIKR collection strain) and 3-day pure live cultures of *P. fuscovaginae* (0335 VNIIKR collection strain) and *P. syringae* pv. *coronafaciens* (0440 collection VNIIKR strain) (Appendix 3 table 3.1) were used. Strains Pf 0335 and Psc 0440 were plated on King B nutrient media, strain Xtt 0337 on L.B nutrient media [77] and Rt 0378 on YPGA nutrient media [76] (Appendix 1 table 1.1).

In Eppendorf micro-tubes (1.5ml), bacteria suspensions were prepared using sterilized PBS (Appendix 2 table 2.1). Visually the initial bacteria suspensions were almost identical in turbidity to pure PBS (Appendix 2 table 2.1).

Using the Koch method [78] the concentration of the bacterial suspensions was determined. 100µl of the 6^{th}, 7^{th} and 8^{th} bacterial suspension dilutions were plated per petri-dish containing their optimal nutrient medium in 3 repeats (9 petri-dishes) using a drigalsky spatula. The petri dishes were sealed with parafilm and incubated for 7 days at + 25°C in an incubator (MIR-254, Panasonic Healthcare Co.Ltd., Japan).

After 7 days the colony forming unit (CFU) count were recorded using the formula 1:

$$X = \bar{a} * {}^{1\,000}/_{V} \quad (1)$$

where "ā" is the average number of colonies, "V" is the volume of the culture plate and "X" is the CFU count.

2.2.4 Molecular genetic methods

2.2.4.1 DNA Extraction

DNA extraction from isolates collected, bacterial suspensions, analytical samples from plant grain samples and analytical samples seed grain samples was carried out using the set “Proba-GS AgroDiagnostics," Russia, in accordance with the manufacturer’s instructions. As follows:

Samples were mixed with 150×(N+1) of the Lysing solution and 20×(N+1) of the pre-suspended sorbent in a separate tube. With N representing the number of samples. Then 170 µl using dispensers (mechanical single-channel dispensers 0.5–10 µl, 2–20 µl, 20–200 µl, and 100–1000 µl BIOHIT, Sartorius, Finland) of the resulting mixture was added to each sample tube and shaken on the vortex. The test tube was heated for 20 minutes at 50°C in a thermostat (solid-state programmable thermostat TT-1 (Gnome), “DNA-Technologies” RF). After this, they were centrifuged (MiniSpin centrifuge, Eppendorf Germany) for 1 minute at 13000 rpm.

Volume 200 µl of washing solution (No. 1) was added to the sediment within the test tubes and shaken on the vortex (microcentrifuge MICROSPIN FV-2400, “BioSan” Latvia) for 3-5 seconds.

The test tubes were then centrifuged for 1 minute at 13000 rpm, and the resulting supernatant was discarded. 200 µl of washing solution (No. 2) was then added to the sediment, shaken on the vortex for 3-5 seconds, and centrifuged for 1 minute at 13000 rpm. The resulting supernatant was discarded.

After this, 200 µl of washing solution (No. 3) was added to the sediment, and the mix was shaken on the vortex for 3-5 seconds. The suspension in tubes were then centrifuged for 1 minute at 13000 rpm, and the resulting supernatant was discarded. With open caps, the test tubes were placed on a thermostat for 5 minutes at 50°C.

After this step, 100 µl of elution solution was added to the sediment and shaken on the vortex for 5–10 seconds. With closed caps, the test tubes were then heated for 5 minutes at 50°C and centrifuged for 1 minute at 13000 rpm.

The supernatant containing DNA was then transferred to a new test tube, stored at -80°C, and used later in various PCR tests.

2.2.4.2 Polymerase Chain Reaction (PCR)

The DNA obtained from the isolates, the bacterial suspensions, and samples of plants and seeds were used in this section. Various PCR and RT (real time) PCR tests were conducted.

2.2.4.2.1 Conventional PCR

All Conventional PCR was performed in separate tubes using an internal positive control (IPC) according to the method described by Mazurin et al., 2012. 2.5 µl per reaction [79]. The size of the PCR product was 714 b.p. The nucleotide sequences of primers:

Mus714F: 5`-CTT CTG GCG TTT CAG AGA CC-3`

Mus714R: 5`-GGC TCC TCT TGT GCA GAT TC-3`

The IPC reaction mixture for classical PCR is presented in Table 2.4.

Table 2.4 Composition of the IPC mixture for classical PCR

Reaction component	Concentration	Quantity, µl
Ultrapure water	-	229.5
Master Mix 5X	10X	250
Primer Mus714F	10 pM	10
Primer Mus714R	10 pM	10
Plasmid Mus714_6	10 pM	0.5
Total	-	250

The primers are suitable for use at annealing temperatures from 53.7 to 64.1 ºC, the amplification product size is 714 bp. Amplification was carried out simultaneously and under the same conditions as for tubes with primers. The composition of the mixtures is shown in table 2.5.

Table 2.5 Reaction mixture composition and amplification conditions for conventional PCR with PSF/PSR primers (714b.p)

Reaction component	Concentration	Quantity, µl

Reaction component	Concentration	Quantity, µl
Ultrapure water	-	15,5
Master Mix 5X	5X	5,0
IPC	10X	2,5
Total	-	23,0
DNA	-	2,0
Final volume	-	25,0
Amplification program: According to the primers		

NB: IPC- Internal Positive Control

PCR was performed on DNA samples using the following tests:

1. Conventional PCR with primers PSF/PSR [5], was used for the identification of bacteria from the *Pseudomonas* genus amongst the DNA samples of all isolated bacterial cultures. The size of the PCR product described by Kazempour was 752 bp (the factual amplicon noted in the study was 610 bp).

The nucleotide sequences of primers:

PSF 5'-AGC CGT AGG GGA ACC TGC GG-'3

PSR 5'- TGA CTG CCA AGG CAT CCA CC-'3.

The reaction mixture and PCR conditions are presented in Table 2.4.

Table 2.4 – Reaction mixture composition and amplification conditions for conventional PCR with PSF/PSR primers (610b.p)

Reaction component	Concentration	Quantity, µl
Ultrapure water	-	16,0
Master Mix 5X	5X	5,0
Direct Primer PsF	10 pM	1,0
Reverse Primer PsR	10 pM	1,0
Total	-	23,0
DNA	-	2,0
Final volume	-	25,0
Amplification program: 95 °C – 10 min., then 25 cycles: 95 °C – 20 sec., 64 °C – 15 sec., 72 °C – 15 sec.; then 72 °C – 2 min..		

1. DNA samples of all isolated bacterial cultures with which an amplicon of 610 b.p. was obtained in PCR with primers PSF/PSR were thereafter tested with SyD1/SyD2 primers when testing for the identification of *Pseudomonas syringae* pv. *syringae* and *Pseudomonas syringae* pv. *atrofaciens* [80]. The PCR products were 1040 bp.

The nucleotide sequences of primers:

SyD1 5'-CAGCGGCGTTGCGTCCATTGC-3'

SyD2 5'-TGCCGCCGACGATGTAGACCAGC-3'

The reaction mixture and PCR conditions are presented in Table 2.5.

Table 2.5 – Composition of the reaction mixture and amplification conditions for conventional PCR with SyD1/SyD2 primers (1040 bp)

Reaction component	Concentration	Quantity, µl
Ultrapure water	-	17,4
Master Mix 5X	5X	5,0
Direct Primer SyD1	10 pM	0,3
Reverse Primer SyD2	10 pM	0,3
Total	-	23,0
DNA	-	2,0
Final volume	-	25,0
Amplification program: 95 °C – 10 min., then 25 cycles: 95 °C – 20 sec., 64 °C – 15 sec., 72 °C – 45 sec.; then 72 °C – 7 min..		

2. DNA samples of isolated bacterial cultures that were negative for the 1 040b.p amplicon from SyD1/SyD2 were then tested with PsyF/PsyR primers [67] for the identification of *Psuedomonas syringae* pv. *syringae*. Analytical samples from seeds were also tested with PsyF/PsyR primers. The PCR product size was 144b.p.

The nucleotide sequences of primers are as follows:

PsyF 5'-ATG ATC GGA GCG GAC AAG-3'

PsyR 5'-GCT CTT GAG GCA AGC ACT-3'

The reaction mixture and PCR conditions are presented in Table 2.6.

Table 2.6 – Composition of the reaction mixture and amplification conditions for conventional PCR with PsyF/PsyR primers (144b.p)

Reaction component	Concentration	Quantity, µl
Ultrapure water	-	17,2
Master Mix 5X	5X	5,0
Direct Primer PsyF	10 pM	0,4
Reverse Primer PsyR	10 pM	0,4
Total	-	23,0
DNA	-	2,0
Final volume	-	25,0
Amplification program: 96 °C – 10 min., then 30 cycles: 94 °C – 30 sec., 61 °C – 30 sec., 72 °C – 30 sec.; then 72 °C – 10 min..		

3. The identification of the DNA samples of all isolated bacterial cultures that tested negative for the 610b.p amplicon from PSF/PSR primers PCR was carried out by sequencing a section 16–23S of the rRNA amplified by PCR with 8UA/519B primers [81] and/or 27f/907r [82]. The PCR products were 500 bp for 8UA/519B primers and 880 bp for 27f/907r primers.

The nucleotide sequences of primers 8UA/519B:

8UA 5 -AGA GTT TGA TCM TGG CTC AG-3

519B 5-GTA TTA CCG CGG CKG CTG-3

The reaction mixture and PCR conditions are presented of 8UA/519B are shown in Table 2.7.

Table 2.7 – Composition of the reaction mixture and amplification conditions for conventional PCR with primers 8UA/519B (500b.p)

Reaction component	Concentration	Quantity, µl
Ultrapure water	-	14,0
Master Mix 5X	5X	5,0
Direct Primer 8UA	10 pM	2,0
Reverse Primer 519B	10 pM	2,0
Total	-	23,0

Reaction component	Concentration	Quantity, µl
DNA	-	2,0
Final volume	-	25,0
Amplification program: 96 °C – 5 min., then 35 cycles: 95 °C – 15 sec., 55 °C – 30 sec., 72 °C – 30 sec.; then 72 °C – 10 min.		

4. The nucleotide sequences of primers 27f/907r:

27f 5’-AGA GTT TGA TCC TGG CTC AG-3’

907r 5’-CCG TCA ATT CCT TTG AGT TT-3’

The composition of the reaction mixture and PCR conditions 27f/907r (880b.p) [82] are shown in Table 2.8.

Table 2.8 – Composition of the reaction mixture and amplification conditions for conventional PCR with 27f/907r primers (880b.p)

Reaction component	Concentration	Quantity, µl
Ultrapure water	-	16,0
Master Mix 5X	5X	5,0
Direct Primer 27f	10 pM	1,0
Reverse Primer 907r	10 pM	1,0
Total	-	23,0
DNA	-	2,0
Final volume	-	25,0
Amplification program: 95 °C – 5 min., then 35 cycles: 95 °C – 15 sec., 58 °C – 30 sec., 72 °C – 60 sec.; then 72 °C – 5 min.		

The amplicons were cleaned using Big Dye Kit, BigDye®XTerminator™ Purification Kit, Hi-Di Formamide and sequenced (2.2.4.3).

5. Conventional PCR with primers Rt 3F/3R targeting hypothetical proteins A6122_2866 of strain NCPPB 1953 *Rathayibacter tritici* [83]. This PCR was run for the identification of *R. tritici* in the DNA samples of bacterial suspensions and analytical samples from plants and seeds. The size of the PCR product is 520b.p.

The nucleotide sequences of primers:

Rt 3F 5’-GTG GGC TGATAG GTG GTG ATGT- 3’

Rt 3R 5’-GCG CCC TTT CTC TAC TGG GTAT- 3’

The reaction mixture and PCR conditions are presented in Table 2.9.

Table 2.9 – Composition of the reaction mixture and amplification conditions for conventional PCR with Rt 3F/3R primers (520b.p)

Reaction component	Concentration	Quantity, µl
Ultrapure water	-	16
Master Mix 5X	5X	5,0
Direct Primer Rt 3F	10 pM	1,0
Reverse Primer Rt 3R	10 pM	1,0
Total	-	23,0
DNA	-	2,0
Final volume	-	25,0
Amplification program: 10 min. at 95 °C, 25 cycles: 20 s at 95 °C, 15 s at 64 °C and 15 s at 72 °C; 2 min. at 72 °C		

For the identification of *Xanthomonas translucens* the primers were designed in this study.

PCR products were accelerated in 1.5% agarose gel using etidium bromide at mode B – 130, mA – 165, W – 40, 50 min. (Elf-4, DNA-Technology, Russia). Amplicons were evaluated using the genetic weight marker GeneRuler 100 bp Plus DNA Ladder.The PCR result was interpreted from electropherograms taken on a gel documentation system (BioRad, USA).

2.2.4.2 Real time PCR (Rt-PCR)

PCR was performed using an internal positive control (IPC) according to the method described by Mazurin et al., 2012. 2.5 µl per reaction [79]. Ready-made commercial mixtures produced by Syntol LLC (Russian Federation) were used as VPC for RT-PCR, 0.1 µl per reaction.

7. Rt-PCR with primers BTRITF2/ BTRITR2 and Probe BTRITP1 (BTRITF2/ BTRITR2/ BTRITP1) targeting 557129-557203 genome of strain NCPPB 1953 (RefSeQ NZ_CP015515.1) of *Rathayibacter tritici* [71]. PCR was

run with DNA of analytical samples from plants and seeds and *Rathayibacter tritici* DNA.

The nucleotide sequences of primers:

BTRITF2 5'-AAT TTG ATC TGT TTG GAA GCT GC-3'

BTRITF2 5'-GCC GTC GTC AAH GCG AT-3'

BTRITP1 5'-6-FAM- CGT GAC GTG GAT AAG TTG ATC AGC CTG A-BHQ1-3'

The reaction mixture and PCR conditions are presented in Table 2.10.

Table 2.10 – Composition of the reaction mixture and amplification conditions for PCR-Rt with BTRITF2/ BTRITR2 primers

Reaction component	Concentration	Quantity, µl
Ultrapure water	-	13,75
Master Mix 5X	5X	5,0
Direct Primer BTRITF2	10 pM	1,875
Reverse Primer BTRITR2	10 pM	1, 875
Probe BTRITP1 (FAM)	5 pM	0,5
Total	-	23,0
DNA	-	2,0
Final volume	-	25,0
Amplification program: 5 min. at 95 °C, 45 cycles: 15 s at 95 °C, 60 s at 60 °C		

8. Rt-PCR was run using the Pseudomonas fuscovaginae-Rt kit, Sintol, Russia for the identification of *Pseudomonas fuscovaginae* in the bacterial suspensions, the DNA of analytical samples from plants and seeds. PCR was carried out in accordance with the manufacturer's instructions using a detection amplifier. Data on the composition of the PCR mixture and the amplification program are shown in Table 2.11.

Table 2.11 – Composition of the reaction mixture and amplification conditions for PCR-Rt with a set of LLC "Syntol"

Reaction component	Quantity, µl
Reaction mixture "A. cit.-VPC»	20,0

Reaction component	Quantity, µl
SynTaq DNA polymerase T+	0,5
Total	20,0
DNA	5,0
Final volume	25,0
Amplification program: 95 ° C – 5 min., then 45 cycles 60 ° C – 40 sec., 95 ° C – 15 sec.	

The result of testing a sample was considered positive if a specific reaction for the target PCR gene was present in the form of an exponential curve using real-time PCR (PCR-Rt). Using real-time PCR (PCR-Rt) the result of testing a sample was considered positive if a specific reaction for the target PCR gene was present in the form of an exponential curve. Negative if there was no specific reaction in the internal positive control reaction (FAM). In other cases, the result of PCR was considered in-applicable.

2.2.4.2.3 Developing PCR tests for the identification of *Xanthomonas translucens*.

Searching for a nucleotide sequence not only corresponding to a region of the *Xanthomonas translucens* genome but that is also suitable for use as a polymerase chain reaction (PCR) target was the main and most challenging task in the development of diagnostic methods based on PCR.

Developing PCR tests for the identification of *Xanthomonas translucens* was done as written by Slovareva O. Yu., Starikova E.V. and Muvingi. M. [84].

Annotated proteins corresponding to the sequences of 10 genomic assemblies of the target bacterium – *X. translucens* were used to search for targets (Table 2) downloaded from the National Center for Biotechnology Information (NCBI) GenBank Database [NCBI, 2021 [site] URL: ftp://ftp.ncbi.nlm.nih.gov/genomes/genbank/bacteria/] in March 2020. The maximum available sample of genomic assemblies of *X. translucens*, presented in Table 2, were used to search for genome regions identical in all assemblies of the target bacterium.

To assess the identity level of selected target genes for *X. translucens* among closely related bacteria, 161 genomic assemblies of 25 other (non-target) species of bacteria of the genus *Xanthomonas* were used that do not belong to species *X. translucens* (Table 2.2) [84].

The cd-hit program (version 4.1) labeled sets of protein sequences for each of the assemblies according to the species and strain and then clustered them, according to a given identity threshold using short “word”-peptides. Grouping or clustering was done according to an identity threshold of 70% with a permissible difference in the length of the sequences of 80% and the length of the “word” of 5 amino acid residues [84].

Using a Python script, clusters that contained only *X translucens* protein sequences and did not contain protein sequences of other analyzed bacterial species were identified from the resulting sets of protein clusters. These clusters of *X. translucens* protein sequences were compared to a database of known protein

sequences (nr) using the blastp program to assess their specificity for *X. translucens*. The following proteins were excluded from the study:

1. Proteins with high (>70% identity) similarity to proteins of other bacterial species not previously included in the analysis,
2. sequences less than 100 amino acid residues in length, were excluded from the analysis.
3. protein sequences that showed strong variability within *X. translucens* species.

Such Protein sequences were excluded as not suitable for the development of universal species markers.

Nucleotide sequences of genes encoding proteins from clusters specific to *X. translucens*, were used to develop primers.

Using the Primer-BLAST program [85] primers were selected. The main parameters for finding primers were chosen: an annealing temperature of 60 ° C, the length of each primer is 20 b.p, the size of the amplification product is at least 200b.p and not more than 1000 b.p. Among the selected pairs of primers preference was given to primers with low self-complementarity. The primers were synthesized in ZAO “Eurogen” (Russia).

Primer pair approbation was satisfactory result in accordance with the specified criteria, and the amplicon size did not exceed 400 b.p, were used to select a probe in order to develop a primer system for the identification of *X. translucens* in real time. The probes were selected manually, analyzing the targets selected for the development of the probes, taking into account the GC composition, annealing temperature and the length of the probe. An oligo calculator was used to determine the annealing temperature [86]. When selecting, they were guided by exceeding the annealing temperature of the probe compared to the annealing temperature of the primers by at least 10°C [84].

Approbation developed for *X. translucens* primers were conducted with DNA from bacterial strains belonging to the genus *Xanthomonas*, including *X. translucens* (Table 2.12).

Table 2.12 – Species of bacteria of the genus Xanthomonas used to verify the specificity of PCR tests

No p/n	Name of the bacterium	No strain in the collection of FGBU "VNIIKR"	Origin
1	*Xanthomonas campestris* pv. *raphani*	0148	NCPPB 1946
2	*Xanthomonas arboricola* pv. *pruni*	0149	AOBC PPSCD
3	*Xanthomonas campestris* pv. *campestris*	0226	*Brassica oleracea*
4	*Xanthomonas oryzae*	0227	NCPPB 3002
5	*Xanthomonas campestris* pv. *campestris*	0228	*Brassica oleracea*
6	*Xanthomonas campestris* pv. *campestris*	0230	*Brassica oleracea*
7	*Xanthomonas campestris* pv. *campestris*	0232	*Brassica oleracea*
8	*Xanthomonas campestris* pv. *campestris*	0234	*Brassica oleracea*
9	*Xanthomonas translucens*	0337	DSMZ 18974
10	*Xanthomonas euvesicatoria*	0338	DSMZ 19128
11	*Xanthomonas perforans*	0343	DSMZ 18975
12	*Xanthomonas gardneri*	0344	DSMZ 19127
13	*Xanthomonas fragariae*	0345	NIB Z125
14	*Xanthomonas fragariae*	0346	NIB Z126
15	*Xanthomonas fragariae*	0347	NIB Z127
16	*Xanthomonas* sp.	0348	NIB Z128
17	*Xanthomonas axonopodis* pv. *allii*	0373	*Allium cepa*
18	*Xanthomonas vesicatoria*	0374	DSMZ 22252
19	*Xanthomonas* sp.	0375	*Vicia*
20	*Xanthomonas oryzae* pv. *oryzicola*	0376	CFBP 2286
21	*Xanthomonas axonopodis* pv. *allii*	0377	CFBP 6107
22	*Xanthomonas axonopodis* pv. *phaseoli*	0386	CFBP 2534
23	*Xanthomonas* sp.	0394	*Trifolium*
24	*Xanthomonas axonopodis* pv.	0399	*Phaseolus vulgaris*

No p/n	Name of the bacterium	No strain in the collection of FGBU "VNIIKR"	Origin
	phaseoli		
25	*Xanthomonas axonopodis* pv. *phaseoli*	0400	*Phaseolus vulgaris*
26	*Xanthomonas gardneri*	0404	*Solanum lycopersicum*
27	*Xanthomonas vesicatoria*	0405	*Solanum lycopersicum*
28	*Xanthomonas campestris*	0406	*Brassica oleracea*
29	*Xanthomonas axonopodis* pv. *allii*	0419	CFBP 6369
30	*Xanthomonas axonopodis* pv. *axonopodis*	0420	CFBP 5141
31	*Xanthomonas phaseoli*	0426	*Trifolium*
32	*Xanthomonas phaseoli*	0427	*Phaseolus vulgaris*
33	*Xanthomonas hyacinthi*	0446	CFBP 1156
34	*Xanthomonas arboricola*	0473	DSMZ 50854
35	*Xanthomonas citri* pv. *glycines*	0483	CFBP 2526

DSMZ – German Collection of Microorganisms and Cell Cultures, Leibniz Association, Germany; AOBC PPSCD – collection of phytopathogens of the laboratory of bacteriology of the Agricultural Plant Protection and Soil Protection Station of baranya region, Pécs, Hungary; NCPPB – National Collection of Phytopathogenic Bacteria, York, UK; CFBP – ***International Microbial Resource Centre (CIRM-CFBP); NIB – National Institute of Biology, Ljubljana, Slovenia***

The composition of the reaction mixture: 75–100 ng of bacterial DNA, forward and reverse primers at a final concentration of 0.4 micromolar, 5 µl of H_2O, 5 µl of the ScreenMix-HS master mix (manufactured by "Evrogen", Russia). PCR was carried out using the following program: initial denaturation – 5 min. at 95 ° C, then 40 cycles: 30 s at 95 ° C, 30 s at 60 ° C and 60 s at 72 ° C, final elongation – 7 min. at 72 °C as written by Slovareva O. Yu, Starikova E.V and Muvingi. M (2021) [84].

PCR detection was carried out by electrophoresis in 1.5% agarose gel using ethidium bromide "AppliChem" (Germany), power source Elf-4, "DNA-

Technology" (Russia), chamber for horizontal electrophoresis SE-1, "Helikon" (Russia) and gel documentation system "BioRad" (USA).

Amplification products that were 200 b.p and longer were subjected to purification from residues of components of the PCR mixture and sequencing [87]. The nucleotide sequence was determined using the Applied Biosystems 3500 genetic analyzer. The analysis of nucleotide sequences was performed using the Bio Edit program [88].

2.2.4.3 Sequencing

Sanger sequencing method [**Error! Reference source not found.**] adapted by Belkin [87] was used for determining nucleotide sequences in DNA. Sequencing was performed using the PCR product at a working concentration, which was determined based on the length of the amplification product. The required amount of water to obtain the working concentration of the PCR product was determined by the formula:

$$y = [\ (x : (\frac{C}{100}) - 1] * 4$$

where y is the amount of water required for dilution; x – DNA concentration; c – size (length) of the PCR product; $x : (\frac{C}{100})$ – number, corresponding to how many times the DNA sample needs to be diluted; 1 – amount of DNA for sequencing, µ; $[(x : (\frac{C}{100}) - 1]$ – amount of water, µl; * 4 – increasing the concentration of the PCR product. If $[x : (\frac{C}{100})] > 2$, then dilution of the concentration is required. If $[x : (\frac{C}{100})] < 2$, then no concentration dilution is required [Sanger et al 1977; Belkin 2019]. The calculated volume of water was added to clean tubes labeled according to the names of the samples, and 4 µl of a sample of the PCR product was added. The contents of the tubes were mixed by vortex and immediately used for sequencing.

For amplification (SEQ-PCR), a Big Dye Kit was used containing the Big Dye 3.1 reagent with labeled dNTPs and Big Dye buffer, as well as primers at a concentration of 0.8 pmol. The reaction mixture for one sample contained 1 µl Big Dye 3.1, 1.5 µl Big Dye buffer, 2 µl primer (forward or reverse) and 4.5 µl water.

Amplification for each sample was carried out in two wells of the plate - in one well with a forward primer, in the second with a reverse primer. The total volume of the reaction mixture was 9 μl; 1 μl of the PCR product was used for the reaction. The amplification plate was tightly sealed with a cover film, the reaction mixture was precipitated, and air bubbles were removed from the mixture. Amplification: 96 °C – 1 min., then 25 cycles: 96 °C – 10 sec., 50 °C – 5 sec., 60 °C – 4 min. After SEQ-PCR, the amplicons were subjected to a purification procedure [**Error! Reference source not found.**; 87].

To purify amplicons, we used the BigDye®XTerminator™ Purification Kit, consisting of Sam Solution and X Terminator Solution. To clean one sample, 45 μl of Sam Solution and 10 μl of X Terminator Solution were mixed. A sample was added to the resulting mixture and shaken on a vortex. Tubes with samples were placed in a thermal shaker and kept at 45 minutes, 1400 rpm, 4 °C. After shaking, if necessary, the tubes were stored at 2–8 °C for no more than a day or at -18–-22 °C, or immediately proceeded to the next step: the contents of the tubes were mixed using a vortex. 25 μl of the sample was taken without touching the sediment and plates were added to the wells for sequencing. Moreover, if there were unfilled wells in one row of the plate, 25 μl of Hi-Di Formamide was added to them. The plate was closed with a special rubber septum and placed in a genetic analyzer [**Error! Reference source not found.**; 87]. The sequencing program was selected based on the length of the PCR product (Table 2.13).

Table 2.13 – List of programs for sequencing

Name	**Number of Base pairs (b.p)**	**Approximate time to complete (mins)**
Short_Read_Seq_Assay_POP7	100–300	30
Rapid_Seq_Assay_POP7	300–500	40
Fast_Seq_Assay_POP7	500–700	65
Std_Seq_Assay_POP7	more than 700	125

Files with sequencing results were analyzed using BioEdit and Unipro UGENE programs. The obtained nucleotide sequences were compared with

sequences posted at NCBI [85]. The result of identification was considered an organism with maximum similarity.

CHAPTER 3
RESULTS

3.1 Plant sample collection of cereals from different regions of the Russian federation (Moscow region, The Republic of Crimea and Stavropol region).

During the collection of samples, key information was recorded about the point of collection of the material (and / or the area occupied under every variety), culture, variety (if such information was provided), the date of collection and the phase of ontogenesis in which the plants were located as shown in Figure 3.1.

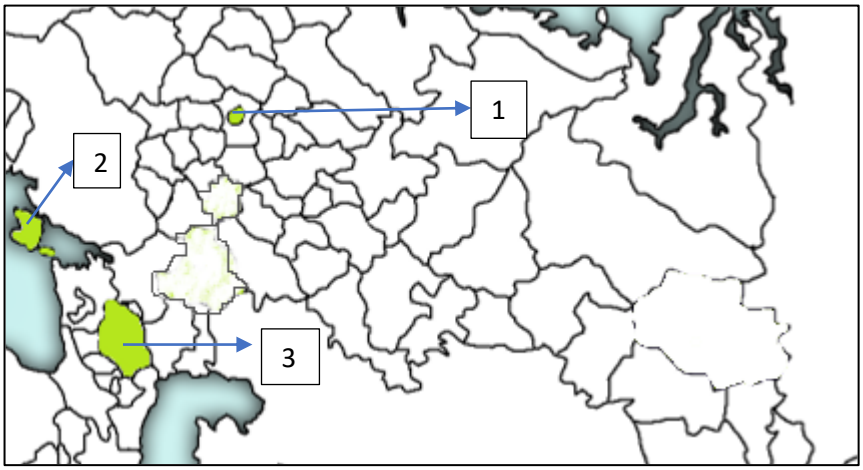

Figure 3.1 Russian Federation's regions from which samples were taken
Key: 1 – Russian State Agrarian University – Moscow Agricultural Academy named after K.A Timiryazev (RSAU), 2 – the Republic of Crimea and 3 – Stavropol region.

Thus, from 2020, the collection of plant material was carried out in Russian State Agrarian University, Stavropol region, and the Republic of Crimea.

For the study in 2020, samples of wheat, triticale and rye plants were taken on May 13, 2020 from variety testing plots and hybridization plots of the field experimental station of the RSAU (appendix 5 figure 5.1).

The Republic of Crimea (3 districts): For the study in 2021, samples of wheat, barley, triticale and oats plants were taken on 1st to 3rd of June 2021 from three districts (appendix 6 figure 6.1): Belogorskij, Krasnogvardejskij and Simferopol`skij.

Stavropol region (8 districts): For the study in 2022, samples of wheat, barley, triticale and oats plants were taken on the 17th to 20th of May 2022. Samples were collected from eight districts (appendix 7 figure 7.1): Kochubeevsky, Budyonnovsky, Sovetsky, Georgievsky, Mineralovodsky, Shpakovsky, Andropovsky, Alexandrovsky, Novoselitsky. In total, 181 plant samples were collected from these three regions.

The folowing grain crops were collected: wheat - 122 plant samples, rye - 3 plant samples, barley - 39 plant samples, oats - 7 plant samples, triticale - 8 samples, and cereal-legume mix - 1 plant sample.

Each sample was assigned a short cipher for the convenience of subsequent analysis (Apppendix 8 table 8.1, Appendix 9 table 9.1 and Appendix 10 table 10.1). The areas from which samples were collected are Stravropol, Moscow and the Republic of Crimea. Wheat, Oat, Triticale and barely are the crop from which the samples were collected. Samples were collected from the three regions of The Russian Federation. The sampling period for winter grain crops was in the booting phase, and for spring crops, in the sprout phase. There were no symptoms of bacterial diseases during sampling of winter crops on plants. Chlorosis was noted on seedlings of spring rye.

A total of 55 samples of grain crops were taken from Timiryazevskaya field experimental station, Russian State Agrarian University – Moscow Agricultural Academy named after K.A. Timiryazev, Moscow see Appendix 8 table 8.1. The sampling period for winter grain crops was in the booting phase, and for spring crops, in the sprout phase. There were no symptoms of bacterial diseases during sampling of winter crops on plants. Chlorosis was noted on seedlings of spring rye.

A total of 60 plant samples were collected from three regions Belogorskij, Krasnogvardejskij, and Simferopolskij of the Republic of Crimea see Appendix 9 table 9.1. The sampling period for winter grain crops was in the milky ripening

phase. There were no symptoms of bacterial diseases during sampling of winter crops on plants.

A total of 66 plant samples were collcted from Kochubeevsky, Budyonnovsky, Soviet, George, Andropovsky, Alexander, Shpakovsky, Novoselitsky and Mineralovodsky of Stavropol region see Appendix 10, Table 10.1. The sampling period for winter grain crops was in the tillering, tillering-out into the tube, earing, ear-flowering and the milky ripening stages. There were no symptoms of bacterial diseases during sampling of winter crops on plants.

3.2 Developing PCR tests for the identification of *Xanthomonas translucens*.

42,570 clusters containing a range of 1 to 343 amino acid sequences were obtained, after clustering 667,416 protein sequences corresponding to genes from 171 genomic assemblies of 26 bacterial species. Among these clusters, 55 included proteins solely from the 10 analyzed assemblies of *Xanthomonas translucens* and did not include any proteins from other species. 6 genes were selected (Table 3.1), following the filtering of sequnces using the established criteria of specificity, length, and variability (as described in the Materials and Methods section).

Table 3.1 – Nucleotide gene sequences used to select primers

No p/n	NCBI accession	Gene coordinates in the genome	Encoded protein	Encoded protein annotation	Names of primer pairs	Length of PCR product, b.p.
1	NZ_FLTU01000142.1	6002-8863	WP_039956369.1	DUF5110 domain-containing protein	1F8/1R8	711
					1F10/1R10	379
2	FLTU01000085.1	5245-7368	WP_009581062.1	TonB-dependent siderophore receptor	2F6/2R6	759
3	NZ_FLTU01000009.1	3500-2202	WP_039955267.1	nucleoside hydrolase	3F3/3R3	246
					3F5/3R5	209
					3F9/3R9	869
4	NZ_LHSI01000001.1	2522179-2523426	WP_009581060.1	ATP-grasp domain-containing protein	4F1/4R1	503
					4F3/4R3	904
5	NZ_ANGG01000457.1	10914-12119	WP_009598496.1	aldose 1-epimerase family protein	5F3/5R3	200
					5F6/5R6	424
6	NZ_ANGG01	4667-3489	WP_009581057.1	MFS transporter	6F6/6R6	970

	000136.1				6F10/6R10	663

The aforementioned sequences were utilized for the purpose of primer design. One to three pairs of primers were chosen for every one of the six sequences. Twelve pairs of primers in all were chosen. Table 3.2 below lists the outcomes of the PCR that was performed using each pair of primers for 35 different *Xanthomonas* bacteria. When an amplification product was present and its size matched that of strain 0337 *Xanthomonas translucens* (a positive control), the PCR result was deemed positive; in this instance, the amplification product's size was shown in table 3.2.

Table 3.2 – PCR results with 35 strains of bacteria of the genus *Xanthomonas* for each pair of the developed primers.

Name of bacteria	Strain	Pair of primers											
		1F8 1R 8	1F10 1R10	2F6 2R 6	3F3 3R 3	3F5 3R5	3F9 3R9	4F1 4R1	4F3 4R 3	5F3 3R3	5F6 5R 6	6F6 6R6	6F10 6R10
		PCR result: amplification fragment size, b.p. (if any) / negative (–) / unreliable (n/a)											
X. campestris pv. *raphani*	0148	–	–	n/a	–	–	n/a	n/a	240	–	–	n/a	n/a
X. arboricola pv. *pruni*	0149	–	–	n/a	–	–	n/a	–	–	–	–	n/a	–
X. campestris pv. *campestris*	0226	–	–	n/a	–	1120	1150	n/a	240	–	–	n/a	n/a
X. oryzae	0227	–	–	–	–	–	–	–	–	–	–	–	–
X. campestris pv. *campestris*	0228	–	–	n/a	–	1120	1150	–	240	–	–	n/a	–
X. campestris pv. *campestris*	0230	–	–	n/a	–	1120	1150	–	240	–	–	n/a	n/a

Name of bacteria	Strain	Pair of primers											
		1F81R8	1F101R10	2F62R6	3F33R3	3F53R5	3F93R9	4F14R1	4F34R3	5F33R3	5F65R6	6F66R6	6F106R10
		PCR result: amplification fragment size, b.p. (if any) / negative (–) / unreliable (n/a)											
X. campestris pv. *campestris*	0232	–	–	n/a	–	n/a	1150	–	240	–	–	n/a	n/a
X. campestris pv. *campestris*	0234	–	–	n/a	–	–	1150	–	240	–	–	n/a	n/a
X. translucens	0337	711	379	759	869	209	246	503	904	424	200	1050	663
X. euvesicatoria	0338	–	–	n/a	869	–	n/a	–	–	n/a	–	470	–
X. perforans	0343	–	n/a	n/a	869	1300	n/a	–	470	1220	–	470	–
X. gardneri	0344	–	–	n/a	869	1300	n/a	–	470	n/a	–	470	–
X. fragariae	0345	260	–	n/a	869	–	–	–	–	n/a	n/a	n/a	–
X. fragariae	0346	260	–	n/a	869	–	–	–	–	n/a	n/a	n/a	–
X. fragariae	0347	260	–	n/a	869	–	–	–	–	n/a	n/a	n/a	–
Xanthomonas sp.	0348	260	–	n/a	869	–	n/a	–	–	n/a	n/a	n/a	–
Xanthomonas sp.	0373	–	–	n/a	n/a	–	–	–	–	–	–	n/a	–
X. vesicatoria	0374	–	n/a	n/a	800	–	–	–	n/a	–	–	–	n/a
Xanthomonas sp.	0375	–	–	n/a	n/a	1120	–	–	–	n/a	–	–	n/a
X. oryzae pv. *oryzicola*	0376	–	–	n/a	869	–	n/a	1200	n/a	1400	–	n/a	n/a
X. axonopodis pv. *allii*	0377	–	–	n/a	869	–	n/a	n/a	n/a	n/a	–	n/a	n/a
X. axonopodis pv. *phaseoli*	0386	–	–	n/a	869	n/a	n/a	–	n/a	–	–	n/a	–

Name of bacteria	Strain	Pair of primers											
		1F81R8	1F101R10	2F62R6	3F33R3	3F53R5	3F93R9	4F14R1	4F34R3	5F33R3	5F65R6	6F66R6	6F106R10
		PCR result: amplification fragment size, b.p. (if any) / negative (–) / unreliable (n/a)											
Xanthomonas sp.	0394	–	–	n/a	n/a	400	n/a	–	n/a	1400	–	n/a	n/a
Xanthomonas sp.	0399	–	–	n/a	869	–	n/a	n/a	–	1120	–	–	–
X. axonopodis pv. *phaseoli*	0400	–	–	n/a	869	n/a	n/a	–	–	1120	–	–	–
X. gardneri	0404	–	–	n/a	–	–	–	–	–	–	–	n/a	–
X. vesicatoria	0405	–	–	n/a	n/a	n/a	n/a	–	n/a	–	–	n/a	n/a
X. campestris	0406	–	–	n/a	n/a	n/a	1150	–	240	1120	–	n/a	–
X. axonopodis pv. *allii*	0419	–	–	n/a	n/a	–	–	–	n/a	–	–	n/a	–
X. axonopodis pv. *axonopodis*	0420	–	–	n/a	869	1300	1150	–	n/a	1340	–	n/a	n/a
X. phaseoli	0426	–	–	n/a	–	–	–	–	–	–	–	n/a	–
X. phaseoli	0427	–	–	n/a	n/a	–	1150	–	–	–	n/a	n/a	–
X. hyacinthi	0446	–	–	n/a	–	–	–	–	–	–	–	n/a	n/a
X. arboricola	0473	–	–	n/a	n/a	–	n/a	n/a	n/a	1120	–	n/a	–
X. citri pv. *glycines*	0483	–	n/a	n/a	n/a	–	n/a	–	–	–	–	n/a	–

The results of DNA testing of the target bacterium (strain 0337 – X. translucens) are shown in green. Pairs of primers, the testing of which showed a satisfactory result, are highlighted in orange. All the strains have been described fully in Table 2.12.

If there was an amplification product present, with a size that was not the same as the control, the length of the acquired fragment was approximately recorded in table 20. In the event that no amplification product was detected, including dimers and nonspecific reaction products, the PCR result was deemed negative (–). The presence of nonspecific reaction products rendered the PCR result unreliable (n/a). When a single length PCR product was present, data regarding the product's size was recorded in Table 3.2.

Using the DNA of strain 0337 *X. translucens* as a template, products of the anticipated length were produced during the course of working with all 12 pairs of primers. Nucleotide sequences that were identical to *X. translucens* sequences identified in the NCBI database by 94.29–99.81%, with a coverage breadth of 95–99%, were found for all 12 PCR products. Low specificity was demonstrated by primers 3F3/3R3, 3F5/3R5, 3F9/3R9, 4F3/4R3, 5F3/5R3, and 6F6/6R6. Figure 3.2 illustrates the potential use of primers 1F8/1R8, 1F10/1R10, 4F1/4R1, 5F6/5R6, and 6F10/6R10 for the species identification of *X. translucens*.

The information in Table 20 demonstrates that the target bacterium, strain 0337 *X. translucens*, was amplified using a set of primers 1F8/1R8, yielding a 711 b.p. PCR result (fig.3.2).

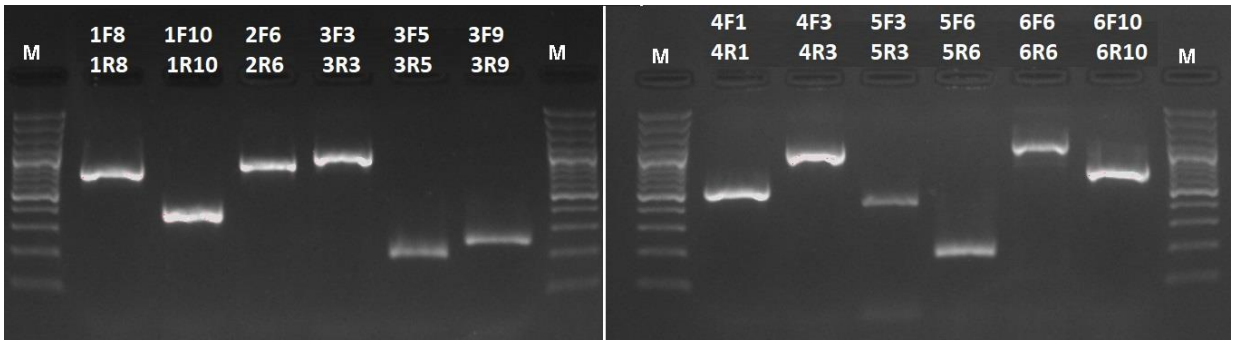

Figure 3.2 – Electrophoregram of PCR results with a strain 0337 *X. translucens* for each pair of primers used in the study. Genetic weight marker GeneRuler 100 b.p Plus DNA Ladder ready-to-use, Thermo Fisher Scientific (USA).

As a result, primers 4F1/4R1 were used in the identification of *X. translucens*. With these primers, 1 PCR product of the expected size is synthesized with *X. translucens* DNA. The presence of PCR products formed during the

amplification of DNA from *X. oryzae* pv. *oryzicola*, strain number 0376 (CFBP 2286) is not alarming as this bacterium affects *Oryza sativa* (rice), which differs in size from *X. translucens*.

Conventional PCR with primers 4F1/4R1, was used for the identification of *X. translucens* [84] targeting the ATP-grasp domain-containing protein. The PCR product was 503b.p.

The nucleotide sequences of primers:

4F1 5'-ATTTGCCGGTCGTTTTGCTC- '3

4R1 5'-GCCGTCAAGAAATCCCTCCA- '3

The reaction mixture and PCR conditions are presented in Table 3.3.

Table 3.3 – Composition of the reaction mixture and amplification conditions for classical PCR with 4F1/4R1 primers (500b.p)

Reaction component	Concentration	Quantity, µl
Ultrapure water	-	18
Master Mix 5X	5X	5,0
Direct Primer 4F1	10 pM	1
Reverse Primer 4R1	10 pM	1
Total	-	23,0
DNA	-	2,0
Final volume	-	25,0
Amplification program: 5 min at 95 °C, 40 cycles: 30 s at 95 °C, 30 s at 61 °C and 30 s at 72 °C; then 72 °C– 7 min.		

PCR detection was performed by electrophoresis in 1.5% agarose gel using etidium bromide.

3.3 **Determining of the optimal nutrient medium for *Rathayibacter tritici*.**

After 5 days the media was fully developed with a yellow color and average diameter of 6mm in size. The bacteria *R. tritici* was tested with the main focus being on the size, shape and number of colonies. In this paper the focus is mainly on the colonies which are visible masses of microorganisms originating from a single mother cell. Thus, a colony constitutes a clone of bacteria all genetically alike [90]. The colonies were circular in shape, with entire margins and a convex elevation (figure 3.3) as noted and described by Anon, 2021 [90].

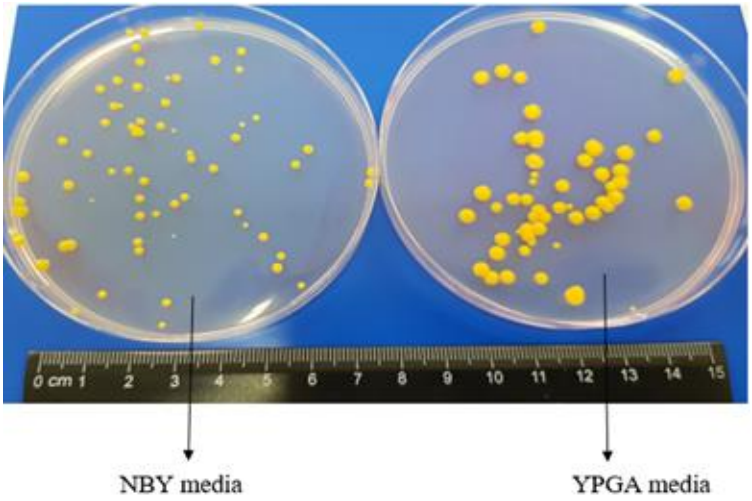

Figure 3.3 – Petri-dishes with 50 µl of 5th dilution *Rathayibacter tritici* on NBY and YPGA after 5 days incubation at 25^{o}C.

The colonies on YPGA were an average size of 3.92 mm whereas NBY had an average size of 2.44 mm (Table 3.4). YPGA media had larger colonies of about 5.60 mm and NBY media had larger colonies of about 3,00 mm and they are also smaller than the medium-sized colonies in YPGA media.

Table 3.4 – The average, standard deviation and confidence level across the 5th dilution and 6th dilution *Rathayibacter tritici* for its colony sizes

Value (Significance level 0,05)	**6th dilution**		**5th dilution**	
	YPGA	**NBY**	**YPGA**	**NBY**
Average	3.92	2.44	3.54	2.15
ST.DEV	0.99	0.81	3.30	0.82
Confidence	0.46	0.37	0.42	0.12

In both 5th and 6th dilution, the average colony size on YPGA was significantly larger than on NBY. The average number of colonies were equal in both medias in 6th dilution (Table 3.5). In 5th dilution, YPGA had significantly more colonies than NBY.

Table 3.5 – The total number of colonies in the petri-dishes across concentrations that is the 5th dilution and 6th dilution

Value (Significance level 0,05)	**6th dilution**		**5th dilution**	
	YPGA	**NBY**	**YPGA**	**NBY**
Total	48	47	586	444
Average	4,8	4,7	58,6	44,4
ST.DEV	2,1	1,4	9,6	10,3
Confidence	0,59	0,41	0,78	0,96

An essential media has water and nutrients specific to the bacteria necessary for bacterial growth as observed by Bonnet et al., (2019) [91] as such by virtue of having bigger colonies and a larger number of colonies YPGA medium is the most essential media to use than NBY medium in propagation of *Rathayibacter tritici*.

3.4 Optimization of seed sample preparation for the subsequent detection and identification of *Rathayibacter tritici, Pseudomonas fuscovaginae, Pseudomonas syringae* and *Xanthomonas translucens* by PCR.

The seeds were soaked in 54ml of PBS and placed on a shaker for 2 hours, as shown in Figure 3.4 as done by Muvingi M., Slovareva O.Yu., and Zargar M. (2022) [92]. The salt buffer (PBS) acted as an extractant ensuring that bacteria was extracted from the grain effectively.

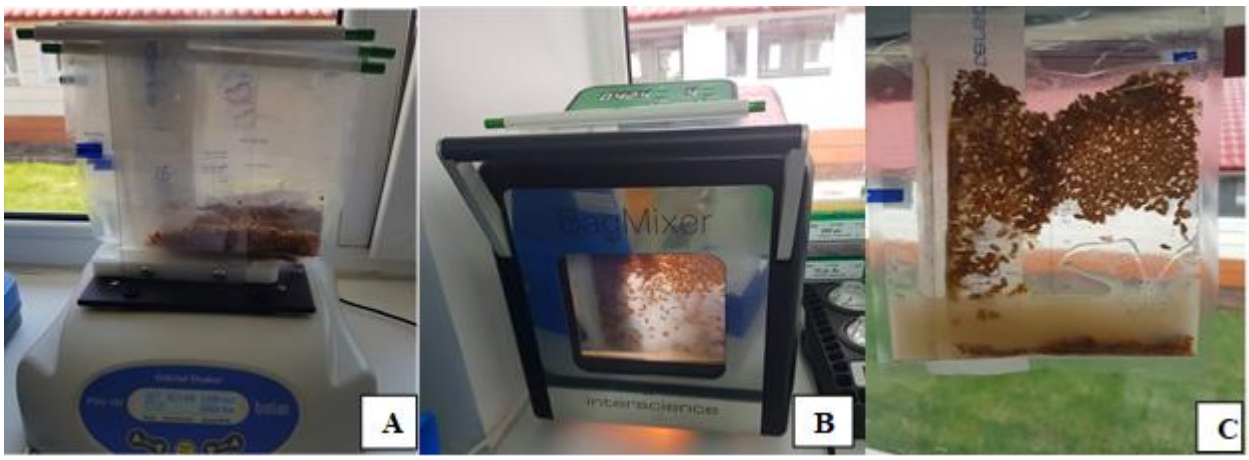

Figure 3.4 A shows the wheat on a shaker for soaking in PBS Buffer, B shows the homogenizer used in the study and C shows the wheat grain sample in PBS after homogenizer treatment.

The outcome shows that, in this manner, soaking the seeds for two hours and treating them with a homogenizer (Fig. 3.4 A and B) is sufficient to efficiently break down and purify every grain in the sample, guaranteeing the release of bacteria into the liquid portion of the sample. Thus, efficiently breaking down every grain in the sample, guaranteeing the release of bacteria into the liquid portion of the sample as shown in Figure 9C [92].

The precipitate was then transferred through funnels 10 (A) into centrifugation tubes, as depicted in Fig. 3.5 (B) illustrates the production of a flour precipitate of 1±0.3 g in each sample after the initial low-speed centrifugation at 5 minutes, 1200 g, and 4 °C.

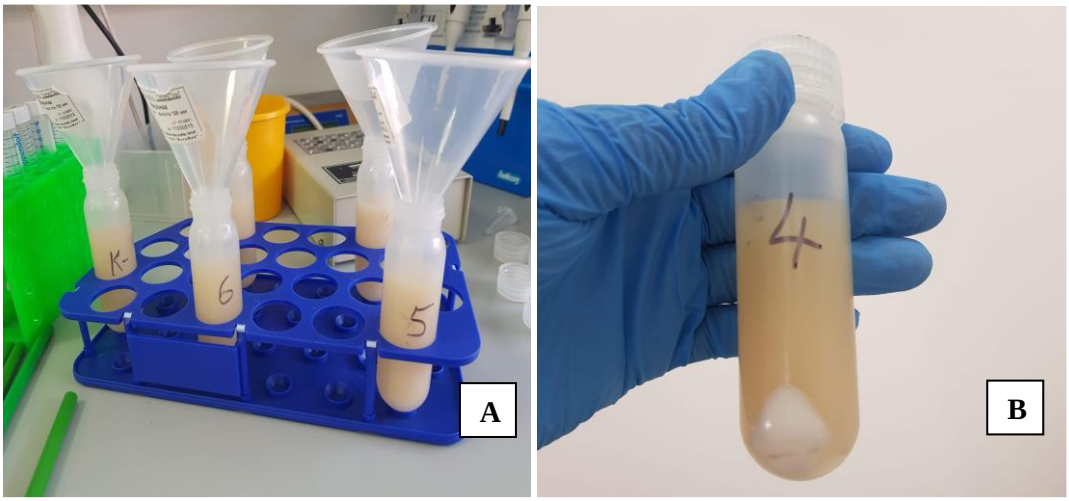

Figure 3.5 – Powdery precipitate after low-rotation centrifugation of the liquid part of a wheat sample treated with a homogenizer in PBS.

For the second centrifugation, the liquid precipitate was carefully transferred into a different centrifudge tube (Figure 3.6(A)). The majority of the starch in the sample, which could have inhibited later PCR testing, could be eliminated by using the low-speed centrifugation stage [92; 93]. A concentrated microbiota contained in the grain sample was obtained by centrifuging the supernatant at 10 minutes, 10,000 g, and 4 °C, as shown in figure 3.6 (B). The amount of flour in the resulting concentrate, figure 3.6 (C), was less than that in figure 3.5 B [92].

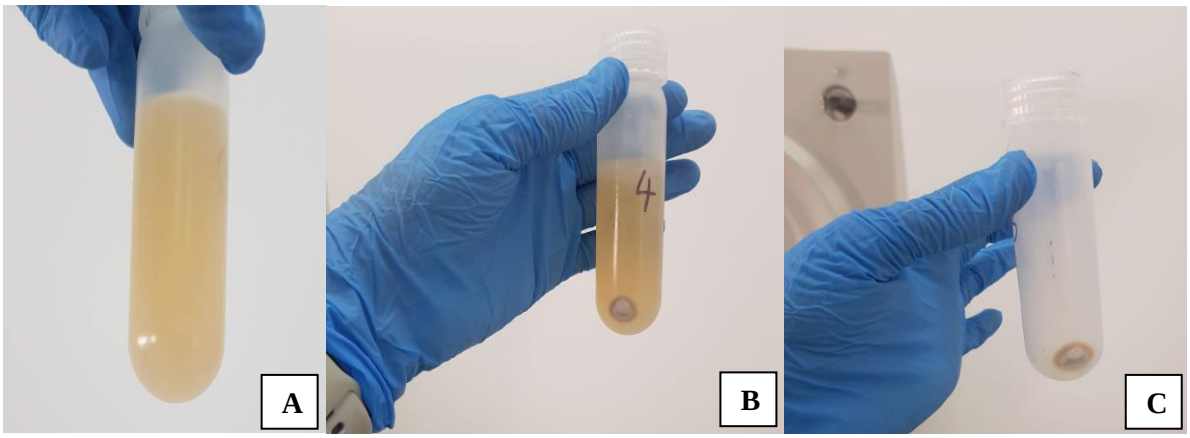

Figure 3.6 – Sediment after high-speed centrifugation of the supernatant obtained after the first low-speed centrifugation.

1ml of PBS was added to the centrifudge tube and placed on the vortex the resulting precipitate was then transferred into eppindorf tubes 200µl were kept for DNA extraction in two repeats and 200µl for inoculation onto nutrient media (figure 3.7) [92].

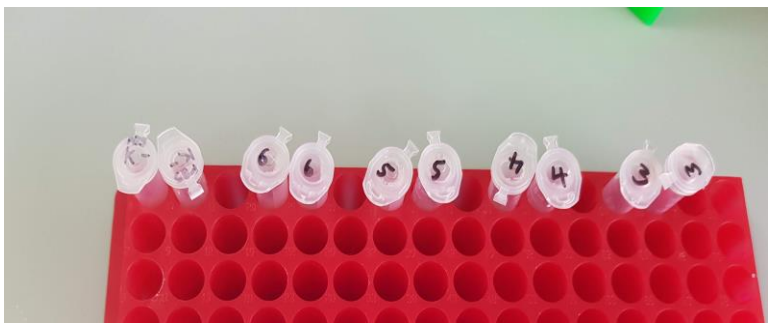

Figure 3.7 – the resulting precipitate and PBS transferred into eppindorf tubes.

After the extraction of DNA from the obtained analytical samples and PCR testing, the effectiveness of the sample preparation method used was evaluated, based on the result of determining the number of CFU/ml in the tested bacterial suspensions (Table. 3.6).

Table 3.6 Result of determination of the number of colony-forming units (CFU / ml) in the tested bacterial suspensions 7 days after plating

Strain	Dilution of the initial suspension				
	2	3	4	5	6
0335	$4,2\times10^7$	$4,2\times10^6$	$4,2\times10^5$	$4,2\times10^4$	$4,2\times10^3$
0440	$3,7\times10^7$	$3,7\times10^6$	$3,7\times10^5$	$3,7\times10^4$	$3,7\times10^3$
0337	$1,5\times10^7$	$1,5\times10^6$	$1,5\times10^5$	$1,5\times10^4$	$1,5\times10^3$
0378	0.6×10^7	0.6×10^6	0.6×10^5	0.6×10^4	0.6×10^3

Since bacterial suspensions of 2, 3, 4 and 5 dilutions were used to inoculate PBS in seed samples in a ratio of 1:9, the concentrations of bacteria in infected seed extracts prior to centrifugation were shown in Table 10 above, respectively $4.2x10^6$, $4.2x10^5$, $4.2x10^4$ and $4.2x10^3$ CFU/ml for strain 0335, $3.7x10^6$, $3.7x10^5$, $3.7x10^4$ and $3.7x10^3$ CFU/ml for strain 0440.

DNA was extracted from the obtained analytical samples and using species specific primers run PCR on the samples. When running rt-PCR record the results as shown in Tables 3.7 and 3.8. PCR testing of samples containing strain 0335 showed the presence of *P. fuscovaginae* in both bacterial suspensions in PBS and infected wheat grain samples (Table 3.7).

Table 3.7 Test result of samples of isolated DNA with the set "Pseudomonas fuscovaginae-PV" (Synthol, Russia)

Test tube ID	Ct, FAM	Ct, HEX	Result
0335-10^6-PBS	27,0	34,0	+
0335-10^5-PBS	29,7	33,8	+
0335-10^4-PBS	30,8	34,1	+
0335-10^3-PBS	31,6	34,2	+
0335-10 6-Extract-1	25,4	34,2	+
0335-10 6-Extract-2	25,2	34,6	+

Test tube ID	Ct, FAM	Ct, HEX	Result
0335-10 5-Extract-1	29,2	34,3	+
0335-10 5-Extract-2	29,4	34,3	+
0335-10 4-Extract-1	32,3	34,3	+
0335-10 4-Extract-2	31,6	34,2	+
0335-10 3-Extract-1	34,3	34,5	+
0335-10 3-Extract-2	35,3	34,5	+
0335-10 7-PBS-PC	22,9	34,1	+
Extract-1-NC		34,4	-
Extract-2-NC		34,6	-
PBS-NC		34,6	-

Note: Ct is the threshold cycle of PCR, FAM is the detection channel of PCR specificity, HEX is the detection channel of internal positive PCR control, "+" is positive, "- "is negative, PBS is phosphate-salt buffer, PC is a positive control sample, NC is a negative control sample

PCR testing of negative control samples for *P. fuscovaginae* (uninfected grain samples and PBS) was negative.

PCR testing of samples containing strain 0378 showed the presence of *R. tritici* in both bacterial suspensions in PBS and infected wheat grain samples (Table 3.8).

Table 3.8 Test result of samples of isolated DNA with the Primer set BTRIT2F / BTRIT2R.

Test tube ID	Ct, FAM	Ct, HEX	Result
0378-10^6-PBS	30.1	30,8	+
0378-10^5-PBS	32,2	31,3	+
0378-10^4-PBS		31,1	-
0378-10^3-PBS		31,6	-
0378-10 6-Extract-1	28,5	30,7	+
0378-10 6-Extract-2	27,7	30,5	+
0378-10 5-Extract-1	31,6	31,2	+
0378-10 5-Extract-2	32,0	31,5	+
0378-10 4-Extract-1		31,6	-

Test tube ID	Ct, FAM	Ct, HEX	Result
0378-10 4-Extract-2		31,5	-
0378-10 3-Extract-1		31,8	-
0378-10 3-Extract-2		31,5	-
0378-10 7-PBS-PC	27,7	31,0	+
Extract-1-NC		34,4	-
Extract-2-NC		34,6	-
PBS-NC		34,6	-

Note: Ct is the threshold cycle of PCR, FAM is the detection channel of PCR specificity, HEX is the detection channel of internal positive PCR control, "+" is positive, "-" is negative, PBS is phosphate-salt buffer, PC is a positive control sample, NC is a negative control sample.

PCR testing of negative control samples for *R. tritici* (uninfected grain samples and PBS) was negative.

In each of the tested samples infected with strain 0440, the bacterium *Pseudomonas syringae* was detected (Fig. 3.8).

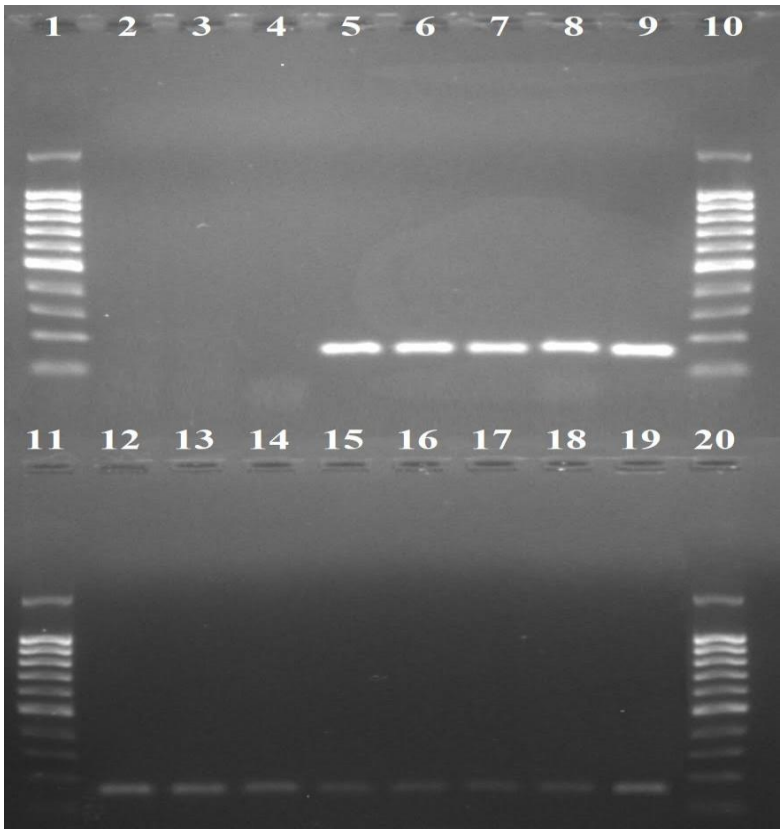

Figure 3.8 Electrophoregram of PCR test result of samples infected with strain 0440 for the presence of *Pseudomonas syringae* with PsyF/PsyR primers

*1, 10, 11 and 20 – DNA length marker, 2, 3 – Negative control sample (Extract), 4 – Negative control sample (buffer), 5–8 – buffer infected with strain 0440 at concentrations of 3.7*10^{6}–3.7*10^{3} respectively, 9, 12 – strain 0440 in extract (3.7*10^{6}), 13, 14 – strain 0440 in extract (3.7*10^{5}), 15, 16 – strain 0440 in extract (3.7*10^{4}), 17,18 – strain 0440 in extract (3,7*10^{5}), 19 – Positive control sample.*

PCR with the DNA of negative control samples when tested with PsyF/PsyR primers showed a negative result. A similar test result of negative control samples was obtained in PCR with primers 4F1/4R1 (Fig. 3.9).

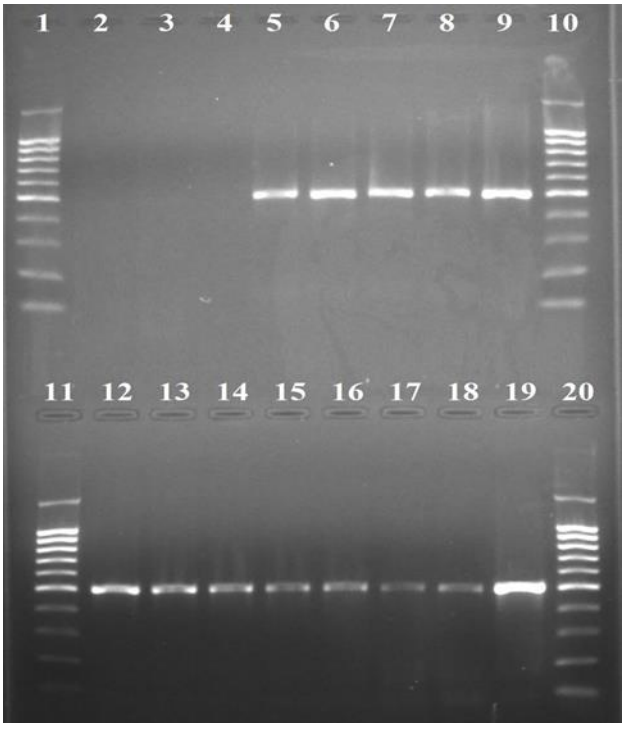

Figure 3.9. An electrophoregram of the result of PCR testing of samples infected with strain 0337 for the presence of *Xanthomonas translucens* with primers 4F1/4R1: *1, 10, 11 and 20 is a marker of DNA length; 2, 3 is a tricate control sample (extract); 4 — about the tricial control sample (buffer); 5–8 – buffer infected with strain 0337 in concentrations of 1.5·106–1.5·103, respectively; 9, 12 – strain 0337 in extract (1.5·106); 13, 14 – strain 0337 in the extract (1,5·105); 15, 16 – strain 0337 in the extract (1.5·104); 17, 18 — strain 0337 in extract (1,5·105); 19 — positive control sample.*

For samples infected with strain 0337, positive results were obtained when tested with primers 4F1/4R1.

Inhibition of PCR with *P. fuscovaginae*-PB was not observed for any of the tested DNA samples isolated from samples contaminated with strains 0335, 0337 and 0440. This result shows that double centrifugation in sample preparation is effective for removing the starch remaining in the sample due to the crushing of the seeds. We are aware of the successful use of the two-stage centrifugation method for removing inhibitory substances from plant material [94], but it has never been used to remove starch in the preparation of wheat grain samples for PCR testing. The result also allows us to conclude that the DNA was effectively isolated by the set “Proba-GS”, AgroDiagnostics (Russia).

As a result of the study seed sample preparation was optimized as follows: Take 25g of wheat grain and place in homogenization bags. Add 60ml of PBS and shake the wheat samples at 100rpm for 2 hours.

After 2 hours, place the bags in a homogenizer (Bag Mixer 400SW, Interscience, France) with the position of the blades closest to the homogenizer door at speed 4 for 5 mins. After place the sample on a shaker for another fifteen minutes.

Transferre the liquid through funnels, into centrifugation tubes. Centrifuge the samples at 1 200g and 4°C for 5 minutes.

Accurately transfere the supernatant into a different centrifudge tube and centrifuge for 10minutes at 10,000g and 4°C for 10minutes.

Accurately remove the supernant and add 1ml of PBS into the centrifudge tube and place on the vortex so as to mix the precipitate and the buffer. Carefuly transfer the resulting analytical samples into eppindorf tubes. The resulting analytical sample can be used for further tests. This method is illustrated in Figure 3.10.

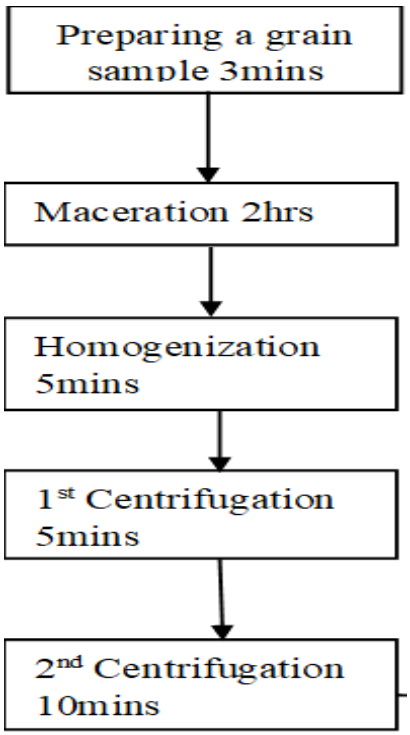

Figure 3.10 Preparation method for seed samples.

Infected seeds are the primary means by which bacteriosis of cereals spreads [99]. The disease's symptoms, if present, are insufficient to identify the pathogen. To effectively manage bacteriosis through phytosanitary measures, grain batches' samples are subjected to laboratory diagnostic testing. Due to its adaptability and quick execution, PCR is the most efficient diagnostic technique [65].

3.5 Identification of *Pseudomonas fuscovaginae, Rathayibacter tritici Pseudomonas syringae* and *Xanthomonas translucens* in plant samples collected from Moscow region, The Republic of Crimea and Stavropol region.

After collection of plant samples from the three regions of the Russian Federation described in Chapter 3 “results” section 3.1, analytical plant samples were prepared and from these DNA was extracted. Extracted DNA was tested for the identification of *Pseudomonas fuscovaginae*, *Rathayibacter tritici*, *Pseudomonas syringae* and *Xanthomonas translucens* as decribed in Chapter 2 “materials and methods” section 2.2.4.2.

3.5.1 *Rathayibacter tritici*

For the “Rt3F/3R” test, the results of testing a sample were considered positive if a 520b.p amplicon was detected, also if the 520b.p amplicon was not detected for the negative control. The results were negative if there was no 520b.p amplicon detected in the testing sample also if the 520b.p amplicon was detected for the positive control. The required condition for the interpretation of a testing sample as negative, was marked by the presence of a 714b.p amplicon in the IPC test for the negative control and testing samples. In other cases, the result of PCR was considered in-applicable.

The following results were obtained after running PCR using Rt3F/3R for the detection of *R. tritici* in the analytical plant samples. A total of 55 DNA samples extracted from analytical plant samples collected in Moscow were tested. The electropherograms of DNA testing with primers Rt3F/3R are shown in Figure 3.11.

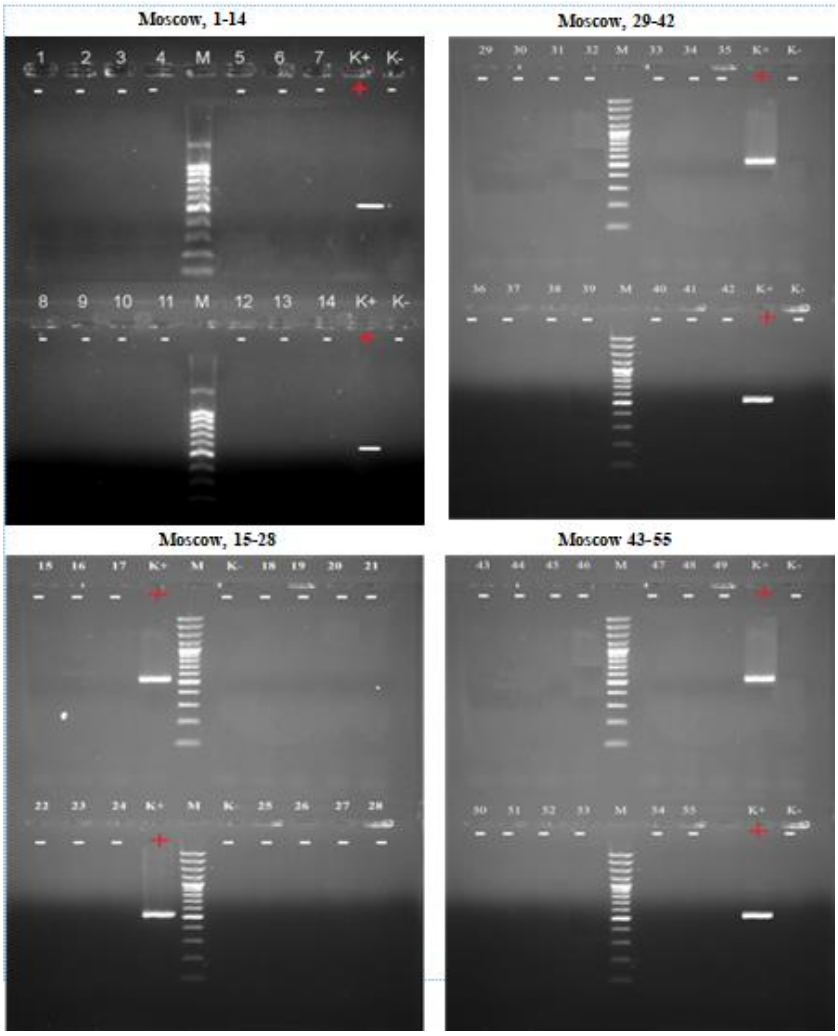

Figure 3.11. Note: (_) means unknown cultivar, + means detected and – means not detected. 1. Winter rye Snezhana, 2. Soft winter wheat Zhiva, 3. Soft winter wheat Alekseevich, 4. Soft winter wheat Urup, 5. Soft winter wheat Morozko, 6. Soft winter wheat Timiryazevskaya Yubilejnaya, 7. Soft winter wheat Moskovskaya 56, 8. Soft winter wheat Biryuza, 9. Soft winter wheat Timiryazevskaya 150, 10. Soft winter wheat Graf, 11 Triticale winter Aleksandr, 12. Winter wheat Don yantarnaya, 13. Triticale winter Victor, 14. Soft winter wheat Vassa, 15. Winter wheat two-grain _, 16. Triticale winter Nemchinovskij 56, 17. Winter wheat Eremeevna, 18. Triticale winter spherical Titus, 19. Soft winter wheat Moskovskaya 39, 20. Triticale winter Valentin 90, 21. Soft winter wheat Dublet, 22. Soft winter wheat Kavalerka, 23. Soft winter wheat Skarlet Zarya, 24. Soft winter wheat Nemchinovskaya 24, 25. Winter durum wheat Pobeda 70, 26. Soft winter wheat Legenda, 27. Soft winter wheat Avesta, 28. Winter rye Verasen', 29. Soft winter wheat Inna, 30. Winter wheat Terra, 31. Triticale winter Timiryazevskaya 150, 32. Soft winter wheat Mel'nica, 33. Soft winter wheat Asket, 34. Soft winter wheat Velena, 35. Soft winter wheat Vanya, 36. Soft winter wheat Artel', 37. Soft winter wheat Nemchinovskaya 85, 38. Soft winter wheat Vidya, 39. Soft winter wheat Don Lira, 40. Soft winter wheat Golubaya, 41. Soft winter wheat Moskovskaya 40, 42. Soft winter wheat Don 107, 43. Soft winter wheat Stepnaya, 44. Soft winter wheat Gubernator Dona, 45. Soft winter wheat Rostovchanka, 46. Soft winter wheat Vekha, 47. Soft winter wheat Nemchinovskaya 57, 48. Soft winter wheat Avgusta, 49. Soft winter wheat Soberbash, 50. Soft winter wheat Anka, 51. Soft winter wheat Prignala, 52. Soft winter wheat Antonina, 53. Soft winter wheat Nemchinovskaya 17, 54. Soft winter wheat Bezostaya 100 and 55. Winter rye _. M — DNA Length Marker 100+ bp DNA ladder (100-1000 b.p. («Evrogen», Russia) (Timiryazev field experimental

station, Russian State Agrarian University — Moscow Agricultural Academy named after K.A. Timiryazev, Moscow, 2020).

The results are interpreted in detail and shown in table 11.1 Appendix 11. *Rathayibacter tritici* DNA was not detected in all 55 analytical plant samples. Positive results like 520b.p amplicon were obtained just for positive control (PC).

Electropherograms of IPC testing of the 55 DNA samples from Moscow are shown in figure 3.12.

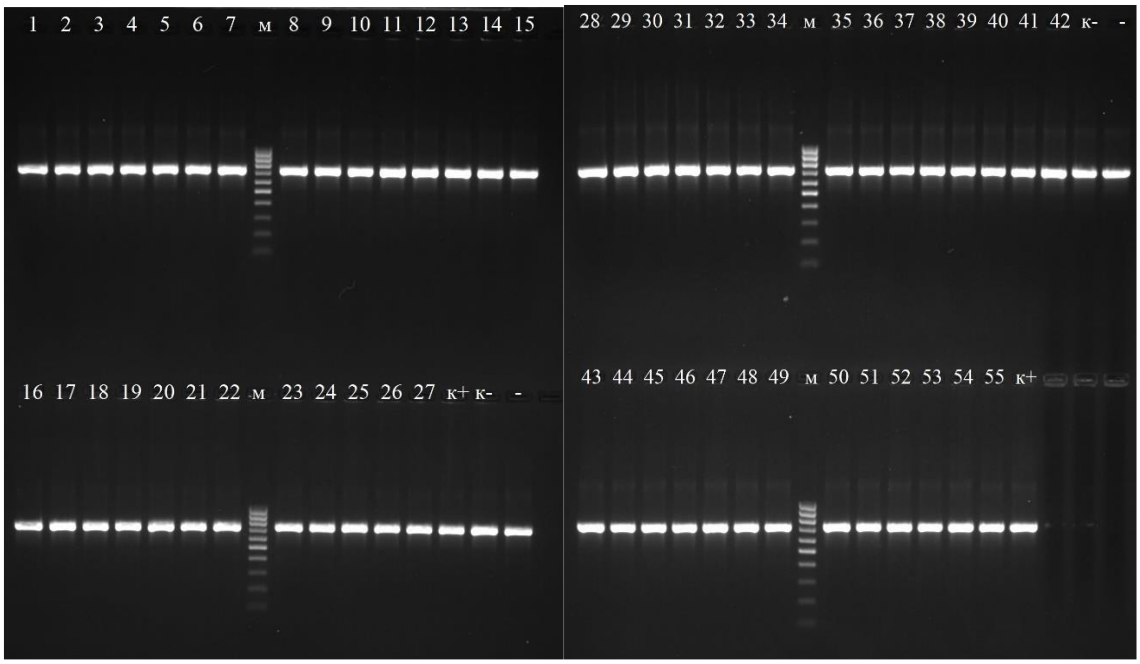

Figure3.12.Note: k+ means positive control, k- means negative conrol, (_) means unknown cultivar and + means detected. 1. Winter rye Snezhana, 2. Soft winter wheat Zhiva, 3. Soft winter wheat Alekseevich, 4. Soft winter wheat Urup, 5. Soft winter wheat Morozko, 6. Soft winter wheat Timiryazevskaya Yubilejnaya, 7. Soft winter wheat Moskovskaya 56, 8. Soft winter wheat Biryuza, 9. Soft winter wheat Timiryazevskaya 150, 10. Soft winter wheat Graf, 11 Triticale winter Aleksandr, 12. Winter wheat Don yantarnaya, 13. Triticale winter Victor, 14. Soft winter wheat Vassa, 15. Winter wheat two-grain _, 16. Triticale winter Nemchinovskij 56, 17. Winter wheat Eremeevna, 18. Triticale winter spherical Titus, 19. Soft winter wheat Moskovskaya 39, 20. Triticale winter Valentin 90, 21. Soft winter wheat Dublet, 22. Soft winter wheat Kavalerka, 23. Soft winter wheat Skarlet Zarya, 24. Soft winter wheat Nemchinovskaya 24, 25. Winter durum wheat Pobeda 70, 26. Soft winter wheat Legenda, 27. Soft winter wheat Avesta, 28. Winter rye Verasen', 29. Soft winter wheat Inna, 30. Winter wheat Terra, 31. Triticale winter Timiryazevskaya 150, 32. Soft winter wheat Mel'nica, 33. Soft winter wheat Asket, 34. Soft winter wheat Velena, 35. Soft winter wheat Vanya, 36. Soft winter wheat Artel', 37. Soft winter wheat Nemchinovskaya 85, 38. Soft winter wheat Vidya, 39. Soft winter wheat Don Lira, 40. Soft winter wheat Golubaya, 41. Soft winter wheat Moskovskaya 40, 42. Soft winter wheat Don 107, 43. Soft winter wheat Stepnaya, 44. Soft winter wheat Gubernator Dona, 45. Soft winter wheat Rostovchanka, 46. Soft winter wheat Vekha, 47. Soft winter wheat Nemchinovskaya 57, 48. Soft winter wheat Avgusta, 49. Soft winter wheat Soberbash, 50. Soft winter wheat Anka, 51. Soft winter wheat Prignala, 52. Soft winter

wheat Antonina, 53. Soft winter wheat Nemchinovskaya 17, 54. Soft winter wheat Bezostaya 100 and 55. Winter rye _. M — DNA Length Marker 100+ bp DNA ladder (100-1000 b.p. («Evrogen», Russia) (Timiryazev field experimental station, Russian State Agrarian University — Moscow Agricultural Academy named after K.A. Timiryazev, Moscow, 2020).

A total of 60 DNA samples extracted from analytical plant samples collected in the Republic of Crimea were tested. Electropherograms of DNA testing with primers Rt3F/3R are shown in figure 3.13.

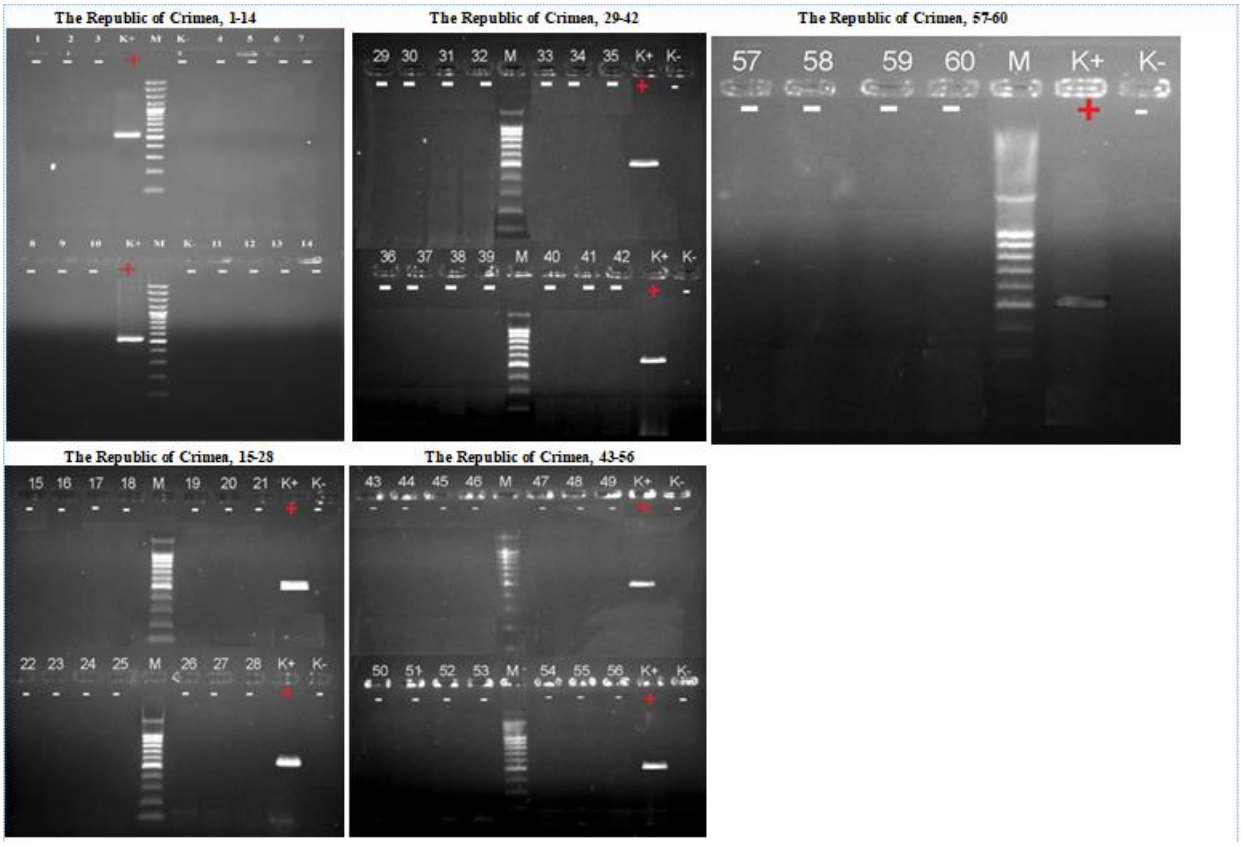

Figure 3.13. Note: (-) means not detected, + means detected and _ cultivar unknown. 1. Winter barley Onega, 2. Winter wheat Aksiniya, 3. Winter wheat Asket, 4. Winter wheat Asket, 5. Winter barley Rajz, 6. Winter wheat Governor of the Don, 7. Winter wheat Governor of the Don, 8. Winter barley Rajz, 9. Winter barley Rajz, 10. Winter wheat Asket, 11, Winter barley Onega, 12. Winter wheat Gubernator Dona, 13. Winter wheat Gubernator Dona, 14. Winter wheat Gubernator Dona, 15. Winter wheat Gubernator Dona, 16. Winter wheat Asket, 17. Winter barley Onega, 18. Winter barley Onega, 19. Winter barley Onega, 20. Winter wheat Asket, 21. Winter wheat Asket, 22. Winter wheat Asket, 23. Winter wheat Asket, 24. Winter wheat Asket, 25. Winter wheat Asket, 26. Winter wheat Asket, 27. Winter wheat Asket, 28. Winter wheat Asket, 29. Winter wheat Asket, 30. Winter wheat Asket, 31. Winter wheat Asket, 32. Winter barley V'yuga, 33. Winter barley Rubezh, 34. Winter barley Sprinter, 35. Winter barley Ob"em. 36. Winter barley Master, 37. Winter barley Espada, 38. Winter wheat Anka, 39. Winter wheat Velena, 40. Winter wheat Vekha, 41. Winter wheat Karavan, 42. Oats Vernyj, 43. Oats Mezmaj, 44. Oats Chernigovskij -nebo, 45. Oats Loshad', 46. Oats Podgornyj, 47. Oats Guzeripl', 48. Winter barley Master, 49. Winter barley Espada, 50. Winter barley V'yuga, 51. Winter barley Rubezh, 52. Winter barley Sprinter, 53. Winter barley Ob"em, 54. Triticale, wheat, barley _, 55. Winter wheat Korona, 56. Winter barley Sprinter, 57. Winter barley Rubezh, 58. Cereal-legume mixture _, 59. Oat_ and 60. Winter wheat

Korona. M — DNA Length Marker 100+ bp DNA ladder (100-1000 b.p. («Evrogen», Russia) (three regions Belogorskij, Krasnogvardejskij, and Simferopolskij of the Republic of Crimea, 2021).

The results are interpreted in detail and shown in table 11.1 Appendix 11. *Rathayibacter tritici* DNA was not detected in all 60 analytical plant samples. Positive results like 520b.p amplicon were obtained just for positive control (PC).

Electropherograms of IPC testing of the 60 DNA samples from the Republic of Crimea are shown in figure 3.14.

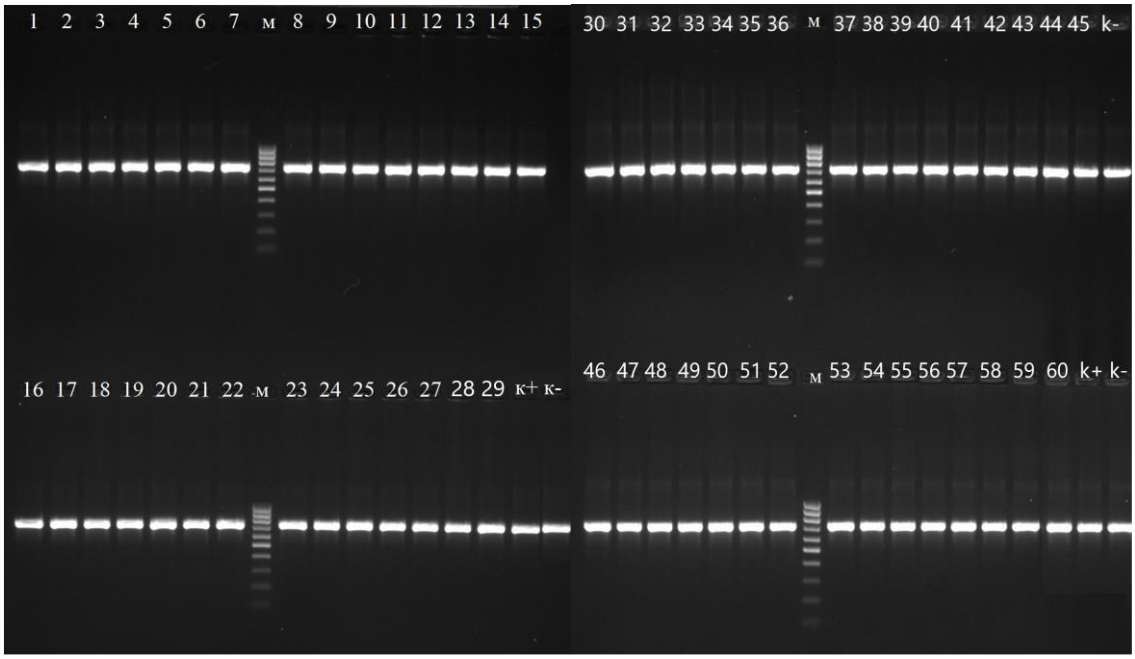

Figure 3.14. Note: k+ means positive control, k- means negative conrol, (_) means unknown cultivar and + means detected. 1. Winter barley Onega, 2. Winter wheat Aksiniya, 3. Winter wheat Asket, 4. Winter wheat Asket, 5. Winter barley Rajz, 6. Winter wheat Governor of the Don, 7. Winter wheat Governor of the Don, 8. Winter barley Rajz, 9. Winter barley Rajz, 10. Winter wheat Asket, 11, Winter barley Onega, 12. Winter wheat Gubernator Dona, 13. Winter wheat Gubernator Dona, 14. Winter wheat Gubernator Dona, 15. Winter wheat Gubernator Dona, 16. Winter wheat Asket, 17. Winter barley Onega, 18. Winter barley Onega, 19. Winter barley Onega, 20. Winter wheat Asket, 21. Winter wheat Asket, 22. Winter wheat Asket, 23. Winter wheat Asket, 24. Winter wheat Asket, 25. Winter wheat Asket, 26. Winter wheat Asket, 27. Winter wheat Asket, 28. Winter wheat Asket, 29. Winter wheat Asket, 30. Winter wheat Asket, 31. Winter wheat Asket, 32. Winter barley V'yuga, 33. Winter barley Rubezh, 34. Winter barley Sprinter, 35. Winter barley Ob"em. 36. Winter barley Master, 37. Winter barley Espada, 38. Winter wheat Anka, 39. Winter wheat Velena, 40. Winter wheat Vekha, 41. Winter wheat Karavan, 42. Oats Vernyj, 43. Oats Mezmaj, 44. Oats Chernigovskij -nebo, 45. Oats Loshad', 46. Oats Podgornyj, 47. Oats Guzeripl', 48. Winter barley Master, 49. Winter barley Espada, 50. Winter barley V'yuga, 51. Winter barley Rubezh, 52. Winter barley Sprinter, 53. Winter barley Ob"em, 54. Triticale, wheat, barley _, 55. Winter wheat Korona, 56. Winter barley Sprinter, 57. Winter barley Rubezh, 58. Cereal-legume mixture _, 59. Oat_ and 60. Winter wheat Korona. M — DNA Length Marker 100+ bp DNA ladder (100-

1000 b.p. («Evrogen», Russia) (three regions Belogorskij, Krasnogvardejskij, and Simferopolskij of the Republic of Crimea, 2021).

A total of 66 DNA samples extracted from analytical plant samples collected in Stavropol region were tested. From Stavropol region 66 DNA samples were tested. Electropherograms of DNA testing with primers Rt3F/3R are shown in figure 3.15.

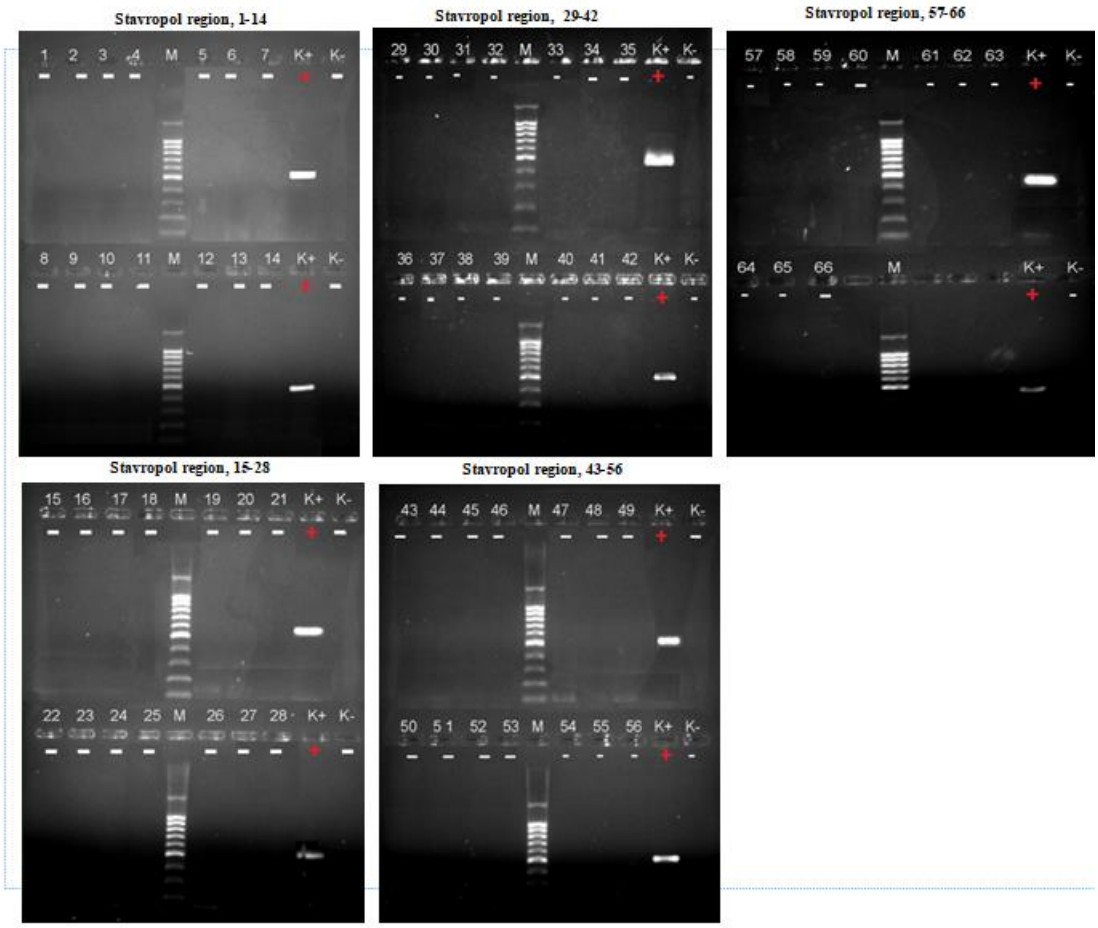

Figure 3.15. Note: (-) means not detected, + means detected and _ cultivar unknown. 1. Winter barley Rubezh, 2. Winter wheat Soberbash, 3. Winter wheat Stil' 18, 4 Winter wheat Tanya, 5. Winter wheat Stil' 18, 6. Winter wheat Tanya, 7. Winter wheat Stil' 18, 8. Winter wheat Tanya, 9. Winter wheat Tanya, 10. Winter wheat Grom, 11. Winter wheat Tanya, 12. Winter wheat Tanya, 13. Winter wheat Tanya, 14. Winter wheat Grom, 15. Winter wheat Tanya, 16. Winter barley Bazal't, 17. Winter wheat Godovshchina, 18. Winter wheat Godovshchina, 19. Winter durum wheat Amazonka, 20. Winter durum wheat Amazonka, 21. Spring durum wheat Yasen'ka, 22. Winter durum wheat Amazonka, 23. Winter durum wheat Amazonka, 24. Winter durum wheat Yahont, 25. Winter durum wheat Agat Donskoj, 26. Winter durum wheat Stepnoj yantar', 27. Winter wheat Centrina, 28. Durum winter wheat Odari, 29. Winter durum wheat Amazonka, 30. Winter wheat Soft Yukka, 31. Winter durum wheat Yahont, 32. Winter durum wheat Amazonka, 33. Durum winter wheat Odari, 34. Winter wheat_, 35. Winter barley + oats_, 36. Winter soft wheat_, 37. Winter barley_, 38. Winter soft wheat_, 39. Winter soft wheat _, 40. Winter soft wheat_, 41. Winter soft wheat_, 42. Winter soft wheat_, 43. Winter barley_, 44. Winter soft wheat_, 45. Winter soft wheat_, 46. Winter barley_, 47. Wheat Chernyava, 48. Winter wheat

Alekseevich, 49. Winter wheat Alekseevich, 50. Winter wheat Chernyava, 51. Winter barley Karrera, 52. Winter barley Karrera, 53. Barley Alekseevich, 54. Winter barley Karrera, 55. Wheat Chernyava. 56. Triticale Grain, 57. Winter wheat Antonina, 58. Spring barley Gris, 59. Spring barley Azimuth, 60. Spring barley Leon, 61. Spring barley Voin, 62. Spring barley Format, 63. Spring barley Shchedrin, 64. Spring barley Vakula, 65. Spring barley Avalon and 66. Winter wheat soft_. M — DNA Length Marker 100+ bp DNA ladder (100-1000 b.p. (Kochubeevskij, Budennovskij, Sovetskij, Georgievskij, Andropovskij, Aleksandrovskij, SHpakovskij, Novoselickij and Mineralovodskij of Stavropol region. 2022).

The results are interpreted in detail and shown in table 11.1 Appendix 11. *Rathayibacter tritici* DNA was not detected in all 66 analytical plant samples. Positive results like 520b.p amplicon were obtained just for positive control (PC).

Electropherograms of IPC testing of the 66 DNA samples from Stavropol region are shown in figure 3.16.

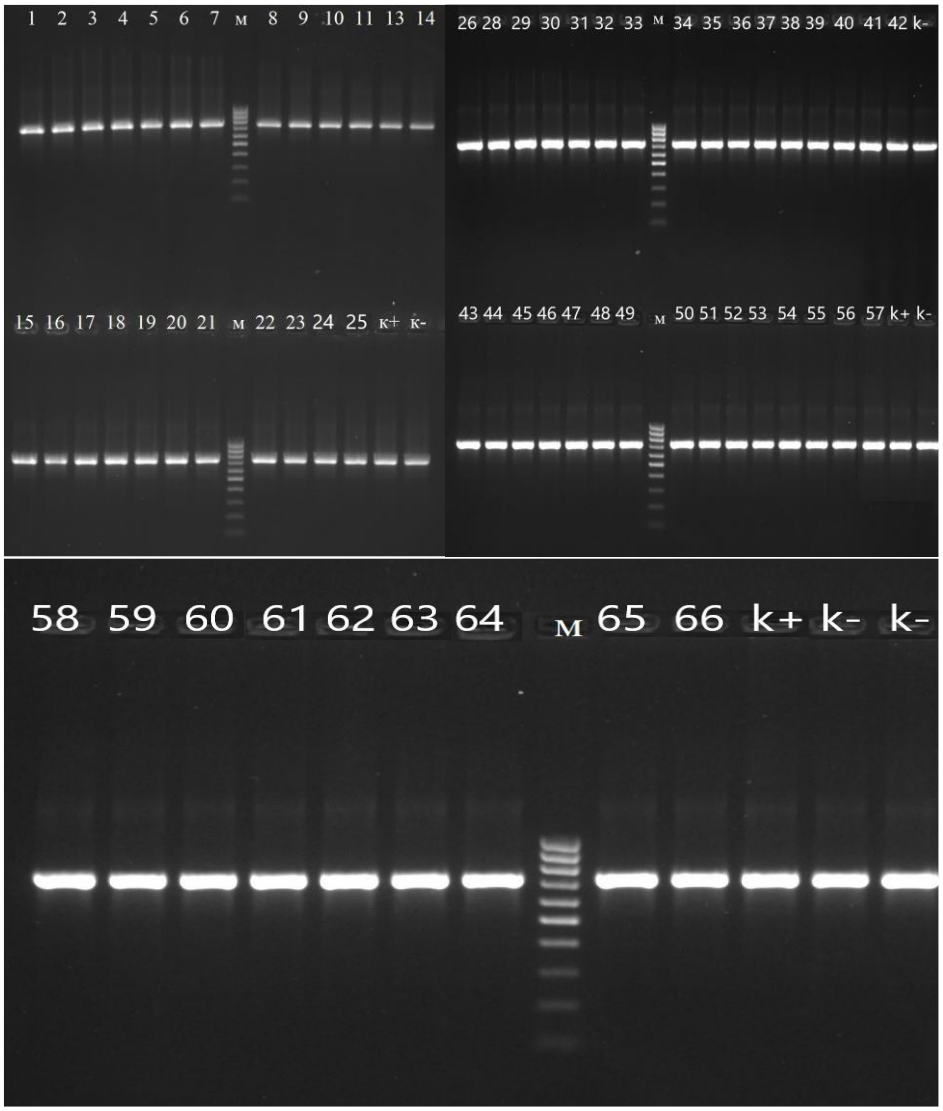

Figure 3.16. Note: k+ means positive control, k- means negative conrol, (_) means unknown cultivar and + means detected. 1. Winter barley Rubezh, 2. Winter wheat Soberbash, 3. Winter wheat Stil' 18, 4 Winter wheat Tanya, 5. Winter wheat Stil' 18, 6. Winter wheat Tanya, 7. Winter wheat Stil' 18, 8.

Winter wheat Tanya, 9. Winter wheat Tanya, 10. Winter wheat Grom, 11. Winter wheat Tanya, 12. Winter wheat Tanya, 13. Winter wheat Tanya, 14. Winter wheat Grom, 15. Winter wheat Tanya, 16. Winter barley Bazal't, 17. Winter wheat Godovshchina, 18. Winter wheat Godovshchina, 19. Winter durum wheat Amazonka, 20. Winter durum wheat Amazonka, 21. Spring durum wheat Yasen'ka, 22. Winter durum wheat Amazonka, 23. Winter durum wheat Amazonka, 24. Winter durum wheat Yahont, 25. Winter durum wheat Agat Donskoj, 26. Winter durum wheat Stepnoj yantar', 27. Winter wheat Centrina, 28. Durum winter wheat Odari, 29. Winter durum wheat Amazonka, 30. Winter wheat Soft Yukka, 31. Winter durum wheat Yahont, 32. Winter durum wheat Amazonka, 33. Durum winter wheat Odari, 34. Winter wheat_, 35. Winter barley + oats_, 36. Winter soft wheat_, 37. Winter barley_, 38. Winter soft wheat_, 39. Winter soft wheat _, 40. Winter soft wheat_, 41. Winter soft wheat_, 42. Winter soft wheat_, 43. Winter barley_, 44. Winter soft wheat_, 45. Winter soft wheat_, 46. Winter barley_, 47. Wheat Chernyava, 48. Winter wheat Alekseevich, 49. Winter wheat Alekseevich, 50. Winter wheat Chernyava, 51. Winter barley Karrera, 52. Winter barley Karrera, 53. Barley Alekseevich, 54. Winter barley Karrera, 55. Wheat Chernyava. 56. Triticale Grain, 57. Winter wheat Antonina, 58. Spring barley Gris, 59. Spring barley Azimuth, 60. Spring barley Leon, 61. Spring barley Voin, 62. Spring barley Format, 63. Spring barley Shchedrin, 64. Spring barley Vakula, 65. Spring barley Avalon and 66. Winter wheat soft_. M — DNA Length Marker 100+ bp DNA ladder (100-1000 b.p. (Kochubeevskij, Budennovskij, Sovetskij, Georgievskij, Andropovskij, Aleksandrovskij, SHpakovskij, Novoselickij and Mineralovodskij of Stavropol region. 2022).

In all the three regions of the Russian Federation *Rathayibacter tritici* was not detected.

3.5.2 *Xanthomonas translucens*

For the “4F1/4R1” test, the results of testing a sample were considered positive if a 503b.p amplicon was detected, also if the 503b.p amplicon was not detected for the negative control. The results were negative if there was no 503b.p amplicon detected in the testing sample also if the 503b.p amplicon was detected for the positive control. The required condition for the interpretation of a testing sample as negative, was marked by the presence of a 714b.p amplicon in the IPC test for the negative control and testing samples. In other cases, the result of PCR was considered in-applicable.

The following results were obtained after running PCR using 4F1/4R1 for the detection of *Xanthomonas translucens* in the analytical plant samples. A total of 55 DNA samples extracted from analytical plant samples collected in Moscow were tested. Electropherograms of DNA testing with primers 4F1/4R1 are shown in figure 3.17.

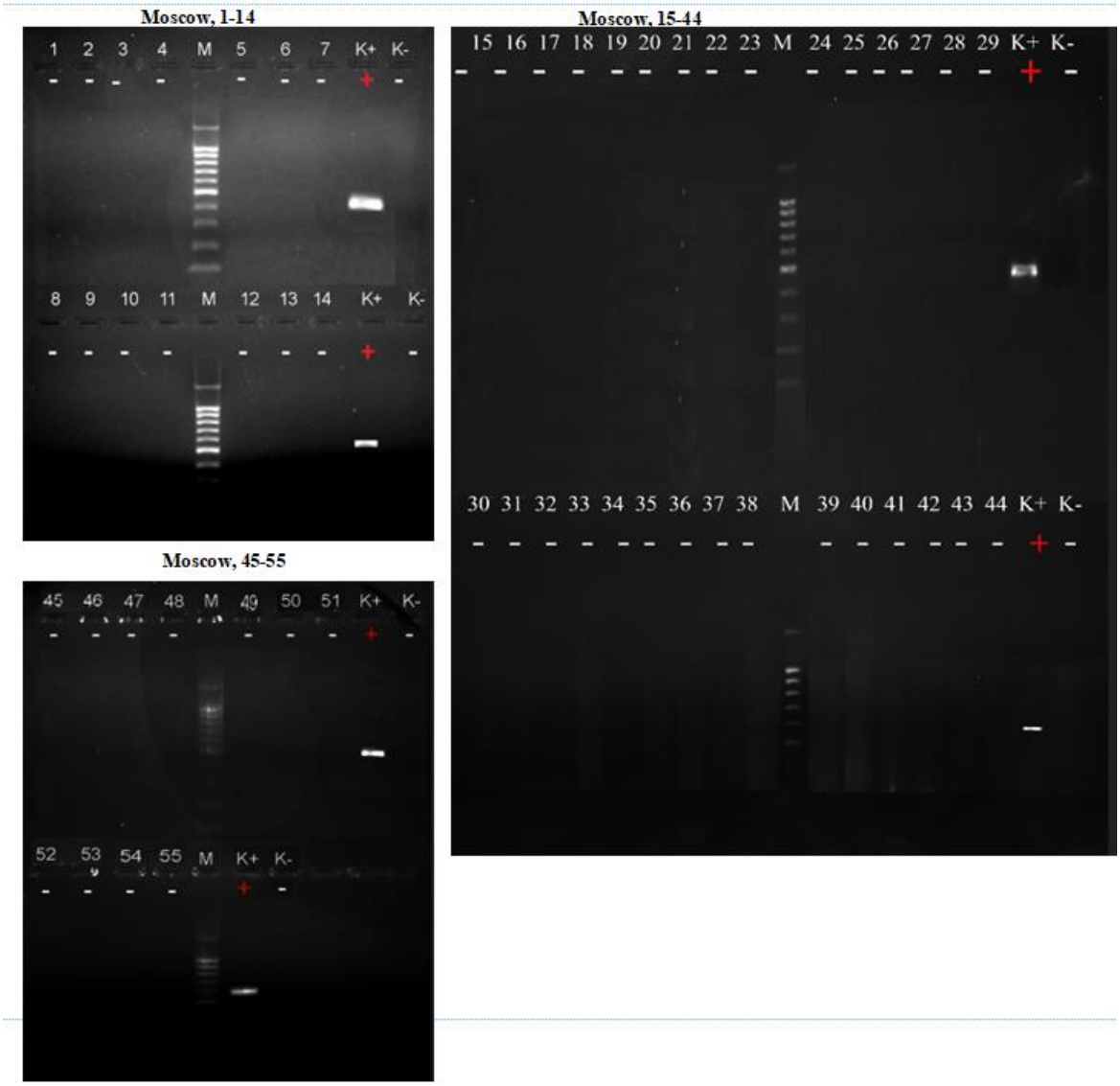

Figure 3.17. Note: (_) means unknown cultivar, + means detected and – means not detected. 1. Winter rye Snezhana, 2. Soft winter wheat Zhiva, 3. Soft winter wheat Alekseevich, 4. Soft winter wheat Urup, 5. Soft winter wheat Morozko, 6. Soft winter wheat Timiryazevskaya Yubilejnaya, 7. Soft winter wheat Moskovskaya 56, 8. Soft winter wheat Biryuza, 9. Soft winter wheat Timiryazevskaya 150, 10. Soft winter wheat Graf, 11 Triticale winter Aleksandr, 12. Winter wheat Don yantarnaya, 13. Triticale winter Victor, 14. Soft winter wheat Vassa, 15. Winter wheat two-grain _, 16. Triticale winter Nemchinovskij 56, 17. Winter wheat Eremeevna, 18. Triticale winter spherical Titus, 19. Soft winter wheat Moskovskaya 39, 20. Triticale winter Valentin 90, 21. Soft winter wheat Dublet, 22. Soft winter wheat Kavalerka, 23. Soft winter wheat Skarlet Zarya, 24. Soft winter wheat Nemchinovskaya 24, 25. Winter durum wheat Pobeda 70, 26. Soft winter wheat Legenda, 27. Soft winter wheat Avesta, 28. Winter rye Verasen', 29. Soft winter wheat Inna, 30. Winter wheat Terra, 31. Triticale winter Timiryazevskaya 150, 32. Soft winter wheat Mel'nica, 33. Soft winter wheat Asket, 34. Soft winter wheat Velena, 35. Soft winter wheat Vanya, 36. Soft winter wheat Artel', 37. Soft winter wheat Nemchinovskaya 85, 38. Soft winter wheat Vidya, 39. Soft winter wheat Don Lira, 40. Soft winter wheat Golubaya, 41. Soft winter wheat Moskovskaya 40, 42. Soft winter wheat Don 107, 43. Soft winter wheat Stepnaya, 44. Soft winter wheat Gubernator Dona, 45. Soft winter wheat Rostovchanka, 46. Soft winter wheat Vekha, 47. Soft winter wheat Nemchinovskaya 57, 48. Soft winter wheat Avgusta, 49. Soft winter wheat Soberbash, 50. Soft winter wheat Anka, 51. Soft winter wheat Prignala, 52. Soft winter wheat Antonina, 53. Soft winter

wheat Nemchinovskaya 17, 54. Soft winter wheat Bezostaya 100 and 55. Winter rye _. M — DNA Length Marker 100+ bp DNA ladder (100-1000 b.p. («Evrogen», Russia) (Timiryazev field experimental station, Russian State Agrarian University — Moscow Agricultural Academy named after K.A. Timiryazev, Moscow, 2020).

The results are interpreted in detail and shown in table 11.1 Appendix 11. *Xanthomonas translucens* DNA was not detected in all 55 analytical plant samples. Positive results like 503b.p amplicon were obtained just for positive control (PC).

Result analysis of the IPC test showed that there was no inhibition in the PCR test as shown in figure 3.12.

A total of 60 DNA samples extracted from analytical plant samples collected in the Repulic of Crimea were tested. Electropherograms of DNA testing with primers 4F1/4R1 are shown in figure 3.18.

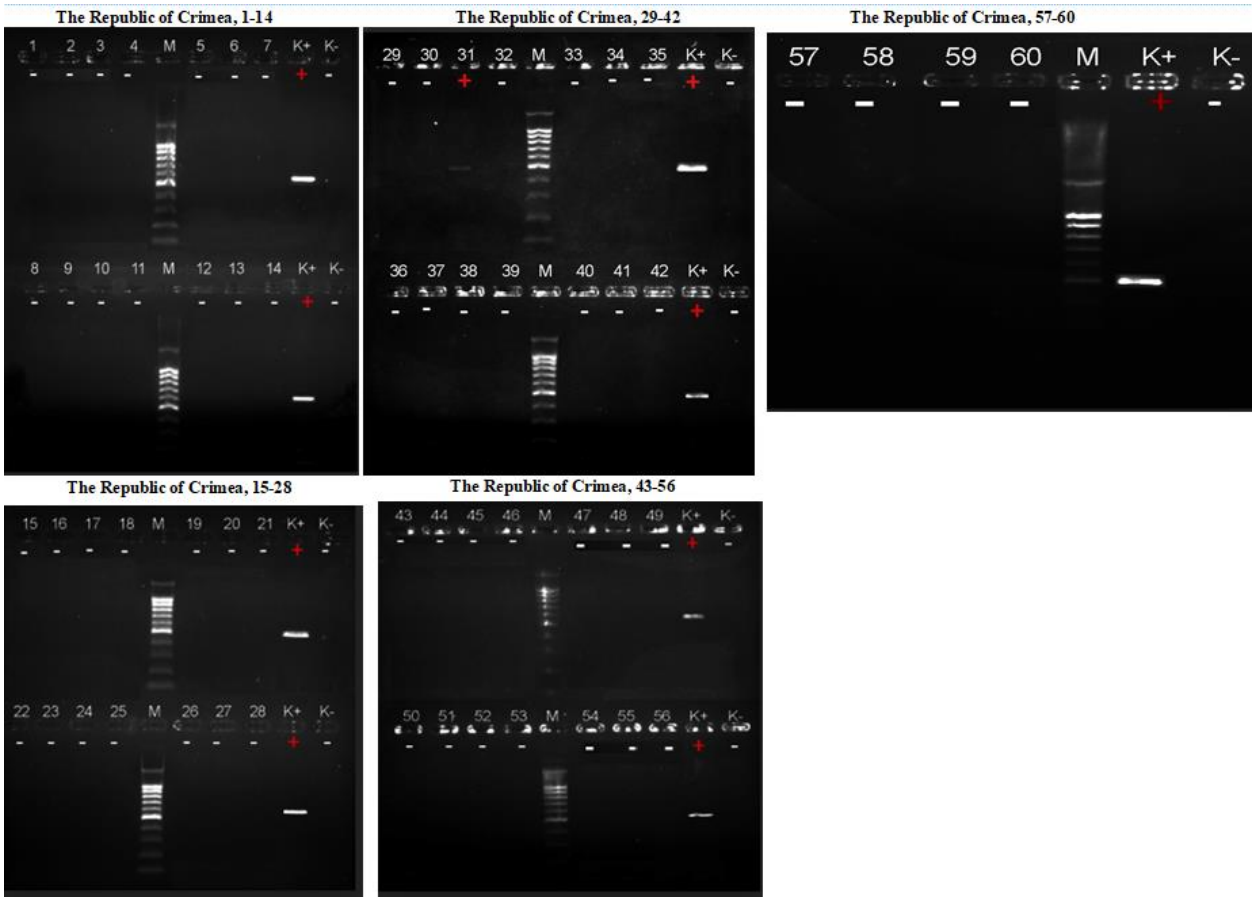

Figure 3.18. Note: (-) means not detected, + means detected and _ cultivar unknown. 1. Winter barley Onega, 2. Winter wheat Aksiniya, 3. Winter wheat Asket, 4. Winter wheat Asket, 5. Winter barley Rajz, 6. Winter wheat Governor of the Don, 7. Winter wheat Governor of the Don, 8. Winter barley Rajz, 9. Winter barley Rajz, 10. Winter wheat Asket, 11, Winter barley Onega, 12. Winter wheat Gubernator Dona, 13. Winter wheat Gubernator Dona, 14. Winter wheat Gubernator Dona, 15. Winter wheat Gubernator Dona, 16. Winter wheat Asket, 17. Winter barley Onega, 18. Winter barley Onega, 19. Winter barley Onega, 20. Winter wheat Asket, 21. Winter wheat Asket, 22. Winter wheat Asket, 23. Winter wheat Asket, 24. Winter wheat Asket, 25. Winter wheat Asket, 26. Winter wheat Asket, 27. Winter wheat

Asket, 28. Winter wheat Asket, 29. Winter wheat Asket, 30. Winter wheat Asket, 31. Winter wheat Asket, 32. Winter barley V'yuga, 33. Winter barley Rubezh, 34. Winter barley Sprinter, 35. Winter barley Ob''em. 36. Winter barley Master, 37. Winter barley Espada, 38. Winter wheat Anka, 39. Winter wheat Velena, 40. Winter wheat Vekha, 41. Winter wheat Karavan, 42. Oats Vernyj, 43. Oats Mezmaj, 44. Oats Chernigovskij -nebo, 45. Oats Loshad', 46. Oats Podgornyj, 47. Oats Guzeripl', 48. Winter barley Master, 49. Winter barley Espada, 50. Winter barley V'yuga, 51. Winter barley Rubezh, 52. Winter barley Sprinter, 53. Winter barley Ob''em, 54. Triticale, wheat, barley _, 55. Winter wheat Korona, 56. Winter barley Sprinter, 57. Winter barley Rubezh, 58. Cereal-legume mixture _, 59. Oat_ and 60. Winter wheat Korona. M — DNA Length Marker 100+ bp DNA ladder (100-1000 b.p. («Evrogen», Russia) (three regions Belogorskij, Krasnogvardejskij, and Simferopolskij of the Republic of Crimea, 2021).

Xanthomonas translucens DNA was detected in DNA sample 21C31-winter wheat cultivar Asket tested positive for *X. translucens*. Positive results like 503b.p amplicon were also obtained for positive control (PC). In the rest of the 59 DNA samples *X. translucens* was not detected also in the negative control samples (NC). The results are interpreted in detail and shown in table 11.1 Appendix 11.

Result analysis of the IPC test showed that there was no inhibition in the PCR test as shown in figure 3.14.

A total of 66 DNA samples extracted from analytical plant samples collected in Stavropol region were tested. Electropherograms of DNA testing with primers 4F1/4R1 are shown in figure 3.19.

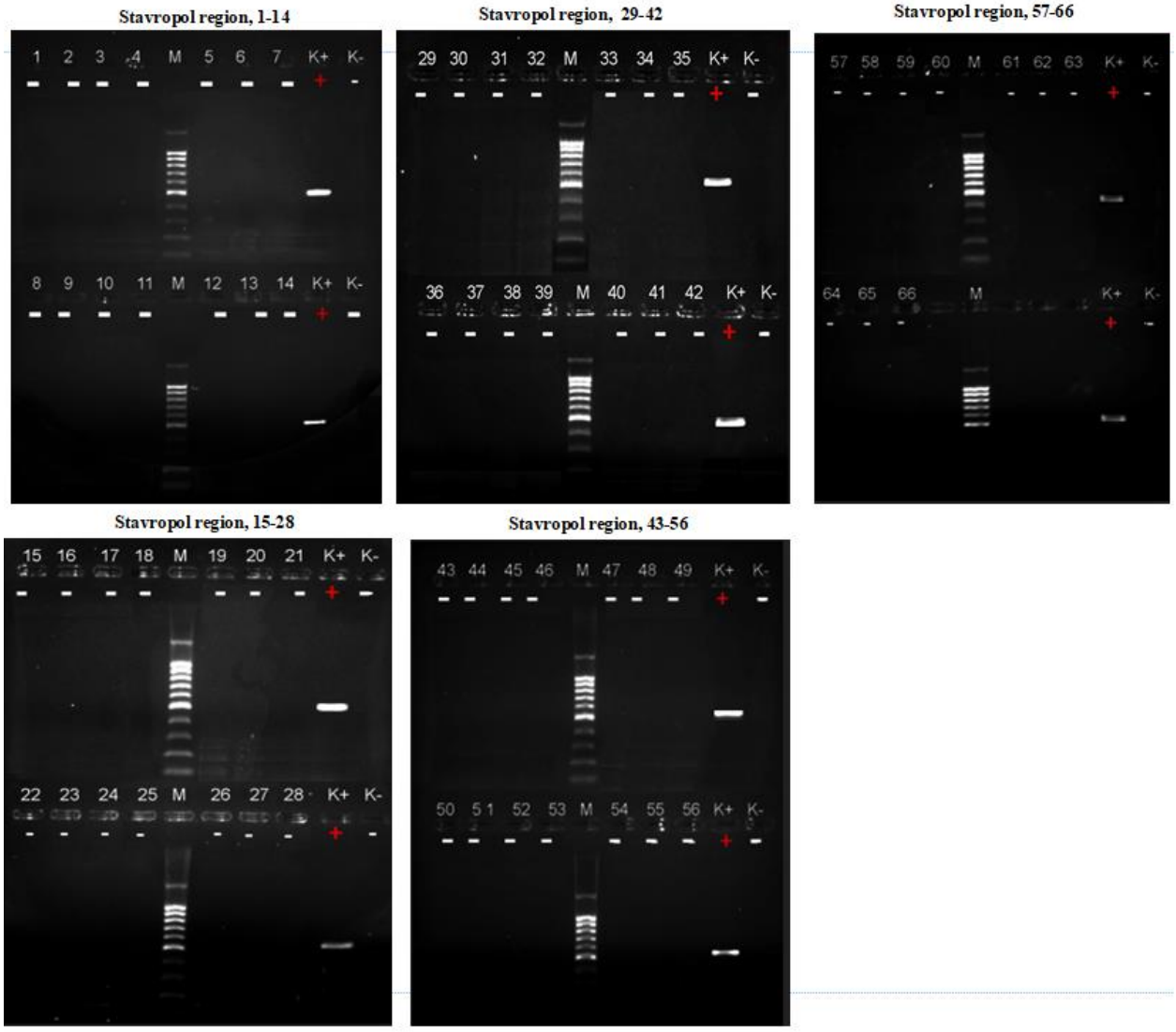

Figure 3.19. Note: (-) means not detected, + means detected and _ cultivar unknown. 1. Winter barley Rubezh, 2. Winter wheat Soberbash, 3. Winter wheat Stil' 18, 4 Winter wheat Tanya, 5. Winter wheat Stil' 18, 6. Winter wheat Tanya, 7. Winter wheat Stil' 18, 8. Winter wheat Tanya, 9. Winter wheat Tanya, 10. Winter wheat Grom, 11. Winter wheat Tanya, 12. Winter wheat Tanya, 13. Winter wheat Tanya, 14. Winter wheat Grom, 15. Winter wheat Tanya, 16. Winter barley Bazal't, 17. Winter wheat Godovshchina, 18. Winter wheat Godovshchina, 19. Winter durum wheat Amazonka, 20. Winter durum wheat Amazonka, 21. Spring durum wheat Yasen'ka, 22. Winter durum wheat Amazonka, 23. Winter durum wheat Amazonka, 24. Winter durum wheat Yahont, 25. Winter durum wheat Agat Donskoj, 26. Winter durum wheat Stepnoj yantar', 27. Winter wheat Centrina, 28. Durum winter wheat Odari, 29. Winter durum wheat Amazonka, 30. Winter wheat Soft Yukka, 31. Winter durum wheat Yahont, 32. Winter durum wheat Amazonka, 33. Durum winter wheat Odari, 34. Winter wheat_, 35. Winter barley + oats_, 36. Winter soft wheat_, 37. Winter barley_, 38. Winter soft wheat_, 39. Winter soft wheat _, 40. Winter soft wheat_, 41. Winter soft wheat_, 42. Winter soft wheat_, 43. Winter barley_, 44. Winter soft wheat_, 45. Winter soft wheat_, 46. Winter barley_, 47. Wheat Chernyava, 48. Winter wheat Alekseevich, 49. Winter wheat Alekseevich, 50. Winter wheat Chernyava, 51. Winter barley Karrera, 52. Winter barley Karrera, 53. Barley Alekseevich, 54. Winter barley Karrera, 55. Wheat Chernyava. 56. Triticale Grain, 57. Winter wheat Antonina, 58. Spring barley Gris, 59. Spring barley Azimuth, 60. Spring barley Leon, 61. Spring barley Voin, 62. Spring barley Format, 63. Spring barley Shchedrin, 64. Spring

barley Vakula, 65. Spring barley Avalon and 66. Winter wheat soft_. M — DNA Length Marker 100+ bp DNA ladder (100-1000 b.p. (Kochubeevskij, Budennovskij, Sovetskij, Georgievskij, Andropovskij, Aleksandrovskij, SHpakovskij, Novoselickij and Mineralovodskij of Stavropol region. 2022).

The results are interpreted in detail and shown in table 11.1 Appendix 11. *Xanthomonas translucens* DNA was not detected in all 66 analytical plant samples. Positive results like 503b.p amplicon were obtained just for positive control (PC).

Result analysis of IPC test showed that there was no inhibition in the PCR test as shown in figure 3.16.

In Moscow and Stavropol region *Xanthomonas translucens* was not detected. It was however detected in one sample of winter wheat cultivar Asket from the Republic of Crimea.

3.5.3 *Pseudomonas syringae*

For the "PSYf/PSYr" test, the results of testing a sample were considered positive if a 144b.p amplicon was detected, also if the 144b.p amplicon was not detected for the negative control. The results were negative if there was no 144b.p amplicon detected in the testing sample also if the 144b.p amplicon was detected for the positive control. The required condition for the interpretation of a testing sample as negative, was marked by the presence of a 714b.p amplicon in the IPC test for the negative control and testing samples. In other cases, the result of PCR was considered in-applicable.

The following results were obtained after running PCR using PSYf/PSYr for the detection of *Pseudomonas syringae* in the analytical plant samples. A total of 55 DNA samples extracted from analytical plant samples collected in Moscow were tested. Electropherograms of DNA testing with primers PSYf/PSYr are shown in figure 3.20.

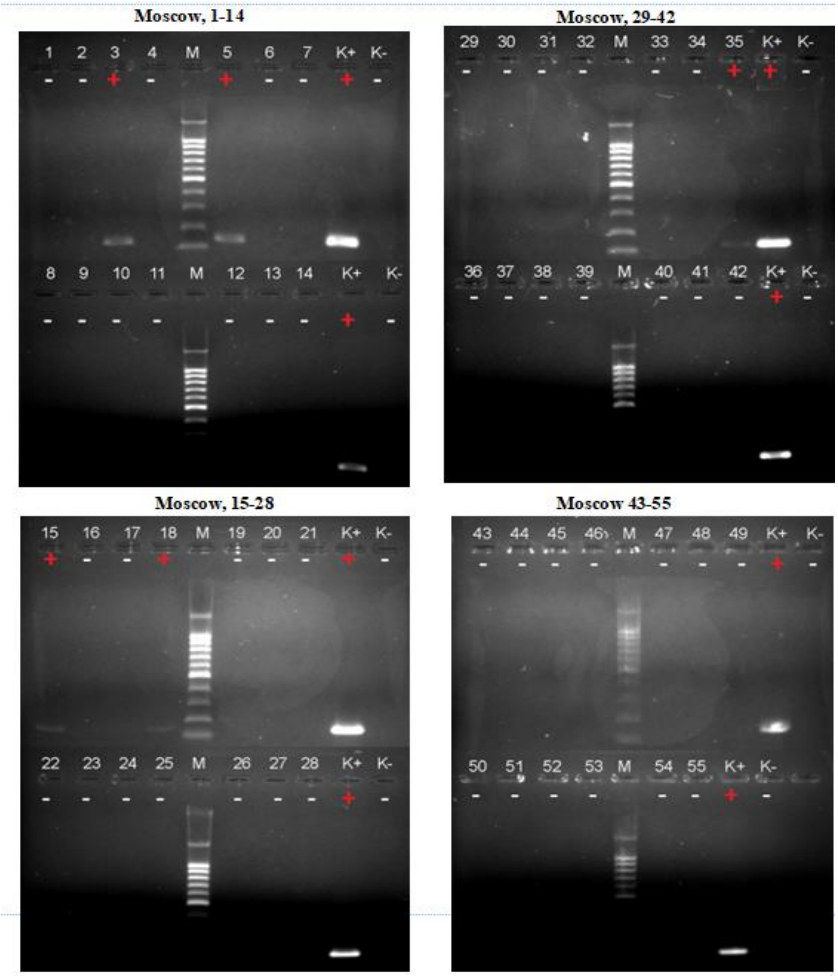

Figure 3.20. Note: (_) means unknown cultivar, + means detected and – means not detected. 1. Winter rye Snezhana, 2. Soft winter wheat Zhiva, 3. Soft winter wheat Alekseevich, 4. Soft winter wheat Urup, 5. Soft winter wheat Morozko, 6. Soft winter wheat Timiryazevskaya Yubilejnaya, 7. Soft winter wheat Moskovskaya 56, 8. Soft winter wheat Biryuza, 9. Soft winter wheat Timiryazevskaya 150, 10. Soft winter wheat Graf, 11 Triticale winter Aleksandr, 12. Winter wheat Don yantarnaya, 13. Triticale winter Victor, 14. Soft winter wheat Vassa, 15. Winter wheat two-grain _, 16. Triticale winter Nemchinovskij 56, 17. Winter wheat Eremeevna, 18. Triticale winter spherical Titus, 19. Soft winter wheat Moskovskaya 39, 20. Triticale winter Valentin 90, 21. Soft winter wheat Dublet, 22. Soft winter wheat Kavalerka, 23. Soft winter wheat Skarlet Zarya, 24. Soft winter wheat Nemchinovskaya 24, 25. Winter durum wheat Pobeda 70, 26. Soft winter wheat Legenda, 27. Soft winter wheat Avesta, 28. Winter rye Verasen', 29. Soft winter wheat Inna, 30. Winter wheat Terra, 31. Triticale winter Timiryazevskaya 150, 32. Soft winter wheat Mel'nica, 33. Soft winter wheat Asket, 34. Soft winter wheat Velena, 35. Soft winter wheat Vanya, 36. Soft winter wheat Artel', 37. Soft winter wheat Nemchinovskaya 85, 38. Soft winter wheat Vidya, 39. Soft winter wheat Don Lira, 40. Soft winter wheat Golubaya, 41. Soft winter wheat Moskovskaya 40, 42. Soft winter wheat Don 107, 43. Soft winter wheat Stepnaya, 44. Soft winter wheat Gubernator Dona, 45. Soft winter wheat Rostovchanka, 46. Soft winter wheat Vekha, 47. Soft winter wheat Nemchinovskaya 57, 48. Soft winter wheat Avgusta, 49. Soft winter wheat Soberbash, 50. Soft winter wheat Anka, 51. Soft winter wheat Prignala, 52. Soft winter wheat Antonina, 53. Soft winter wheat Nemchinovskaya 17, 54. Soft winter wheat Bezostaya 100 and 55. Winter rye _. M — DNA Length Marker 100+ bp DNA ladder (100-1000 b.p. («Evrogen», Russia) (Timiryazev field experimental

station, Russian State Agrarian University — Moscow Agricultural Academy named after K.A. Timiryazev, Moscow, 2020).

Pseudomonas syringae DNA was detected in 5 analytical plant samples as shown in Appendix 11 table 11. Positive results like 144b.p amplicon were also obtained for positive control (PC). In the rest of the 50 DNA samples *P. syringae* was not detected also in the negative control samples (NC). The results are interpreted in detail and shown in table 11.1 Appendix 11. That is 9% of the DNA samples.

Result analysis of the IPC test showed that there was no inhibition in the PCR test as shown in figure 3.12.

A total of 60 DNA samples extracted from analytical plant samples collected in the Republic of Crimea were tested. Electropherograms of DNA testing with primers PSYf/PSYr are shown in figure 3.21.

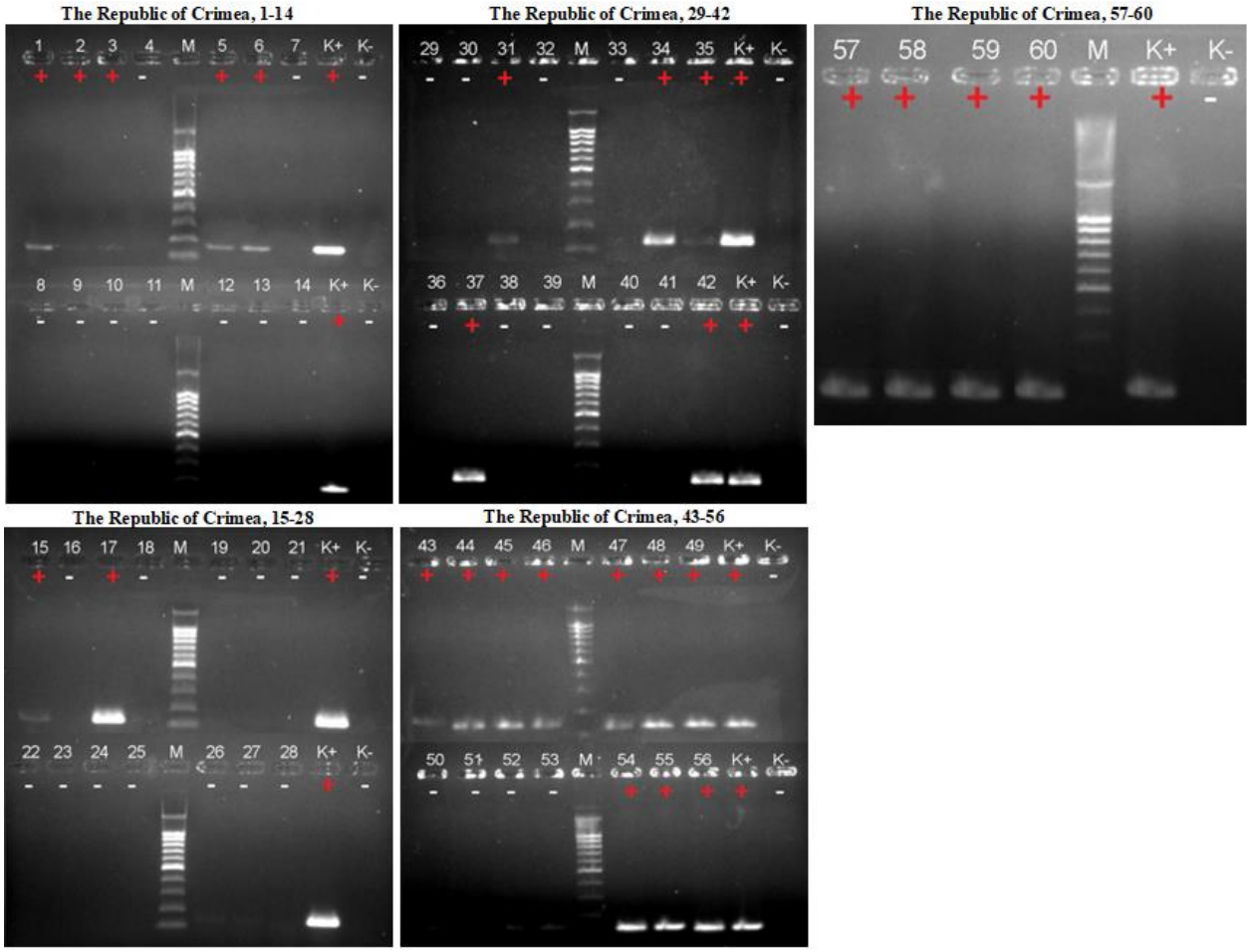

Figure 3.21. Note: (-) means not detected, + means detected and _ cultivar unknown. 1. Winter barley Onega, 2. Winter wheat Aksiniya, 3. Winter wheat Asket, 4. Winter wheat Asket, 5. Winter barley Rajz, 6. Winter wheat Governor of the Don, 7. Winter wheat Governor of the Don, 8. Winter barley Rajz,

9. Winter barley Rajz, 10. Winter wheat Asket, 11, Winter barley Onega, 12. Winter wheat Gubernator Dona, 13. Winter wheat Gubernator Dona, 14. Winter wheat Gubernator Dona, 15. Winter wheat Gubernator Dona, 16. Winter wheat Asket, 17. Winter barley Onega, 18. Winter barley Onega, 19. Winter barley Onega, 20. Winter wheat Asket, 21. Winter wheat Asket, 22. Winter wheat Asket, 23. Winter wheat Asket, 24. Winter wheat Asket, 25. Winter wheat Asket, 26. Winter wheat Asket, 27. Winter wheat Asket, 28. Winter wheat Asket, 29. Winter wheat Asket, 30. Winter wheat Asket, 31. Winter wheat Asket, 32. Winter barley V'yuga, 33. Winter barley Rubezh, 34. Winter barley Sprinter, 35. Winter barley Ob"em. 36. Winter barley Master, 37. Winter barley Espada, 38. Winter wheat Anka, 39. Winter wheat Velena, 40. Winter wheat Vekha, 41. Winter wheat Karavan, 42. Oats Vernyj, 43. Oats Mezmaj, 44. Oats Chernigovskij -nebo, 45. Oats Loshad', 46. Oats Podgornyj, 47. Oats Guzeripl', 48. Winter barley Master, 49. Winter barley Espada, 50. Winter barley V'yuga, 51. Winter barley Rubezh, 52. Winter barley Sprinter, 53. Winter barley Ob"em, 54. Triticale, wheat, barley _, 55. Winter wheat Korona, 56. Winter barley Sprinter, 57. Winter barley Rubezh, 58. Cereal-legume mixture _, 59. Oat_ and 60. Winter wheat Korona. M — DNA Length Marker 100+ bp DNA ladder (100-1000 b.p. («Evrogen», Russia) (three regions Belogorskij, Krasnogvardejskij, and Simferopolskij of the Republic of Crimea, 2021).

Pseudomonas syringae DNA was detected in 26 analytical plant samples as shown in Appendix 11 table 11.1. Positive results like 144b.p amplicon were also obtained for positive control (PC). In the rest of the 34 DNA samples *P. syringae* was not detected also in the negative control samples (NC). The results are interpreted in detail and shown in table 11.1 Appendix 11. That is 43% of the analytical plant DNA samples.

Result analysis of the IPC test showed that there was no inhibition in the PCR test as shown in figure 3.14.

A total of 66 DNA samples extracted from analytical plant samples collected in Stavropol region were tested. Electropherograms of DNA testing with primers PSYf/PSYr are shown in figure 3.22.

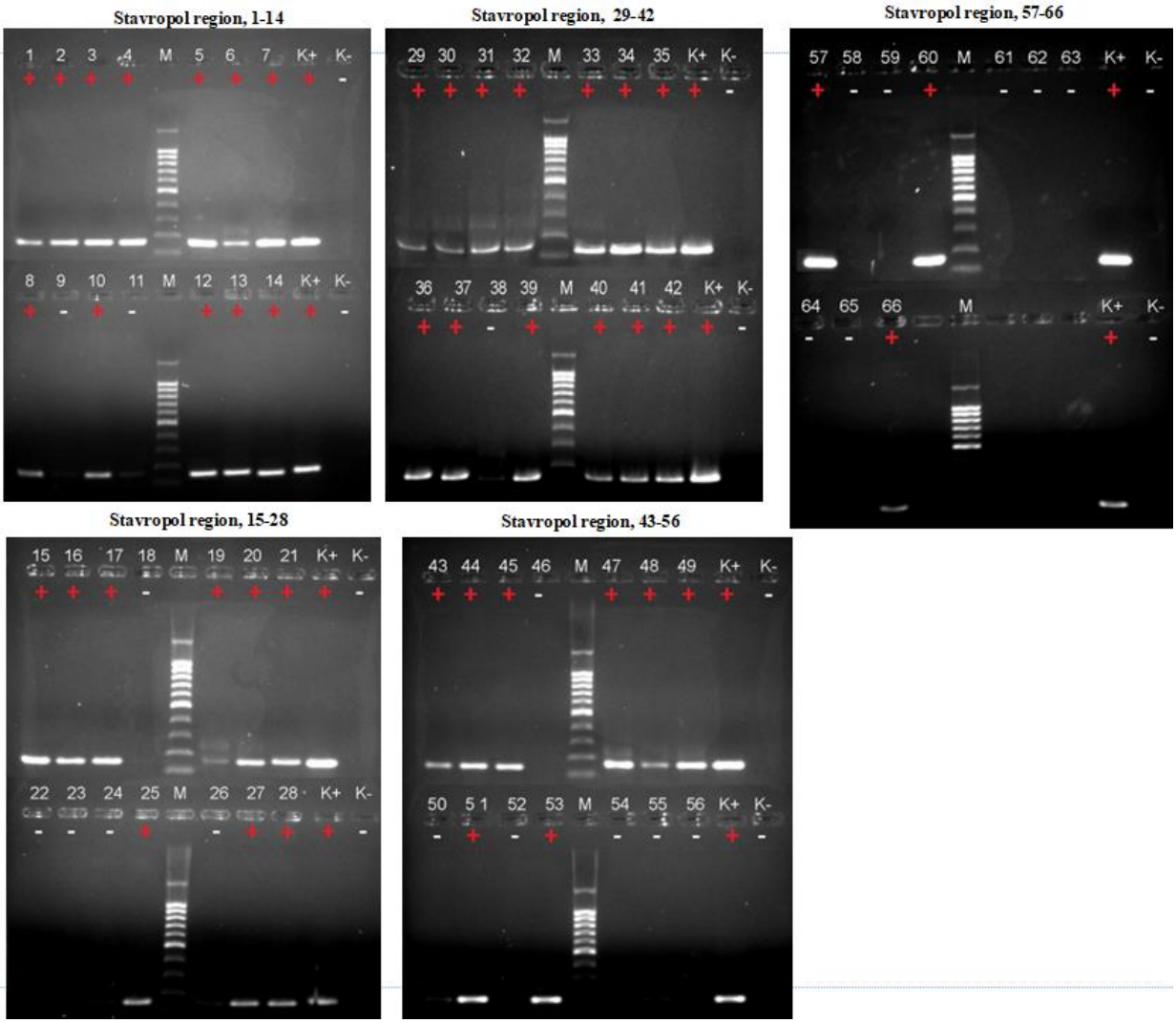

Figure 3.22. Note: (-) means not detected, + means detected and _ cultivar unknown. 1. Winter barley Rubezh, 2. Winter wheat Soberbash, 3. Winter wheat Stil' 18, 4 Winter wheat Tanya, 5. Winter wheat Stil' 18, 6. Winter wheat Tanya, 7. Winter wheat Stil' 18, 8. Winter wheat Tanya, 9. Winter wheat Tanya, 10. Winter wheat Grom, 11. Winter wheat Tanya, 12. Winter wheat Tanya, 13. Winter wheat Tanya, 14. Winter wheat Grom, 15. Winter wheat Tanya, 16. Winter barley Bazal't, 17. Winter wheat Godovshchina, 18. Winter wheat Godovshchina, 19. Winter durum wheat Amazonka, 20. Winter durum wheat Amazonka, 21. Spring durum wheat Yasen'ka, 22. Winter durum wheat Amazonka, 23. Winter durum wheat Amazonka, 24. Winter durum wheat Yahont, 25. Winter durum wheat Agat Donskoj, 26. Winter durum wheat Stepnoj yantar', 27. Winter wheat Centrina, 28. Durum winter wheat Odari, 29. Winter durum wheat Amazonka, 30. Winter wheat Soft Yukka, 31. Winter durum wheat Yahont, 32. Winter durum wheat Amazonka, 33. Durum winter wheat Odari, 34. Winter wheat_, 35. Winter barley + oats_, 36. Winter soft wheat_, 37. Winter barley_, 38. Winter soft wheat_, 39. Winter soft wheat _, 40. Winter soft wheat_, 41. Winter soft wheat_, 42. Winter soft wheat_, 43. Winter barley_, 44. Winter soft wheat_, 45. Winter soft wheat_, 46. Winter barley_, 47. Wheat Chernyava, 48. Winter wheat Alekseevich, 49. Winter wheat Alekseevich, 50. Winter wheat Chernyava, 51. Winter barley Karrera, 52. Winter barley Karrera, 53. Barley Alekseevich, 54. Winter barley Karrera, 55. Wheat Chernyava. 56. Triticale Grain, 57. Winter wheat Antonina, 58. Spring barley Gris, 59. Spring barley Azimuth, 60. Spring barley Leon, 61. Spring barley Voin, 62. Spring barley Format, 63. Spring barley Shchedrin, 64. Spring barley Vakula, 65. Spring barley Avalon and 66. Winter wheat soft_. M — DNA Length Marker 100+ bp

DNA ladder (100-1000 b.p. (Kochubeevskij, Budennovskij, Sovetskij, Georgievskij, Andropovskij, Aleksandrovskij, Shpakovskij, Novoselickij and Mineralovodskij of Stavropol region. 2022).

Pseudomonas syringae DNA was detected in 45 analytical plant samples as shown in Appendix 11 table 11.1. Positive results like 144b.p amplicon were also obtained for positive control (PC). In the rest of the 21 DNA samples *P. syringae* was not detected also in the negative control samples (NC). The results are interpreted in detail and shown in table 11.1 Appendix 11. That is 68% of the samples.

Result analysis of IPC test showed that there was no inhibition in the PCR test as shown in figure 3.16.

Pseudomonas syringae was detected in the following percentages 9%, 43% and 68% of the analytical plant samples in Moscow, the Republic of Crimea and Stavropol region respectively as shown in figure 3.23.

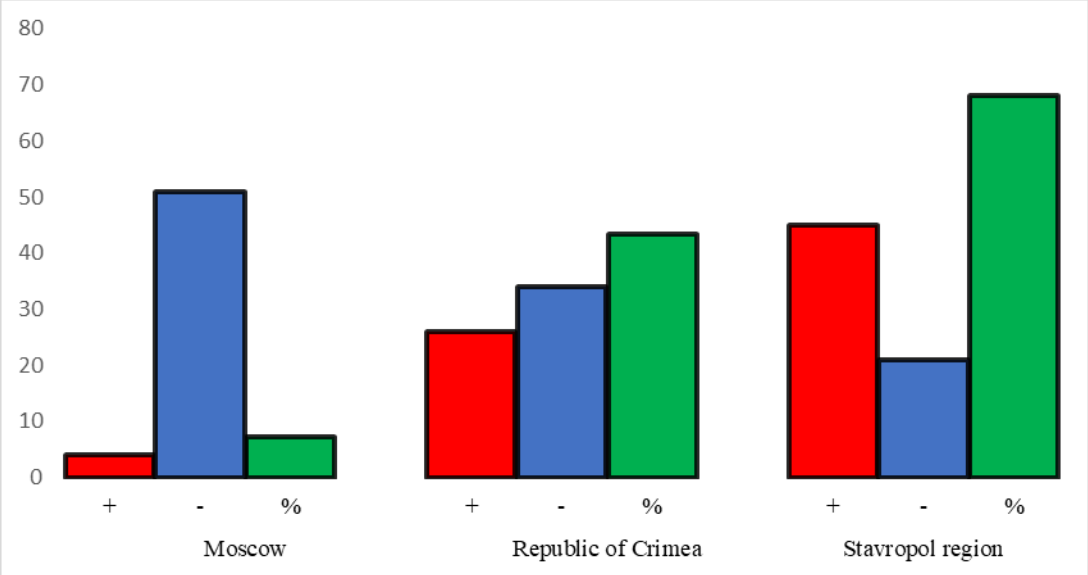

Figure 3.24 Results of *P. syringae* detection in the analytical plant samples. N.B "+" analytical plant samples in which *P. syringae* was detected "-" analytical plant samples in which *P. syringae* was not detected and "%" the analytical plant samples in which *P. syringae* was detected as a percentage.

As shown in Figure 3.23 the detection percentage of *P. syringae* in Stavropol region is 25% higher than in the Republic of Crimea and 57% higher than in Moscow. The percentage of detection in the Republic of Crimea is 34% higher than that of Moscow but lower than Stavropol region. Moscow had the lowest percentage detection of the three regions as shown in figure 3.23.

41% of all the 181 analytical plant samples tested positive for the presence of *P. syringae*. In the rest of the 59% DNA samples *P. syringae* was not detected.

In all the three regions of the Russian Federation *Pseudomonas syringae* was detected.

3.5.4 *Pseudomonas fuscovaginae*

For test “Pseudomonas fuscovaginae-Rt”, the results of testing a sample were considered positive if a specific reaction for the target PCR gene marked by an exponential curve was present in the PCR specificity detection channel (FAM), also specific reaction should be negative for the negative control. The results were negative if there was no specific reaction in the PCR specificity detection channel (FAM) of the testing sample. The presence of a specific reaction marked by an exponential curve in the inner positive control HEX channel for the negative control and testing samples was considered a negative result. In other cases, the result of PCR was considered in-applicable. Figure 3.24 below shows the exponential curves in the FAM channel (A, A^2 and A^3) and HEX channel (B, B^2 and B^3).

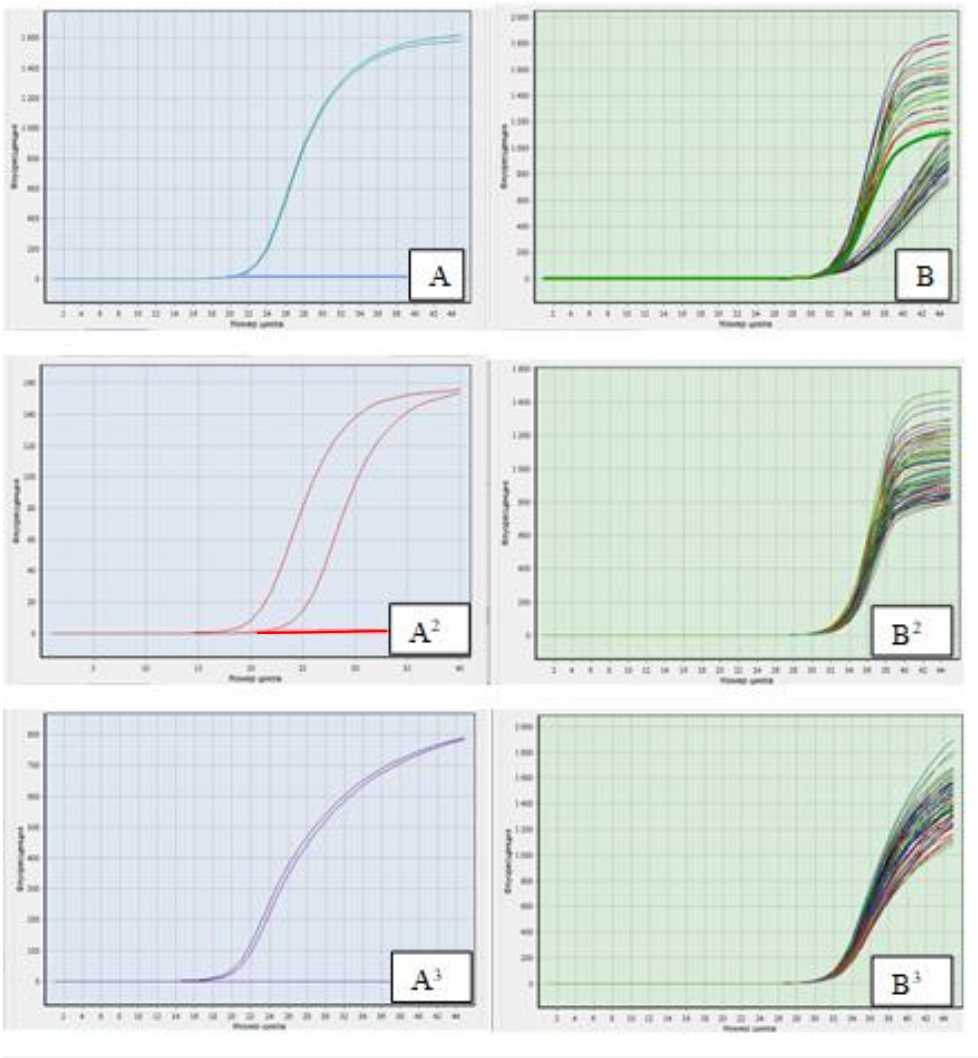

Figure 3.24 showing the results of "Pseudomonas fuscovaginae-Rt" PCR-Rt test. NB A- FAM Moscow B- HEX Moscow, A^2 - FAM Crimea B^2- HEX Crimea and A^3- FAM Stavropol B^3- HEX Stavropol.

A total of 55 DNA samples extracted from analytical plant samples collected in Moscow were tested. Figure 3.24 shows the exponential curves in the FAM Moscow (A) and HEX Moscow (B). The results are interpreted in detail and shown in table 11.1 Appendix 11. *Pseudomonas fuscovaginae* DNA was not detected in all the 55 DNA samples analytical plant samples. Positive results were obtained just for positive control (PC) as shown in Figure 3.24 A.

A total of 60 DNA samples extracted from analytical plant samples collected in the Republic of Crimea were tested. Figure 3.24 shows the exponential curves in the FAM Crimea (A^2) and HEX Crimea (B^2). The results are interpreted in detail and shown in table 11.1 Appendix 11. *Pseudomonas fuscovaginae* DNA was not

detected in all the 60 DNA samples analytical plant samples. Positive results were obtained just for positive control (PC) as shown in Figure 3.24 A[2].

A total of 66 DNA samples extracted from analytical plant samples collected in Stavropol region were tested. The results are interpreted in detail and shown in table 11.1 Appendix 11. Figure 3.24 shows the exponential curves in the FAM Stavropol (A[3]) and HEX Stavropol (B[3]). *Pseudomonas fuscovaginae* DNA was not detected in all the 66 DNA samples analytical plant samples. Positive results were obtained just for positive control (PC) as shown in Figure 3.24 A[3].

In all three regions of the Russian Federation *Pseudomonas fuscovaginae* was not detected.

3.6 Identification of bacterial isolates in samples collected from Moscow, the Republic of Crimea and Stavropol region

The results of the identification of bacterial isolates are shown by each region in sections 3.6.1, 3.6.2 and 3.6.3.

3.6.1 Timiryazevskaya field experimental station (Moscow)

A total of 177 bacterial isolates were obtained from the analytical plant samples. As a result of PCR with PSF/PSR primers, an amplicon with a length of 610 bp was obtained for 60 tested DNA samples of bacterial cultures (Fig. 3.25).

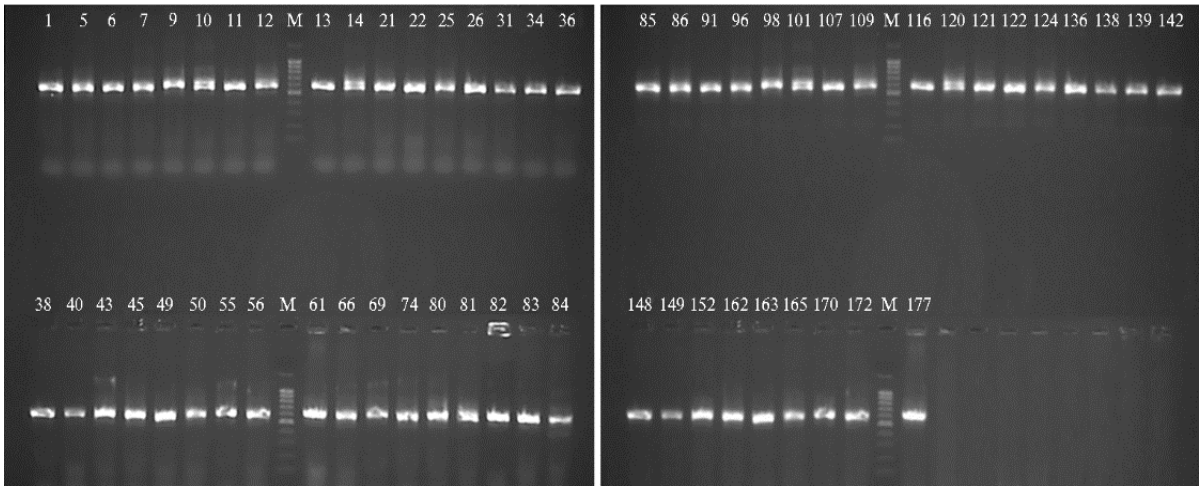

Fig. 3.25. PCR products with primers PSF/PSR (610 bp) obtained for DNA samples of bacterial isolates from cereal varieties: 1 – Snezhana; 5 – Zhivoj; 6, 7 – Alekseevich; 9-12 – Morozko; 13, 14 – Timiryazevskaya yubilejnaya; 21 – Moskovskaya 56; 22 —Biryuzovyj; 25, 26 – Timiryazevka 150; 31 – Aleksandr; 34, 36, 38 — Donskoj yantar'; 40 – Viktor; 43 – Triticum dicoccum Schrank (bez nazvaniya); 45 – Nemchinovskij 56; 49 – Eremeevna; 50 – Tit; 55, 56 – Moskovskaya 39; 61 – Dublet; 66-69 – Kavaler; 74 –

Alaya zarya; 80 – Nemchinovskaya 24; 81, 82 – Pobeda 70; 83, 84 – Legenda; 85, 86 – Avesta; 91, 96, 98 – Verasen; 101 – Inna; 107 – Terra; 109 – Timiryazevskaya 150; 116 – Stan; 120-122 – Asket; 124 – Velena; 136 – Videya; 138, 139 — Donskaya lira; 142 – Sineva; 148 – Don 107; 149 – Step'; 152 – rostovchanin; 162 – Soberbash; 163, 165 – Anka; 170 – Antonina; 172 – Nemchinovskaya 17; 177 — Secale corne L. (un-titled);M — DNA Length Marker 100+ bp DNA ladder (100-1000 b.p. («Evrogen », Russia) (Timiryazev field experimental station, Russian State Agrarian University — Moscow Agricultural Academy named after K.A. Timiryazev, Moscow, 2020).

As a result of PCR with primers SyD1/SyD2, the 1040 bp amplicon was obtained for eight tested DNA samples of bacterial cultures: 11,12 – Morozko; 82 – Pobeda 70; 101 – Inna; 107 – Terra; 116 – Sten; 152 – Rostovchanka and 170 – Antonina as shown in (Fig. 3.26).

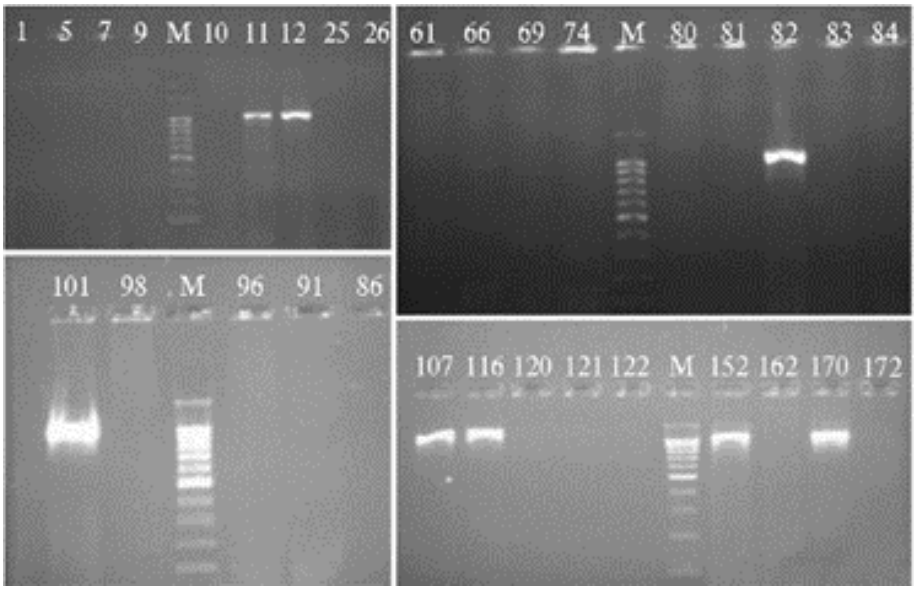

Fig. 3.26. PCR products with primers SyD1/ SyD2 (1040 bp) obtained for DNA samples of bacterial isolates from cereal varieties: 1 — Snezhana; 5 – Zhiva; 7 – Alekseevich; 9-12 – Morozko; 25, 26 – Timiryazevka 150; 61 – Dublet; 66, 69 – Kavaleriya; 74 – Alayazar; 80 – Nemchinovskaya 24; 81, 82 – Pobeda 70; 83, 84 – Legenda; 86 – Avesta; 91, 96, 98 – Verasen; 101 – Inna; 107 – Terra; 116 – Sten; 120-122 – Asketichnyj; 152 – Rostovchanka; 162 – Soberbash; 170 – Antonina; 172 – Nemchinovskaya 17; M — DNA Length Marker 100+ bp DNA ladder (100-1000 b.p. (“Evrogen”, Russia) (Timiryazev field experimental station, Russian State Agrarian University — Moscow Agricultural Academy named after K.A. Timiryazev, Moscow, 2020).

Using primers 8UA/519B 500 bp amplicons were obtained for these remaining 103 bacterial culture DNA samples: 2, 3 — Snezhana; 8 – Urup; 17-20 – Timiryazevskaya yubilejnaya; 23-24 – Biryuza; 27 – Timiryazevka 150; 28-30 – graf; 32, 33, 35, 37 – donskoj yantar'; 39 – Viktor; 35(2), 36(2) – Donskoj yantar'; 41-42 – Vassa; 44 – Nemchinovskij 56; 47-48 – Eremeevna; 51-52 – Tit; 53-54,

57-58 – Moskovskaya 39; 59-60 – Valentin 90; 62-65 – Dublet; 67-68 – Kavalerka; 70-73, 75-76 – Alaya zarya; 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 – Asket; 123 – Velena; 125 – Vanya; 126-129, 131-132 – Artel'; 133-134 – Nemchinovskaya 85; 135 – Videya; 137 – Donskaya lira; 140-141 – Sineva; 143, 145-146 – Moskovskaya 40; 147 – Don 107; 150 – Gubernator Dona; 151 – Rostovchanka; 153 – Vekha; 154-155 – Nemchinovskaya 57; 156, 158-159 – Avgusta; 160-161 – Soberbash; 164 – Anka; 166-169 – Kraj; 171 – Nemchinovskaya 17; 173-175 – Bezostaya 100; 176, 178 – spring rye Secale cereale L. (un-titled). These are shown in Fig. 3.27.

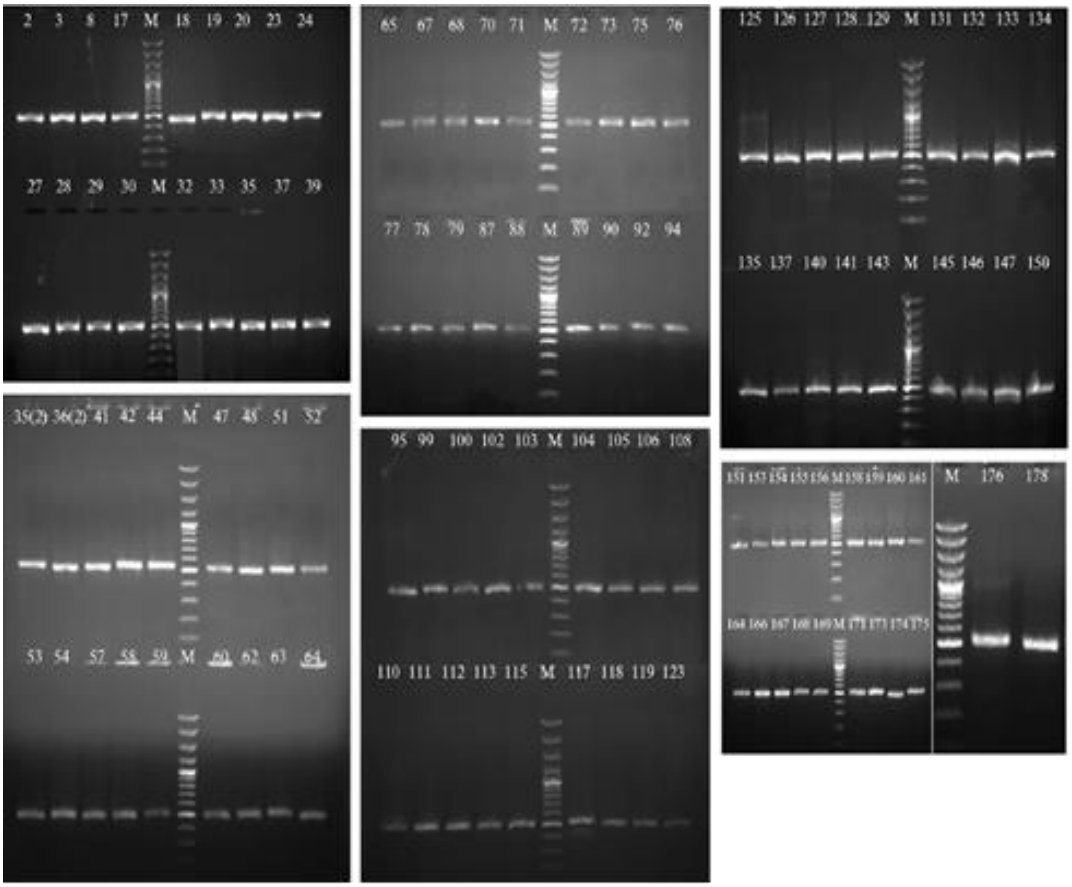

Fig. 3.27. PCR products with primers 8UA/519B (500 bp) obtained for DNA samples of bacterial isolates from cereal varieties: 2, 3 — Snezhana; 8 – Urup; 17-20 – Timiryazevskaya yubilejnaya; 23-24 – Biryuza; 27 – Timiryazevka 150; 28-30 – graf; 32, 33, 35, 37 – donskoj yantar'; 39 – Viktor; 35(2), 36(2) – Donskoj yantar'; 41-42 – Vassa; 44 – Nemchinovskij 56; 47-48 – Eremeevna; 51-52 – Tit; 53-54, 57-58 – Moskovskaya 39; 59-60 – Valentin 90; 62-65 – Dublet; 67-68 – Kavalerka; 70-73, 75-76 – Alaya zarya; 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 – Asket; 123 – Velena; 125 – Vanya; 126-129, 131-132 – Artel'; 133-134 – Nemchinovskaya 85; 135 – Videya; 137 – Donskaya lira; 140-141 – Sineva; 143, 145-146 –

Moskovskaya 40; 147 – Don 107; 150 – Gubernator Dona; 151 – Rostovchanka; 153 – Vekha; 154-155 – Nemchinovskaya 57; 156, 158-159 – Avgusta; 160-161 – Soberbash; 164 – Anka; 166-169 – Kraj; 171 – Nemchinovskaya 17; 173-175 – Bezostaya 100; 176, 178 – spring rye Secale cereale L. (un-titled); M — DNA Length Marker 100+ bp DNA ladder (100-1000 b.p. ("Evrogen", Russia) (Timiryazev field experimental station, Russian State Agrarian University — Moscow Agricultural Academy named after K.A. Timiryazev, Moscow, 2020).

The results of identification of each isolated strain are presented in Appendix 12 Table 12.1. A total of 168 isolates have been identified using Sanger sequencing method [89] adapted by Belkin [87].

The highest frequency of occurrence was observed in bacteria of the genus *Pseudomonas*: 70,9% (Table 3.9).

Table 3.9. Bacteria species and genera occurrence frequencies of analytical plant samples from Timiryazevskaya field experimental station, Russian State Agrarian University - Moscow Agricultural Academy named after K.A. Timiryazev, Moscow, 2020.

Genus	%	Species	%
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.B % means the frequency of occurrence, - indicates absence

A total of 52 strains of bacteria of the genus *Pseudomonas* have been isolated, which is 31% of all isolated strains (figure 3.31).

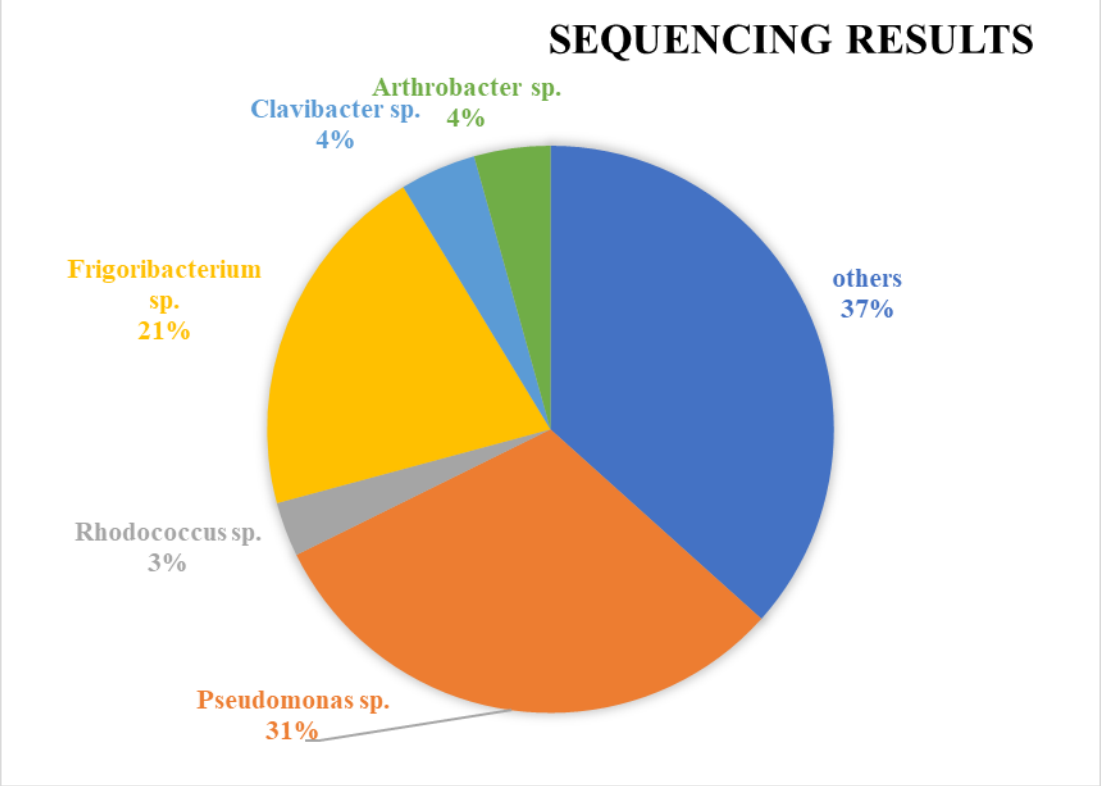

Figure 3.31 shows the sequencing results of the bacteria from the analytical plant samples collected from Timiryazevskaya field experimental station (Moscow).

Among identified *Pseudomonas* bacteria, 9 were belonged to the species *Pseudomonas syringae*, the object of this study: *Pseudomonas syringae* pv. *aptata*

(isolate M5-9), *Pseudomonas syringae* pv. *atrofaciens* (isolate M5-10, M45-143, M52-160), *Pseudomonas syringae* pv. *lapsa* (isolate M30-101), *Pseudomonas syringae* pv. *syringae* (isolate M32-109, M39-130, M40-134) and *Pseudomonas syringae* (isolate M34-117) (appendix 12 table 12.1).

The genus *Frigoribacterium* was represented next in terms of frequency of occurrence: 36,4% (Table 28). Bacteria of the genus *Frigoribacterium* are common representatives of the plant microbiota, facilitating their growth and adaptation [96]. A total of 33 strains of bacteria of the genus *Frigoribacterium* have been isolated, which is 21% of all isolated strains (figure 3.31). Among them identified 4 strains of *Frigoribacterium faeni* making up 2.5% of all the identified strains. This species is characterized by psychrophilic properties. Cryophiles or psychrophiles are extremophilic organisms that can thrive and reproduce in frigid temperatures like 2℃ temperate temperatures like + 20℃ [97].

Bacteria of the genus *Clavibacter*, which was presented at the third place after *Pseudomonas* and *Frigoribacterium* (the frequency of occurrence was 16,4%, as shown in table 28), is characterized by gram positive anaerobic properties. It causes leaf blight and bacterial wilt in cereal grain [98]. A total of 12 strains of bacteria of the genus *Clavibacter* have been isolated, which is 4% of all isolated strains (figure 3.31). Among them identified 10 strains of *Clavibacter michiganensis.* This species is characterized as the causative agent that may result in notable yield decreases of up to 50% due to bacterial blight and wilt [95].

Bacteria of the genus *Arthrobacter*, with a frequency of occurrence was 12.7%, as shown in table 28), is commonly known for phosphate solubilization and thus enhancing plant growth. Bacteria in this genus are aerobic, gram-stain-positive, non-spore-forming bacteria that is soil borne [100]. A total of 8 strains of bacteria of the genus *Arthrobacter* have been isolated, which is 4% of all isolated strains (figure 3.31). Among them 1 strain of *Arthrobacter chlorophenolicus* was identified. This species is characterized as co-metabolic bio degrader of bromophenol [101].

The next bacteria of genus *Rhodococcus* in the fourth place (as shown in table 28, the frequency of occurrence was 10.9%) is characterized as anaerobic

gram positive. Most of the bacteria in this genus are beneficial with the exception of *Rhodococcus fascians* which is plant-pathogenic and *Rhodococcus equi* which is livestock-pathogenic [102]. A total of 9 strains of bacteria of the genus *Rhodococcus* have been isolated, which is 3% of all isolated strains (figure 3.31). Among them identified were one strain of *Rhodococcus cerastii* and one strain of *Rhodococcus yunnanensis* and two strains of *Rhodococcus fascians*. *Rhodococcus fascians* is characterized as causing leafy galls and deformed growths in infected plants [103].

The next bacteria of genus *Curtobacterium* in the fifth place (as shown in table 28, the frequency of occurrence was 7.3%) is characterized as an endophyte in plants [104]. A total of 5 strains of bacteria of the genus *Curtobacterium* have been isolated, which is 1.9% of all isolated strains (figure 3.31).

The next bacteria of genus *Sphingomonas* as shown in table 28, the frequency of occurrence was 5.5% is characterized as being beneficial to plant growth by improving drought tolerance and improving plant growth [105]. A total of three strains of bacteria of the genus *Sphingomonas* have been isolated, which is 1.9% of all isolated strains (figure 3.31).

The next bacteria of genus *Bacillus* in the seventh place (as shown in table 28, the frequency of occurrence was 5.5%) are beneficial bacteria that are used as biological control agents towards plant pathogens by the producing broad range of antimicrobials [106]. A total of three strains of bacteria of the genus *Bacillus* have been isolated, which is 1.8% of all isolated strains (figure 3.31). Among them identified 1 strain of *Bacillus pumilis*. This species is characterized as beneficial to plant growth as it helps with drought tolerance and helps with plants growing in soils with high salinity [107].

The next bacteria of genus *Erwinia* in the eighth place (as shown in table 28, the frequency of occurrence was 3.6%) is characterized as gram-negative bacteria that is short rodded, non-spore forming facultative and anaerobic. Some of the strains are known to be beneficial to plant growth with most of them being harmful [108]. A total of 2 strains of bacteria of the genus *Erwinia* have been isolated, which is 1.2% of all isolated strains (figure 3.31). Among them 1 strain of

Erwinia amylovora was identified. This species is known for causing causes fire blight in apple and pear trees [109].

The next bacteria of genus *Frondihabitans* in the nineth place (as shown in table 28, the frequency of occurrence was 3.6%) is characterized as irregular-rod shaped, gram-positive and aerobic [110]. A total of two strains of bacteria of the genus *Rhodococcus* have been isolated, which is 1.23% of all isolated strains (figure 3.31).

In the analytical plant samples etxtracted from Moscow 5.4% of the identified pathogens are the phytopathogens of interest in this study.

1. *Pseudomonas syringae* pv. *aptata* 1 isolate,
2. *Pseudomonas syringae* pv. *atrofaciens* 3 isolates (Quarantine phytopathogen),
3. *Pseudomonas syringae* pv. *lapsa* 1 isolate,
4. *Pseudomonas syringae* pv. *syringae* 3 isolates, and
5. *Pseudomonas syringae* 1 isolate.

3.6.2 The Republic of Crimea

A total of 102 bacterial isolates were obtained from the analytical plant samples. As a result of PCR with 8UA/519B primers, an amplicon with a length of 500 bp was obtained for 60 tested DNA samples of bacterial cultures (Fig. 3.32).

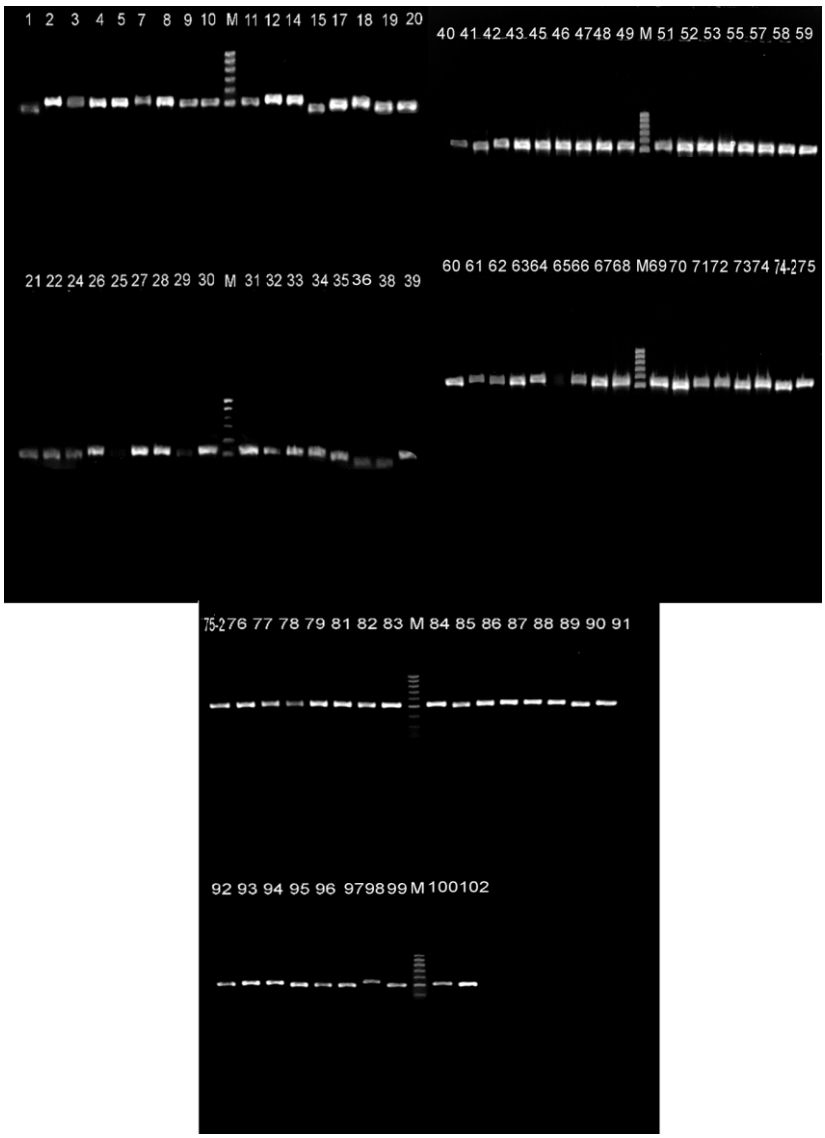

Figure 3.32 PCR products with primers 8UA/519B (500 bp) obtained for DNA samples of bacterial isolates from cereal varieties:1-5 7-9 Winter Barley Onega, 10-12 Winter wheat Aksiniya, 14-15, 17 Winter wheat Asket, 18-22, 24 Winter barley Vosxod, 25-30 Winter wheat Gubernator Dona, 31-35 Winter barley Vosxod, 36, 38, 39 Winter wheat Asket, 41-42 Winter barley Onega, 43, 45-53, 55, 57-60 Winter wheat Gubernator Dona, 61-64 Winter wheat Asket, 66-75-1 Winter barley Onega, 75-2-79, 81-82 Winter wheat Asket, 83-85 Winter barley Rubezh, 86 Winter barley Espada, 87 Winter wheat Anka, 88 Winter wheat Karavan, 89, 90 Oats Verny`j, 91, 92 Oats Skakun, 93, 94 Oats Podgorny`j, 95-96 Winter barley Master, 97 Winter barley Rubezh, 98.1, 98.2 Winter barley Toma, 99 Triticale, 100 Cereal legume, and 102 Winter wheat Korona; M Molecular weight marker GeneRuler 100 bp Plus (100-1000 bp), ("Thermo Fisher Scientific, USA) (Belogorskij, Krasnogvardejskij and Simferopol`skij of the Republic of Crimea 2021).

As a result of PCR with 27F/907R primers, the 880 bp amplicon was obtained for 61 tested samples of DNA from bacterial cultures (Fig. 3.33)

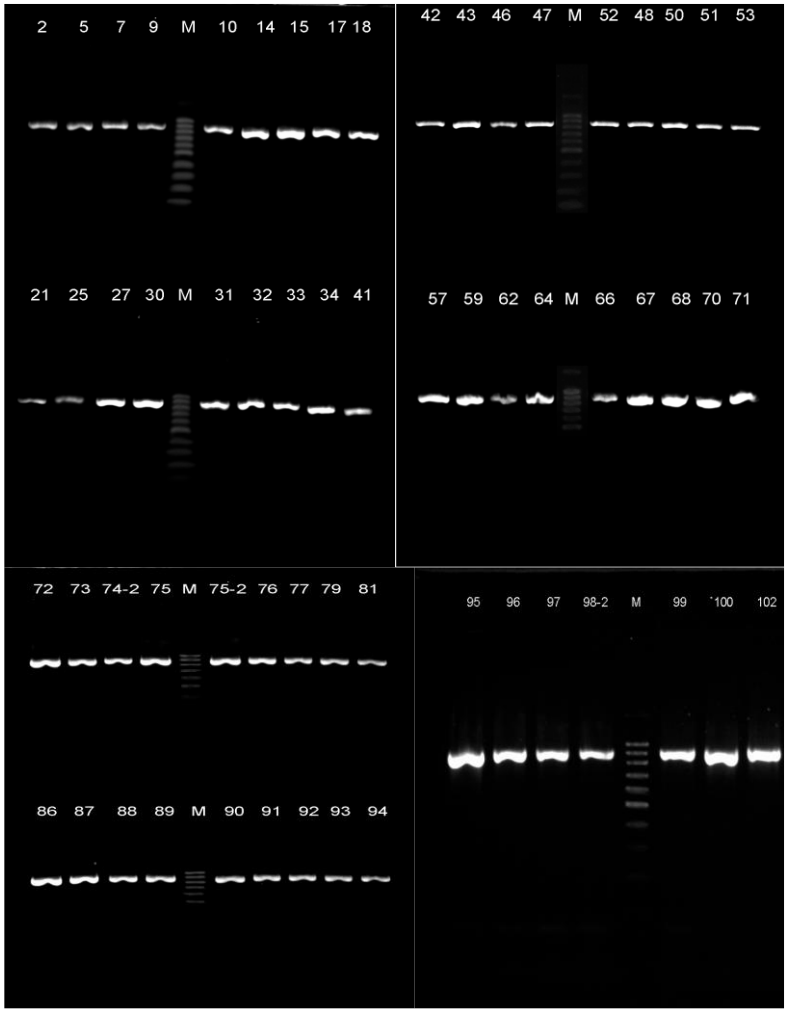

Figure 3.33; PCR products with primers 27F/907R (880 bp) obtained for DNA samples of bacterial isolates from cereal varieties:2 5 7 9 Winter Barley Onega, 10 Winter wheat Aksiniya, 14,15, 17 Winter wheat Asket, 18 25 Winter barley Vosxod, 27, 30 Winter wheat Gubernator Dona, 31-34 Winter barley Vosxod, 41 42 Winter barley Onega, 43, 46-48, 50, 51, 53, 57,59 Winter wheat Gubernator Dona, 62, 64 Winter wheat Asket, 66-68, 70-73, 74-2, 75-1 Winter barley Onega, 75-2, 76,77, 79, 81 Winter wheat Asket, 86 Winter barley Espada, 87 Winter wheat Anka, 88 Winter wheat Karavan, 89, 90 Oats Verny`j, 91, 92 Oats Skakun, 93, 94 Oats Podgorny`j, 95-96 Winter barley Master, 97 Winter barley Rubezh, 98.2 Winter barley Toma, 99 Triticale, 100 Cereal legume, and 102Winter wheat Korona; M Molecular weight marker GeneRuler 100 bp Plus (100-1000 bp), ("Thermo Fisher Scientific, USA) (Belogorskij, Krasnogvardejskij and Simferopol`skij of the Republic of Crimea 2021).

As a result of PCR with primers PSF/PSR, the 610 bp amplicon was obtained for four tested DNA samples of bacterial cultures: 19 Winter barley Vosxod, 63 Winter wheat Asket, 78 Winter wheat Asket and 101 Oat (no name) as shown in (Fig. 3.34).

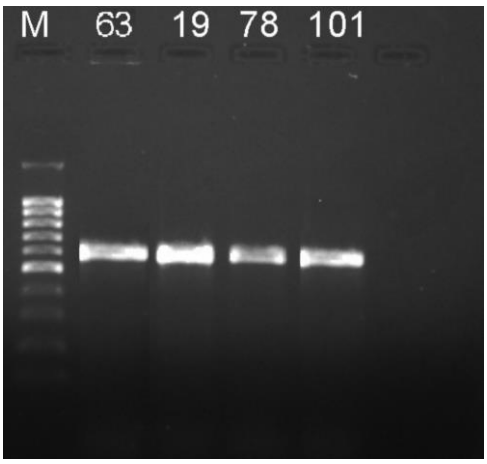

Figure 3.34; PCR products with primers PSF/PSR (610 bp) obtained for DNA samples of bacterial isolates from cereal varieties:19 Winter barley Vosxod, 63 Winter wheat Asket, 78 Winter wheat Asket, 101 Oat (no name); M Molecular weight marker GeneRuler 100 bp Plus (100-1000 bp), ("Thermo Fisher Scientific, USA) (Belogorskij, Krasnogvardejskij and Simferopol`skij of the Republic of Crimea 2021).

The products of the PCR were as follows for 8UA/519B primer set amplicon sizes obtained were 500bp, for 27F/907R primer set amplicon sizes obtained were 880bp and for PSF/PSR primer set amplicon sizes of 610bp were obtained. Appendix 13 table 13.1 shows the presence and absence of amplicons upon testing with the three pairs of primers.

The results of identification of each isolated strain are presented in appendix 14 table 14.1. A total of 86 isolates have been identified.

Bacterial morphology is extremely diverse. Of the 60 samples extracted from the the Republic of Crimea, bacterial isolates from 38 plant samples were processed and identified. This owing to the fact that the colonies collcetd from the 38 samples ahd similar morphology to those from the other 22 plant samples. Colony morphology, color shape and elevation were studied. This morphology is determined by shape, color, edges, profile and form. Colonies with similar morphology to those selected as a result were not collcted but linked to the specific isolate with which they had similar morphologies see table 3.10. Ultimately bacterial morphology is dictated by the net-like peptidoglycan (PG) sacculus [111]. Table 3.10 shows the samples that were not collcted and the samples with which isolates shared similar morphology.

Table 3.10 The samples of grain crops excluded from selection (Belogorskij, Krasnogvardejskij and Simferopol`skij of the Republic of Crimea 2021)

Sample	District	Coordinates	Crop	Cultivar	Isolate №	Result of identification
C22	Krasnogvardejskij	45.527476, 34.193599	Winter wheat	Asket	74	*Pantoea vagans*
C23	Krasnogvardejskij	45.527476, 34.193599	Winter wheat	Asket	78	*Pseudomonas* sp.
C29	Krasnogvardejskij	45.527476, 34.193599	Winter wheat	Asket	79	*Pantoea agglomerans*
C30	Krasnogvardejskij	45.527476, 34.193599	Winter wheat	Asket	93	*Pantoea agglomerans*
C31	Krasnogvardejskij	45.527476, 34.193599	Winter wheat	Asket	79	*Pantoea agglomerans*
C32	Krasnogvardejskij	45.527476, 34.193599	Winter barley	Buran	86	Uncultured bacterium
C34	Krasnogvardejskij	45.527476, 34.193599	Winter barley	Sprinter	88	*Rosenbergiella* sp.
C35	Krasnogvardejskij	45.527476, 34.193599	Winter barley	Toma	86	Uncultured bacterium
C36	Krasnogvardejskij	45.527476, 34.193599	Winter barley	Master	88	*Rosenbergiella* sp.
C39	Krasnogvardejskij	45.527476, 34.193599	Winter wheat	Velena	88	*Rosenbergiella* sp.
C40	Krasnogvardejskij	45.527476, 34.193599	Winter wheat	Vexa	88	*Rosenbergiella* sp.
C43	Krasnogvardejskij	45.527476, 34.193599	Oats	Mezmaj	101	*Pseudomonas* sp.
C44	Krasnogvardejskij	45.527476, 34.193599	Oats	Chernigovskij	99	*Arthrobacter* sp.
C47	Krasnogvardejskij	45.527476, 34.193599	Oats	Guzeripl`	93	*Pantoea agglomerans*
C49	Krasnogvardejskij	45.527476, 34.193599	Winter barley	E`spada	101	*Pseudomonas* sp.
C50	Krasnogvardejskij	45.527476, 34.193599	Winter barley	Buran	98	*Enterococcus mundtii*
C52	Krasnogvardejskij	45.527476, 34.193599	Winter barley	Sprinter	93	*Pantoea agglomerans*
C55	Simferopol`skij	45.056814, 34.058398	Winter barley	Korona	101	*Pseudomonas* sp.
C56	Simferopol`skij	45.056814, 34.058398	Winter barley	Sprinter	101	*Pseudomonas* sp.
C57	Simferopol`skij	45.056814, 34.058398	Winter barley	Rubezh	101	*Pseudomonas* sp.

Bacterial colonies that were similar to those collected.

The highest frequency of occurrence was observed in bacteria of the genera *Pantoea* and *Erwinia* (Table 3.11).

Table 3.11. Bacteria species and genera occurrence frequencies of analytical plant samples from Belogorskij, Krasnogvardejskij and Simferopol`skij of the Republic of Crimea 2021.

Genus	**Frequency %**	**Species**	**Frequency %**
Pantoea	42.11	*Pantoea agglomerans*	21.05
		Pantoea ananatis	2.63
		Pantoea vagans	2.63
		Pantoea pleuroti	2.63
Erwinia	23.68	*Erwinia rhapontici*	5.26
		Erwinia aphidicola	5.26
Rosenbergiella	18.42	-	-
Frigoribacterium	18.42	-	-
Stenotrophomonas	13.16	*Stenotrophomonas maltophilia*	2.63
Pseudomonas	13.16	*Pseudomonas poae*	2.63
Ochrobactrum	13.16	-	-
Arthrobacter	10.53	-	-
Uncultured bacterium	7.89	Uncultured bacterium -	7.89
Exiguobacterium	5.26	-	-
Curtobacterium	5.26	-	-
Microbacterium	5.26	-	-
Clavibacter	5.26	*Clavibacter michiganensis*	5.26
Bacteria	5.26	Bacteria	5.26
Rathayibacter	2.63	*Rathayibacter festucae*	2.63
Enterococcus	5.26	*Enterococcus mundtii*	5.26
Agrococcus	2.63	*Agrococcus jenensis*	2.63
Plantibacter	5.26	*Plantibacter flavus*	2.63
Enterobacter	2.63	-	-

Note – means species were not identified within the given genus frequency of the various bacteria identified. For each identified species and genus, the frequency of occurrence (A) was calculated using the formula [112]: A = B/C*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.

A total of 6 strains of bacteria of the genus *Pseudomonas* have been isolated, which is 6.31% of all isolated strains (figure 3.35).

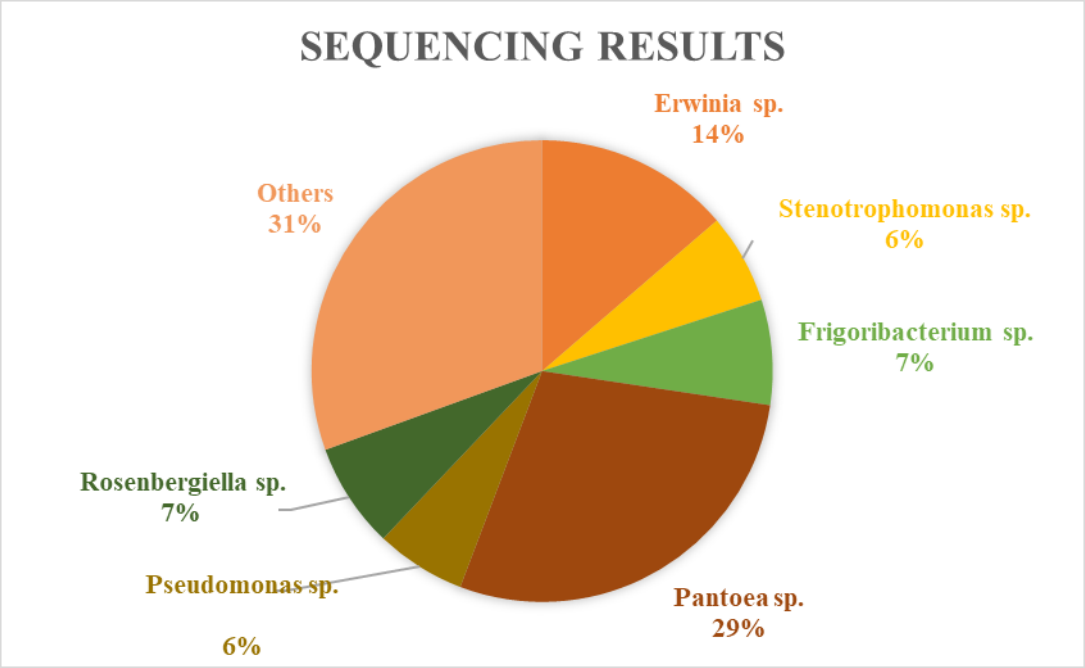

Figure 3.35 shows the sequencing results of the bacteria from the analytical plant samples collected from the Republic of Crimea.

Among identified 27 bacteria identified belonged to the *Pantoea* genus. The species identified included *Pantoea agglomerans*, *P. ananatis*, *P. vagans* and *P. pleuroti* (appendix 14 table 14.1).

The genus *Pantoea* was represented the most in terms of frequency of occurrence: 42% (Table 3.11). Bacteria of the genus *Pantoea* are unique as it includes both beneficial and phytopathogenic microbiota [113; 114]. As shown in appendix 14 table 14.1 a total of 27 strains of bacteria of the genus *Pantoea* have been isolated, which is 29% of all isolated strains (figure 3.35). Among them identified 8 strains of *Pantoea agglomerans* making up 8.4% of all the identified strains. This species is characterized as an an endophyte and epiphyte. Endophytes are able to enter deeper inside plants, whereas, epiphytes remain on the outside of plant organs [115]. Some pathogenic *P. agglomerans* strains cause bacterial blight in cereal crops [113; 114]. Beneficial strains of *P. agglomerans* are popular for being beneficial to the rhizosphere [113; 114].

The 29% of *Pantoea* also compromised of *P. ananatis*, *P. vagans* and *P. pleuroti* (appendix 14 table 14.1). Each of these strains were represented by one strain therefore a frequency of occurrence of 2.63% (table 3.11) and identification

percentage of 1.1% as shown in figure 3.35 for each of the three. On wheat leaves, *P. ananatis* causes brownish lesions with distinct edges and yellow haloes. In addition to feeding on barley and oats, the cereal leaf beetle (CLB, *Oulema melanopus*) is a vector of *P. ananatis*. Insects spread the pathogen through their mouth secretions [116]. *P. vagans* is a beneficial bacterium. It regulates *Erwinia amylovora*, which infects apple and pear plants and results in fire blight [117]. *P. pleuroti* known for causing bacterial blight disease [118].

The genus *Erwinia* was represented second most 13 strains were identified with a frequency of occurrence 24% (Table 3.11). Bacteria of the genus *Erwinia* include both pathogenic and beneficial bacteria [119; 120]. The genus *Erwinia* occupied 14% of all the bacteri identified. The species *Erwinia aphidicola* and *Erwinia rhapontici* were found to have a frequency of occurrence of 5.26%. *Erwinia rhapontici* had an identification percentage of 2.11% this bacterium is a phytopathogen that causes pink seed and soft rot [120]. *Erwinia rhapontici* is monitored by the phytosanitary laws of Brazil, Sudan, Colombia, and Mali since it is listed on the A1 list of the Euro-Mediterranean Plant Protection Organisation (EPPO), indicating that it is an organism that is either missing or has not been discovered in Brazil [3]. *Erwinia aphidicola* also had an identification percentage of 2.11%. when this phytopathogen affects seeds, it results in poor germination of seed [121;122].

The genera *Frigoribacterium* and *Rosenbergiella* were represented next in terms of frequency of occurrence: 18.42% (Table 3.11). A total of 7 strains of bacteria of the genus *Frigoribacterium* have been identified, which is 7% of all isolated strains (figure 3.35). Seven strains of the identified bacteria belong to the genus *Rosenbergiella*. This genus of bacteria is oxidase-negative, gram-negative, facultatively anaerobic, rod-shaped, motile, and grows best at 28–30 °C. Its maximum temperature is 36 °C. On nutrient medium, it develops colonies with yellow-orange pigmentation [123]. They are known to generate large amounts of indole acetic acid, or IAA, which enhances plant germination and growth [124].

The genera *Pseudomonas*, *Ochrobactrum* and *Stenotrophomonas* were represented next in terms of frequency of occurrence: 13.16% (Table 3.11).

A total of 6 strains of bacteria of the genus *Stenotrophomonas* have been isolated, which is 6.31% of all isolated strains (figure 3.35). The genus *Stenotrophomonas* is a soil borne pathogen that also affects humans. It usually attacks internal tissue like root and stem vascular tissues and also exeternal areas like the rhizosphere [125]. One strain was identified as *Stenotrophomonas maltophilia*, that is 1.1%. This bacterium is a beneficial bacterium to the ecosystem and to the overall health of the plant [126]. This bacterium is usually found in association with plants like oilseed, wheat, potatoes, various weeds and maize amongst others [125].

A total of 6 strains of bacteria of the genus *Pseudomonas* have been isolated, which is 6.31% of all isolated strains (figure 3.35). The genus *Pseudomonas* has more than 150 species. This genus of bacteria has rod-shaped, facultative aerobes that are gramm negative [127]. With the exception of oats, triticale, and barley, 20% of the bacteria found in grains are from this genus, which is reason for concern. Phytopathogens as *Pseudomonas putida* and *Pseudomonas syringae* are included in the *Pseudomonas* genus. [128; 122]. Not all bacteria in the *Pseudomonas* genus are classified as quarantine pathogens. Several species within this genus—such as *Pseudomonas syringae* in Mexico, Taiwan, and Colombia—are classified as quarantine pathogens. Whereas in other nations like Great Britain, *P. syringae* is currently regulated but not classified as a quarantine pathogen. One strain of *Pseudomonas poae* was identified that is an identification percentage of 1.1% (figure 3.35). Its frequency of occurrence was 2.63% and *Pseudomonas poae* is a soil beneficial bacterium [129].

A total of 5 strains of bacteria of the genus *Ochrobactrum* have been identified, which is 5.26% of all identified strains (figure 3.35). Commercially, *Ochrobactrum* bacteria are helpful and are utilised to modify soils. Additionally resistant to cold, these bacteria are helpful in the nitrogen fixation process by degrading ammonia [130].

In the analytical plant samples etxtracted from the Republic of Crimea none of the identified pathogens are the phytopathogens of interest in this study.

3.6.2.1 The study of the bacterial microbiota composition of cereals cultivated after Sunflower and Black Fallow.

Analyisis of the results obtained from the Republic of Crimea, a study was conducted on the microbiota composistion after different cultivations. The results of the identification of bacterial isolates isolated from winter barley and wheat plants are presented in the table 3.12.

Table 3.12– Results of identification of bacterial isolates from cereal samples.

Culture	**Sort**	**Predecessor**	
		Sunflower	**Black Fallow**
Winter barley	Buran	*Pedobacter terrae*	*Frigoribacterium faeni*
Winter barley	Rubezh	*Microbacterium foliorum*	*Microbacterium ginsengisoli*
Winter barley	Sprinter	*Rosenbergiella sp.*	*Pseudomonas* sp.
Winter barley	Toma	*Pedobacter terrae*	*Frigoribacterium faeni*
Winter barley	Master	*Rosenbergiella* sp.	*Curtobacterium flaccumfaciens, Clavibacter michiganesis*
Winter barley	Espada	*Pedobacter terrae*	*Pseudomonas* sp.
Winter wheat	Ascetic	*Pantoea agglomerans, Pseudomonas* sp.	*Rosenbergiella nectarea, Pantoea agglomerans, Frigoribacterium faeni, Pantoea* sp.

In a winter barley sample, the Buran, Toma and Espada variety grown after sunflower, the predominant bacterium, which, when isolated on the YDC medium, displaced all other species, was a bacterium identified as *Pedobacter terrae* (Table 34). *Pedobacter terrae* is a gram-negative palo-shaped bacterium that was first isolated from soil in a study by Yoon et al. in 2007 [131]. They contained a species of *Frigoribacterium faeni* (Table 23), a bacterium isolated from water [132], whose growth activity prevailed compared to other microbiota agents in the sample.

In a sample of Barley of the Espada variety grown after black steam, the suppressive bacterial cultured component was *Pseudomonas* sp. *Pseudomonas* sp. are aerobic Gram-negative rod-shaped bacteria released from soil and other habitats [133].

In a sample of winter barley of the Rubezh variety, grown after sunflower, the predominant bacterium was the species Microbacterium foliorum (Table 3.12). The species is a rod-shaped gram-positive and aerobic bacterium previously extracted from the cereal phyllosphere Russell et al., 2019 and used to increase drought resistance and stimulate growth plants [134]. No species *Microbacterium foliorum* was found in a barley sample of Rubezh variety grown after black steam. In this sample, the species *Microbacterium ginsengisoli* (Table 3.12) has been identified. This bacterium is heterotrophic, gram-positive, strictly aerobic, immobile and has a rod-shaped shape [135].

In a sample of winter barley of the Sprinter and Master varieties grown after sunflower, a representative of the genus *Rosenbergiella* (Table 3.12). *Rosenbergiella* sp. are rod-shaped, gram-negative, facultatively anaerobic, oxidase-negative, motile bacteria previously isolated by Halpern et al., 2013 from the nectar of the flowers *Citrus paradisi* (grapefruit) and *Amygdalus communis* (almonds) (Halpern M et al 2013). In a sample of barley of the Sprinter variety, grown on a black vapor, growth suppressing all other bacteria was noted in a representative of *Pseudomonas* sp. (Table 3.12). In a sample of barley of the Master variety, the species *Curtobacterium flaccumfaciens* and *Clavibacter michiganesis* (Table 3.12) were identified, characterized by phytopathogenic properties [137; 138].

In a sample of winter wheat variety Ascetic, grown after sunflower, *Pantoea agglomerans* and *Pseudomonas* sp. (Table 3.12) *Pantoea agglomerans* is a seed-carried phytopathogen [139]. A phytopathogenic species, *Pantoea agglomerans*, was also found in a sample of this variety grown in black steam, which probably contaminated the seeds before sowing. In addition, *Pseudomonas* sp., *Pantoea* sp., *Rosenbergiella nectarea* and *Frigoribacterium faeni* (Table 3.12).

3.6.3 Stavropol region

A total of 165 bacterial isolates were obtained from the analytical plant samples. As a result of PCR with PSF/PSR primers, an amplicon with a length of 610 bp was obtained for 4 tested DNA samples of bacterial cultures (Fig. 3.36).

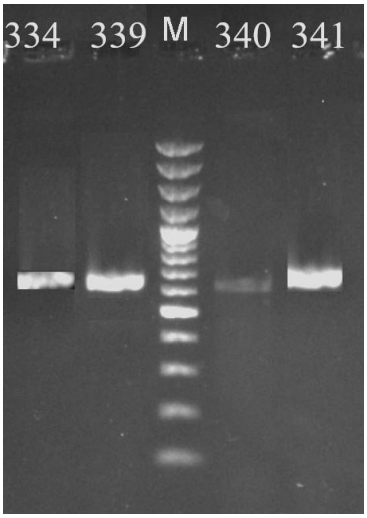

Figure 3.36 PCR products with primers PSF/PSR (610 bp) obtained for DNA samples of bacterial isolates from cereal varieties: 334, 339, 340 and 341; M Molecular weight marker GeneRuler 100 bp Plus (100-1000 bp), ("Thermo Fisher Scientific, USA) (Kochubeevsky, Budyonnovsky, Soviet, George, Andropovsky, Alexander, Shpakovsky, Novoselitsky and Mineralovodsky of Stavropol region 2022).

As a result of PCR with primers SyD1/SyD2, the 1040 bp amplicon was obtained for three tested DNA samples of bacterial cultures: 344, 360 and 472 as shown in (Fig. 3.37).

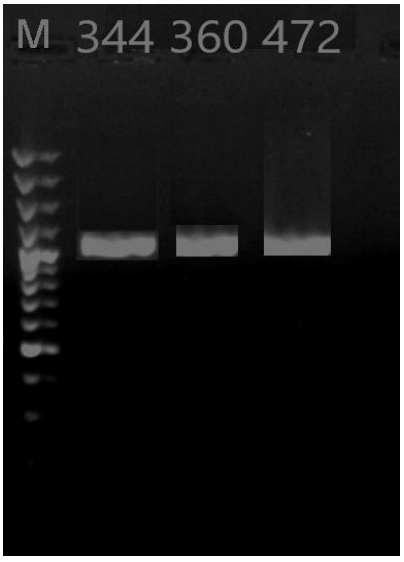

Figure 3.37 PCR products with primers SyD1/SyD2 (1040 bp) obtained for DNA samples of bacterial isolates from cereal varieties: 344, 360 and 472; M Molecular weight marker GeneRuler 100 bp Plus (100-1000 bp), ("Thermo Fisher Scientific, USA) (Kochubeevsky, Budyonnovsky, Soviet, George, Andropovsky, Alexander, Shpakovsky, Novoselitsky and Mineralovodsky of Stavropol region 2022).

Using primers 8UA/519B 500 bp amplicons were obtained for these remaining 157 bacterial culture DNA samples.

A total of 164 isolates have been identified. The results of identification of each isolated strain are presented in more detail in appendix 15 table 15.1. Table 3.13 shows a summary of the sequencing results.

Table 3.13 Identification results of bacterial isolates in Stravropol Region

Sample No.	**Result of identification**
22S28	*Bacillus* sp., *Pseudomonas* sp.
22S51	*Rhodococcus* sp., *Arthrobacter* sp., *Pseudomonas* sp.
22S39	*Rhodococcus* sp., *Pantoea sp., Curtobacterium sp., Arthrobacter sp.* (=*Paenarthrobacter* sp.), *Microbacterium sp.*
22S1	*Sphingomonas* sp., *Sphingomonas* sp., *Frigoribacterium* sp., *Pseudarthrobacter equi, Clavibacter* sp., *Pseudomonas* sp., *Curtobacterium flaccumfaciens, Achromobacter* sp., *Arthrobacter* sp., *Rathayibacter festucae, Pseudomonas poae, Rhodococcus fascians, Microbacterium* sp., *Pseudomonas syringae, Rhodococcus* sp., *Microbacterium hydrocarbonoxydans, Paenarthrobacter* sp., *Clavibacter* sp., *Rhodococcus* sp., *Frigoribacterium* sp., *Rathayibacter festucae, Achromobacter* sp.,*Pseudomonas syringae, Microbacterium* sp., *Rathayibacter* sp.,*Luteimonas* sp., *Paenarthrobacter* sp. (=*Arthrobacter* sp.) *Rhodococcus* sp., *Rathayibacter festucae, Frigoribacterium* sp., *Pedobacter petrophilus, Plantibacter* sp.
22S2	*Paeniglutamicibacter* sp., *Clavibacter* sp., *Pseudomonas syringae, Plantibacter* sp., *Gordonia* sp., *Pseudomonas* sp., *Rhodococcus* sp., *Clavibacter michiganensis, Paeniglutamicibacter terrestris, Pantoea* sp.
22S4	*Sphingobacterium faecium, Microbacterium* sp., *Achromobacter* sp., *Pseudomonas syringae, Gordonia* sp.
22C6	*Agrococcus citreus, Rhodococcus* sp., *Pseudomonas poae, Clavibacter michiganensis, Arthrobacter* sp., *Pseudarthrobacter* sp., *Glutamicibacter bergerei, Frigoribacterium* sp., *Microbacterium* sp.
22S8	*Rhodococcus* sp., *Paenarthrobacter* sp., *Microbacterium hydrocarbonoxydans, Pseudomonas poae, Pseudomonas* sp., *Rahnella* sp., *Paeniglutamicibacter* sp.
22S9	*Rhodococcus* sp., *Pseudomonas poae, Frigoribacterium* sp., *Rhodococcus fascians, Okibacterium* sp., *Microbacterium* sp., *Rhodococcus* sp.
22S11	*Frigoribacterium* sp., *Agrococcus citreus, Paeniglutamicibacter terrestris, Plantibacter* sp., *Paenarthrobacter* sp. (=*Arthrobacter sp.*), *Rhodococcus* sp., *Frigoribacterium* sp., *Pseudomonas syringae, Curtobacterium flaccumfaciens, Plantibacter* sp., *Plantibacter* sp., *Clavibacter* sp., *Microbacterium* sp., *Sphingobacterium* sp.
22S18	*Mycobacterium frederiksbergense, Rhodococcus* sp., *Achromobacter* sp., *Pseudomonas poae, Arthrobacter* sp., *Curtobacterium flaccumfaciens, Microbacterium* sp., *Frigoribacterium* sp., *Paenarthrobacter* sp. (=*Arthrobacter* sp.), *Knoellia aerolata, Bacillus pumilus*
22S13	*Bacillus* sp., *Paeniglutamicibacter* sp., *Sphingobacterium faecium, Pseudomonas syringae, Plantibacter* sp., *Staphylococcus equirum, Rhodococcus* sp., *Microbacterium* sp.
22S14	*Paeniglutamicibacter sulfureus, Rhodococcus fascians, Pseudomonas poae, Pantoea* sp.
22S16	*Pseudoclavibacter* sp., *Sphingobacterium* sp., *Frigoribacterium* sp., *Paeniglutamicibacter* sp., *Sphingobacterium faecium, Achromobacter* sp., *Rhodococcus sp., Rhizobium* sp.
22S17	*Microbacterium* sp., *Rhodococcus* sp., *Staphylococcus equirum, Bacillus* sp., *Pseudarthrobacter* sp., *Pseudomonas poae, Sphingobacterium* sp.

22S20	*Bacillus* sp., *Microbacterium* sp., *Plantibacter* sp.
22S22	*Rhodococcus* sp., *Bacillus* sp.
22S26	*Microbacterium* sp., *Bacillus* sp., *Pseudomonas poae, Rhodococcus* sp.
22S24	*Microbacterium* sp., *Achromobacter* sp., *Pseudomonas poae, Pseudarthrobacter equi, Arthrobacter* sp., *Curtobacterium* sp., *Exiguobacterium* sp., *Rhodococcus* sp.
22S29	*Frigoribacterium* sp., *Rhodococcus* sp., *Bacillus* sp., *Paenarthrobacter* sp. (=*Arthrobacter* sp.), *Achromobacter* sp., *Rathayibacter* sp.
22S33	*Staphylococcus equirum, Paeniglutamicibacter terrestris, Psychrobacillus psychrodurans, Paenarthrobacter* sp. (=*Arthrobacter* sp.), *Staphylococcus* sp.
22S32	*Frigoribacterium* sp., *Rhodococcus* sp., *Clavibacter* sp., *Pantoea* sp., *Rhodococcus* sp., *Paenarthrobacter* sp. (=*Arthrobacter* sp.)
22S34	*Frigoribacterium* sp., *Pseudoclavibacter* sp., *Rhodococcus* sp., *Agrococcus citreus, Pseudomonas poae*
22S36	*Glutamibacter* sp., *Rhodococcus* sp., *Paeniglutamicibacter* sp., *Exiguobacterium* sp.
22S38	*Rhodococcus* sp., *Bacillus pumilus, Glutamicibacter bergerei, Agrococcus citreus*
22S40	*Curtobacterium* sp., *Rhodococcus* sp., *Microbacterium hydrocarbonoxydans, Pseudomonas poae, Curtobacterium flaccumfaciens*
22S43	*Rhodococcus* sp., *Rhodococcus fascians, Frigoribacterium* sp., *Microbacterium* sp., *Pseudomonas poae, Pantoea* sp.
22S46	*Rhodococcus cerastii, Rhodococcus* sp., *Clavibacter* sp. *Pseudomonas syringae*
22S57	*Agrococcus* sp., *Rhodococcus* sp., *Bacillus pumilus, Paeniglutamicibacter* sp., *Microbacterium* sp., *Paenarthrobacter* sp. (=*Arthrobacter* sp.) *Arthrobacter* sp., *Pseudomonas poae, Pseudarthrobacter equi, Agrococcus jenensis, Frigoribacterium* sp., *Agreia* sp., *Rhodococcus* sp.
22S58	*Paenarthrobacter* sp., *Gordonia* sp., *Microbacterium* sp.
22S52	*Okibacterium* sp., *Rhodococcus* sp., *Pantoea* sp., *Pseudomonas syringae, Plantibacter* sp., *Frigoribacterium* sp., *Pseudomonas poae, Achromobacter* sp., *Microbacterium* sp.
22S59	*Microbacterium* sp., *Pseudomonas syringae., Stenotrophomonas* sp.
22S53	*Plantibacter* sp., *Pseudomonas syringae, Rhodococcus* sp., *Pseudomonas poae, Frigoribacterium* sp., *Pseudoclavibacter* sp., *Plantibacter* sp.
22S60	*Exiguobacterium* sp., *Frigoribacterium* sp., *Microbacterium* sp., *Paenibacillus lautus*
22S56	*Paenarthrobacter* sp. (=*Arthrobacter* sp.), *Exiguobacterium* sp., *Pseudomonas poae, Rhodococcus* sp.
22S65	*Rhodococcus* sp., *Pseudoclavibacter* sp., *Pseudomonas poae, Gordonia* sp., *Labedella* sp., *Microbacterium* sp., *Frigoribacterium* sp.
22S54	*Paenarthrobacter* sp. (=*Arthrobacter* sp.), *Labedella* sp., *Plantibacter* sp., *Pseudomonas poae, Pseudarthrobacter equi*
22S45	*Chryseobacterium* sp., *Exiguobacterium* sp., *Rhodococcus* sp., *Microbacterium* sp.
22S50	*Plantibacter* sp., *Paenibacillus lautus, Exiguobacterium* sp., *Pseudarthrobacter equi, Rhizobium* sp., *Agreia* sp.
22S48	*Plantibacter* sp., *Rhodococcus* sp., *Labedella* sp., *Exiguobacterium* sp., *Paenarthrobacter* sp. (=*Arthrobacter* sp.), *Rhodococcus* sp., *Pseudomonas poae, Rathayibacter festucae, Chryseobacterium* sp.
22S35	*Frigoribacterium* sp., *Plantibacter* sp., *Bacillus* sp.
22S55	*Rhodococcus* sp., *Glutamibacter* sp., *Rhizobium* sp., *Paenarthrobacter* sp. (=*Arthrobacter* sp.), *Pseudomonas poae*
22S37	*Paenarthrobacter* sp. (=*Arthrobacter* sp.), *Pseudomonas poae, Arthrobacter* sp., *Pseudomonas* sp., *Rhodococcus*

	fascians
22S66	*Paenarthrobacter* sp. (=*Arthrobacter* sp.), *Achromobacter* sp., *Pseudomonas syringae*, *Exiguobacterium* sp.

The highest frequency of occurrence was observed in bacteria of the genus *Rhodococcus*: 48.5% (Table 3.14).

Table 3.14. Bacteria species and genera occurrence frequencies of analytical plant samples from Kochubeevsky, Budyonnovsky, Soviet, George, Andropovsky, Alexander, Shpakovsky, Novoselitsky and Mineralovodsky of Stavropol region.

Genus	**Frequency %**	**Species**	**Frequency %**
Rhodococcus	48.48	*Rhodococcus fascians*	4.55
		Rhodococcus cerastii	1.52
Microbacterium	24.24	*Microbacterium hydrocarbonoxydans*	1.52
Frigoribacterium	19.70	-	-
Paenarthrobacter	18.18	*Paenarthrobacter aurescens*	1.52
Plantibacter	12.12	-	-
Clavibacter	9.09	*Clavibacter michiganensis*	3.03
Pseudomonas	7.58	*Pseudomonas syringae*	4.55
		Pseudomonas poae	4.55
Bacillus	4.55	*Bacillus pumilis*	1.52
Curtobacterium	4.55	*Curtobacterium flaccumfaciens*	1.52
Rathayibacter	3.03	*Rathayibacter festucae*	4.55
Achromobacter	3.03	-	-
Arthrobacter	3.03	-	-
Paeniglutamicibacter	3.03	-	-
Pantoea	3.03	-	-
Pseudarthrobacter	3.03	-	-
Okibacterium	3.03	-	-
Sphingobacterium	3.03	*Sphingobacterium faecium*	1.52
Pseudoclavibacter	3.03	-	-
Rhizobium	3.03	-	-
Exiguobacterium	3.03	-	-
Agreia	3.03	-	-
Chryseobacterium	3.03	-	-
Sphingomonas	1.52	-	-
Pseudoarthrobacter	1.52	*Pseudoarthrobacter equi*	1.52
Luteimonas	1.52	-	-
Pedobacter	1.52	*Pedobacter petrophilus*	1.52
Paeniglutamicibacter	1.52	*Paeniglutamicibacter terrestris*	1.52

		Paeniglutamicibacter sulfureus	1.52
Agrococcus	1.52	*Agrococcus citreus*	1.52
		Agrococcus jenesis	1.52
Glutamibacter	1.52	*Glutamibacter bergerei*	1.52
Rahnella	1.52	-	-
Mycobacterium	1.52	*Mycobacterium frederiksbergense*	1.52
Knoellia	1.52	*Knoellia aerolata*	1.52
Staphylococcus	1.52	*Staphylococcus equorum*	1.52
Psychrobacillus	1.52	*Psychrobacillus psychodurans*	1.52
Stenotrophomonas	1.52	-	-
Paenibacillus	1.52	*Paenibacillus lautus*	1.52
Labedella	1.52	-	-

Note – means species were not identified within the given genus frequency of the various bacteria identified. For each identified species and genus, the frequency of occurrence (A) was calculated using the formula [112]: A = B/C*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.

A total of 32 strains of bacteria of the genus *Rhodococcus* have been isolated, which is 19% of all isolated strains (figure 3.38).

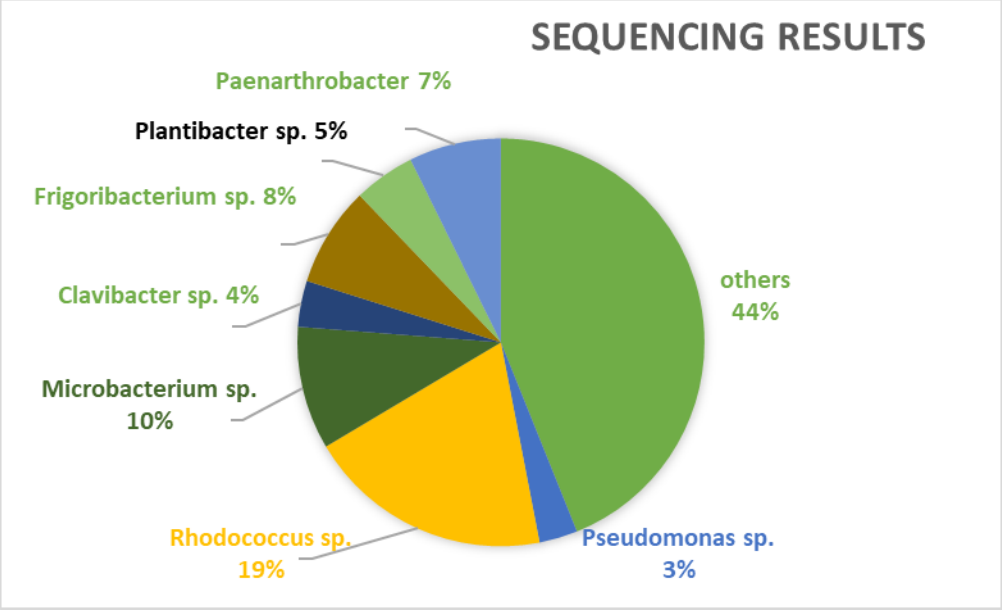

Figure 3.38 shows the sequencing results of the bacteria from the analytical plant samples collected from Stavropol region.

In the analytical plant samples etxtracted from Stavropol region 2% of the identified pathogens are the phytopathogens of interest in this study.

Pseudomonas syringae 3 isolates.

3.6.4 General findings

Analytical samples extracted from the plant samples collected were used to for bacteria isolation on CRL, YDC and R2A nutrient media (appendix 1 table 1) as shown in table 3.15.

Table 3.15: The samples collected from the three regions of the Russian Federation.

Sampling region	**Number of plant samples**	**Nutrient media**	**Number of colony morphotypes (N)**	**Number of isolates selected**	**Variety of morphotypes (N/M)**
Moscow	55	CRL	177	168	2,7
Republic of Crimea	60	YDC	102	102	1,7
Stavropol Region	66	R2A	513	164	7,8

Key CRL, YDC and R2A nutrient media

Various colonies were collected from the analytical samples. Isolated colonies were selected as shown in table 3.15, and from these DNA was extracted.

Various universal nutrient media were used and the characterists of R2A nutrient media makes it the best in this study. On R2A nutrient media, bacterial colonies are characterized by compactness, appear quickly, but do not have aggressive growth, do not secrete mucus. Screening can be carried out even after 2-4 weeks, since individual colonies will still be distinguishable on the cup at a moderate concentration of the plated suspension (Fig. 3.39).

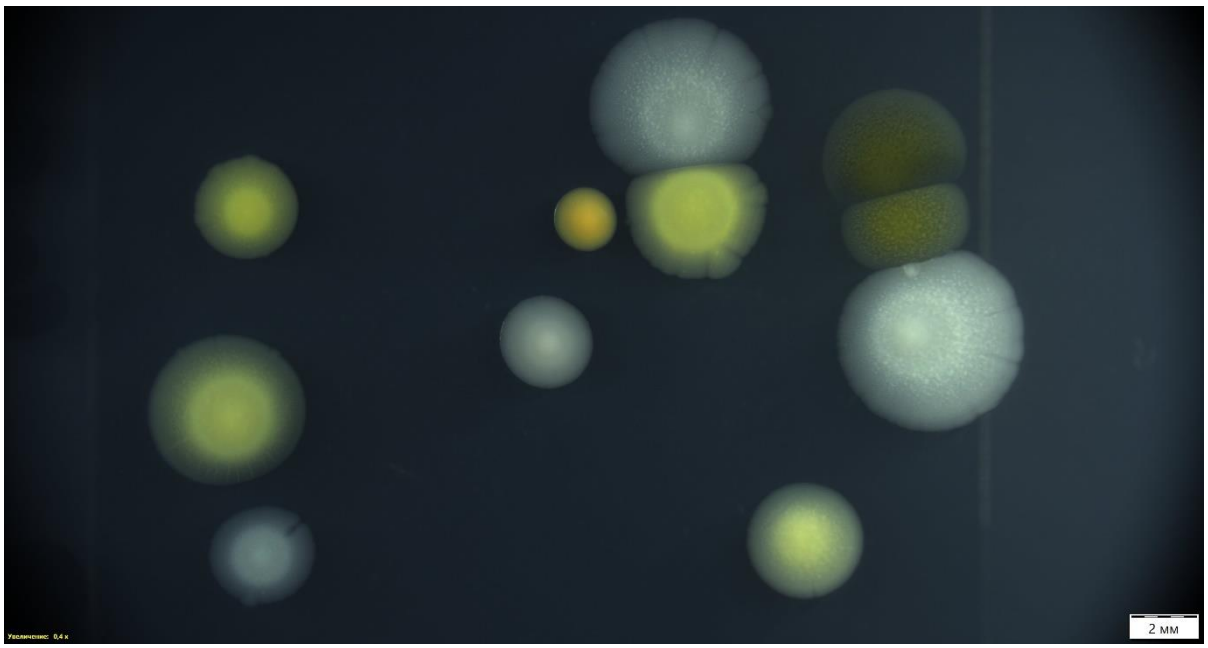

Figure 3.39 – Bacterial colonies from a sample of sample S18 on R2Anutrient media after 21 days of incubation at 25 ° C.

Using the Olympus macroscope, macro images of each of the isolates were taken. Figures 3.40 – 3.44 show photographs of the colonies of the identified isolates. The photographs contain images of *Pseudomonas syringae* colonies, which can serve as a visual material for comparison when isolating bacteria.

Figure 3.40 – Colonies of isolate S1 *Pseudomonas poae* on R2A nutrient media

Figure 3.41 – Colonies of isolate S1 *Pseudomonas syringae* pv. *atrofaciens* on R2A nutrient media.

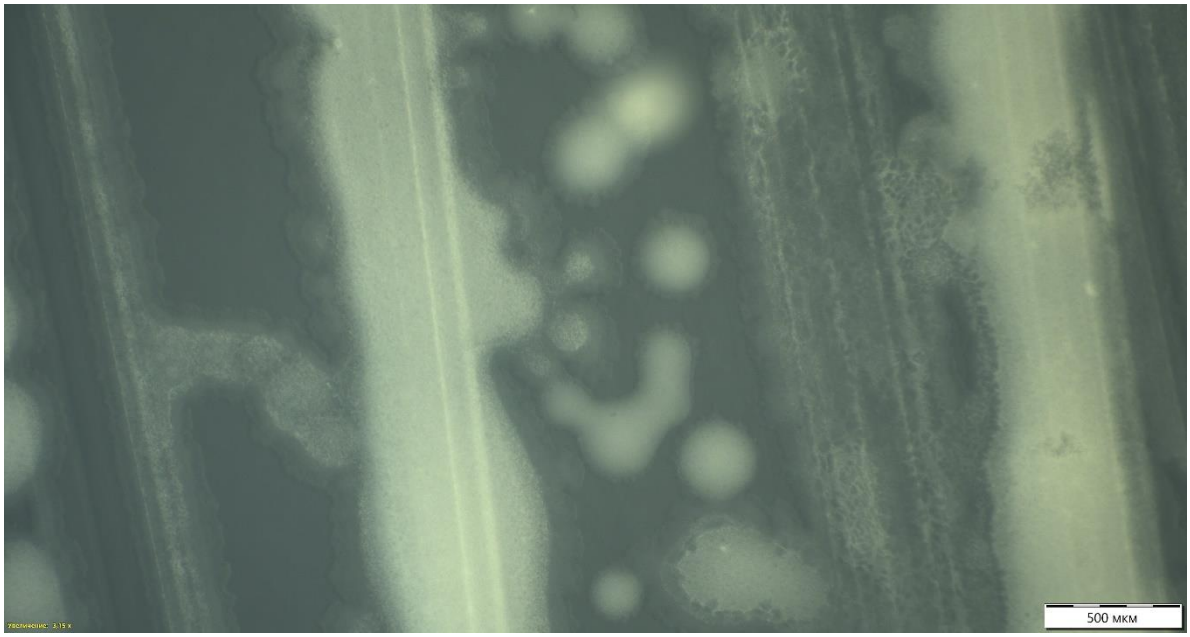

Figure 3.42 – Isolate colonies S47 *Pseudomonas syringae* pv. *atrofaciens* on R2A nutrient media.

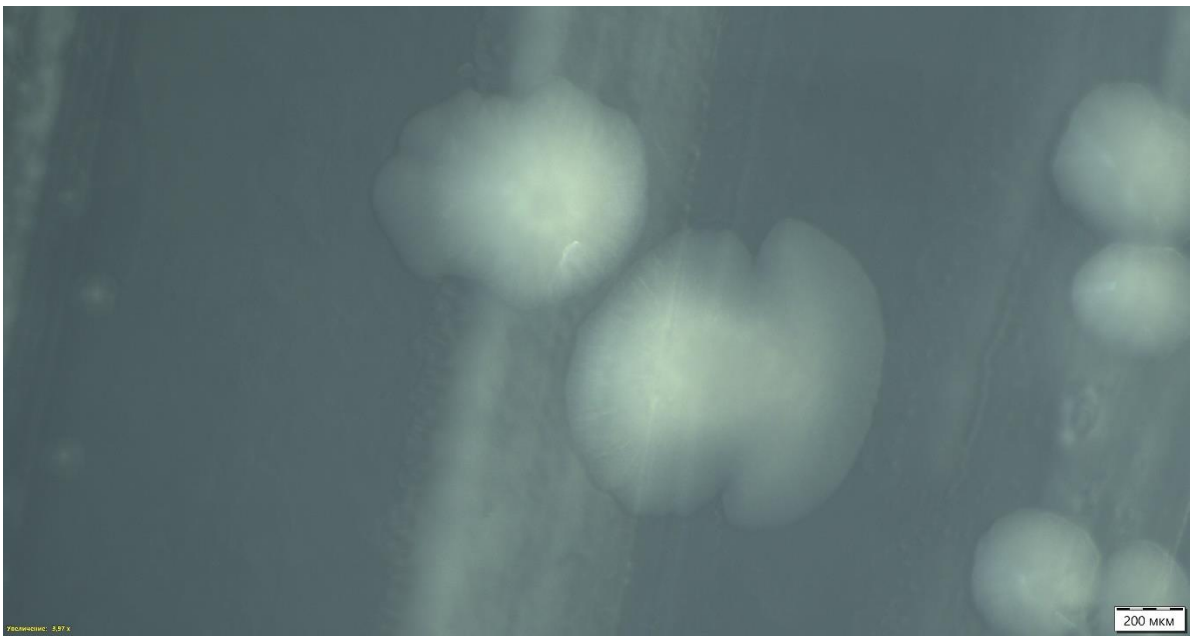

Figure 3.43 – Isolate colonies S1 *Pseudomonas syringae* pv. *atrofaciens* on R2A nutrient media.

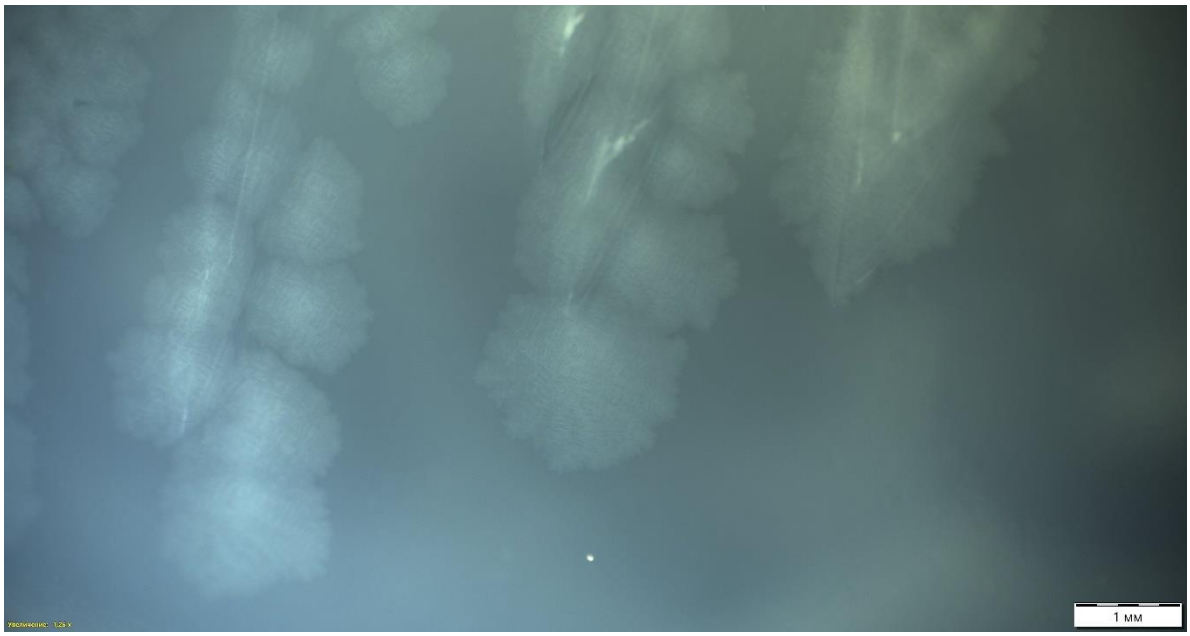

Figure 3.44 – Colonies of isolate S1 *Pseudomonas poae* R2A nutrient media.

The following universal primers were used in this study: 8UA/519B (500b.p) and 27f/907r primers (880b.p). Of these two the best set of primers was 27f/907r as it produced amplicons that have longer fragements that made sequencing more efficient.

Thus, in our study, we studied the microbiota of local crops of grain crops (Moscow in 2020, The Republic of Crimea in 2021 and Stavropol region in 2022) and identified field isolates that can be used in the formation of a single collection of cereal bacteria in order to create species-specific primers, which will be a key part of the developed regulatory documents for the detection and identification of quarantine and export-important microbiome pathogens of grain crops. These regulatory documents, the need for which is very high, will be used by phytosanitary laboratories carrying out their activities in the field of phytosanitary control. Considering the regional peculiarities of soil-climatic and agrotechnical conditions and the biodiversity of isolates [140; 141; 142], we believe that type strains from foreign collections of microorganisms (if available) are less suitable for these purposes.

CONCLUSION

- Samples of grain crops were taken in three regions of the Russian Federation: Moscow (2020), the Republic of Crimea (2021) and the Stavropol region (2022). In these three regions, 181 samples of wheat, rye, barley, oats and triticale plants were collected.
- Unique genetic targets for the causative agent of black bacteriosis of cereal crops *X. translucens* have been found, and 5 new PCR tests for the identification of the phytopathogen have been developed on their basis: 1F8/1R8, 1F10/1R10, 4F1/4R1, 5F6/5R6 and 6F10/6R10. New PCR tests can be part of the solution to the problem of establishing the phytosanitary condition of batches of Russian grain products.
- It has been established that the optimal nutrient medium for the cultivation of *R. tritici* is the YPGA nutrient medium.
- The method of preparing grain samples for subsequent PCR identification of *R. tritici, X. translucens, P. fuscovaginae* and *P. syringae* has been optimized, the use of which, in conjunction with PCR tests, will allow the identification of phytopathogens within 6 hours.
- For the first time in the Russian Federation, using molecular genetic

diagnostic methods, a study of plant samples of cereal crops for the content of phytopathogenic bacteria was carried out. As a result of testing samples from phytocenoses in Moscow, the Republic of Crimea and the Stavropol region, *R. tritici* and *P. fuscovaginae* were not detected, *X. translucens* was found in one sample of wheat from the Krasnogvardeysky district of the Republic of Crimea, and *P. syringae* was found in all three regions, the total frequency of occurrence was 41%.

- For the first time, a large-scale study of the components of the cultured bacterial microbiota of cereal crops using PCR and sequencing was carried out. A variety of bacteria included in the microbiome of cereal crops was found.

The identified isolates made it possible to form a collection of pathogenic and non-pathogenic bacteria isolated from cereal crops. The collection can be further used for scientific developments, production activities and in the educational process.

The results of the research were used in the development of methodological recommendations of the FSBI "VNIIKR" for the detection and identification of pathogens of bacteriosis of grain crops, which are currently put into operation and recommended for use by testing laboratories in the Russian Federation.

LIST OF ABBREVIATIONS AND SYMBOLS

In this manuscript, the following abbreviations and symbols are applied:

°C – degree Celsius

μl – microliter

μm – micrometer

g – is the acceleration of rotor rotation

OCR – Open Reading Frames

PBS – phosphate buffered saline or phosphate-salt buffer

pmol – picomol

pmol/μl – picomol/microliter

DNA – deoxyribonucleic acid

EAEU – Eurasian Economic Union

EPPO – European and Mediterranean Plant Protection Organization

FAO – Food and Agriculture Organization

UN – United Nations

FSBI – Federal State Budgetary Institution

VNIIKR – All-Russian Plant Quarantine Institute

CFU/ml – colony-forming units/milliliter

MR – methodological recommendations

rpm – revolutions per minute

b.p. – a pair of bases

PCR – polymerase chain reaction

PCR-Rt – Real time PCR

Russia – The Russian Federation

HS – Commodity Nomenclature of Foreign Economic Activity of the Eurasian Economic Union

NA – Nutritive agar;

YDC – Yeast Extract-Dextrose-Calcium Carbonate;

SNR medium – Sorbitol Neutral Red medium;

KB – King B medium;

SPTPsjA – serine-potassium tellurite-based

Psj – selective agar;

PPGA – potato-peptone-glucose agar;

YPGA – yeast-peptone-glucose agar

NBY – nutrient broth with yeast extract.

LB – Luria-Bertani

CRL –

YDC – Yeast extract-dextrose

R2A – Reasoner's 2A

Ct – threshold cycle of PCR,

FAM – is the detection channel of PCR specificity,

HEX – is the detection channel of internal positive PCR control,

"+" – is positive

"-" – is negative

AOI – any other information

PC – is a positive control sample

IPC – internal positive control

NCO – is a negative control sample

RT – real time PCR

GLOSSARY

An amplicon is a stretch of DNA that is copied during the amplification process

Amplification is the selective copying of a specific section of DNA

Analytical sample – a part of the sample selected and specially prepared for analysis.

Analytical plant sample - a part of the plant sample selected and specially prepared for analysis.

Analytical seed sample - a part of the seed sample selected and specially prepared for analysis.

Analytical plant DNA sample is the DNA extracted from the analytical plant samples

Analytical seed DNA sample is the DNA extracted from the analytical seed samples

Bacteriosis is a disease caused by a pathogenic bacterium

Bacterial isolates are laboratory samples extracted from plated analytical samples

A gene is a section of the DNA molecule that is a structural and functional unit of heredity and carries information about a certain trait or function of an organism

A genome is a set of genes that are characteristic of the haploid (single) set of chromosomes of a particular species of organism

DNA template is a DNA sequence that needs to be "read" during sequencing (PCR product, plasmids, various regions of genomes, both small and complete genomes, transcriptomes)

DNA polymerase is an enzyme involved in the synthesis of the daughter DNA molecule

Identification is the establishment of the identity of an unknown object with a known one

Isolate is a pure culture of a microorganism isolated on a nutrient medium

Isolation – isolation of a pure culture of a microorganism on a culture medium

A sample is a part of a batch of products that has been selected according to the sampling procedure

Lysis is the dissolution of cells and their systems under the influence of various agents

Lipase is an enzyme that catalyzes the hydrolysis of lipid substrates

Macerate is a substance formed as a result of maceration

Maceration is soaking, as a result of which the plant tissue softens and the bacteria are released into the buffer

A sample is a part of a batch of products selected in accordance with regulatory documents and representative of all significant properties of the source material

A pathogen is a microorganism capable of causing a pathological condition of another living creature

Pathogenicity is the genetically fixed ability of a microorganism to cause a certain disease in the host organism.

Repeatability (P) is a criterion of the effectiveness of a method, showing the stability of the results of the method when it is repeatedly applied by one person, on the same equipment and in the most limited time

A primer is a short fragment of nucleic acid that serves as a starting point for DNA replication

Sample – a part of a batch of products separated according to the procedure of sample preparation and sampling

Protease is an enzyme from the class of hydrolases that cleave the peptide bond between amino acids in proteins

Sequencing is the general name for methods that establish the sequence of nucleotides in a DNA molecule

Screening is a technique used to selectively isolate target bacterial species

Screening tests (selection tests) – tests for rapid preliminary detection of the pathogen

The substrate is a nutrient medium and a habitat for microorganisms

A supernatant is a liquid above the sediment

A phytopathogen is a microorganism capable of causing a pathological state of a plant

Phytotoxins are toxins that are formed and produced by plants

A strain is a pure culture of a microorganism isolated at a specific time and place

Gene expression is the process by which the hereditary information of a gene is converted into a functional product, such as RNA or protein

Exudate is a liquid containing bacterial cells secreted on the surface of plant tissues affected by a phytopathogen

Extract – concentration of plant/seed material of bacterial cells in water-salt buffer

Molecular phylogenetics is the science of using molecular data (DNA and protein sequences) to infer the phylogenetic relationships among species.

HEX channel is the PCR specificity detection channel

FAM channel is the

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Appendix

Appendix 1

Table 1.1 – Composition of components per 1 liter of nutrient media used

Nutrient Media	
Component	**Amount of substance**
CRL	
Peptone	12,0 g
Glycerin	10,0 g
Bacteriological agar	18,0 g
Magnesium sulfate ($MgSO_4$)	0,7 g
Potassium phosphate two-substituted anhydrous (K_2HPO_4)	2,0 g
Potassium phosphate monosubstituted anhydrous (KH_2PO_4)	2,0 g
Glucose	2,5 g
Yeast Extract	2,0 g
Meat Peptone	2,0 g
Saccharose	15,0 g
Sodium chloride (NaCl)	2,0 g
Calcium carbonate ($CaCO_3$)	20,0 g
Distilled water	Up to 1000 ml
CRL.2 has a similar CRL composition, excluding $CaCO_3$	
mCRL.2 has a composition similar to CRL.2 medium + alcohol (70%) solution of cycloheximide 200 mg per 1 liter of medium and an aqueous solution of 2,3,5-triphenyltetrasolium chloride (TTX) 50 mg / l, sterilized by filtration	
YDC	
Glucose	5,0 g
Yeast Extract	10,0 g
Calcium carbonate ($CaCO_3$)	20,0 g
Bacteriological agar	15,0 g
YPGA	
Peptone	5,0 g
Bacteriological agar	18,0 g
Glucose	10 g
Yeast Extract	5,0 g
LB	

Nutrient Media	
Component	**Amount of substance**
Tripton	10 g
Yeast Extract	5,0 g
Sodium chlorinewithty	10 g
Bacteriological agar	18,0 g
R2A	
Yeast Extract	0,5 g
Protease Peptone	0,5 g
Casamic acids	0,5 g
Glucose	0,5 g
Starch	0,5 g
Sodium phosphate double-substituted anhydrous (Na_2HPO_4)	0,3 g
Magnesium sulfate anhydrous ($MgSO_4$)	0.024 g
Sodium malic acid (C_3H_3NaO)	0,3 g
Bacteriological agar	18,0 g

Each nutrient media was made in a separate container. The ingredientsare stirred, adjustedor, if necessary, pH to 7.0–7.2 and sterilized at a temperature of 121 ˚C for 15 minutes. The finished media were stored at a temperature of 2 to 4 ˚C were used for 1 month.

Appendix 2

Buffer solutions

Table 2.1 – Components of the buffer solutions

No p/n	Component	Amount of substance
Phosphate buffer 50 mM (PB), pH 7.0		
1	Sodium phosphate 2-w used (Na2HPO4)	4.26 g
2	Potassium phosphate 1-w b/v (KH_2PO_4)	2.27 g
3	Distilled water	Up to 1000 ml

Phosphate-buffered saline 10mM (PBS), pH 7.0–7.2		
1	Sodium phosphate 2-z 12-v (Na2HPO4 x 12H2O)	2,9 g
2	Potassium phosphate 1-z 2-in (KH_2PO_4 x $2H_2O$)	0,2 g
3	Sodium chloride (NaCl)	8,0 g
4	Potassium chloride (KCl)	0,2 g
5	Distilled water	Up to 1000 ml

The ingredients are dissolved, brought to a pH of 7.0-7.2 and sterilized at a temperature of 121 ° C for 15 minutes.

Appendix 3

Table 3.1 – Bacterial Strains used in the research

Bacterium	Source	Country	Isolated from	Isolator and date of isolation	Strain number in VNIIKR collection	Other collection numbers	Article
Pseudomonas syringae pv. *syringae*	AOBCPP SCD	Hungary Pécs	Prunus avium	H.B. Дренова 2007	0222	n/a	van Hall 1902
Pseudomonas syringae pv. *coronafaciens*	CIRM-CFBP Collection for Plant associated Bacteria, France CFBP 2216	United Kingdom	Avena sativa	Lelliott R.A. 1958	0440	Collection numbers: ICMP 3113,LMG 5060,NCPPB 600	Elliott 1920 Young, Dye & Wilkie 1978
Xanthomonas translucens pv.	German Collection of Microorga	United States of America Minnesot	Hordeum vulgare	Reddy C.S. 1933	0337	NCPPB 973,ICMP 5752,ATCC 19319,ICPB	ex Jones et al. 1917 Vauterin et al. 1995

translucens	nisms and Cell Cultures, Leibniz Association, Germany DSMZ 18974	a				XT2,PDDCC 5752,VdM 130,CFBP 2054,LMG 876	
Rathayibacter tritici	CIRM-CFBP Collection for Plant associated Bacteria, France CFBP collection (France) 1385	Egypt	Triticum aestivum	1952	0378	Collection numbers: ATCC 11403,CIP 104038,DSM 7486,ICMP 2626,LMG 3728,NCPPB 1857	Carlson and Vidaver 1982 Zgurskaya et al. 1993, comb. nov.
Pseudomonas fuscovaginae	German Collection of Microorganisms and Cell Cultures, Leibniz Association, Germany DSMZ 7231-0205-001	n/a	Oryza sativa	December 2018	0335	n/a	n/a

Note: *All strains were used in 1- the Detection of Pseudomonas fuscovaginae, Rathayibacter tritici Pseudomonas syringae and Xanthomonas translucens in grain samples 2- Identification of Pseudomonas fuscovaginae,*

Rathayibacter tritici Pseudomonas syringae and Xanthomonas translucens in plant samples collected from Moscow region, the Republic of Crimea and Stavropol region. 3- Rathayibacter tritici was used in Determining of the optimal nutrient medium for Rathayibacter tritici. 4- Xanthomonas translucens was used in Developing PCR tests for the identification of Xanthomonas translucens.

Appendix 4

Plant sample preparation for the subsequent detection and identification of bacteria by PCR.

The samples were prepared and the studies were carried out in accordance with the scheme presented in Figure 4.1. The scheme was developed by the authors specifically for this study and was used for the entire period of research work.

The work used classical methods of microbiology and molecular diagnostics; their brief description is given in the corresponding titles. In the case of using your own design of the experiment, a detailed description of the operations performed is given.

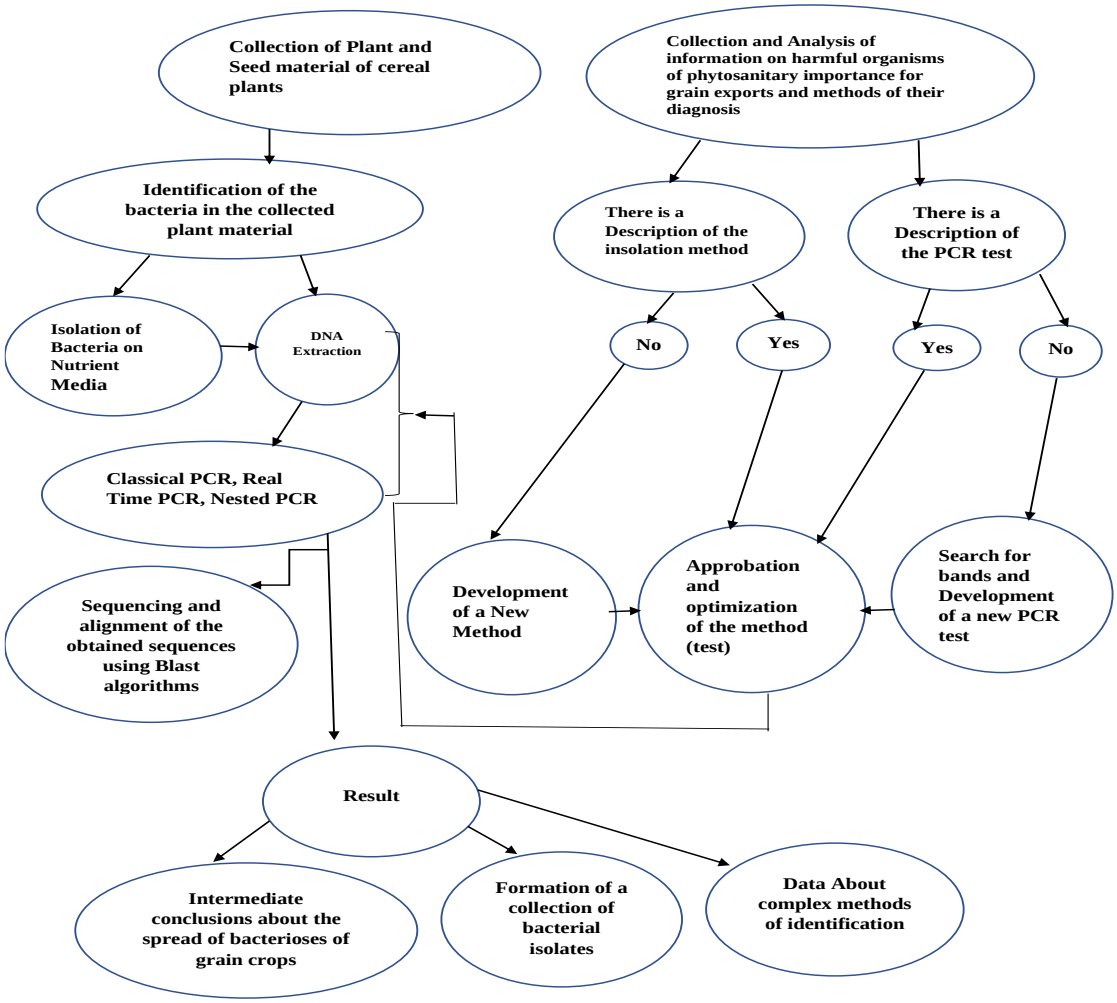

Figure a1 – Scheme of research on the study of bacteriosis of grain crops and methods of their diagnosis

Appendix 5

Plant grain sample collection maps showing the areas from which samples were collected

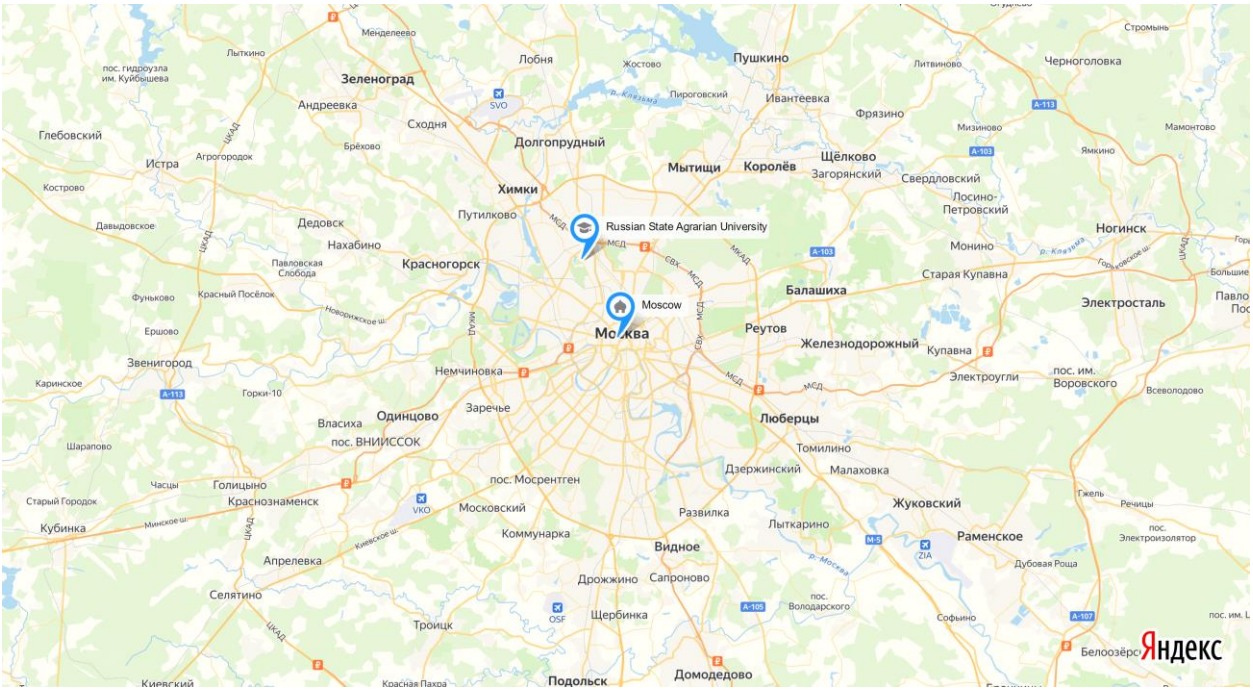

Figure a2 –Area in Moscow where samples were collected, in which the collection of plant grain samples were collected in 2020.

Appendix 6

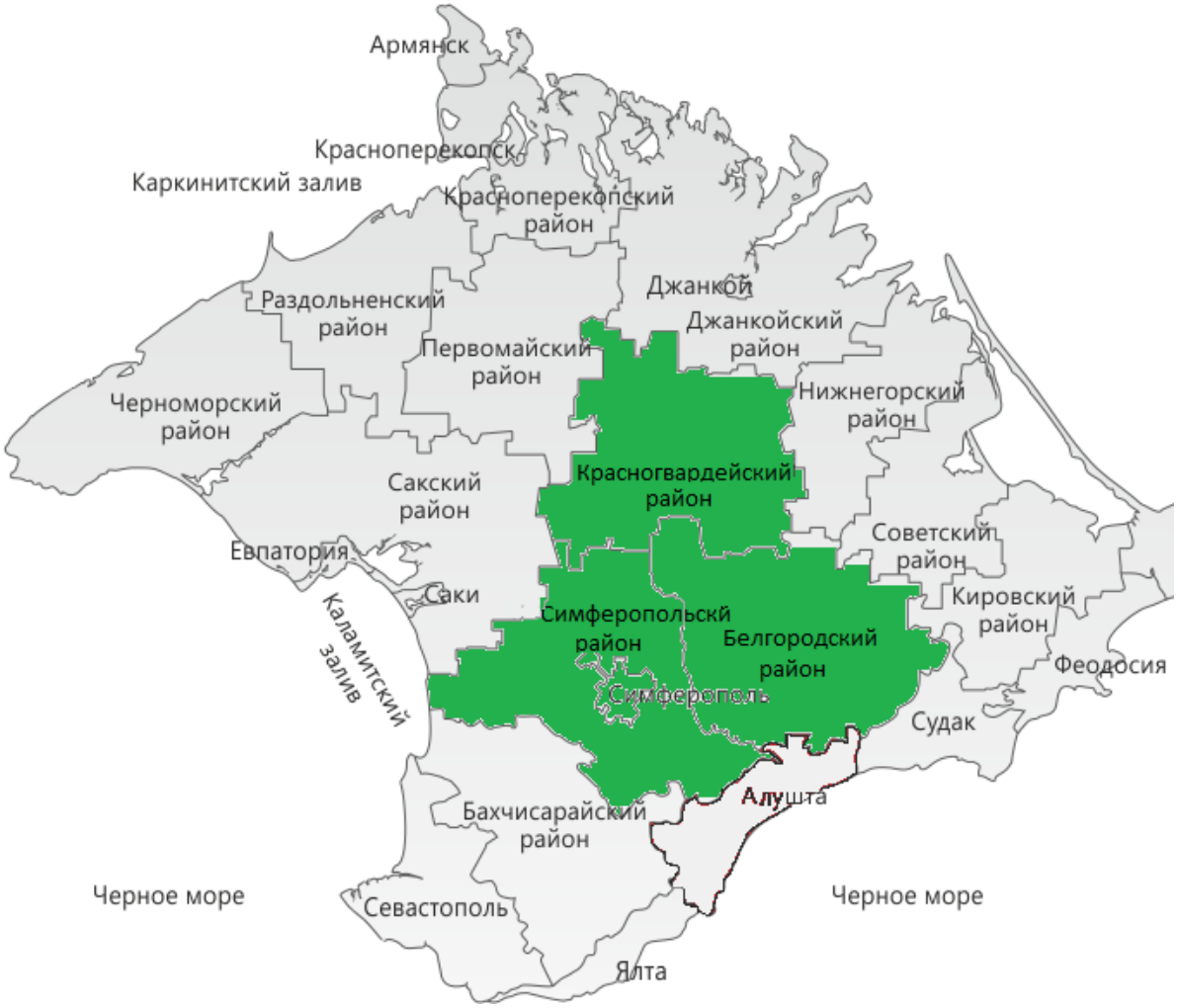

Figure a3 – Districts of the Republic of Crimea, in which the collection of plant grain samples were collected in 2021.

Appendix 7

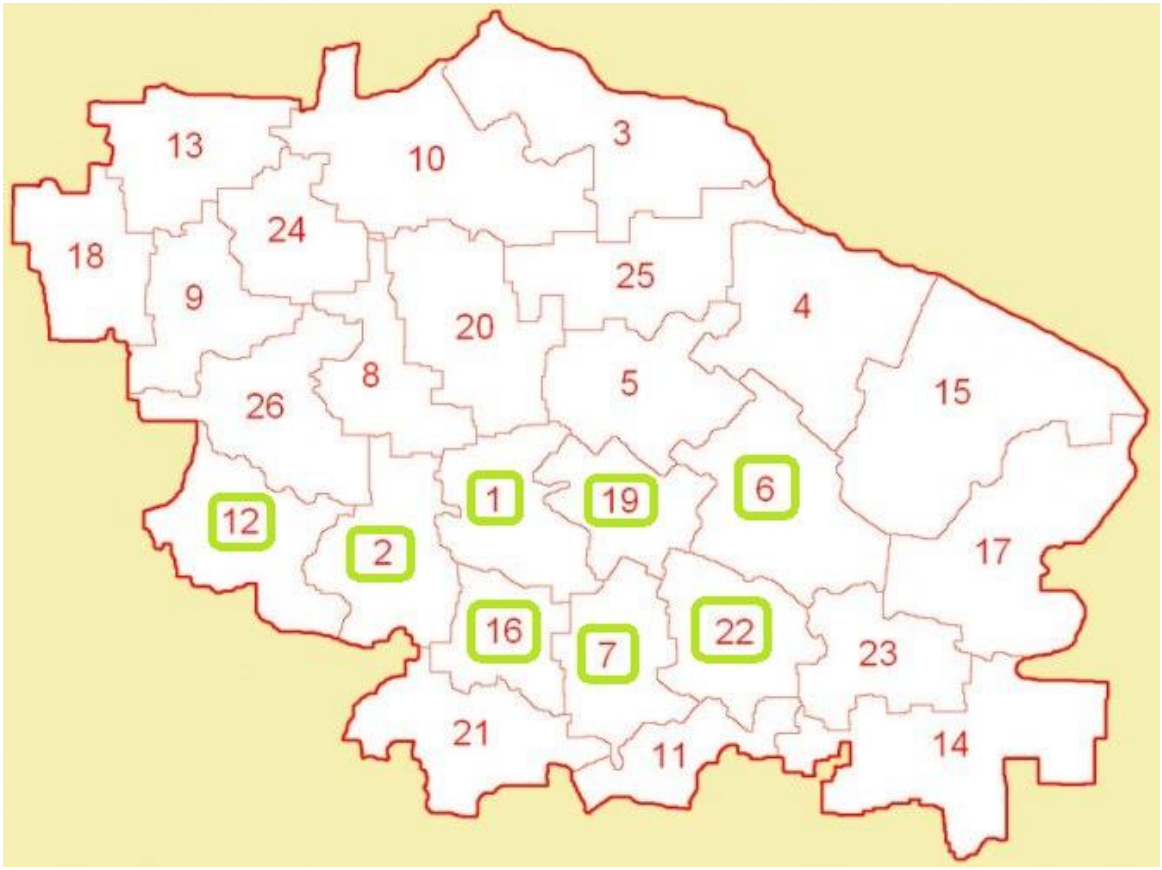

Figure a4 - Districts of the Stavropol Territory, in which the collection of plant grain samples were collected in 2022.

1. Aleksandrovskiy district, 2. Andropovskiy district, 6. Budyonnovsky district, 7. Georgievskiy district, 12. Kochubeevsky district, 16. Mineralovodsky district, 19. Novoselitsky district, 22. Sovetsky District

Appendix 8

Table 8.1 Results of harvesting samples of plant material of cereals in Moscow

No p/n	Region	District	Coordinates	Culture	Sort	Square	Phase of ontogeny	Collection date	Sample cipher
1.	Moscow	Experimental field of RSAU-MSA	Unknown	Winter rye	Snezhana	25 m^2	Tillering-out into the tube	13.05.2020	M1
2.	Moscow	Experimental field of RSAU-MSA	Unknown	Soft winter wheat	ZHiva	25 m^2	Tillering-out into the tube	13.05.2020	M2
3.	Moscow	Experimental field of RSAU-MSA	Unknown	Soft winter wheat	Alekseevich	25 m^2	Tillering-out into the tube	13.05.2020	M3
4.	Moscow	Experimental field of RSAU-MSA	Unknown	Soft winter wheat	Urup	25 m^2	Tillering-out into the tube	13.05.2020	M4
5.	Moscow	Experimental field of RSAU-MSA	Unknown	Soft winter wheat	Morozko	25 m^2	Tillering-out into the tube	13.05.2020	M5
6.	Moscow	Experimental field of RSAU-MSA	Unknown	Soft winter wheat	Timiryazevskaya YUbilejnaya	25 m^2	Tillering-out into the tube	13.05.2020	M6
7.	Moscow	Experimental field of RSAU-MSA	Unknown	Soft winter wheat	Moskovskaya 56	25 m^2	Tillering-out into the tube	13.05.2020	M7
8.	Moscow	Experimental field of RSAU-MSA	Unknown	Soft winter wheat	Biryuza	25 m^2	Tillering-out into the tube	13.05.2020	M8
9.	Moscow	Experimental field	Unknown	Soft winter wheat	Timiryazevska	25 m^2	Tillering-out	13.05.2020	M9

No p/n	Region	District	Coordinates	Culture	Sort	Square	Phase of ontogeny	Collection date	Sample cipher
		of RSAU-MSA			ya 150		into the tube		
10.	Moscow	Experimental field of RSAU-MSA	Unknown	Soft winter wheat	Graf	25 m^2	Tillering-out into the tube	13.05.2020	M10
11.	Moscow	Experimental field of RSAU-MSA	Unknown	Triticale winter	Aleksandr	25 m^2	Tillering-out into the tube	13.05.2020	M11
12.	Moscow	Experimental field of RSAU-MSA	Unknown	Winter wheat	Donskoj yantar'	25 m^2	Tillering-out into the tube	13.05.2020	M12
13.	Moscow	Experimental field of RSAU-MSA	Unknown	Triticale winter	Viktor	25 m^2	Tillering-out into the tube	13.05.2020	M13
14.	Moscow	Experimental field of RSAU-MSA	Unknown	Soft winter wheat	Vassa	25 m^2	Tillering-out into the tube	13.05.2020	M14
15.	Moscow	Experimental field of RSAU-MSA	Unknown	Winter wheat two-grain	–	25 m^2	Tillering-out into the tube	13.05.2020	M15
16.	Moscow	Experimental field of RSAU-MSA	Unknown	Triticale winter	Nemchinovskij 56	25 m^2	Tillering-out into the tube	13.05.2020	M16
17.	Moscow	Experimental field of RSAU-MSA	Unknown	Winter wheat	Eremeevna	25 m^2	Tillering-out into the tube	13.05.2020	M17
18.	Moscow	Experimental field of RSAU-MSA	Unknown	Triticale winter spherical	Tit	25 m^2	Tillering-out into the tube	13.05.2020	M18

No p/n	Region	District	Coordinates	Culture	Sort	Square	Phase of ontogeny	Collection date	Sample cipher
19.	Moscow	Experimental field of RSAU-MSA	Unknown	Soft winter wheat	Moskovskaya 39	1 m^2	Tillering-out into the tube	13.05.2020	M19
20.	Moscow	Experimental field of RSAU-MSA	Unknown	Triticale winter	Valentin 90	1 m^2	Tillering-out into the tube	13.05.2020	M20
21.	Moscow	Experimental field of RSAU-MSA	Unknown	Soft winter wheat	Duplet	1 m^2	Tillering-out into the tube	13.05.2020	M21
22.	Moscow	Experimental field of RSAU-MSA	Unknown	Soft winter wheat	Kavalerka	1 m^2	Tillering-out into the tube	13.05.2020	M22
23.	Moscow	Experimental field of RSAU-MSA	Unknown	Soft winter wheat	Alaya zarya	1 m^2	Tillering-out into the tube	13.05.2020	M23
24.	Moscow	Experimental field of RSAU-MSA	Unknown	Soft winter wheat	Nemchinovskaya 24	1 m^2	Tillering-out into the tube	13.05.2020	M24
25.	Moscow	Experimental field of RSAU-MSA	Unknown	Winter durum wheat	Pobeda 70	1 m^2	Tillering-out into the tube	13.05.2020	M25
26.	Moscow	Experimental field of RSAU-MSA	Unknown	Soft winter wheat	Legenda	1 m^2	Tillering-out into the tube	13.05.2020	M26
27.	Moscow	Experimental field of RSAU-MSA	Unknown	Soft winter wheat	Avesta	1 m^2	Tillering-out into the tube	13.05.2020	M27
28.	Moscow	Experimental field	Unknown	Winter rye	Verasen'	1 m^2	Tillering-out	13.05.2020	M28

No p/n	Region	District	Coordinates	Culture	Sort	Square	Phase of ontogeny	Collection date	Sample cipher
		of RSAU-MSA					into the tube		
29.	Moscow	Experimental field of RSAU-MSA	Unknown	Soft winter wheat	Inna	1 m^2	Tillering-out into the tube	13.05.2020	M29
30.	Moscow	Experimental field of RSAU-MSA	Unknown	Winter wheat	Terra	1 m^2	Tillering-out into the tube	13.05.2020	M30
31.	Moscow	Experimental field of RSAU-MSA	Unknown	Triticale winter	Timiryazevskaya 150	1 m^2	Tillering-out into the tube	13.05.2020	M31
32.	Moscow	Experimental field of RSAU-MSA	Unknown	Soft winter wheat	Stan	1 m^2	Tillering-out into the tube	13.05.2020	M32
33.	Moscow	Experimental field of RSAU-MSA	Unknown	Soft winter wheat	Asket	1 m^2	Tillering-out into the tube	13.05.2020	M33
34.	Moscow	Experimental field of RSAU-MSA	Unknown	Soft winter wheat	Velena	1 m^2	Tillering-out into the tube	13.05.2020	M34
35.	Moscow	Experimental field of RSAU-MSA	Unknown	Soft winter wheat	Vanya	1 m^2	Tillering-out into the tube	13.05.2020	M35
36.	Moscow	Experimental field of RSAU-MSA	Unknown	Soft winter wheat	Artel'	1 m^2	Tillering-out into the tube	13.05.2020	M36
37.	Moscow	Experimental field of RSAU-MSA	Unknown	Soft winter wheat	Nemchinovskaya 85	1 m^2	Tillering-out into the tube	13.05.2020	M37

No p/n	Region	District	Coordinates	Culture	Sort	Square	Phase of ontogeny	Collection date	Sample cipher
38.	Moscow	Experimental field of RSAU-MSA	Unknown	Soft winter wheat	Videya	1 m^2	Tillering-out into the tube	13.05.2020	M38
39.	Moscow	Experimental field of RSAU-MSA	Unknown	Soft winter wheat	Donskaya lira	1 m^2	Tillering-out into the tube	13.05.2020	M39
40.	Moscow	Experimental field of RSAU-MSA	Unknown	Soft winter wheat	Sineva	1 m^2	Tillering-out into the tube	13.05.2020	M40
41.	Moscow	Experimental field of RSAU-MSA	Unknown	Soft winter wheat	Moskovskaya 40	1 m^2	Tillering-out into the tube	13.05.2020	M41
42.	Moscow	Experimental field of RSAU-MSA	Unknown	Soft winter wheat	Don 107	1 m^2	Tillering-out into the tube	13.05.2020	M42
43.	Moscow	Experimental field of RSAU-MSA	Unknown	Soft winter wheat	Step'	1 m^2	Tillering-out into the tube	13.05.2020	M43
44.	Moscow	Experimental field of RSAU-MSA	Unknown	Soft winter wheat	Gubernator Dona	1 m^2	Tillering-out into the tube	13.05.2020	M44
45.	Moscow	Experimental field of RSAU-MSA	Unknown	Soft winter wheat	Rostovchanka	1 m^2	Tillering-out into the tube	13.05.2020	M45
46.	Moscow	Experimental field of RSAU-MSA	Unknown	Soft winter wheat	Vekha	1 m^2	Tillering-out into the tube	13.05.2020	M46
47.	Moscow	Experimental field	Unknown	Soft winter wheat	Nemchinovska	1 m^2	Tillering-out	13.05.2020	M47

No p/n	Region	District	Coordinates	Culture	Sort	Square	Phase of ontogeny	Collection date	Sample cipher
		of RSAU-MSA			ya 57		into the tube		
48.	Moscow	Experimental field of RSAU-MSA	Unknown	Soft winter wheat	Avgusta	1 m^2	Tillering-out into the tube	13.05.2020	M48
49.	Moscow	Experimental field of RSAU-MSA	Unknown	Soft winter wheat	Soberbash	1 m^2	Tillering-out into the tube	13.05.2020	M49
50.	Moscow	Experimental field of RSAU-MSA	Unknown	Soft winter wheat	Anka	1 m^2	Tillering-out into the tube	13.05.2020	M50
51.	Moscow	Experimental field of RSAU-MSA	Unknown	Soft winter wheat	Gurt	1 m^2	Tillering-out into the tube	13.05.2020	M51
52.	Moscow	Experimental field of RSAU-MSA	Unknown	Soft winter wheat	Antonina	1 m^2	Tillering-out into the tube	13.05.2020	M52
53.	Moscow	Experimental field of RSAU-MSA	Unknown	Soft winter wheat	Nemchinovska ya 17	1 m^2	Tillering-out into the tube	13.05.2020	M53
54.	Moscow	Experimental field of RSAU-MSA	Unknown	Soft winter wheat	Bezostaya 100	1 m^2	Tillering-out into the tube	13.05.2020	M54
55.	Moscow	Experimental field of RSAU-MSA	Unknown	Winter rye	–	25 m^2	Sprouts	13.05.2020	M55

Samples were collected from the Timiryazevskaya field experimental station, Russian State Agrarian University - Moscow Agricultural Academy named after K.A. Timiryazev, Moscow. The sampling period for winter grain crops was in the booting

phase, and for spring crops, in the sprout phase. There were no symptoms of bacterial diseases during sampling of winter crops on plants. Chlorosis was noted on seedlings of spring rye. A total of 55 samples of grain crops were taken see table t.5 above. 47 Wheat plant samples (marked in yellow), 2 Rye plant samples (marked in purple) and 6 Triticale plant samples (marked in turkoise blue).

Appendix 9

Table 9.1 Results of harvesting samples of plant material of cereals in The Republic of Crimea

No p/n	Region	District	Coordinates	Culture	Sort	Square	Phase of ontogeny	Collection date	Sample cipher
1.	Republic of Crimea	Belogorsky	45.081419, 34.373821	Winter barley	Onega	110 ha	Milk ripeness	01.06.2021	C1
2.	Republic of Crimea	Belogorsky	45.079287, 34.371427	Winter wheat	Aksiniya	107 ha	Milk ripeness	01.06.2021	C2
3.	Republic of Crimea	Belogorsky	45.067908, 34.372111	Winter wheat	Asket	17 ha	Milk ripeness	01.06.2021	C3
4.	Republic of Crimea	Belogorsky	45.077597, 34.356295	Winter wheat	Asket	13 ha	Milk ripeness	01.06.2021	C4
5.	Republic of Crimea	Belogorsky	45.061996, 34.360063	Winter barley	Voskhod	12 ha	Milk ripeness	01.06.2021	C5
6.	Republic of Crimea	Belogorsky	45.055425, 34.351367	Winter wheat	Gubernator Dona	37 ha	Milk ripeness	01.06.2021	C6

7.	Republic of Crimea	Belogorsky	45.046134, 34.337614	Winter wheat	Gubernator Dona	60 ha	Milk ripeness	01.06.2021	C7
8.	Republic of Crimea	Belogorsky	45.043243, 34.334407	Winter barley	Voskhod	22 ha	Milk ripeness	01.06.2021	C8
9.	Republic of Crimea	Belogorsky	45.036303, 34.335786	Winter barley	Voskhod	20 ha	Milk ripeness	01.06.2021	C9
10.	Republic of Crimea	Belogorsky	45.033814, 34.350337	Winter wheat	Asket	24 ha	Milk ripeness	01.06.2021	C10
11.	Republic of Crimea	Belogorsky	45.033775, 34.349101	Winter barley	Onega	25 ha	Milk ripeness	01.06.2021	C11
12.	Republic of Crimea	Belogorsky	45.034224, 34.349356	Winter wheat	Gubernator Dona	26 ha	Milk ripeness	01.06.2021	C12
13.	Republic of Crimea	Belogorsky	45.034234, 34.350154	Winter wheat	Gubernator Dona	22 ha	Milk ripeness	01.06.2021	C13
14.	Republic of Crimea	Belogorsky	45.038558, 34369283	Winter wheat	Gubernator Dona	16 ha	Milk ripeness	01.06.2021	C14
15.	Republic of Crimea	Belogorsky	45.038714, 34.369855	Winter wheat	Gubernator Dona	20 ha	Milk ripeness	01.06.2021	C15
16.	Republic of Crimea	Belogorsky	45.050091, 34.37571	Winter wheat	Asket	14 ha	Milk ripeness	01.06.2021	C16
17.	Republic of	Belogorsky	45.050322,	Winter barley	Onega	27 ha	Milk	01.06.2021	C17

	Crimea		34.375661				ripeness		
18.	Republic of Crimea	Belogorsky	45.053527, 34.3769	Winter barley	Onega	37 ha	Milk ripeness	01.06.2021	C18
19.	Republic of Crimea	Belogorsky	45.053798, 34.376878	Winter barley	Onega	53 ha	Milk ripeness	01.06.2021	C19
20.	Republic of Crimea	Krasnogvardeysky	45.527476, 34.193599	Winter wheat	Asket	25 m^2	Milk ripeness	02.06.2021	C20
21.	Republic of Crimea	Krasnogvardeysky	45.527476, 34.193599	Winter wheat	Asket	25 m^2	Milk ripeness	02.06.2021	C21
22.	Republic of Crimea	Krasnogvardeysky	45.527476, 34.193599	Winter wheat	Asket	25 m^2	Milk ripeness	02.06.2021	C22
23.	Republic of Crimea	Krasnogvardeysky	45.527476, 34.193599	Winter wheat	Asket	25 m^2	Milk ripeness	02.06.2021	C23
24.	Republic of Crimea	Krasnogvardeysky	45.527476, 34.193599	Winter wheat	Asket	25 m^2	Milk ripeness	02.06.2021	C24
25.	Republic of Crimea	Krasnogvardeysky	45.527476, 34.193599	Winter wheat	Asket	25 m^2	Milk ripeness	02.06.2021	C25
26.	Republic of Crimea	Krasnogvardeysky	45.527476, 34.193599	Winter wheat	Asket	25 m^2	Milk ripeness	02.06.2021	C26
27.	Republic of Crimea	Krasnogvardeysky	45.527476, 34.193599	Winter wheat	Asket	25 m^2	Milk ripeness	02.06.2021	C27

28.	Republic of Crimea	Krasnogvardeysky	45.527476, 34.193599	Winter wheat	Asket	25 m^2	Milk ripeness	02.06.2021	C28
29.	Republic of Crimea	Krasnogvardeysky	45.527476, 34.193599	Winter wheat	Asket	25 m^2	Milk ripeness	02.06.2021	C29
30.	Republic of Crimea	Krasnogvardeysky	45.527476, 34.193599	Winter wheat	Asket	25 m^2	Milk ripeness	02.06.2021	C30
31.	Republic of Crimea	Krasnogvardeysky	45.527476, 34.193599	Winter wheat	Asket	25 m^2	Milk ripeness	02.06.2021	C31
32.	Republic of Crimea	Krasnogvardeysky	45.527476, 34.193599	Winter barley	Buran	25 m^2	Milk ripeness	02.06.2021	C32
33.	Republic of Crimea	Krasnogvardeysky	45.527476, 34.193599	Winter barley	Rubezh	25 m^2	Milk ripeness	02.06.2021	C33
34.	Republic of Crimea	Krasnogvardeysky	45.527476, 34.193599	Winter barley	Sprinter	25 m^2	Milk ripeness	02.06.2021	C34
35.	Republic of Crimea	Krasnogvardeysky	45.527476, 34.193599	Winter barley	Toma	25 m^2	Milk ripeness	02.06.2021	C35
36.	Republic of Crimea	Krasnogvardeysky	45.527476, 34.193599	Winter barley	Master	25 m^2	Milk ripeness	02.06.2021	C36
37.	Republic of Crimea	Krasnogvardeysky	45.527476, 34.193599	Winter barley	Espada	25 m^2	Milk ripeness	02.06.2021	C37
38.	Republic of	Krasnogvardeysky	45.527476,	Winter wheat	Anka	25 m^2	Milk	02.06.2021	C38

	Crimea		34.193599				ripeness		
39.	Republic of Crimea	Krasnogvardeysky	45.527476, 34.193599	Winter wheat	Velena	25 m^2	Milk ripeness	02.06.2021	C39
40.	Republic of Crimea	Krasnogvardeysky	45.527476, 34.193599	Winter wheat	Vekha	25 m^2	Milk ripeness	02.06.2021	C40
41.	Republic of Crimea	Krasnogvardeysky	45.527476, 34.193599	Winter wheat	Karavan	25 m^2	Milk ripeness	02.06.2021	C41
42.	Republic of Crimea	Krasnogvardeysky	45.527476, 34.193599	Oats	Vernyj	25 m^2	Milk ripeness	02.06.2021	C42
43.	Republic of Crimea	Krasnogvardeysky	45.527476, 34.193599	Oats	Mezmaj	25 m^2	Milk ripeness	02.06.2021	C43
44.	Republic of Crimea	Krasnogvardeysky	45.527476, 34.193599	Oats	CHernigov-skij	25 m^2	Milk ripeness	02.06.2021	C44
45.	Republic of Crimea	Krasnogvardeysky	45.527476, 34.193599	Oats	Skakun	25 m^2	Milk ripeness	02.06.2021	C45
46.	Republic of Crimea	Krasnogvardeysky	45.527476, 34.193599	Oats	Podgor-nyj	25 m^2	Milk ripeness	02.06.2021	C46
47.	Republic of Crimea	Krasnogvardeysky	45.527476, 34.193599	Oats	Guzeripl'	25 m^2	Milk ripeness	02.06.2021	C47
48.	Republic of Crimea	Krasnogvardeysky	45.527476, 34.193599	Winter barley	Master	25 m^2	Milk ripeness	02.06.2021	C48

49.	Republic of Crimea	Krasnogvardeysky	45.527476, 34.193599	Winter barley	Espada	25 m^2	Milk ripeness	02.06.2021	C49
50.	Republic of Crimea	Krasnogvardeysky	45.527476, 34.193599	Winter barley	Buran	25 m^2	Milk ripeness	02.06.2021	C50
51.	Republic of Crimea	Krasnogvardeysky	45.527476, 34.193599	Winter barley	Rubezh	25 m^2	Milk ripeness	02.06.2021	C51
52.	Republic of Crimea	Krasnogvardeysky	45.527476, 34.193599	Winter barley	Sprinter	25 m^2	Milk ripeness	02.06.2021	C52
53.	Republic of Crimea	Krasnogvardeysky	45.527476, 34.193599	Winter barley	Toma	25 m^2	Milk ripeness	02.06.2021	C53
54.	Republic of Crimea	Simferopol	45.056814, 34.058398	Triticale, wheat, barley	–	89 ha	Milk ripeness	03.06.2021	C54
55.	Republic of Crimea	Simferopol	45.057161, 34.055664	Winter wheat	Korona	54 ha	Milk ripeness	03.06.2021	C55
56.	Republic of Crimea	Simferopol	45.055086, 34.054119	Winter barley	Sprinter	68 ha	Milk ripeness	03.06.2021	C56
57.	Republic of Crimea	Simferopol	45.056207, 34.074969	Winter barley	Rubezh	80 ha	Milk ripeness	03.06.2021	C57
58.	Republic of Crimea	Simferopol	45.054792, 34.083356	Cereal-legume mixture	–	43 ha	Milk ripeness	03.06.2021	C58
59.	Republic of	Simferopol	45.056775,	Oat	–	16 ha	Milk	03.06.2021	C59

	Crimea		34. 083748				ripeness		
60.	Republic of Crimea	Simferopol	45.060749, 34.096627	Winter wheat	Korona	56 ha	Milk ripeness	03.06.2021	C60

Samples were collected from three regions Belogorskij, Krasnogvardejskij, and Simferopolskij of the Republic of Crimea. The sampling period for winter grain crops was in the milky ripening phase. There were no symptoms of bacterial diseases during sampling of winter crops on plants. A total of 60 samples of grain crops were taken see Appendix 9 table 9.1 above. 29 Wheat plant samples (marked in yellow), 22 Barley plant samples (marked in red), 7 Oat plant samples (marked in green), 1 Cereal legume mix plant sample (marked in dark blue) and 1 Triticale plant samples (marked in turkoise blue).

Appendix 10

Table 10.1 Results of harvesting samples of plant material of cereals in Stavropol region.

No p/n	Region	District	Coordinates	Culture	Sort	Square	Phase of ontogeny	Collection date	Sample cipher
1.	Stavropol Region	Kochubeevsky	44.505689 41.793055	Winter barley	Rubezh	103 ha	Earing	17.05.2022	S1
2.	Stavropol Region	Kochubeevsky	44.504559 41.786457	Winter wheat	Soberbash	63 ha	Tillering-out into the tube	17.05.2022	S2
3.	Stavropol Region	Kochubeevsky	44.502055 41.772699	Winter wheat	Stil' 18	72 ha	Tillering-out into the tube	17.05.2022	S3
4.	Stavropol	Kochubeevsky	44.500683	Winter wheat	Tanya	40 ha	Tillering-out	17.05.2022	S4

	Region		41.767048				into the tube		
5.	Stavropol Region	Kochubeevsky	44.500102 41.759162	Winter wheat	Stil' 18	76 ha	Tillering-out into the tube	17.05.2022	S5
6.	Stavropol Region	Kochubeevsky	44.500929 41.757024	Winter wheat	Tanya	123 ha	Tillering	17.05.2022	S6
7.	Stavropol Region	Kochubeevsky	44.496347 41.741366	Winter wheat	Stil' 18	68 ha	Tillering	17.05.2022	S7
8.	Stavropol Region	Kochubeevsky	44.496332 41.738324	Winter wheat	Tanya	67 ha	Tillering-out into the tube	17.05.2022	S8
9.	Stavropol Region	Kochubeevsky	44.590153 41.848965	Winter wheat	Tanya	68 ha	Tillering-out into the tube	17.05.2022	S9
10.	Stavropol Region	Kochubeevsky	44.620376 41.838994	Winter wheat	Grom	51 ha	Tillering-out into the tube	17.05.2022	S10
11.	Stavropol Region	Kochubeevsky	44.640756 41.834345	Winter wheat	Tanya	68 ha	Tillering-out into the tube	17.05.2022	S11
12.	Stavropol Region	Kochubeevsky	44.650636 41.830988	Winter wheat	Tanya	33 ha	Tillering-out into the tube	17.05.2022	S12
13.	Stavropol Region	Kochubeevsky	44.657482 41.827884	Winter wheat	Tanya	40 ha	Tillering-out into the tube	17.05.2022	S13
14.	Stavropol Region	Kochubeevsky	44.595188 41.851614	Winter wheat	Grom	44 ha	Tillering	17.05.2022	S14

15.	Stavropol Region	Kochubeevsky	44.56021, 41.882643	Winter wheat	Tanya	58 ha	Tillering-out into the tube	17.05.2022	S15
16.	Stavropol Region	Kochubeevsky	44.507824 41.836163	Winter barley	Bazal't	12 ha	Earing	17.05.2022	S16
17.	Stavropol Region	Kochubeevsky	44.497287 41.840748	Winter wheat	YUbilejnaya	58 ha	Ear-flowering	17.05.2022	S17
18.	Stavropol Region	Kochubeevsky	44.568092 41.888319	Winter wheat	YUbilejnaya	78 ha	Tillering-out into the tube	17.05.2022	S18
19.	Stavropol Region	Budyonnovsky	44.652388 44.168094	Winter durum wheat	Amazonka	127 ha	Earing	18.05.2022	S19
20.	Stavropol Region	Budyonnovsky	44.653062 44.168906	Winter durum wheat	Amazonka	135 ha	Earing	18.05.2022	S20
21.	Stavropol Region	Budyonnovsky	44.641606 44.186155	Spring durum wheat	YAsenka	46 ha	Tillering-out into the tube	18.05.2022	S21
22.	Stavropol Region	Budyonnovsky	44.644446 44.201798	Winter durum wheat	Amazonka	86 ha	Earing	18.05.2022	S22
23.	Stavropol Region	Budyonnovsky	44.640502 44.216323	Winter durum wheat	Amazonka	87 ha	Earing	18.05.2022	S23
24.	Stavropol Region	Budyonnovsky	44.641738 44.21795	Winter durum wheat	YAhont	1,2 ha	Earing	18.05.2022	S24
25.	Stavropol	Budyonnovsky	44.642119	Winter durum	Agat Donskoj	1,2 ha	Earing	18.05.2022	S25

	Region		44.218391	wheat					
26.	Stavropol Region	Budyonnovsky	44.642454 44.218675	Winter durum wheat	Stepnoj yantar'	1,2 ha	Earing	18.05.2022	S26
27.	Stavropol Region	Budyonnovsky	44.64263 44.218879	Winter wheat	Centrina	1,2 ha	Earing	18.05.2022	S27
28.	Stavropol Region	Budyonnovsky	44.64278, 44.219009	Durum winter wheat	Odari	1,2 ha	Earing	18.05.2022	S28
29.	Stavropol Region	Budyonnovsky	44.64287 44.219124	Winter durum wheat	Amazonka	82 ha	Earing	18.05.2022	S29
30.	Stavropol Region	Budyonnovsky	44.655838 44.238452	Winter wheat	Myagkaya YUkka	205 ha	Earing	18.05.2022	S30
31.	Stavropol Region	Budyonnovsky	44.655317 44.239605	Winter durum wheat	YAhont	197 ha	Earing	18.05.2022	S31
32.	Stavropol Region	Budyonnovsky	44.654837 44.237914	Winter durum wheat	Amazonka	105 ha	Earing	18.05.2022	S32
33.	Stavropol Region	Budyonnovsky	44.668511 44.21715	Durum winter wheat	Odari	164 ha	Earing	18.05.2022	S33
34.	Stavropol Region	Budyonnovsky	44.53891, 44.001035	Winter wheat	Unknown	Unknown	Earing	19.05.2022	S34
35.	Stavropol Region	Soviet	44.488121 43.930737	Winter barley + oats	Unknown	Unknown	Milk ripeness	19.05.2022	S35

36.	Stavropol Region	Soviet	44.463615 43.903353	Winter soft wheat	Unknown	Unknown	Ear-flowering	19.05.2022	S36
37.	Stavropol Region	Soviet	44.383855 43.836491	Winter barley	Unknown	Unknown	Earing	19.05.2022	S37
38.	Stavropol Region	Soviet	44.346468 43.798814	Winter soft wheat	Unknown	Unknown	Earing	19.05.2022	S38
39.	Stavropol Region	Soviet	44.313342 43.764606	Winter soft wheat	Unknown	Unknown	Earing	19.05.2022	S39
40.	Stavropol Region	Soviet	44.283472 43.702031	Winter soft wheat	Unknown	Unknown	Earing	19.05.2022	S40
41.	Stavropol Region	George	44.236059 43.539996	Winter soft wheat	Unknown	Unknown	Earing	19.05.2022	S41
42.	Stavropol Region	George	44.2092 43.444823	Winter soft wheat	Unknown	Unknown	Earing	19.05.2022	S42
43.	Stavropol Region	George	44.210055 43.444459	Winter barley	Unknown	Unknown	Earing	19.05.2022	S43
44.	Stavropol Region	George	44.152053 43.388061	Winter soft wheat	Unknown	Unknown	Earing	19.05.2022	S44
45.	Stavropol Region	Andropovsky	44.461885 42.577464	Winter soft wheat	Unknown	Unknown	Earing	20.05.2022	S45
46.	Stavropol	Andropovsky	44.5186	Winter barley	Unknown	Unknown	Earing	20.05.2022	S46

	Region		42.560239						
47.	Stavropol Region	Alexander	44.805984 42.947121	Wheat	CHernyava		Tillering-out into the tube	20.05.2022	S47
48.	Stavropol Region	Alexander	44.807363 42.947422	Winter wheat	Alekseevich	142 ha	Earing	20.05.2022	S48
49.	Stavropol Region	Alexander	44.805684, 42.975595	Winter wheat	Alekseevich	152 ha	Tillering-out into the tube	20.05.2022	S49
50.	Stavropol Region	Alexander	44.817837 42.974403	Winter wheat	CHornyava	48 ha	Earing	20.05.2022	S50
51.	Stavropol Region	Alexander	44.814897, 42.988211	Winter barley	Karrera	181 ha	Earing	20.05.2022	S51
52.	Stavropol Region	Alexander	44.818337 42.986134	Winter barley	Karrera	63 ha	Earing	20.05.2022	S52
53.	Stavropol Region	Alexander	44.816788 42.995267	Barley	Alekseevich	69 ha	Earing	20.05.2022	S53
54.	Stavropol Region	Alexander	44.827182 43.012524	Winter barley	Karrera	136 ha	Earing	20.05.2022	S54
55.	Stavropol Region	Alexander	44.830326 43.009871	Wheat	CHernyava	72 ha	Earing	20.05.2022	S55
56.	Stavropol Region	Shpakovsky	44.849529 42.017492	Triticale	Hleborob	93 ha	Earing	20.05.2022	S56

57.	Stavropol Region	Novoselitsky	44.846789 43.17077	Winter wheat	Antonina	32 ha	Ear-flowering	20.05.2022	S57
58.	Stavropol Region	Novoselitsky	44.859364 43.173279	Spring barley	gris	73 ha	Tillering-out into the tube	20.05.2022	S58
59.	Stavropol Region	Novoselitsky	44.864025 43.178836	Spring barley	Azimut	demonstrator. plot 50*8	Tillering-out into the tube	20.05.2022	S59
60.	Stavropol Region	Novoselitsky	44.864113 43.17892	Spring barley	Leon	demonstrator. plot 50*8	Tillering-out into the tube	20.05.2022	S60
61.	Stavropol Region	Novoselitsky	44.864199 43.178999	Spring barley	Ratnik	demonstrator. plot 50*8	Tillering-out into the tube	20.05.2022	S61
62.	Stavropol Region	Novoselitsky	44.864276 43.17908	Spring barley	Format	demonstrator. plot 50*8	Tillering-out into the tube	20.05.2022	S62
63.	Stavropol Region	Novoselitsky	44.86432 43.179148	Spring barley	SHCHedryj	demonstrator. plot 50*8	Tillering-out into the tube	20.05.2022	S63
64.	Stavropol Region	Novoselitsky	44.864378 43.179188	Spring barley	Vakula	demonstrator. plot 50*8	Tillering-out into the tube	20.05.2022	S64
65.	Stavropol Region	Novoselitsky	44.86452 43.179334	Spring barley	Avalon	demonstrator. plot 50*8	Tillering-out into the tube	20.05.2022	S65
66.	Stavropol Region	Mineralovodsky	44.434236 43.165855	Winter wheat soft	Unknown	Unknown	Earing	20.05.2022	S66

Samples were collcted from Kochubeevsky, Budyonnovsky, Soviet, George, Andropovsky, Alexander, Shpakovsky, Novoselitsky and Mineralovodsky of Stavropol region. The sampling period for winter grain crops was in the tillering, tillering-out into the tube, earing, ear-flowering and the milky ripening stages. There were no symptoms of bacterial diseases during sampling of winter crops on plants. A total of 66 samples of grain crops were taken see table t10 above. 47 Wheat plant samples (marked in yellow), 17 Barley plant samples (marked in red), 1 Barley + Oat plant samples (marked in green), and 1 Triticale plant samples (marked in turkoise blue).

Appendix 11

Table 11.1 The result of PCR-testing with plant sample DNA

Sample cipher	Result of PCR/ Test											
20M1	*X. t*	4F1/4R1	-	*P. f*	Syntol	-	*R. t*	Rt3F/3R	-	*P. s*	PSYf/PSYr	-
20M2	*X. t*	4F1/4R1	-	*P. f*	Syntol	-	*R. t*	Rt3F/3R	-	*P. s*	PSYf/PSYr	-
20M3	*X. t*	4F1/4R1	-	*P. f*	Syntol	-	*R. t*	Rt3F/3R	-	*P. s*	PSYf/PSYr	+
20M4	*X. t*	4F1/4R1	-	*P. f*	Syntol	-	*R. t*	Rt3F/3R	-	*P. s*	PSYf/PSYr	-
20M5	*X. t*	4F1/4R1	-	*P. f*	Syntol	-	*R. t*	Rt3F/3R	-	*P. s*	PSYf/PSYr	+
20M6	*X. t*	4F1/4R1	-	*P. f*	Syntol	-	*R. t*	Rt3F/3R	-	*P. s*	PSYf/PSYr	-
20M7	*X. t*	4F1/4R1	-	*P. f*	Syntol	-	*R. t*	Rt3F/3R	-	*P. s*	PSYf/PSYr	-
20M8	*X. t*	4F1/4R1	-	*P. f*	Syntol	-	*R. t*	Rt3F/3R	-	*P. s*	PSYf/PSYr	-
20M9	*X. t*	4F1/4R1	-	*P. f*	Syntol	-	*R. t*	Rt3F/3R	-	*P. s*	PSYf/PSYr	-
20M10	*X. t*	4F1/4R1	-	*P. f*	Syntol	-	*R. t*	Rt3F/3R	-	*P. s*	PSYf/PSYr	-
20M11	*X. t*	4F1/4R1	-	*P. f*	Syntol	-	*R. t*	Rt3F/3R	-	*P. s*	PSYf/PSYr	-
20M12	*X. t*	4F1/4R1	-	*P. f*	Syntol	-	*R. t*	Rt3F/3R	-	*P. s*	PSYf/PSYr	-
20M13	*X. t*	4F1/4R1	-	*P. f*	Syntol	-	*R. t*	Rt3F/3R	-	*P. s*	PSYf/PSYr	-
20M14	*X. t*	4F1/4R1	-	*P. f*	Syntol	-	*R. t*	Rt3F/3R	-	*P. s*	PSYf/PSYr	-
20M15	*X. t*	4F1/4R1	-	*P. f*	Syntol	-	*R. t*	Rt3F/3R	-	*P. s*	PSYf/PSYr	+
20M16	*X. t*	4F1/4R1	-	*P. f*	Syntol	-	*R. t*	Rt3F/3R	-	*P. s*	PSYf/PSYr	-
20M17	*X. t*	4F1/4R1	-	*P. f*	Syntol	-	*R. t*	Rt3F/3R	-	*P. s*	PSYf/PSYr	-
20M18	*X. t*	4F1/4R1	-	*P. f*	Syntol	-	*R. t*	Rt3F/3R	-	*P. s*	PSYf/PSYr	+
20M19	*X. t*	4F1/4R1	-	*P. f*	Syntol	-	*R. t*	Rt3F/3R	-	*P. s*	PSYf/PSYr	-
20M20	*X. t*	4F1/4R1	-	*P. f*	Syntol	-	*R. t*	Rt3F/3R	-	*P. s*	PSYf/PSYr	-
20M21	*X. t*	4F1/4R1	-	*P. f*	Syntol	-	*R. t*	Rt3F/3R	-	*P. s*	PSYf/PSYr	-
20M22	*X. t*	4F1/4R1	-	*P. f*	Syntol	-	*R. t*	Rt3F/3R	-	*P. s*	PSYf/PSYr	-
20M23	*X. t*	4F1/4R1	-	*P. f*	Syntol	-	*R. t*	Rt3F/3R	-	*P. s*	PSYf/PSYr	-
20M24	*X. t*	4F1/4R1	-	*P. f*	Syntol	-	*R. t*	Rt3F/3R	-	*P. s*	PSYf/PSYr	-
20M25	*X. t*	4F1/4R1	-	*P. f*	Syntol	-	*R. t*	Rt3F/3R	-	*P. s*	PSYf/PSYr	-
20M26	*X. t*	4F1/4R1	-	*P. f*	Syntol	-	*R. t*	Rt3F/3R	-	*P. s*	PSYf/PSYr	-
20M27	*X. t*	4F1/4R1	-	*P. f*	Syntol	-	*R. t*	Rt3F/3R	-	*P. s*	PSYf/PSYr	-
20M28	*X. t*	4F1/4R1	-	*P. f*	Syntol	-	*R. t*	Rt3F/3R	-	*P. s*	PSYf/PSYr	-
20M29	*X. t*	4F1/4R1	-	*P. f*	Syntol	-	*R. t*	Rt3F/3R	-	*P. s*	PSYf/PSYr	-
20M30	*X. t*	4F1/4R1	-	*P. f*	Syntol	-	*R. t*	Rt3F/3R	-	*P. s*	PSYf/PSYr	-
20M31	*X. t*	4F1/4R1	-	*P. f*	Syntol	-	*R. t*	Rt3F/3R	-	*P. s*	PSYf/PSYr	-
20M32	*X. t*	4F1/4R1	-	*P. f*	Syntol	-	*R. t*	Rt3F/3R	-	*P. s*	PSYf/PSYr	-

Sample cipher	Result of PCR/ Test											
20M33	*X. t*	4F1/4R1	-	*P. f*	Syntol	-	*R. t*	Rt3F/3R	-	*P. s*	PSYf/PSYr	-
20M34	*X. t*	4F1/4R1	-	*P. f*	Syntol	-	*R. t*	Rt3F/3R	-	*P. s*	PSYf/PSYr	-
20M35	*X. t*	4F1/4R1	-	*P. f*	Syntol	-	*R. t*	Rt3F/3R	-	*P. s*	PSYf/PSYr	+
20M36	*X. t*	4F1/4R1	-	*P. f*	Syntol	-	*R. t*	Rt3F/3R	-	*P. s*	PSYf/PSYr	-
20M37	*X. t*	4F1/4R1	-	*P. f*	Syntol	-	*R. t*	Rt3F/3R	-	*P. s*	PSYf/PSYr	-
20M38	*X. t*	4F1/4R1	-	*P. f*	Syntol	-	*R. t*	Rt3F/3R	-	*P. s*	PSYf/PSYr	-
20M39	*X. t*	4F1/4R1	-	*P. f*	Syntol	-	*R. t*	Rt3F/3R	-	*P. s*	PSYf/PSYr	-
20M40	*X. t*	4F1/4R1	-	*P. f*	Syntol	-	*R. t*	Rt3F/3R	-	*P. s*	PSYf/PSYr	-
20M41	*X. t*	4F1/4R1	-	*P. f*	Syntol	-	*R. t*	Rt3F/3R	-	*P. s*	PSYf/PSYr	-
20M42	*X. t*	4F1/4R1	-	*P. f*	Syntol	-	*R. t*	Rt3F/3R	-	*P. s*	PSYf/PSYr	-
20M43	*X. t*	4F1/4R1	-	*P. f*	Syntol	-	*R. t*	Rt3F/3R	-	*P. s*	PSYf/PSYr	-
20M44	*X. t*	4F1/4R1	-	*P. f*	Syntol	-	*R. t*	Rt3F/3R	-	*P. s*	PSYf/PSYr	-
20M45	*X. t*	4F1/4R1	-	*P. f*	Syntol	-	*R. t*	Rt3F/3R	-	*P. s*	PSYf/PSYr	-
20M46	*X. t*	4F1/4R1	-	*P. f*	Syntol	-	*R. t*	Rt3F/3R	-	*P. s*	PSYf/PSYr	-
20M47	*X. t*	4F1/4R1	-	*P. f*	Syntol	-	*R. t*	Rt3F/3R	-	*P. s*	PSYf/PSYr	-
20M48	*X. t*	4F1/4R1	-	*P. f*	Syntol	-	*R. t*	Rt3F/3R	-	*P. s*	PSYf/PSYr	-
20M49	*X. t*	4F1/4R1	-	*P. f*	Syntol	-	*R. t*	Rt3F/3R	-	*P. s*	PSYf/PSYr	-
20M50	*X. t*	4F1/4R1	-	*P. f*	Syntol	-	*R. t*	Rt3F/3R	-	*P. s*	PSYf/PSYr	-
20M51	*X. t*	4F1/4R1	-	*P. f*	Syntol	-	*R. t*	Rt3F/3R	-	*P. s*	PSYf/PSYr	-
20M52	*X. t*	4F1/4R1	-	*P. f*	Syntol	-	*R. t*	Rt3F/3R	-	*P. s*	PSYf/PSYr	-
20M53	*X. t*	4F1/4R1	-	*P. f*	Syntol	-	*R. t*	Rt3F/3R	-	*P. s*	PSYf/PSYr	-
20M54	*X. t*	4F1/4R1	-	*P. f*	Syntol	-	*R. t*	Rt3F/3R	-	*P. s*	PSYf/PSYr	-
20M55	*X. t*	4F1/4R1	-	*P. f*	Syntol	-	*R. t*	Rt3F/3R	-	*P. s*	PSYf/PSYr	-
21C1	*X. t*	4F1/4R1	-	*P. f*	Syntol	-	*R. t*	Rt3F/3R	-	*P. s*	PSYf/PSYr	+
21C2	*X. t*	4F1/4R1	-	*P. f*	Syntol	-	*R. t*	Rt3F/3R	-	*P. s*	PSYf/PSYr	+
21C3	*X. t*	4F1/4R1	-	*P. f*	Syntol	-	*R. t*	Rt3F/3R	-	*P. s*	PSYf/PSYr	+
21C4	*X. t*	4F1/4R1	-	*P. f*	Syntol	-	*R. t*	Rt3F/3R	-	*P. s*	PSYf/PSYr	-
21C5	*X. t*	4F1/4R1	-	*P. f*	Syntol	-	*R. t*	Rt3F/3R	-	*P. s*	PSYf/PSYr	+
21C6	*X. t*	4F1/4R1	-	*P. f*	Syntol	-	*R. t*	Rt3F/3R	-	*P. s*	PSYf/PSYr	+
21C7	*X. t*	4F1/4R1	-	*P. f*	Syntol	-	*R. t*	Rt3F/3R	-	*P. s*	PSYf/PSYr	-
21C8	*X. t*	4F1/4R1	-	*P. f*	Syntol	-	*R. t*	Rt3F/3R	-	*P. s*	PSYf/PSYr	-
21C9	*X. t*	4F1/4R1	-	*P. f*	Syntol	-	*R. t*	Rt3F/3R	-	*P. s*	PSYf/PSYr	-
21C10	*X. t*	4F1/4R1	-	*P. f*	Syntol	-	*R. t*	Rt3F/3R	-	*P. s*	PSYf/PSYr	-

Sample cipher	Result of PCR/ Test											
21C11	*X. t*	4F1/4R1	-	*P. f*	Syntol	-	*R. t*	Rt3F/3R	-	*P. s*	PSYf/PSYr	-
21C12	*X. t*	4F1/4R1	-	*P. f*	Syntol	-	*R. t*	Rt3F/3R	-	*P. s*	PSYf/PSYr	-
21C13	*X. t*	4F1/4R1	-	*P. f*	Syntol	-	*R. t*	Rt3F/3R	-	*P. s*	PSYf/PSYr	-
21C14	*X. t*	4F1/4R1	-	*P. f*	Syntol	-	*R. t*	Rt3F/3R	-	*P. s*	PSYf/PSYr	-
21C15	*X. t*	4F1/4R1	-	*P. f*	Syntol	-	*R. t*	Rt3F/3R	-	*P. s*	PSYf/PSYr	+
21C16	*X. t*	4F1/4R1	-	*P. f*	Syntol	-	*R. t*	Rt3F/3R	-	*P. s*	PSYf/PSYr	-
21C17	*X. t*	4F1/4R1	-	*P. f*	Syntol	-	*R. t*	Rt3F/3R	-	*P. s*	PSYf/PSYr	+
21C18	*X. t*	4F1/4R1	-	*P. f*	Syntol	-	*R. t*	Rt3F/3R	-	*P. s*	PSYf/PSYr	-
21C19	*X. t*	4F1/4R1	-	*P. f*	Syntol	-	*R. t*	Rt3F/3R	-	*P. s*	PSYf/PSYr	-
21C20	*X. t*	4F1/4R1	-	*P. f*	Syntol	-	*R. t*	Rt3F/3R	-	*P. s*	PSYf/PSYr	-
21C21	*X. t*	4F1/4R1	-	*P. f*	Syntol	-	*R. t*	Rt3F/3R	-	*P. s*	PSYf/PSYr	-
21C22	*X. t*	4F1/4R1	-	*P. f*	Syntol	-	*R. t*	Rt3F/3R	-	*P. s*	PSYf/PSYr	-
21C23	*X. t*	4F1/4R1	-	*P. f*	Syntol	-	*R. t*	Rt3F/3R	-	*P. s*	PSYf/PSYr	-
21C24	*X. t*	4F1/4R1	-	*P. f*	Syntol	-	*R. t*	Rt3F/3R	-	*P. s*	PSYf/PSYr	-
21C25	*X. t*	4F1/4R1	-	*P. f*	Syntol	-	*R. t*	Rt3F/3R	-	*P. s*	PSYf/PSYr	-
21C26	*X. t*	4F1/4R1	-	*P. f*	Syntol	-	*R. t*	Rt3F/3R	-	*P. s*	PSYf/PSYr	-
21C27	*X. t*	4F1/4R1	-	*P. f*	Syntol	-	*R. t*	Rt3F/3R	-	*P. s*	PSYf/PSYr	-
21C28	*X. t*	4F1/4R1	-	*P. f*	Syntol	-	*R. t*	Rt3F/3R	-	*P. s*	PSYf/PSYr	-
21C29	*X. t*	4F1/4R1	-	*P. f*	Syntol	-	*R. t*	Rt3F/3R	-	*P. s*	PSYf/PSYr	-
21C30	*X. t*	4F1/4R1	-	*P. f*	Syntol	-	*R. t*	Rt3F/3R	-	*P. s*	PSYf/PSYr	-
21C31	*X. t*	4F1/4R1	+	*P. f*	Syntol	-	*R. t*	Rt3F/3R	-	*P. s*	PSYf/PSYr	+
21C32	*X. t*	4F1/4R1	-	*P. f*	Syntol	-	*R. t*	Rt3F/3R	-	*P. s*	PSYf/PSYr	-
21C33	*X. t*	4F1/4R1	-	*P. f*	Syntol	-	*R. t*	Rt3F/3R	-	*P. s*	PSYf/PSYr	-
21C34	*X. t*	4F1/4R1	-	*P. f*	Syntol	-	*R. t*	Rt3F/3R	-	*P. s*	PSYf/PSYr	+
21C35	*X. t*	4F1/4R1	-	*P. f*	Syntol	-	*R. t*	Rt3F/3R	-	*P. s*	PSYf/PSYr	+
21C36	*X. t*	4F1/4R1	-	*P. f*	Syntol	-	*R. t*	Rt3F/3R	-	*P. s*	PSYf/PSYr	-
21C37	*X. t*	4F1/4R1	-	*P. f*	Syntol	-	*R. t*	Rt3F/3R	-	*P. s*	PSYf/PSYr	-
21C38	*X. t*	4F1/4R1	-	*P. f*	Syntol	-	*R. t*	Rt3F/3R	-	*P. s*	PSYf/PSYr	-
21C39	*X. t*	4F1/4R1	-	*P. f*	Syntol	-	*R. t*	Rt3F/3R	-	*P. s*	PSYf/PSYr	-
21C40	*X. t*	4F1/4R1	-	*P. f*	Syntol	-	*R. t*	Rt3F/3R	-	*P. s*	PSYf/PSYr	-
21C41	*X. t*	4F1/4R1	-	*P. f*	Syntol	-	*R. t*	Rt3F/3R	-	*P. s*	PSYf/PSYr	-
21C42	*X. t*	4F1/4R1	-	*P. f*	Syntol	-	*R. t*	Rt3F/3R	-	*P. s*	PSYf/PSYr	+
21C43	*X. t*	4F1/4R1	-	*P. f*	Syntol	-	*R. t*	Rt3F/3R	-	*P. s*	PSYf/PSYr	+

Sample cipher	Result of PCR/ Test											
21C44	*X. t*	4F1/4R1	-	*P. f*	Syntol	-	*R. t*	Rt3F/3R	-	*P. s*	PSYf/PSYr	+
21C45	*X. t*	4F1/4R1	-	*P. f*	Syntol	-	*R. t*	Rt3F/3R	-	*P. s*	PSYf/PSYr	+
21C46	*X. t*	4F1/4R1	-	*P. f*	Syntol	-	*R. t*	Rt3F/3R	-	*P. s*	PSYf/PSYr	+
21C47	*X. t*	4F1/4R1	-	*P. f*	Syntol	-	*R. t*	Rt3F/3R	-	*P. s*	PSYf/PSYr	+
21C48	*X. t*	4F1/4R1	-	*P. f*	Syntol	-	*R. t*	Rt3F/3R	-	*P. s*	PSYf/PSYr	+
21C49	*X. t*	4F1/4R1	-	*P. f*	Syntol	-	*R. t*	Rt3F/3R	-	*P. s*	PSYf/PSYr	+
21C50	*X. t*	4F1/4R1	-	*P. f*	Syntol	-	*R. t*	Rt3F/3R	-	*P. s*	PSYf/PSYr	-
21C51	*X. t*	4F1/4R1	-	*P. f*	Syntol	-	*R. t*	Rt3F/3R	-	*P. s*	PSYf/PSYr	-
21C52	*X. t*	4F1/4R1	-	*P. f*	Syntol	-	*R. t*	Rt3F/3R	-	*P. s*	PSYf/PSYr	-
21C53	*X. t*	4F1/4R1	-	*P. f*	Syntol	-	*R. t*	Rt3F/3R	-	*P. s*	PSYf/PSYr	-
21C54	*X. t*	4F1/4R1	-	*P. f*	Syntol	-	*R. t*	Rt3F/3R	-	*P. s*	PSYf/PSYr	+
21C55	*X. t*	4F1/4R1	-	*P. f*	Syntol	-	*R. t*	Rt3F/3R	-	*P. s*	PSYf/PSYr	+
21C56	*X. t*	4F1/4R1	-	*P. f*	Syntol	-	*R. t*	Rt3F/3R	-	*P. s*	PSYf/PSYr	+
21C57	*X. t*	4F1/4R1	-	*P. f*	Syntol	-	*R. t*	Rt3F/3R	-	*P. s*	PSYf/PSYr	+
21C58	*X. t*	4F1/4R1	-	*P. f*	Syntol	-	*R. t*	Rt3F/3R	-	*P. s*	PSYf/PSYr	+
21C59	*X. t*	4F1/4R1	-	*P. f*	Syntol	-	*R. t*	Rt3F/3R	-	*P. s*	PSYf/PSYr	+
21C60	*X. t*	4F1/4R1	-	*P. f*	Syntol	-	*R. t*	Rt3F/3R	-	*P. s*	PSYf/PSYr	+
22S1	*X. t*	4F1/4R1	-	*P. f*	Syntol	-	*R. t*	Rt3F/3R	-	*P. s*	PSYf/PSYr	+
22S2	*X. t*	4F1/4R1	-	*P. f*	Syntol	-	*R. t*	Rt3F/3R	-	*P. s*	PSYf/PSYr	+
22S3	*X. t*	4F1/4R1	-	*P. f*	Syntol	-	*R. t*	Rt3F/3R	-	*P. s*	PSYf/PSYr	+
22S4	*X. t*	4F1/4R1	-	*P. f*	Syntol	-	*R. t*	Rt3F/3R	-	*P. s*	PSYf/PSYr	+
22S5	*X. t*	4F1/4R1	-	*P. f*	Syntol	-	*R. t*	Rt3F/3R	-	*P. s*	PSYf/PSYr	+
22S6	*X. t*	4F1/4R1	-	*P. f*	Syntol	-	*R. t*	Rt3F/3R	-	*P. s*	PSYf/PSYr	+
22S7	*X. t*	4F1/4R1	-	*P. f*	Syntol	-	*R. t*	Rt3F/3R	-	*P. s*	PSYf/PSYr	+
22S8	*X. t*	4F1/4R1	-	*P. f*	Syntol	-	*R. t*	Rt3F/3R	-	*P. s*	PSYf/PSYr	+
22S9	*X. t*	4F1/4R1	-	*P. f*	Syntol	-	*R. t*	Rt3F/3R	-	*P. s*	PSYf/PSYr	-
22S10	*X. t*	4F1/4R1	-	*P. f*	Syntol	-	*R. t*	Rt3F/3R	-	*P. s*	PSYf/PSYr	+
22S11	*X. t*	4F1/4R1	-	*P. f*	Syntol	-	*R. t*	Rt3F/3R	-	*P. s*	PSYf/PSYr	-
22S12	*X. t*	4F1/4R1	-	*P. f*	Syntol	-	*R. t*	Rt3F/3R	-	*P. s*	PSYf/PSYr	+
22S13	*X. t*	4F1/4R1	-	*P. f*	Syntol	-	*R. t*	Rt3F/3R	-	*P. s*	PSYf/PSYr	+
22S14	*X. t*	4F1/4R1	-	*P. f*	Syntol	-	*R. t*	Rt3F/3R	-	*P. s*	PSYf/PSYr	+
22S15	*X. t*	4F1/4R1	-	*P. f*	Syntol	-	*R. t*	Rt3F/3R	-	*P. s*	PSYf/PSYr	+
22S16	*X. t*	4F1/4R1	-	*P. f*	Syntol	-	*R. t*	Rt3F/3R	-	*P. s*	PSYf/PSYr	+

Sample cipher	Result of PCR/ Test											
22S17	*X. t*	4F1/4R1	-	*P. f*	Syntol	-	*R. t*	Rt3F/3R	-	*P. s*	PSYf/PSYr	+
22S18	*X. t*	4F1/4R1	-	*P. f*	Syntol	-	*R. t*	Rt3F/3R	-	*P. s*	PSYf/PSYr	-
22S19	*X. t*	4F1/4R1	-	*P. f*	Syntol	-	*R. t*	Rt3F/3R	-	*P. s*	PSYf/PSYr	+
22S20	*X. t*	4F1/4R1	-	*P. f*	Syntol	-	*R. t*	Rt3F/3R	-	*P. s*	PSYf/PSYr	+
22S21	*X. t*	4F1/4R1	-	*P. f*	Syntol	-	*R. t*	Rt3F/3R	-	*P. s*	PSYf/PSYr	+
22S22	*X. t*	4F1/4R1	-	*P. f*	Syntol	-	*R. t*	Rt3F/3R	-	*P. s*	PSYf/PSYr	-
22S23	*X. t*	4F1/4R1	-	*P. f*	Syntol	-	*R. t*	Rt3F/3R	-	*P. s*	PSYf/PSYr	-
22S24	*X. t*	4F1/4R1	-	*P. f*	Syntol	-	*R. t*	Rt3F/3R	-	*P. s*	PSYf/PSYr	-
22S25	*X. t*	4F1/4R1	-	*P. f*	Syntol	-	*R. t*	Rt3F/3R	-	*P. s*	PSYf/PSYr	+
22S26	*X. t*	4F1/4R1	-	*P. f*	Syntol	-	*R. t*	Rt3F/3R	-	*P. s*	PSYf/PSYr	-
22S27	*X. t*	4F1/4R1	-	*P. f*	Syntol	-	*R. t*	Rt3F/3R	-	*P. s*	PSYf/PSYr	+
22S28	*X. t*	4F1/4R1	-	*P. f*	Syntol	-	*R. t*	Rt3F/3R	-	*P. s*	PSYf/PSYr	+
22S29	*X. t*	4F1/4R1	-	*P. f*	Syntol	-	*R. t*	Rt3F/3R	-	*P. s*	PSYf/PSYr	+
22S30	*X. t*	4F1/4R1	-	*P. f*	Syntol	-	*R. t*	Rt3F/3R	-	*P. s*	PSYf/PSYr	+
22S31	*X. t*	4F1/4R1	-	*P. f*	Syntol	-	*R. t*	Rt3F/3R	-	*P. s*	PSYf/PSYr	+
22S32	*X. t*	4F1/4R1	-	*P. f*	Syntol	-	*R. t*	Rt3F/3R	-	*P. s*	PSYf/PSYr	+
22S33	*X. t*	4F1/4R1	-	*P. f*	Syntol	-	*R. t*	Rt3F/3R	-	*P. s*	PSYf/PSYr	+
22S34	*X. t*	4F1/4R1	-	*P. f*	Syntol	-	*R. t*	Rt3F/3R	-	*P. s*	PSYf/PSYr	+
22S35	*X. t*	4F1/4R1	-	*P. f*	Syntol	-	*R. t*	Rt3F/3R	-	*P. s*	PSYf/PSYr	+
22S36	*X. t*	4F1/4R1	-	*P. f*	Syntol	-	*R. t*	Rt3F/3R	-	*P. s*	PSYf/PSYr	+
22S37	*X. t*	4F1/4R1	-	*P. f*	Syntol	-	*R. t*	Rt3F/3R	-	*P. s*	PSYf/PSYr	+
22S38	*X. t*	4F1/4R1	-	*P. f*	Syntol	-	*R. t*	Rt3F/3R	-	*P. s*	PSYf/PSYr	-
22S39	*X. t*	4F1/4R1	-	*P. f*	Syntol	-	*R. t*	Rt3F/3R	-	*P. s*	PSYf/PSYr	+
22S40	*X. t*	4F1/4R1	-	*P. f*	Syntol	-	*R. t*	Rt3F/3R	-	*P. s*	PSYf/PSYr	+
22S41	*X. t*	4F1/4R1	-	*P. f*	Syntol	-	*R. t*	Rt3F/3R	-	*P. s*	PSYf/PSYr	+
22S42	*X. t*	4F1/4R1	-	*P. f*	Syntol	-	*R. t*	Rt3F/3R	-	*P. s*	PSYf/PSYr	+
22S43	*X. t*	4F1/4R1	-	*P. f*	Syntol	-	*R. t*	Rt3F/3R	-	*P. s*	PSYf/PSYr	+
22S44	*X. t*	4F1/4R1	-	*P. f*	Syntol	-	*R. t*	Rt3F/3R	-	*P. s*	PSYf/PSYr	+
22S45	*X. t*	4F1/4R1	-	*P. f*	Syntol	-	*R. t*	Rt3F/3R	-	*P. s*	PSYf/PSYr	+
22S46	*X. t*	4F1/4R1	-	*P. f*	Syntol	-	*R. t*	Rt3F/3R	-	*P. s*	PSYf/PSYr	-
22S47	*X. t*	4F1/4R1	-	*P. f*	Syntol	-	*R. t*	Rt3F/3R	-	*P. s*	PSYf/PSYr	+
22S48	*X. t*	4F1/4R1	-	*P. f*	Syntol	-	*R. t*	Rt3F/3R	-	*P. s*	PSYf/PSYr	+
22S49	*X. t*	4F1/4R1	-	*P. f*	Syntol	-	*R. t*	Rt3F/3R	-	*P. s*	PSYf/PSYr	+

Sample cipher	Result of PCR/ Test											
22S50	*X. t*	4F1/4R1	-	*P. f*	Syntol	-	*R. t*	Rt3F/3R	-	*P. s*	PSYf/PSYr	-
22S51	*X. t*	4F1/4R1	-	*P. f*	Syntol	-	*R. t*	Rt3F/3R	-	*P. s*	PSYf/PSYr	+
22S52	*X. t*	4F1/4R1	-	*P. f*	Syntol	-	*R. t*	Rt3F/3R	-	*P. s*	PSYf/PSYr	-
22S53	*X. t*	4F1/4R1	-	*P. f*	Syntol	-	*R. t*	Rt3F/3R	-	*P. s*	PSYf/PSYr	+
22S54	*X. t*	4F1/4R1	-	*P. f*	Syntol	-	*R. t*	Rt3F/3R	-	*P. s*	PSYf/PSYr	-
22S55	*X. t*	4F1/4R1	-	*P. f*	Syntol	-	*R. t*	Rt3F/3R	-	*P. s*	PSYf/PSYr	-
22S56	*X. t*	4F1/4R1	-	*P. f*	Syntol	-	*R. t*	Rt3F/3R	-	*P. s*	PSYf/PSYr	-
22S57	*X. t*	4F1/4R1	-	*P. f*	Syntol	-	*R. t*	Rt3F/3R	-	*P. s*	PSYf/PSYr	+
22S58	*X. t*	4F1/4R1	-	*P. f*	Syntol	-	*R. t*	Rt3F/3R	-	*P. s*	PSYf/PSYr	-
22S59	*X. t*	4F1/4R1	-	*P. f*	Syntol	-	*R. t*	Rt3F/3R	-	*P. s*	PSYf/PSYr	-
22S60	*X. t*	4F1/4R1	-	*P. f*	Syntol	-	*R. t*	Rt3F/3R	-	*P. s*	PSYf/PSYr	+
22S61	*X. t*	4F1/4R1	-	*P. f*	Syntol	-	*R. t*	Rt3F/3R	-	*P. s*	PSYf/PSYr	-
22S62	*X. t*	4F1/4R1	-	*P. f*	Syntol	-	*R. t*	Rt3F/3R	-	*P. s*	PSYf/PSYr	-
22S63	*X. t*	4F1/4R1	-	*P. f*	Syntol	-	*R. t*	Rt3F/3R	-	*P. s*	PSYf/PSYr	-
22S64	*X. t*	4F1/4R1	-	*P. f*	Syntol	-	*R. t*	Rt3F/3R	-	*P. s*	PSYf/PSYr	-
22S65	*X. t*	4F1/4R1	-	*P. f*	Syntol	-	*R. t*	Rt3F/3R	-	*P. s*	PSYf/PSYr	-
22S66	*X. t*	4F1/4R1	-	*P. f*	Syntol	-	*R. t*	Rt3F/3R	-	*P. s*	PSYf/PSYr	+

55 analytical DNA plant samples were tested from Timiryazevka- Moscow indicated in yellow, 60 analytical DNA plant samples were tested form The Republic of Crimea indicated in purple and 66 analytical DNA plant samples were tested in Stavropol region highlighted in blue. *Xanthomonas translucens* – *X. t*, *Pseudomonas* fuscovaginae – *P. f*, *Pseudomonas syringae*– *P. s* and *Rathayibacter tritici*– *R. t.* The absence of the bacteria is marked by - whilst the presence of the bacteria was marked by + and highlighted in green.

Appendix 12

Table 12.1 the PCR identification results of the bacterial isolates from Timiryazevskaya field experimental station (Moscow).

No p/n	Sample cipher	Isolate cipher	Primers	*Result of identification*
1.	M1	M1-1	PSF/PSR	*Pseudomonas sp.*
2.	M1	M1-2	8UA/519B	*Rhodococcus sp.*
3.	M1	M1-3	8UA/519B	*Rhodococcus sp.*
4.	M2	M2-4	PSF/PSR	*Pseudomonas trivialis*
5.	M3	M3-5	PSF/PSR	*Pseudomonas sp.*
6.	M3	M3-6	PSF/PSR	*Pseudomonas poae*
7.	M4	M4-7	8UA/519B	*Erwinia sp.*
8.	M5	M5-8	PSF/PSR	*Pseudomonas viridiflava*
9.	M5	M5-9	PSF/PSR	*Pseudomonas syringae pv. aptata*
10.	M5	M5-10	SyD1/SyD2	*Pseudomonas syringae pv. atrofaciens*
11.	M5	M5-11	PsyF/PsyR	*Pseudomonas sp.*
12.	M6	M6-12	PSF/PSR	*Pseudomonas sp.*
13.	M6	M6-13	PSF/PSR	*Pseudomonas sp.*
14.	M6	M6-14	8UA/519B	*Frigoribacterium sp.*
15.	M6	M6-15	8UA/519B	*Frigoribacterium sp.*
16.	M6	M6-16	8UA/519B	*Clavibacter michiganensis*
17.	M6	M6-17	8UA/519B	*Kineococcus sp.*
18.	M7	M7-18	PSF/PSR	*Pseudomonas sp.*
19.	M8	M8-19	PSF/PSR	*Pseudomonas sp.*
20.	M8	M8-20	8UA/519B	*Pantoea agglomerans*
21.	M8	M8-21	8UA/519B	*Pantoea agglomerans*
22.	M9	M9-22	PSF/PSR	*Pseudomonas graminis*
23.	M9	M9-23	PSF/PSR	*Pseudomonas poae*
24.	M9	M9-24	8UA/519B	*Uncultured bacterium*
25.	M10	M10-25	8UA/519B	*Curtobacterium sp.*
26.	M10	M10-26	8UA/519B	*Arthrobacter sp.*
27.	M10	M10-27	8UA/519B	*Streptomyces sp.*
28.	M11	M11-28	PSF/PSR	*Pseudomonas sp.*
29.	M12	M12-29	8UA/519B	*Frigoribacterium faeni*
30.	M12	M12-30	PSF/PSR	*Paucimonas lemoignei*
31.	M12	M12-31	8UA/519B	*Uncultured Enterobacteriaceae bacterium*
32.	M12	M12-32	8UA/519B	*Uncultured soil bacterium*
33.	M12	M12-33	PSF/PSR	*Pantoea ananatis*
34.	M12	M12-34	8UA/519B	*Frigoribacterium sp.*
35.	M12	M12-35	8UA/519B	*Salinibacterium sp.*
36.	M12	M12-36	PsyF/PsyR	*Pseudomonas sp.*

37.	M12	M12-37	8UA/519B	*Frigoribacterium sp.*
38.	M13	M13-38	8UA/519B	*Frigoribacterium sp.*
39.	M13	M13-39	PSF/PSR	*Pseudomonas sp.*
40.	M14	M14-40	8UA/519B	*Frigoribacterium sp.*
41.	M14	M14-41	8UA/519B	*Frigoribacterium sp.*
42.	M15	M15-42	PSF/PSR	*Pseudomonas sp.*
43.	M16	M16-43	8UA/519B	*Dyadobacter sp.*
44.	M16	M16-44	8UA/519B	*Serratia plymuthica*
45.	M17	M17-45	8UA/519B	*Staphylococcus warneri*
46.	M17	M17-46	8UA/519B	*Frigoribacterium sp.*
47.	M17	M17-47	8UA/519B	*Sanguibacter sp.*
48.	M17	M17-48	PSF/PSR	*Pseudomonas sp.*
49.	M18	M18-49	PSF/PSR	*Pseudomonas sp.*
50.	M18	M18-50	8UA/519B	*Curtobacterium flaccumfaciens*
51.	M18	M18-51	8UA/519B	*Frigoribacterium sp.*
52.	M19	M19-52	8UA/519B	*Frigoribacterium sp.*
53.	M19	M19-53	8UA/519B	*Clavibacter michiganesis*
54.	M19	M19-54	PSF/PSR	*Pseudomonas trivialis*
55.	M19	M19-55	PsyF/PsyR	*Pseudomonas sp.*
56.	M19	M19-56	8UA/519B	*Frigoribacterium sp.*
57.	M19	M19-57	8UA/519B	*Frigoribacterium sp.*
58.	M20	M20-58	8UA/519B	*Clavibacter michiganensis*
59.	M20	M20-59	8UA/519B	*Frigoribacterium faeni*
60.	M21	M21-60	8UA/519B	*Frigoribacterium sp.*
61.	M21	M21-61	8UA/519B	*Frigoribacterium sp.*
62.	M21	M21-62	8UA/519B	*Arthrobacter sp.*
63.	M21	M21-63	8UA/519B	*Curtobacterium flaccumfaciens*
64.	M22	M22-64	PSF/PSR	*Pseudomonas poae*
65.	M22	M22-65	8UA/519B	*Phycicocous sp.*
66.	M22	M22-66	8UA/519B	*Frigoribacterium sp.*
67.	M22	M22-67	PSF/PSR	*Pseudomonas graminis*
68.	M23	M23-68	8UA/519B	*Oerskovia sp.*
69.	M23	M23-69	8UA/519B	*Cellulomonas sp.*
70.	M23	M23-70	8UA/519B	*Oerskovia paurametabola*
71.	M23	M23-71	8UA/519B	*Microbacterium phyllosphaerae*
72.	M23	M23-72	8UA/519B	*Frigoribacterium faeni*
73.	M23	M23-73	8UA/519B	*Microbacterium sp.*
74.	M24	M24-74	8UA/519B	*Microbacteriaceae bacterium*
75.	M24	M24-75	8UA/519B	*Frondihabitans sp.*
76.	M24	M24-76	8UA/519B	*Curtobacterium sp.*
77.	M25	M25-77	PSF/PSR	*Pseudomonas sp.*

78.	M25	M25-78	PsyF/PsyR	*Pseudomonas sp.*
79.	M26	M26-79	PSF/PSR	*Pseudomonas graminis*
80.	M26	M26-80	PSF/PSR	*Pseudomonas poae*
81.	M27	M27-81	PSF/PSR	*Pseudomonas poae*
82.	M27	M27-82	PSF/PSR	*Pseudomonas poae*
83.	M27	M27-83	8UA/519B	*Clavibacter michiganensis subsp. michiganensis*
84.	M27	M27-84	8UA/519B	*Frigoribacterium faeni*
85.	M27	M27-85	8UA/519B	*Sphingomonas sp.*
86.	M27	M27-86	8UA/519B	*Clavibacter michiganensis subsp. michiganensis*
87.	M28	M28-87	PSF/PSR	*Pseudomonas trivialis*
88.	M28	M28-88	8UA/519B	*Micrococcus luteus*
89.	M28	M28-89	8UA/519B	*Staphylococcus sp.*
90.	M28	M28-90	8UA/519B	*Staphylococcus sp.*
91.	M28	M28-91	PsyF/PsyR	*Pseudomonas graminis*
92.	M28	M28-92	8UA/519B	*Arthrobacter sp.*
93.	M28	M28-93	8UA/519B	*Staphylococcus sp.*
94.	M29	M29-94	8UA/519B	*Clavibacter michiganensis*
95.	M29	M29-95	PsyF/PsyR	*Pseudomonas graminis*
96.	M29	M29-96	8UA/519B	*Bacillus pumilis*
97.	M29	M29-97	8UA/519B	*Microbacterium sp.*
98.	M30	M30-98	8UA/519B	*Arthrobacter sp.*
99.	M30	M30-99	8UA/519B	*Rhodococcus sp.*
100.	M30	M30-100	8UA/519B	*Clavibacter michiganensis*
101.	M30	M30-101	SyD1/SyD2	*Pseudomonas syringae pv. lapsa*
102.	M30	M30-102	8UA/519B	*Frigoribacterium sp.*
103.	M31	M 31-103	8UA/519B	*Uncultured bacterium*
104.	M31	M 31-104	8UA/519B	*Frigoribacterium sp.*
105.	M31	M 31-105	8UA/519B	*Rathayibacter festucae*
106.	M32	M 32-106	8UA/519B	*Rhodococcus fascians*
107.	M32	M 32-107	8UA/519B	*Arthrobacter sp.*
108.	M32	M 32-108	8UA/519B	*Phycicoccus sp.*
109.	M32	M 32-109	SyD1/SyD2	*Pseudomonas syringae pv. syringae*
110.	M32	M 32-110	8UA/519B	*Frigoribacterium sp.*
111.	M33	M 33-111	8UA/519B	*Frigoribacterium sp.*
112.	M33	M 33-112	8UA/519B	*Bacillus sp.*
113.	M33	M 33-113	PSF/PSR	*Pseudomonas viridiflava*
114.	M33	M 33-114	PSF/PSR	*Pseudomonas chlororaphis*
115.	M33	M 33-115	PSF/PSR	*Pseudomonas sp.*
116.	M34	M 34-116	8UA/519B	*Rhodococcus sp.*
117.	M34	M 34-117	PsyF/PsyR	*Pseudomonas syringae*
118.	M35	M 35-118	8UA/519B	*Plantibacter sp.*

119.	M36	M 36-119	8UA/519B	*Arthrobacter sp.*
120.	M36	M 36-120	8UA/519B	*Frigoribacterium sp.*
121.	M36	M 36-121	8UA/519B	*Curtobacterium sp.*
122.	M36	M 36-122	8UA/519B	*Frigoribacterium sp.*
123.	M36	M 36-123	8UA/519B	*Rhizosphere soil bacterium*
124.	M36	M 36-124	8UA/519B	*Sphingomonas sp.*
125.	M37	M 37-125	8UA/519B	*Clavibacter michiganensis subsp. michiganensis*
126.	M37	M 37-126	8UA/519B	*Frigoribacterium sp.*
127.	M38	M 38-127	8UA/519B	*Frigoribacterium sp.*
128.	M38	M 38-128	PSF/PSR	*Pseudomonas sp.*
129.	M39	M39-129	8UA/519B	*Plantibacter sp.*
130.	M39	M39-130	SyD1/SyD2	*Pseudomonas syringae pv. syringae*
131.	M39	M39-131	PSF/PSR	*Pseudomonas sp.*
132.	M40	M40-132	8UA/519B	*Frigoribacterium sp.*
133.	M40	M40-133	8UA/519B	*Arthrobacter chlorophenolicus*
134.	M40	M40-134	SyD1/SyD2	*Pseudomonas syringae pv. syringae*
135.	M41	M41-135	8UA/519B	*Clavibacter michiganesis*
136.	M41	M41-136	8UA/519B	*Arthrobacter sp.*
137.	M41	M41-137	8UA/519B	*Clavibacter michiganesis*
138.	M42	M42-138	8UA/519B	*Frigoribacterium sp.*
139.	M42	M42-139	PSF/PSR	*Pseudomonas sp.*
140.	M43	M43-140	PSF/PSR	*Pseudomonas sp.*
141.	M44	M44-141	8UA/519B	*Frigoribacterium sp.*
142.	M45	M45-142	8UA/519B	*Rhodococcus sp.*
143.	M45	M45-143	SyD1/SyD2	*Pseudomonas syringae pv. atrofaciens*
144.	M46	M46-144	8UA/519B	*Erwinia amylovora*
145.	M47	M47-145	8UA/519B	*Microbacterium foliorum*
146.	M47	M47-146	8UA/519B	*Rhodococcus fascians*
147.	M48	M48-147	8UA/519B	*Clavibacter sp.*
148.	M48	M48-148	8UA/519B	*Bacillus sp.*
149.	M48	M48-149	8UA/519B	*Frigoribacterium sp.*
150.	M49	M49-150	8UA/519B	*Clavibacter sp.*
151.	M49	M49-151	8UA/519B	*Rhodococcus cerastii*
152.	M49	M49-152	8UA/519B	*Phycicoccus sp.*
153.	M50	M50-153	PSF/PSR	*Pseudomonas trivialis*
154.	M50	M50-154	8UA/519B	*Frigoribacterium sp.*
155.	M50	M50-155	PsyF/PsyR	*Pseudomonas sp.*
156.	M51	M51-156	8UA/519B	*Pseudomonas sp.*
157.	M51	M51-157	8UA/519B	*Agreria sp.*
158.	M51	M51-158	8UA/519B	*Frondihabitans sp.*
159.	M51	M51-159	8UA/519B	*Sphingomonas sp.*

160.	M52	M52-160	SyD1/SyD2	*Pseudomonas syringae pv. atrofaciens*
161.	M53	M53-161	8UA/519B	*Rhodococcus yunnanensis*
162.	M53	M53-162	PsyF/PsyR	*Pseudomonas sp.*
163.	M54	M54-163	8UA/519B	*Athrobacter sp.*
164.	M54	M54-164	8UA/519B	*Micrococcus sp.*
165.	M54	M54-165	8UA/519B	*Frigoribacterium sp.*
166.	M55	M55-166	8UA/519B	*Actinomycetales bacterium*
167.	M55	M55-167	PSF/PSR	*Pseudomonas sp.*
168.	M55	M55-168	8UA/519B	*Pseudoclavibacter helvolus*

Appendix 13

Table 13.1 The PCR and Electrophoresis results of the isolates collected from the samples of grain crops from Belogorskij, Krasnogvardejskij and Simferopol`skij of the Republic of Crimea with primers 8UA/519B (500bp), 27F/907R (880bp) and PSF/PSR (610bp).

Isolate №	500bp	880bp	610bp	Isolate №	500bp	880bp	610bp	Isolate №	500bp	880bp	610bp
1	+	-	-	37	-	+	-	74-1	+	-	-
2	+	+	-	38	+	-	-	74-2	+	+	-
3	+	+	-	39	+	-	-	75-1	+	+	-
4	-	+	-	41	+	-	-	75-2	+	+	-
5	+	+	-	42	-	+	-	76	+	+	-
7	+	+	-	43	+	+	-	77	+	-	-
8	-	+	-	45	-	+	-	78	+	-	+
9	+	+	-	46	+	+	-	79	+	+	-
10	+	+	-	47	+	+	-	81	+	+	-
11	+	-	-	48	+	+	-	82	+	+	-
12	+	-	-	49	+	-	-	83	-	+	-
14	+	+	-	50	+	+	-	84	-	+	-
15	+	+	-	51	+	+	-	85	+	-	-
17	+	+	-	52	+	+	-	86	+	+	-
18	+	+	-	53	+	+	-	87	+	+	-
19	+	-	+	55	+	-	-	88	+	+	-
20	+	-	-	57	+	+	-	89	+	+	-
21	+	+	-	58	+	-	-	90	+	+	-
22	+	-	-	59	+	+	-	91	+	+	-
24	+	-	-	60	+	-	-	92	+	+	-
25	-	+	-	61	+	-	-	93	+	+	-
26	+	-	-	62	-	+	-	94	+	+	-
27	+	+	-	63	-	-	+	95	+	+	-
28	+	+	-	64	+	+	-	96	+	+	-
29	+	-	-	66	+	+	-	97	+	+	-
30	+	+	-	67	+	+	-	98-1	+	-	-
31	+	-	-	68	+	+	-	98-2	+	+	-
32	+	+	-	69	+	-	-	99	+	+	-
33	+	+	-	70	+	+	-	100	+	+	-
34	+	+	-	71	+	+	-	101	+	-	+

35	+	+	-	72	+	+	-	102	+	+	-
36	-	+	-	73	+	+	-	102	+	+	-

N o t e. + means the amplicon size desired was obtained, – indicates the absence of the desired amplicon size and the numbers represent the isolates collected. As a result of PCR with 8UA/519B primers, the 500 bp amplicon was obtained for 92 tested samples of DNA from bacterial cultures, PCR with 27F/907R primers, the 880 bp amplicon was obtained for 61 tested samples of DNA from bacterial cultures and PCR with PSF/PSR primers, the 610 bp amplicon was obtained for 4 tested samples of DNA from bacterial cultures.

Appendix 14.1

Table 14.1 the PCR identification results of the bacterial isolates from the Republic of Crimea

No p/n	Sample cipher	Isolate cipher	Primers	Result of identification
1.	C1	C1-1	8UA-519B	*Ochrobactrum* sp.
2.	C1	C1-2	27f-509r	*Erwinia aphidicola*
3.	C1	C1-3	27f-509r	*Rathayibacter festucae*
4.	C1	C1-4	27f-509r	*Arthrobacter* sp.
5.	C1	C1-5	27f-509r	*Arthrobacter* sp.
6.	C1	C1-7	27f-509r	*Stenotrophomonas maltophilia*
7.	C1	C1-8	27f-509r	*Erwinia* sp.
8.	C1	C1-9	27f-509r	Bacteria
9.	C2	C2-10	27f-509r	*Erwinia* sp.
10.	C2	C2-11	27f-509r	*Pseudoclavibacter terrae*
11.	C2	C2-12	27f-509r	*Frigoribacterium* sp
12.	C3	C3-14	8UA-519B	*Pantoea* sp.
13.	C3	C3-15	8UA-519B	*Pantoea* sp.
14.	C4	C4-17	27f-509r	*Ochrobactrum* sp.
15.	C5	C5-18	27f-509r	*Frigoribacterium* sp.
16.	C5	C5-19	27f-509r	*Pantoea agglomerans*
17.	C5	C5-20	27f-509r	*Frigoribacterium* sp.
18.	C5	C5-21	27f-509r	*Pseudomonas poae*
19.	C5	C5-22	8UA-519B	*Plantibacter flavus*
20.	C5	C5-24	8UA-519B	*Frigoribacterium* sp.
21.	C6	C6-25	8UA-519B	*Erwinia rhapontici*
22.	C6	C6-26	8UA-519B	*Erwinia rhapontici*
23.	C6	C6-27	27f-509r	*Arthrobacter* sp.
24.	C7	C7-28	8UA-519B	*Pantoea* sp.
25.	C7	C7-29	27f-509r	*Erwinia* sp.
26.	C7	C7-30	27f-509r	*Clavibacter michiganensis*
27.	C8	C8-31	8UA-519B	*Pantoea agglomerans*
28.	C9	C9-32	27f-509r	*Erwinia aphidicola*
29.	C9	C9-33	8UA-519B	*Erwinia* sp.
30.	C9	C9-34	27f-509r	*Pantoea* sp.
31.	C9	C9-35	27f-509r	*Ochrobactrum* sp.
32.	C9	C9-36	27f-509r	*Pantoea* sp.
33.	C9	C9-37	27f-509r	*Erwinia* sp.
34.	C10	C10-38	27f-509r	*Ochrobactrum* sp.
35.	C10	C10-39	27f-509r	*Pantoea agglomerans*

No p/n	Sample cipher	Isolate cipher	Primers	Result of identification
36.	C11	C11-41	27f-509r	*Pantoea* sp.
37.	C11	C11-42	27f-509r	Uncultured bacterium
38.	C12	C12-43	8UA-519B	*Ochrobactrum* sp.
39.	C12	C12-45	27f-509r	*Pantoea ananatis*
40.	C13	C13-46	8UA-519B	*Pantoea agglomerans*
41.	C13	C13-47	8UA-519B	*Enterobacter* sp.
42.	C13	C13-48	27f-509r	*Rosenbergiella* sp.
43.	C13	C13-49	27f-509r	*Pantoea agglomerans*
44.	C13	C13-50	27f-509r	*Stenotrophomonas* sp.
45.	C13	C13-51	27f-509r	*Erwinia* sp.
46.	C14	C14-52	27f-509r	*Rosenbergiella* sp.
47.	C14	C14-53	27f-509r	*Erwinia* sp.
48.	C15	C15-55	8UA-519B	*Rosenbergiella* sp.
49.	C15	C15-57	27f-509r	*Erwinia* sp.
50.	C15	C15-58	27f-509r	*Pantoea* sp.
51.	C15	C15-59	27f-509r	*Pantoea* sp.
52.	C15	C15-60	27f-509r	*Pantoea* sp.
53.	C16	C16-61	8UA-519B	Uncultured bacterium
54.	C16	C16-62	27f-509r	*Erwinia* sp.
55.	C16	C16-63	PSF/PSR	*Pseudomonas* sp.
56.	C16	C16-64	8UA-519B	*Exiguobacterium* sp.
57.	C17	C17-66	27f-509r	*Exiguobacterium* sp.
58.	C17	C17-67	8UA-519B	*Stenotrophomonas* sp.
59.	C17	C17-68	8UA-519B	*Stenotrophomonas* sp.
60.	C18	C18-69	8UA-519B	*Pseudomonas* sp.
61.	C18	C18-70	27f-509r	*Plantibacter* sp.
62.	C18	C18-71	27f-509r	*Stenotrophomonas* sp.
63.	C18	C18-72	27f-509r	*Stenotrophomonas* sp.
64.	C18	C18-73	27f-509r	*Agrococcus jenensis*
65.	C19	C19-74.1	8UA-519B	*Pantoea vagans*
66.	C19	C19-74.2	27f-509r	*Frigoribacterium* sp.
67.	C19	C19-75.1	27f-509r	*Rosenbergiella* sp.
68.	C20	C20-75.2	27f-509r	*Pantoea* sp.
69.	C21	C21-76	27f-509r	*Pantoea* sp.
70.	C24	C24-77	8UA-519B	*Pantoea* sp.
71.	C24	C24-78	8UA-519B	*Pseudomonas* sp.
72.	C26	C26-79	27f-509r	*Pantoea agglomerans*
73.	C28	C28-81	27f-509r	*Plantibacter* sp.
74.	C28	C28-82	27f-509r	Bacteria

No p/n	Sample cipher	Isolate cipher	Primers	Result of identification
75.	C33	C33-83	27f-509r	*Pantoea pleuroti*
76.	C33	C33-84	27f-509r	*Pantoea* sp.
77.	C33	C33-85	8UA-519B	*Microbacterium* sp.
78.	C37	C37-86	27f-509r	Uncultured bacterium
79.	C38	C38-87	27f-509r	*Rosenbergiella* sp.
80.	C41	C41-88	27f-509r	*Rosenbergiella* sp.
81.	C42	C42-89	27f-509r	*Pantoea* sp.
82.	C42	C42-90	27f-509r	*Curtobacterium* sp.
83.	C45	C45-91	27f-509r	*Rosenbergiella* sp.
84.	C45	C45-92	8UA-519B	*Pseudomonas* sp.
85.	C46	C46-93	27f-509r	*Pantoea agglomerans*
86.	C46	C46-94	27f-509r	*Pantoea* sp.
87.	C48	C48-95	27f-509r	*Curtobacterium* sp.
88.	C48	C48-96	27f-509r	*Clavibacter michiganensis*
89.	C51	C51-97	27f-509r	*Microbacterium* sp.
90.	C53	C53-98.1	27f-509r	*Enterococcus mundtii*
91.	C53	C53-98.2	27f-509r	*Frigoribacterium* sp.
92.	C54	C54-99	27f-509r	*Arthrobacter* sp.
93.	C58	C58-100	27f-509r	*Frigoribacterium* sp.
94.	C59	C59-101	27f-509r	*Pseudomonas* sp.
95.	C60	C60-102	27f-509r	*Pantoea agglomerans*

Appendix 15

Table 15.1 the PCR identification results of the bacterial isolates from Stavropol

No.	Sample No.	Isolate code	Primers	Identification result
22	22S28	22S28-106	27f/907	*Bacillus* sp.
22	22S28	22S28-107	27f/907	*Pseudomonas* sp.
22	22S51	22S51-109	27f/907	*Rhodococcus* sp.
22	22S39	22S39-143	27f/907	*Rhodococcus* sp.
22	22S39	22S39-144	27f/907	*Curtobacterium* sp.
22	22S39	22S39-145	27f/907	*Rhodococcus* sp.
22	22S39	22S39-146	27f/907	*Arthrobacter* sp. (= *Paenarthrobacter* sp.)
22	22S39	22S39-147	27f/907	*Microbacterium* sp.
22	22S39	22S39-148	27f/907	*Arthrobacter aurescens* (= *Paenarthrobacter aurescens*)
22	22S1	22S1-330	27f/907	*Sphingomonas* sp.
22	22S1	22S1-331	27f/907	*Frigoribacterium* sp.
22	22S1	22S1-332	27f/907	*Pseudarthrobacter equi*
22	22S1	22S1-333	27f/907	*Clavibacter* sp.
22	22S1	22S1-334	PSF/PSR	*Pseudomonas* sp.
22	22S1	22S1-335	27f/907	*Curtobacterium flaccumfaciens*
22	22S1	22S1-336	27f/907	*Achromobacter* sp.
22	22S1	22S1-337	27f/907	*Arthrobacter* sp.
22	22S1	22S1-338	27f/907	*Rathayibacter festucae*
22	22S1	22S1-339	PSF/PSR	*Pseudomonas poae*
22	22S1	22S1-340	PSF/PSR	*Pseudomonas poae*
22	22S1	22S1-341	PSF/PSR	*Pseudomonas poae*
22	22S1	22S1-342	27f/907	*Rhodococcus fascians*
22	22S1	22S1-343	27f/907	*Microbacterium* sp.
22	22S1	22S1-344	SyD1/SyD2	*Pseudomonas syringae*
22	22S1	22S1-345	27f/907	*Frigoribacterium* sp.
22	22S1	22S1-347	27f/907	*Frigoribacterium* sp.
22	22S1	22S1-349	27f/907	*Rhodococcus* sp.
22	22S1	22S1-351	27f/907	*Microbacterium hydrocarbonoxydans*
22	22S1	22S1-352	27f/907	*Paenarthrobacter* sp.
22	22S1	22S1-353	27f/907	*Clavibacter* sp.
22	22S1	22S1-355	27f/907	*Rhodococcus* sp.
22	22S1	22S1-356	27f/907	*Frigoribacterium* sp.
22	22S1	22S1-357	27f/907	*Rathayibacter festucae*
22	22S1	22S1-359	27f/907	*Achromobacter* sp.

22	22S1	22S1-360	SyD1/SyD2	*Pseudomonas syringae*
22	22S1	22S1-361	27f/907	*Microbacterium* sp.
22	22S1	22S1-362	27f/907	*Rathayibacter* sp.
22	22S1	22S1-364	27f/907	*Luteimonas* sp.
22	22S1	22S1-367	27f/907	*Paenarthrobacter* sp. (= *Arthrobacter* sp.)
22	22S1	22S1-368	27f/907	*Rhodococcus* sp.
22	22S1	22S1-369	27f/907	*Rhodococcus* sp.
22	22S1	22S1-370	27f/907	*Rathayibacter festucae*
22	22S1	22S1-371	27f/907	*Frigoribacterium* sp.
22	22S1	22S1-372	27f/907	*Pedobacter petrophilus*
22	22S1	22S1-373	27f/907	*Plantibacter* sp.
22	22S2	22S2-374	27f/907	*Paeniglutamicibacter* sp.
22	22S2	22S2-375	27f/907	*Pseudomonas* sp.
22	22S2	22S2-377	27f/907	*Rhodococcus* sp.
22	22S2	22S2-378	27f/907	*Rhodococcus* sp.
22	22S2	22S2-379	27f/907	*Rhodococcus* sp.
22	22S2	22S2-380	27f/907	*Clavibacter michiganensis*
22	22S2	22S2-381	27f/907	*Paeniglutamicibacter terrestris*
22	22S2	22S2-382	27f/907	*Clavibacter* sp.
22	22S2	22S2-383	27f/907	*Pantoea* sp.
22	22S4	22S4-384	27f/907	*Sphingobacterium faecium*
22	22S6	22S6-386	27f/907	*Agrococcus citreus*
22	22S6	22S6-387	27f/907	*Clavibacter michiganensis*
22	22S6	22S6-388	27f/907	*Pseudarthrobacter* sp.
22	22S6	22S6-389	27f/907	*Pseudarthrobacter* sp.
22	22S6	22S6-390	27f/907	*Glutamibacter bergerei*
22	22S6	22S6-391	27f/907	*Microbacterium* sp.
22	22S6	22S6-392	27f/907	*Microbacterium* sp.
22	22S8	22S8-393	27f/907	*Rhodococcus* sp.
22	22S8	22S8-394	27f/907	*Pseudomonas* sp.
22	22S8	22S8-395	27f/907	*Rahnella* sp.
22	22S8	22S8-396	27f/907	*Paeniglutamicibacter* sp.
22	22S9	22S9-397	27f/907	*Rhodococcus* sp.
22	22S9	22S9-398	27f/907	*Rhodococcus* sp.
22	22S9	22S9-399	27f/907	*Frigoribacterium* sp.
22	22S9	22S9-400	27f/907	*Frigoribacterium* sp.
22	22S9	22S9-401	27f/907	*Rhodococcus fascians*
22	22S9	22S9-402	27f/907	*Okibacterium* sp.
22	22S9	22S9-403	27f/907	*Microbacterium* sp.
22	22S9	22S9-404	27f/907	*Rhodococcus* sp.
22	22S11	22S11-406	27f/907	*Frigoribacterium* sp.

22	22S11	22S11-407	27f/907	*Plantibacter* sp.
22	22S11	22S11-408	27f/907	*Clavibacter* sp.
22	22S11	22S11-409	27f/907	*Microbacterium* sp.
22	22S11	22S11-411	27f/907	*Sphingobacterium* sp.
22	22S18	22S18-413	27f/907	*Mycobacterium frederiksbergense*
22	22S18	22S18-417	27f/907	*Paenarthrobacter* sp. (= *Arthrobacter* sp.)
22	22S18	22S18-420	27f/907	*Knoellia aerolata*
22	22S18	22S18-421	27f/907	*Rhodococcus* sp.
22	22S18	22S18-423	27f/907	*Rhodococcus* sp.
22	22S18	22S18-424	27f/907	*Bacillus pumilus*
22	22S18	22S18-425	27f/907	*Arthrobacter* sp.
22	22S13	22S13-426	27f/907	*Bacillus* sp.
22	22S13	22S13-427	27f/907	*Plantibacter* sp.
22	22S13	22S13-428	27f/907	*Staphylococcus equorum*
22	22S13	22S13-429	27f/907	*Rhodococcus* sp.
22	22S13	22S13-430	27f/907	*Microbacterium* sp.
22	22S14	22S14-431	27f/907	*Paeniglutamicibacter sulfureus*
22	22S16	22S16-432	27f/907	*Pseudoclavibacter* sp.
22	22S16	22S16-433	27f/907	*Rhodococcus* sp.
22	22S16	22S16-434	27f/907	*Rhodococcus* sp.
22	22S16	22S16-435	27f/907	*Rhizobium* sp.
22	22S17	22S17-436	27f/907	*Microbacterium* sp.
22	22S17	22S17-437	27f/907	*Sphingobacterium* sp.
22	22S20	22S20-438	27f/907	*Bacillus* sp.
22	22S20	22S20-439	27f/907	*Plantibacter* sp.
22	22S22	22S22-441	27f/907	*Rhodococcus* sp.
22	22S26	22S26-444	27f/907	*Microbacterium* sp.
22	22S26	22S26-445	27f/907	*Rhodococcus* sp.
22	22S24	22S24-446	27f/907	*Microbacterium* sp.
22	22S24	22S24-447	27f/907	*Curtobacterium* sp.
22	22S24	22S24-448	27f/907	*Exiguobacterium* sp.
22	22S24	22S24-449	27f/907	*Rhodococcus* sp.
22	22S29	22S29-451	27f/907	*Frigoribacterium* sp.
22	22S29	22S29-452	27f/907	*Rathayibacter* sp.
22	22S33	22S33-453	27f/907	*Staphylococcus equorum*
22	22S33	22S33-454	27f/907	*Psychrobacillus psychodurans*
22	22S33	22S33-455	27f/907	*Paenarthrobacter* sp. (= *Arthrobacter* sp.)
22	22S33	22S33-456	27f/907	*Staphylococcus* sp.
22	22S32	22S32-457	27f/907	*Frigoribacterium* sp.
22	22S32	22S32-458	27f/907	*Rhodococcus* sp.
22	22S32	22S32-459	27f/907	*Paenarthrobacter* sp. (= *Arthrobacter* sp.)

22	22S34	22S34-460	27f/907	*Frigoribacterium* sp.
22	22S36	22S36-461	27f/907	*Glutamibacter* sp.
22	22S38	22S38-462	27f/907	*Rhodococcus* sp.
22	22S40	22S40-463	27f/907	*Curtobacterium* sp.
22	22S40	22S40-464	27f/907	*Rhodococcus* sp.
22	22S43	22S43-465	27f/907	*Rhodococcus* sp.
22	22S43	22S43-466	27f/907	*Pantoea* sp.
22	22S43	22S43-467	27f/907	*Frigoribacterium* sp.
22	22S43	22S43-468	27f/907	*Rhodococcus* sp.
22	22S46	22S46-469	27f/907	*Rhodococcus cerastii*
22	22S46	22S46-470	27f/907	*Rhodococcus* sp.
22	22S46	22S46-471	27f/907	*Clavibacter* sp.
22	22S46	22S46-472	SyD1/SyD2	*Pseudomonas syringae*
22	22S57	22S57-473	27f/907	*Agrococcus* sp.
22	22S57	22S57-474	27f/907	*Agrococcus jenensis*
22	22S57	22S57-475	27f/907	*Agreia* sp.
22	22S57	22S57-476	27f/907	*Rhodococcus* sp.
22	22S58	22S58-477	27f/907	*Paenarthrobacter* sp.
22	22S58	22S58-478	27f/907	*Microbacterium* sp.
22	22S52	22S52-479	27f/907	*Okibacterium* sp.
22	22S52	22S52-481	27f/907	*Microbacterium* sp.
22	22S52	22S52-482	27f/907	*Rhodococcus* sp.
22	22S59	22S59-483	27f/907	*Microbacterium* sp.
22	22S59	22S59-484	27f/907	*Microbacterium* sp.
22	22S59	22S59-485	27f/907	*Stenotrophomonas* sp.
22	22S53	22S53-487	27f/907	*Plantibacter* sp.
22	22S53	22S53-489	27f/907	*Pseudoclavibacter* sp.
22	22S53	22S53-490	27f/907	*Plantibacter* sp.
22	22S60	22S60-491	27f/907	*Exiguobacterium* sp.
22	22S60	22S60-492	27f/907	*Paenibacillus lautus*
22	22S56	22S56-493	27f/907	*Paenarthrobacter* sp. (= *Arthrobacter* sp.)
22	22S65	22S65-494	27f/907	*Rhodococcus* sp.
22	22S65	22S65-495	27f/907	*Labedella* sp.
22	22S65	22S65-496	27f/907	*Microbacterium* sp.
22	22S54	22S54-497	27f/907	*Paenarthrobacter* sp. (= *Arthrobacter* sp.)
22	22S45	22S45-498	27f/907	*Chryseobacterium* sp.
22	22S50	22S50-499	27f/907	*Plantibacter* sp.
22	22S50	22S50-500	27f/907	*Rhizobium* sp.
22	22S50	22S50-501	27f/907	*Agreia* sp.
22	22S48	22S48-502	27f/907	*Plantibacter* sp.
22	22S48	22S48-503	27f/907	*Chryseobacterium* sp.

22	22S35	22S35-505	27f/907	*Frigoribacterium* sp.
22	22S55	22S55-506	27f/907	*Rhodococcus* sp.
22	22S55	22S55-507	27f/907	*Paenarthrobacter* sp. (= *Arthrobacter* sp.)
22	22S37	22S37-508	27f/907	*Paenarthrobacter* sp. (= *Arthrobacter* sp.)
22	22S37	22S37-509	27f/907	*Pseudomonas* sp.
22	22S37	22S37-510	27f/907	*Rhodococcus fascians*
22	22S66	22S66-512	27f/907	*Paenarthrobacter* sp. (= *Arthrobacter* sp.)