Identification of Ralstonia Solanacearum in Kyrgyzstan's Potato Fields and the Possibility of Using Biocontrol Agents Against this Pathogen

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Abstract— *In this study, we have used well-known, efficient methods and bioassay for systematic screening of R. solanacearum for identification of its phenotype and biochemical profile, as well as for pathogenicity and virulence. As a result, an aggressive race — Biovar 3 — was most isolated from the potato fields of the Issyk-Kul region, especially in fields where the Picasso variety was grown. The isolated indigenous strains of Streptomyces diastatochromogenesstrain sk-6 and Streptomyces bambergiensis strain k1-3 has the potential to be used as a biocontrol agent for the management of the bacterial wilt of potatoes, as indicated by the reduced percentage wilt incidence. Root zone and soil application of Streptomyces diastatochromogenesstrain sk-6 and Streptomyces bambergiensis strain k1-3 at a dose of 10^8 cell/ml significantly reduced disease incidence and increased the growth of potato plants. The disease's progress was reduced by 60% and 56% in plants inoculated with Streptomyces diastatochromogenesstrain sk-6 and Streptomyces bambergiensis strain k1-3, respectively.*

Keywords— bacterial wilt of potatoes, biocontrol, R. solanacearum,Streptomyces diastatochromogenes, Streptomyces bambergiensis.

I. INTRODUCTION

Ralstonia solanacearum is a soil-borne pathogen that naturally infects roots. It exhibits a strong and tissue-specific tropism within the host, specifically invading and highly multiplying in the xylem vessels (Smith, 1896, Yabuuchi et al., 1995). It causes a wilt disease in more than 450 plant species of 54 botanical families across the globe (Allenet al., 2005, Agrios, 1997, Stevenson, 2001). *Ralstonia solanacearum* has been studied intensively both biochemically and genetically, and has long been recognized as a model system for the analysis of pathogenicity (Staskawicz et al., 2001). It is well adapted to life in soil in the absence of host plants (Granada and Sequeira, 1983), thereby providing a good system to investigate functions governing adaptation to such an ecological niche. Considering the genetic diversity among the strains responsible for wilting disease in different plants, the pathogen is now termed as *Ralstonia solanacearum* species complex (Genin and Denny, 2012). In a traditional way, this pathogen has been classified into five races with respect to their host specificity and six biovars according to their biochemical properties (French et al., 1995).

The first signs of the disease are shown in the beginning of the flowering and tuber formation. Plants suddenly wilt; the leaves turn yellow, shrivel, and droop. The lower basal part of the stem softens and rots. A typical feature of brown rot is the splitting of the stems; the crosscut of them follows a drop of bacterial exudates. Subsequently, the bacteria penetrate into the stolon, then into young tubers, causing browning of the vascular ring. From sections of the affected vessels and tubers follows brown mucus (Daughtrey, 2003). Bacterial wilt occurs mainly in tropic, sub-tropic and warm temperature zones (Jaunet and Wang, 1999). However, this disease has spread to more temperate areas (Kim et al., 2003).

Ralstonia solanacearum is a *b-Proteobacterium*, whose complete genome sequence was presented by analysis of strain GMI1000. The 5.8-megabase (Mb) genome is organized into two replicons: a 3.7-Mb chromosome and a 2.1-Mb megaplasmid. The genome encodes many proteins potentially associated with a role in pathogenicity (Salanoubat et al., 2002).

As the disease is widely distributed, it has a wide host range and is mainly soil-borne; it is difficult to control with chemicals and cultural practices (Grimaultet al., 1993). Biological control strategies may either help development of alternative management measures or be integrated with other practices for effective disease management at the field level. Several microorganisms have been tried out with variable success for biological control of bacterial wilt (Shekhawat et al., 1993). Effective microorganisms (EM) are a mixture of beneficial microorganisms, which can increase the crop yield and also protect against plant pathogens (Higa, 1999, Lwin and Ranamukhaarachch, 2006). It is a mixed culture of photosynthetic bacteria, *Azotobacter*, *Streptomyces*, and *Lactobacillus spp*., which improve crop yield by increasing photosynthesis, nitrogen fixation, controlling soil diseases, and accelerating decomposition of lignin material in the soil (Hussain et al., 1993).

Biocontrol of bacterial wilt by plant growth-promoting rhizobacteria provided disease control and increased yields in greenhouses (Guoet al.,2004, Lemessa and Zeller, 2007). Research on microbial antagonists, such as *Candida ethanolica* has shown promise for bacterial wilt control (Lwin and Ranamukhaarachch, 2006). Toyota and Kimura (1996) reported the suppressive effect of some antagonistic bacteria on *R. solanacearum*. Three antagonists isolated from soil *(Bacillus megaterium, Enterobacter cloacae, Pichia guillermondii*) and *Candida ethanolica* showed high potential for disease suppression and also increased fruit weight, biomass, and plant height (Nguyen and Ranamukhaarachchi,2010).

In Kyrgyzstan, the potato (*Solanum tuberosum*) is a staple product for the population.Recently, farms in different regions have started to grow the varieties such as Picasso, Sante, and Nevskiy, which were imported from Russia and other countries of the world, besides local potato varieties. Approximately 32% of potato yields are lost every year due to viral, bacterial, fungal, and pest attacks to potato tubers and potato plants [An overview of the emergence and spread of major pests and diseases, 2011). Brown, slimy bacterial bacterios of potatoes (bacterial wilt, or wilt) caused by *Ralstonia solanacearum potatoes* (RS) is a relatively a new disease in the fields of Kyrgyzstan. There are still no data and records of the scientists and experts on the biology and distribution of this disease in the potato crop regions of Kyrgyzstan. There are suggestions that this bacterial disease was brought with imported planting material to Kyrgyzstan from neighboring countries. So, the disease has been found in Russia in 1999 by a quarantine inspection only in an area of 0.06 hectares, planted with the imported variety Sante, then the infestation of potato was found in many regions of Russia: in the Urals, Far East, and Western and Eastern Siberia (http://www.kartofel.org/bolezn/bacteria/bacwilt.htm). There is a particular threat to potato production (especially seed production) because of asymptomatic cases of these bacterial diseases, as apparently healthy tubers have a margin hidden (latent) infection and pose a threat to crops the following year. This makes it important to be able to identify the disease in the contaminated material. Still, the prevalence and host range of races and biovars of *Ralstonia solanacearum* is unknown in the potato-cultivated regions of Kyrgyzstan, but it is becoming increasingly clear that this species causes disease in the vegetation period and in storage after harvesting. Biological control of *Ralstonia solanacearum* is still in its research phase in Kyrgyzstan. The objective of this study was to distinguish the biovars of *Ralstonia solanacearum* by using biochemical and ELISA tests, PCR analysis, to determine the prevalence of pathogen races in commercial potato fields of Kyrgyzstan and to develop the biocontrol agents to reduce the harmfulness of latent infection of seed tubers.

II. MATERIAL AND METHODS

2.1 Origin of isolates

For direct isolation of *Ralstonia solanacearum*, the potato tubers of Picasso, Sante, and Nevskiy varieties were used, which were collected in the fall 2012 and 2013 from Issuk-Kul and Chy regions of country. All isolates from potato fields came from individual tubers of different plants. Each tuber was placed in an individual plastic bag after harvest.

2.2 Cultural characterization

The infected part of tubers was cut using a sterile, sharp knife. A suspension from plant ooze and exudates was prepared in sterile distilled water and then streaked onto Kelman's tetrazolium chloride (TZC) agar and 2% sucrose peptone agar (SPA). After incubation at 28°C for 24 to 36 hours, chartered colonies of *Ralstonia solanacearum* were selected on mediums. Isolates of *R. solanacearum* were maintained in sterile distilled water for the identification steps and stored at room temperature. Pure cultures were tested by biochemical and enzyme-linked immunosorbent assay (ELISA) methods. A mobility, gram negative reaction, catalase, amylolytic and lecithinase activity, liquefaction of gelatin, saccharolytic enzymes, the formation of indole, and other biochemical properties, were determined. For pigment formation tests, the liquid mediums of meat-peptone broth and tryptophan broth were used. More consistent results were obtained when L-tyrosine was added to the medium. Denitrification ability was tested using the semi-solid medium: 10% peptone, 5% NaCL, 2.0% KNO_3, 3.0 % Bacto Agar, and Hiss reagent.

2.3 The biovars test

The pathogen species is subdivided into races based on host range. To identify the biovar of pathogen species we have used a biochemical method based on the utilization of the disaccharides cellobiose, lactose, and maltose, and oxidation of the hexose alcohols dulcitol, mannitol, and sorbitol (French et al.,1995, Staskawicz et al., 2001).

2.4 Accumulation of *Ralstonia solanacearum* isolates in the host-plant tissue

Healthy potato tubers of different varieties were used for accumulation the pathogen culture in the host cell. The tubers were washed thoroughly with water, and then were sterilized in 96% ethyl alcohol. After that, they were thoroughly rinsed in

sterile water, cut into pieces, and placed in Petri dishes on wet, sterile filter paper. Bacterial suspensions at a concentration of 10^8 CFU/ml were infiltrated into potato slices. Inoculated slices were incubated at the optimal temperature (28°C) for the bacteria. The optimum moisture ensured the rapid growth of bacteria.

2.5 Pathogenesis assays on potato seedlings and plants

Three potato (*Solanum tuberosum*) cultivars were used for pathogenicity tests: Picasso (highly sensitive), Sante (medium resistance) and Nevskiy (high resistance).Three-week-old plants grown in soil were inoculated by soil drench without root severing. The concentration of bacterial inoculums was 10^8 CFU/ml. The experiment was repeated at least twice, giving a total of six test plants. Inoculated plants were kept in a room with natural light and a mean temperature of 25–27°C. The percentage of plants showing wilting symptoms was recorded during 28 days.

2.6 Immunoblot ELISA test (Agdia).

The *Ralstonia solanacearum* (RS) ELISA test was used with plant samples exhibiting symptoms of Rs and with bacterial culture samples. According to the protocol of DAS ELISA of Agdia, the samples were added to a microplate coated with monoclonal antibodies to EPS of Rs. If EPS is present in the sample, it is bound by antibodies and captured on the microplate during the incubation period. After incubation, the plate was washed to remove the unbound sample. An enzyme conjugate solution, containing a monoclonal antibody conjugated to peroxidase, is added and bound to any captured EPS. After incubation the plate is washed to remove any unbound conjugate. This final binding creates a sandwich of the target analyst between the two specific antibodies. Wells in which a blue color developed indicated positive results. Wells in which there was no significant color development indicated a negative result. Test results were valid only if positive control wells gave a positive result and buffer wells remained colorless.

2.7 Characteristics of biocontrol agents

The antagonistic microorganisms: *Streptomyces* species and *Trichoderma lignorium* used in this study were obtained from the laboratory collection of *Phytopathology* Laboratory (Plant Protection Department, Faculty of Agriculture, Kyrgyz-Turkish Manas University, Kyrgyzstan). *Streptomyces diastatochromogenesstrain* sk-6, *Streptomyces bambergiensis strain* sk-2-2 and*S. cirratus SK 2-8* were isolated from the rhizosphere of wild plants in the elevated mountain ecosystem of Son-Kul place (3400 m above sea level).The 16S rRNA genes of these strains were PCR amplified with 27f and 1522r primers, and PKS genes were screened by polyketide synthetase primers. *Trichoderma lignorium* was isolated from soil where red beets were grown. They had been selected as active antagonistic organisms after successive screenings against gram positive and gram negative bacteria, as well as pathogen fungi.

2.8 Evaluation of *Streptomyces species* and *Trichodermalignorium* as potential antagonists against the pathogen *Ralstonia solanacearum* in vitro

For screening the antagonists for activity against *Ralstonia solanacearum* we have used several in vitro methods: the cross-culture method, the filter paper method, and the perforated agar plate method. In the cross-culture method, each antagonist was streaked across the Petri plates, and after 72 hours, *R. solanacearum* was applied as streaks perpendicular to the antagonist. Plates were incubated at 28°C for 24–48 hours, after which interactions were examined and the distance of the inhibition zones was measured and recorded. In the filter paper method, the pathogen was poured onto Petri plates and the antagonist was introduced as filter paper disks 5 mm in diameter. The filter paper disks were dried at 40°C for one hour, and then placed on cultured pathogen plates. Plates were incubated for 24–48 hours at 28°C before interactions were examined. In the perforated agar plate method (also known as the bore-hole method) the pathogen was poured as a thin layer onto the plate, and then an 8 mm hole was made in the agar in which the antagonists were introduced. Plates were incubated for 24–48 hours at 30°C before examining antagonist-pathogen interactions.

2.9 Effects of antagonistic strains on potato wilt

Soil and potato seedlings were treated with the antagonists and their effects studied in indoor conditions. Microbial suspensions of antagonists and *R. solanacearum* were adjusted to a concentration of 10^8 CFU/ml by measurement at a wavelength of 585 nm (UV/VIS Spectrophotometer, JENWAY, UK, 2011).

The Picasso potato (highly sensitive) cultivar was used for evaluation of antagonistic actinomycetes strains *Streptomyces diastatochromogenesstrain* sk-6 and *Streptomyces bambergiensis* strain k1-3 in vivo tests. A sufficient volume of soil autoclaved at 121°C for 30 minutes was used to fill pots 18 cm tall and 30×13 cm in diameter, to a height of 15 cm. Picasso

cultivar healthy potato tubers were planted in soil. Three-week-old seedlings were watered with 20 ml (10^8cell/ml) of pathogen suspension/per plant every day. Then the wilt-susceptible seedlings were watered every day with 20 ml (10^8cell/ml) of antagonist suspension over seven days. In control pots, the wilt-susceptible seedlings were watered with 20ml plain water. The experiment was repeated at least twice, giving a total of six test plants in each variant. Inoculated plants were kept in a room with natural light and a mean temperature of 28°C. The percentage of plants showing the wilting symptom was recorded during 28 days. Disease record: The percentage wilt incidence (PWI) was calculated as follows:

$$\frac{\text{Total no. of plants receiving that treatment}}{\text{No. of plants wilted in a treatment}} \times 100\%$$

2.10 Statistical analysis

Data were analyzed following GLIM program of Royal Society of London (Crawley, 1995). Significant differences between two mean values due to treatments or varieties and their interaction at a crop growth stage were computed by comparing their significant levels at $P<0.05$.

III. RESULTS

3.1 Origin of isolates and organism characteristics

We analyzed potato tubers of Picasso, Sante, and Nevskiy varieties. *Ralstonia solanacearum* — as a pathogen of bacterial wilt — was obtained from the Picasso variety. Twelve isolates from the potato fields of Issuk-Kul and seven isolates from Chy regions were identified as *Ralstonia solanacearum* species.

Large, elevated, fluidal, and white colonies of isolated bacteria were grown after two days on the TZC medium, and white, fluidal with whorls characteristic colonies appeared on SPA. The organism was capable of growing at 28–36°C temperatures aerobically and it does not form endospores. The bacteria take the form of slightly thick sticks with dimensions of 0.7–0.9 microns, it is gram negative, motile, and non-encapsulated. Cells of obtained isolates *Ralstonia solanacearum* were motile when viewed microscopically, indicating its ability to be virulent. The isolates were catalase and oxidase positive. New isolates of *Ralstonia solanacearum* were able to reduce nitrate to nitrite. Changing the medium color to red and the formation of a layer of foam from an intensive gas release indicates a complete reduction of nitrate and denitrification (Fig.1).

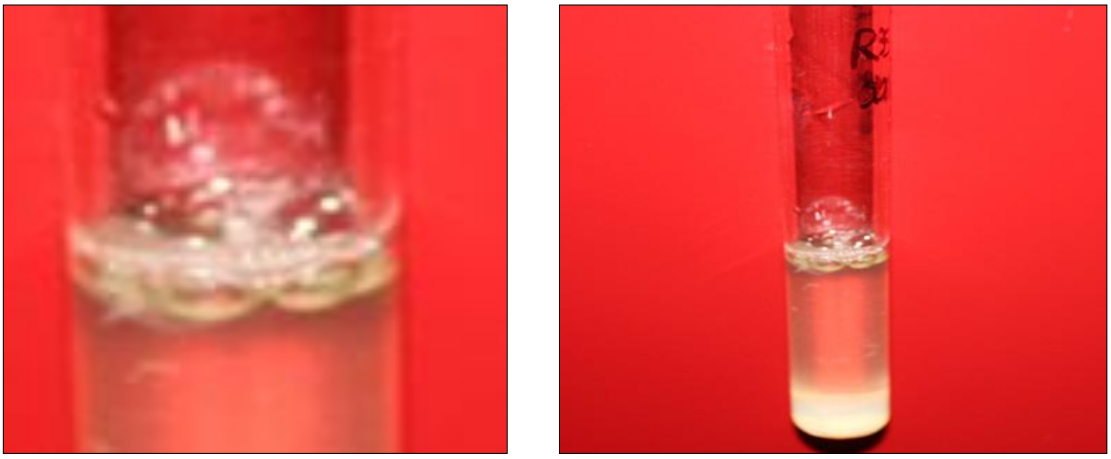


FIG.1. FORMATION OF A LAYER OF FOAM FROM AN INTENSIVE GAS RELEASE, INDICATING A COMPLETE REDUCTION OF NITRATE AND DENITRIFICATION BY *RALSTONIA SOLANACEARUM*

3.2 The biovars test

Specific host range and distribution of *Ralstonia solanacearum* depends on the race and the biovars of the pathogen. In Table 1, the data related to the relationship of race, biovars, host range, and geographic distribution of *Ralstonia solanacearum* are summarized. It is known the five races of potato brown rot. The most dangerous is Race 3, which affects the potatoes at low temperatures. The infection persists for a long time in plant debris and potato tubers (in a latent form), and it is common in temperate regions. Its main sources are infected soil, crop residues, and weeds of the genus *Solanaceae* (Kim et al., 2003).

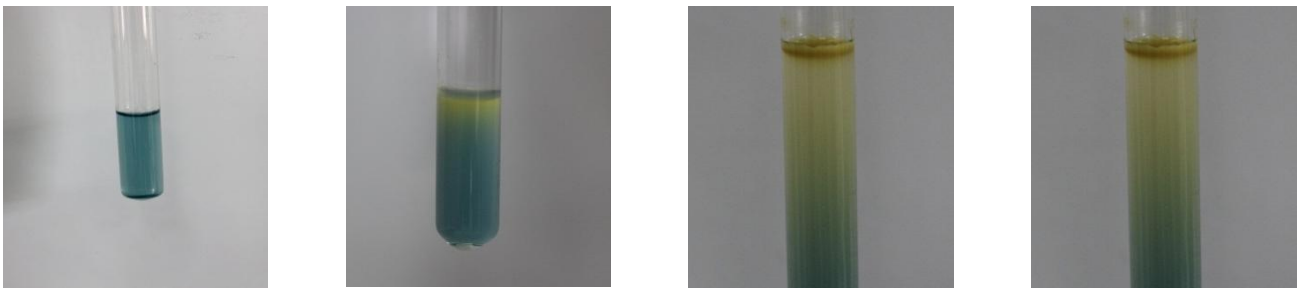
TABLE 1
RACES AND BIOVARS OF *RALSTONIA SOLANACEARUM*. (ADAPTED FROM DAUGHTREY, 2003)

Race	Host Range	Geographic Distribution	Biovar
1	Wide	Asia, Australia, the Americas	3,4 1
2	Banana, other Musa spp.	Caribbean, Brazil, Worldwide	1
3	Potato, some other Solanaceae, Geranium; a few other species	Worldwide, except US and Canada	2
4	Ginger	Asia	3,4
5	Mulberry	China	5

Isolated races *of Ralstonia solanacearum* by biochemical characteristics were classified as a 3-biotype, so they were able to oxidize the disaccharides cellobiose, lactose, and maltose and the hexose alcohols dulcitol, mannitol, and sorbitol. Table 2 illustrates the classification into biovars based on this method. When bromomethylBlau was used as an indicator the medium becomes yellow as a result of oxidation, and when Andred indicator was used the medium changes to red. Transformation of this substrate by isolates occurred slowly, for example as shown in Fig. 3 in the presence of bromomethylBlau indicating that an oxidation of dulcitol occurred only after 12 days.

TABLE 2
CLASSIFICATION OF *RALSTONIA SOLANACEARUM* INTO BIOVARS. (ADAPTED FROM FRENCH ET AL, 1995)

Physiological Tests	**Biovars**				
	1	**2**	**3**	**4**	**5**
Utilization of disaccharides Cellobiose	–	+	+	–	+
Lactose	–	+	+	–	+
Maltose	–	+	+	–	+
Oxidation of alcohols Dulcitol	–	–	+	+	–
Mannitol	–	–	+	+	–
Sorbitol	–	–	+	+	–



2 days 4-5 days 7-9 days 10-12 days

FIG. 3. AN OXIDATION OF DULCITOL BY *RALSTONIA SOLANACEARUM* ISOLATES IN THE PRESENCE OF BROMOMETHYLBLAU INDICATOR

3.3 Accumulation of *Ralstonia solanacearum* isolates in the host-plant tissue

In many cases, *Ralstonia solanacearum* bacteria are closely interrelated with secondary pathogens such as the causative agent of soft rot *Erwinia carotovora* var. *atroseptica*(Fegan and Prior,2005).This creates some difficulties for the isolation of a pure culture of *Ralstonia solanacearum* from the affected tissue. For the accumulation of the culture of the pathogen in the host cell and to determinate its virulence, bacterial suspensions of *Ralstonia solanacearum* at a concentration of 10^8 CFU/ml were infiltrated into sterile healthy potato slices. They were incubated at lower temperatures (22°C), in a moisture chamber. The optimum moisture level ensured the rapid growth of bacteria. The organism quickly began to multiply in infected host cells. On the third day, dark ringed circles appeared on potato slices. Gradually, a rotting of the entire surface of potato slices started. In five days, there was a complete decay, with the release of odors and the tissues turning into mucous (Fig. 4). Of all the varieties tested, only Picasso showed high sensitivity to rotting at low temperatures. These results allowed us to identify which varieties are more resistant or more susceptible to this disease. It is important to provide advice to farmers on which varieties are the best to grow in different climatic zones of the Republic. This test has additionally confirmed that obtained *Ralstonia solanacearum* new isolates are belong to biovar 3, which can survive at low temperatures. Some researchers have noted in their results that high temperatures and high soil-moisture levels generally favor *Ralstonia solanacearum*, the exception being certain Race 3 strains that are pathogenic in potatoes and are able to grow well at lower temperatures (Frenchet al., 1995).

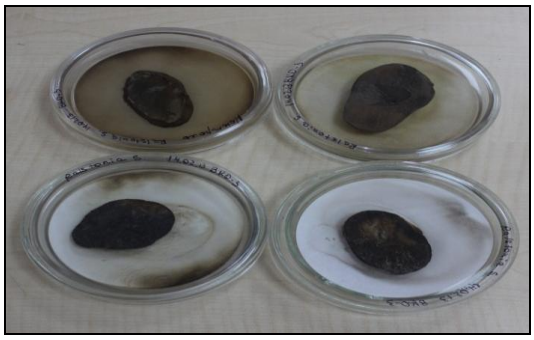


FIG.4. ROTTED POTATO TUBERS OF THE PICASSO VARIETY AT FIVE DAYS AFTER INFILTRATION OF A PATHOGEN SUSPENSION

3.4 Pathogenesis assays on potato seedlings and plants

Three potato (*Solanum tuberosum*) cultivars were used for pathogenicity tests: Picasso (highly sensitive), Sante (medium resistance), and Nevskiy (high resistance). The symptoms of disease in the Picasso variety plants began to appear between three to six days. The first symptoms of the disease were wilting leaves on the ends of branches. During disease development, the leaves turn chlorosis and eventually become necrotic. The part of the stem close to the ground in infected plants turns gray-brown. This is a characteristic symptom of potatoes' brown rot (Fig.5 A and B). In the variety of Sante, the symptoms of disease began to appear within two weeks, and the lower leaves turned browned and dry, yellowed and chlorosis. Stems stood relatively straight for long time, and then four weeks later started to bend. The Nevskiy variety was resistant to the pathogen-infected dose. Within six weeks there were no signs of disease. The specific symptoms were wilting of the leaves at the end of the day with recovery at night, the edges of the leaves turning black, and curling was observed within five to ten days, but no symptoms were observed on control plants treated with sterile water.

FIG.5: A CLOSE TO THE GROUND PART OF THE STEM OF INFECTED AN INFECTED PLANT BECAME GREY BROWN; INFECTED PLANTS SHOW YELLOWING, WILTING, AND BROWNING OF LOWER LEAVES, FOLLOWED BY NECROSIS.

3.5 Immunoblot ELISA test (Agdia).

Detection and identification of the pathogen by ELISA (Agdia product, USA) performed directly from diseased potato stems and leaves at a concentration of 10^3–10^4 cells/ml. Wells in which a blue color developed indicated positive results. The bacterium was re-isolated from the infected leaves and stems, and identified as described above (Fig.6).

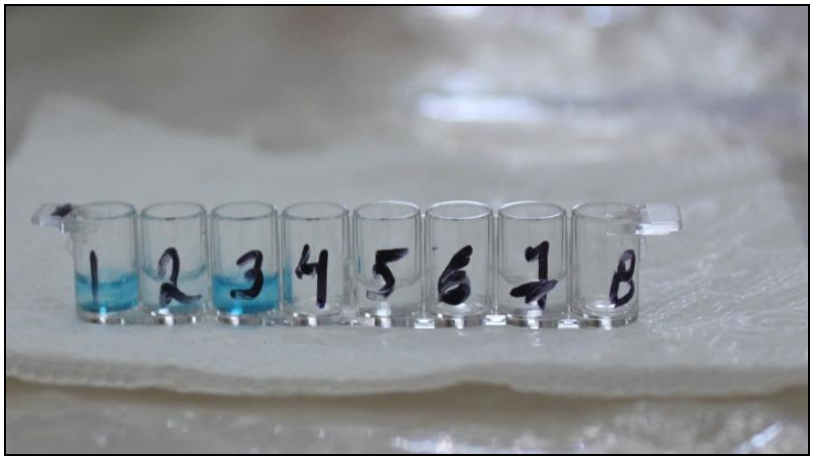


FIG.6. WELLS WHERE A BLUE COLOR DEVELOPED INDICATED POSITIVE RESULTS FROM DISEASED POTATO STEMS AND LEAVES AT A CONCENTRATION OF 10^3–10^4 CELLS/ML.

3.6 Evaluation of *Streptomyces species* and *Trichoderma lignorium* as potential antagonists against the pathogen *Ralstonia solanacerum* in vitro

Primary and secondary screening by the cross-culture method have showed that the strain *Streptomyces diastochromogenes SK-6,6* inhibited the growth of *Ralstonia solanacearum* in 72 hours and the zone of lyses was 8.2 ± 1.25 mm. *Streptomyces bambergiensis strain SK-2-2* showed an inhibition zone — 7.9 ± 1.25 mm in the same time. *Streptomyces cirratus*SK-2 and *Trichoderma lignorium* strains had no biological effects on *Ralstonia solanacearum* cultures (Table 3). When using the filter paper and perforated agar plate methods, the antagonistic effects of *Streptomyces diastochromogenes SK-6,6* and *Streptomyces bambergiensis* strain *SK2-2* demonstrated itself not only by suppressing the growth of the pathogen culture but by the expression of hyper-parasitism, so that in 120 hours the growth of *Ralstonia solanacearum* was completely inhibited by these antagonists (Fig.7). *Streptomyces cirratus* SK-2 and *Trichoderma lignorium*strains had no biological effects on *Ralstonia solanacearum* cultures in vitro bioassays. These results meant that for the following tests in soil conditions, only two strains of *Streptomyces genus* were used:*Streptomyces diastochromogenes SK-6,6*and *Streptomyces bambergiensis Sk-2-2*.

TABLE 3
MEAN DISTANCE OF INHIBITION ZONE BETWEEN EACH ANTAGONIST AND *R. SOLANACEARUM* FOR THE THREE METHODS TESTED (N=3).

Treatment	Inhibition zone (mm) for the three methods tested at 72 and 120 hours		
	Cross streak	**Filter paper disk**	**Perforated agar plate**
Streptomyces bambergiensis strain s k -2-2	8.2 ± 1.25	Hyper-parasitism	Hyper-parasitism
Streptomyces bambergiensis strains k -1-3	7.9 ± 1.21	Hyper-parasitism	Hyper-parasitism
*Streptomycescirratus*SK 2	No inhibition zone	No effect	No effect
Trichoderma lignorium	No inhibition zone	No effect	No effect

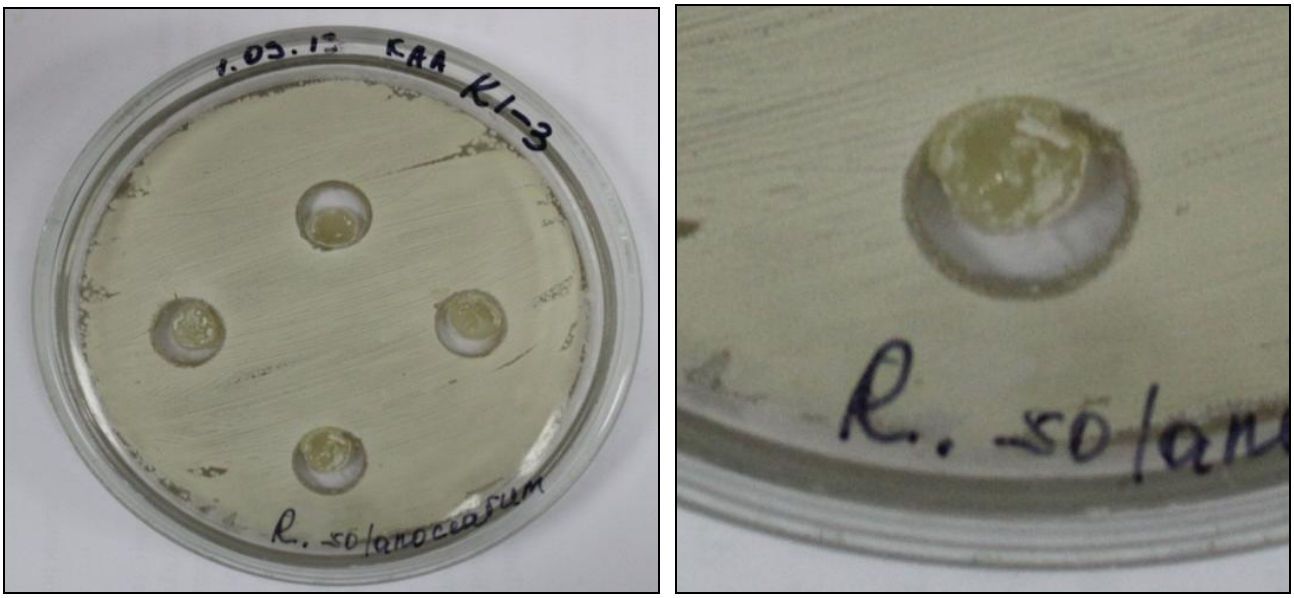


FIG.5. HYPER-PARASITIC EFFECT OF *STREPTOMYCES BOMBARGIENSIS* (K1-3) IN 120 HOURS TO *R. SOLANACEARUM* CULTURE

3.7 Effects of antagonistic strains on potato wilt in vivo

Picasso potato (high sensitivity) cultivar (*Solanum tuberosum*) was used for evaluation of antagonistic actinomycetes strains *Streptomyces diastatochromogenesstrain* sk-6 and *Streptomyces bambergiensisstrain* k1-3 in vivo tests. Soil and potato seedlings were treated with the antagonists and their effects studied in soil conditions. Root zone and soil application of *Streptomyces diastatochromogenesstrain* sk-6 and *Streptomyces bambergiensisstrain* k1-3 at a dose of 10^8 cell/ml significantly reduced disease incidence and increased the growth of potato plants. The disease's progress was reduced by 60% and 56% in plants inoculated with *Streptomyces diastatochromogenesstrain sk-6 and Streptomyces bambergiensis strain k1-3,* respectively (table 4). The study revealed that sk-6 and k1-3 strains are promising strains whose effectiveness under field conditions and their mode of action should be investigated.

TABLE 4
EFFECT OF STREPTOMYCES STRAINS CELL SUSPENSION APPLIED ON PERCENTAGE WILT INCIDENCE (PWI) OF POTATOES IN POT EXPERIMENT

Treatments	Percentage wilt incidence
Root + Soil treatment with *Streptomyces diastatochromogenesstrain* **sk-6 (10^8cell/ml)** + *R. solanacearum* **(10^8cell/ml)**	34.56 (33.75)
Root + Soil treatment with *Streptomyces bambergiensis* strain *sk-1-3* **(10^8cell/ml)**+ *R. solanacearum* **(10^8cell/ml)**	35.7 (36.2)
Inoculated control (only R. Solanacearum, **(10^8cell/ml)**)	100.00 (99.95)
Uninoculated control (No *R. solanacearum*)	0.00 (0.05)

FIG.6. A. RECOVERY OF WILT POTATO PLANT WATERED BY BIOLOGICAL AGENT; B. IN THE CONTROL VARIANT WITH WILT SYMPTOMS

In this study, we have used well-known, efficient methods and bioassay for systematic screening of *R. solanacearum* for identification of its phenotype and biochemical profile, as well as for pathogenicity and virulence. As a result, an aggressive race — Biovar 3 — was most isolated from the potato fields of the Tup district of the Issyk-Kul region, especially in fields where the Picasso variety was grown. This area is characterized by a more wet and temperate climate than other areas of the Issyk-Kul region. The low percentage of affection with this pathogenic agent was noted in the Sante variety. The pathogen was no almost obtained from the Nevskiy variety plants and tubers. In this region, the pathogens were isolated from growing plants with characteristic symptoms and tubers after harvest in storage, they were available for sale. In Chy region, where the climate is hot and the humidity is relatively low (An overview of the emergence and spread of major pests and diseases, 2011), pathogenic races of *R. solanacearum* were obtained from the Picasso and Santa potato varieties. In this region, essential isolates were obtained from the tubers for sale, or in storage. We have not found *R. solanacearum* species to be causative agents of wilt in local potato varieties (red and white crumbly) grown in mountainous areas of the Kochkor district. This indicates that the disease has penetrated into Kyrgyzstan from neighboring countries, together with planting material.

Our results have revealed for the first time in Kyrgyzstan the presence of the *Ralstonia solanacearum* bacterium as a pathogen of bacterial wilt (quarantine for the country object) in the potato fields of the Issyk-Kul and Chy regions. Our results have allowed us to determine which varieties are most susceptible to the disease and to which district its wide dissemination constitutes the biggest threat. This is important for informing farmers which varieties they should buy for planting. The areas in which commercial varieties have not yet been introduced should be remaining zones clean from this disease.

IV. CONCLUSION

The isolated indigenous strains of *Streptomyces diastatochromogenes strain sk-6 and Streptomyces bambergiensis strain k1-3* has the potential to be used as a biocontrol agent for the management of the bacterial wilt of potatoes, as indicated by the reduced percentage wilt incidence. The most suitable method of application of the antagonist suspension was found to be the root + soil method. Besides biocontrol properties, the antagonist suspension applied by the root + soil method also showed best performance in physiological and biochemical parameters indicating plant growth. However, the effective biocontrol agent can be applied under field conditions or further commercialized only when immobilized in certain carriers. Thus, formulations of the biocontrol agent should be prepared for easy application, storage, commercialization, and field use.

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