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SCREENING BACTERIAL ANTAGONISTS TO COMMON SCAB DISEASE

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ABSTRACT

Common scab caused by *Streptomyces* species is an important disease worldwide. Huge economic losses are faced because of the disease every year and control options are scant. This study was conducted to develop effective biocontrol agents, which have capability of enhancing potato yield. A total of 1048 bacterial isolates from former studies were tested against *S. scabiei* in-vitro and 22 out of these strains were found to be effective. Most effective four strains (KBA-10, K-19B, TV-91C and RK-92) were chosen for field trials. All four strains can fix nitrogen and dissolve phosphate. According to the result, KBA-10, TV-91C, RK-92 and K-19B have biocontrol efficacy of 60.35%, 37.2%, 33.5% and 18.7%, respectively. They increased potato yield 40.0%, 41.1%, 25.52% and 20.42 %, respectively, compared to control. Increases provided by KBA-10, K-19B and TV-91C were found to be statistically significant. Biocontrol efficacies of K-19B, TV-91C and RK-92 were of lesser degree, compared to KBA-10. Additionally, KBA-10 generated an extensive rise (71.7%) in tuber number. KBA-10 can be a good biocontrol agent for reducing potato scab disease and enhancing crop yield. Moreover, KBA-10 can be used in tuber seed production.

Keywords: Common scab, potato, biological control, *Streptomyces scabiei.*

INTRODUCTION

Potato is a widely cultivated plant and some researchers enounced that potato can contribute to reducing food shortages in the world. However, potato is susceptible to diseases caused by bacteria, fungi or viruses (Agrios, 2005). Common scab disease caused by *Streptomyces* species is one of them.

Streptomycetes are spore-forming gram-positive bacteria found in soil in large numbers and are a specialized bacterial group with high G+C content. Some of them can produce numerous antibiotics (Kieser *et al*., 2000). *Streptomyces* species can also cause symptoms in tuber and root crops including beet, radish, turnip, peanut, sweet potato and carrot. Additionally, they can harm seedlings of some monocotyledonous or dicotyledonous plants (Wanner, 2009). Most important damages of *Streptomyces* species occur in potato and they cause significant quality loss in tubers. They lead to different symptoms like netted scab, pitted scab or surface scab (Stead and Wale, 2004). Farmers generally do not harvest potato tubers, which are affected by common scab. In addition to decreasing yield, these tubers serve as inoculum source for next vegetation period.

Streptomyces damage in potato was reported in many countries including USA and Canada (Lambert and Loria, 1989a, b; Goyer *et al*. 1996; Wanner, 2007; St-Onge *et al*., 2008; Jiang *et al*., 2012), France (Bouchek-Mechiche *et al*., 2000), Japan (Miyajima *et al*., 1998), Korea (Park *et al*., 2003), United Kingdom (Thwaites *et al*., 2010), Uruguay (Lapaz *et al*., 2012), Norway (Dees *et al*., 2013), Germany (Leiminger *et al*., 2013), Spain and Netherlands (Flores-Gonzalez *et al*., 2008), Iran (Cao *et al*., 2012), Algeria (Bencheikh and Setti, 2007), Finland and Switzerland (Lethonen *et al*., 2004), Turkey (Karahan, 2006; Karagoz, 2013).

There are different strategies for coping with the disease, which involve chemical, biological, and cultural means or use resistant varieties. However, commercially unimportant varieties have resistance and none of them are fully resistant (Zadina *et al*., 1975), and cultural practices such as control of the soil pH and irrigation, are recommended, but it is generally difficult to establish desired disease control using these methods (Tomihama *et al*., 2016). Therefore, it is important to develop new control strategies.

Biological control including environmentally friendly applications is one of the most important disease control approaches. Since it has no deleterious effects on soil and warm-blooded organisms, this approach is a popular one among researchers. There are some studies aimed to control common scab disease by nonpathogenic *Streptomyces* sp. or other nonpathogenic bacteria (Han *et al*., 2005; Hiltunen *et al*., 2009; Meng *et al*., 2013; Wanner *et al*., 2014; Arseneault *et al*., 2015).

In this study, we aimed to assess the biocontrol efficiency of four bacterial strains against common scab disease and favorable effects of these strains on plant growth in field conditions.

MATERIALS AND METHODS

Bacterial strains: One thousand and forty eight bacterial strains isolated from under-ground or aboveground parts of wild and cultivated plants in several studies were evaluated for their antagonistic potential against *Streptomyces scabiei* on petri plate assays. Most of the strains have ability of nitrogen fixing and / or phosphate solubilizing (Karagoz *et al*.,2012; Karagoz and Kotan, 2010; Cakmakci *et al*., 2010; Erman *et al*., 2009). All the 1048 strains were deposited in culture collection of Ataturk University, Agricultural Faculty, Department of Plant Protection. Pathogen strain, *Streptomyces scabiei* KS-196, was isolated from symptomatic potatoes in field. Pathogenicity tests were performed. The strain was also identified using classical and molecular methods and was deposited in GenBank with KR422360 accession number.

In-vitro petri assays: Pathogen strain was grown on Oat Meal Agar (OMA) for one week at 28 ± 2 ºC. Then one loop spore was transferred to Oat Meal Broth (OMB) and incubated in same conditions at 200 rpm. After the incubation period, bacterial cells were harvested by centrifugation at 8000 x g for 10 min and were rinsed twice with sterile distilled water (sdH_2O). Bacterial density was adjusted to ~ 1 X 10^6 cfu ml^{-1} with serial dilutions. Candidate antagonistic strains were grown on Nutrient Agar (NA) medium for 24 h, at 26 ± 2 ºC. Single colony was transferred to Nutrient Broth (NB) and incubated in same conditions at 200 rpm. Then bacteria were harvested by centrifugation at 5000 x g for 5 min and were rinsed twice with sdH_2O. Bacterial density was adjusted to OD_{600} = 0.5 with sdH_2O.

Inhibitory effects of the candidate antagonistic strains were defined with agar disc diffusion method (Kimura *et al*., 1998). Briefly, 50 µl of pathogen solution was pipetted on OMA and was spread with sterile swab. Then 10 µl of candidate bacteria solution was pipetted on the 6 mm sterile disc, placed on center of the pathogen inoculated OMA. The plates were incubated for one week at 28 ± 2 ºC. After the incubation period, inhibition zones around the discs were recorded.

Identification of candidate antagonistic strains: Twenty-two out of 1048 strains exhibited antagonistic effect against *Streptomyces scabiei* and all strains were identified by Microbial Identification System (MIS). Analysis of whole cell fatty acid of bacterial strains were conducted according to the method described by the manufacturer`s manual (Sherlock Microbial Identification System version 5.5 MIDI, Inc., Newark, DE, USA). All strains were grown on Difco Trypticase Soy Agar (TSA) for 48 h at 26 °C. Approximately 40 mg cells (wet weight) were transferred to 13x100 mm glass tubes fitted with Teflon-lined screw caps and fatty acids methyl esters (FAMEs) were extracted. FAMEs were separated by gas chromatography (HP6890, Hewlett Packard, Palo Alto, CA, USA) with a fused-silica capillary column (25 m x 0. 2 mm) with cross-linked 5% phenyl methyl silicone. A FAME profile and similarity index of each bacterial strain was identified by comparing the commercial databases (TSBA 50) with the MIS software package. Additionally, four strains used in field trials were identified by 16S rDNA sequencing. Sequencing was performed via next generation sequencing (Intergen, C.O, Ankara, TURKEY). Sequence data were edited and analyzed using the BioEdit Sequence Alignment Editor 7.2.5.0 software. All sequence data obtained were confirmed by BLAST searching and were deposited in GenBank.

Pathogenicity tests of candidate antagonistic strains: Pathogenicity tests were also performed for the four strains chosen for field study. Potato tubers (*Solanum tuberosum* cv. *agria*) were surface sterilized with 0.1% mercuric chloride and washed extensively with sdH_2O. Then tubers were inoculated with 0.2 ml candidate strain suspension and were put inside sterile polysterilene bags. Tubers were incubated to evaluate symptoms occurring at 26 ± 2 ºC for one week at humid conditions.

Field trials: Four antagonistic bacterial strains (KBA-10, K-19B, TV-91C and RK-92), which strongly reduced *Streptomyces scabies* growth on petri plates and have ability of solubilizing phosphate and fixing nitrogen were selected for evaluating the disease suppressing and potato yield in field conditions.

Potato tubers (*Solanum tuberosum* cv. agria) having about five eyes were surface sterilized with 0.1% mercuric chloride and washed extensively with sdH_2O. Potato tubers were dipped in antagonistic bacterial suspension (~ 2 × 10^9 cfu/ml), which were grown in NB and re-suspended in sdH_2O, for five hours. Then, potato tubers were dipped in the pathogen inoculums, which were grown in oat meal broth at 28 C for 7 d (~ 10^6 cfu ml^{-1}) and re-suspended in sdH_2O, for one h. Then tubers were seeded to seedbed. Control treatment was performed by using only pathogen *S. scabiei* inoculums. The experiment was conducted in completely randomized design with 10 replicates. Plants were regularly watered. Second and third inoculums of antagonistic bacteria maintained at 2 × 10^9 cfu ml^{-1} were given to root area of the plants by sterile syringe after 25 and 50 days of germination (1 ml for each plant).

Evaluation of yields and infection rate: Plants were harvested after 110 days and the data was taken for potato yield (kg) and number of potato tubers in each seedbed. Data were analyzed by SPSS 18.0 and increases compared to the control application were recorded as percentage.

The infection rate was measured at the time of harvest by determining of common scab at tuber skin according to the following formula (Han *et al*., 2005) for

each seed bed and average of the infection rates of total 10 seed beds were recorded as infection rate

Infection rate (100 %)

= Number of potatoes with lesions over 0.5 cm / Number of total potatoes

Then, % biocotrol efficacies of the strains were evaluated by Abbott's formula:

Biocontrol efficacy (%)

= (1- infection rate of treatments / infection rate of control) X 100

RESULTS

Antagonistic activities of 1048 isolates were evaluated against *S. scabiei* in Petri plates. 22 out of these strains were shown to possess antagonistic effect in various degrees. It is observed that inhibition zones of the strains were between 12 and 50 mm. According to the MIS identification results, 15 of the effective strains were defined as *Bacillus* sp., 3 as *Pantoea* sp., 3 as *Pseudomona*s sp. and 1 as *Paenibacillus* sp.

The strains (KBA-10, K-19B, TV-91C and RK-92) chosen for field trials were also identified by 16S rDNA sequencing. Partial sequnce of KBA10, K-19B, TV-91C and RK-92 were deposited in GenBank with MG547766, MG547768, MG547767 and MG547769 accession numbers, respectively. MIS identification results, 16S rDNA sequencing identification results, nitrogen fixing and phosphate solubilizing abilities and inhibition zones in Petri plates were presented in Table 1.

Pathogenicity on potato of four strains chosen for field trials were also evaluated. None of them caused symptoms on potato tuber. Then, these four strains were used for field trials.

All applications reduced the infection rate to varying extent. Infection rates of potatoes were defined as 34.3 %, 28.0 %, 22.8 %, 21.5 % and 13.6 % for control, K-19B, RK-92, TV-91C and KBA-10 applications, respectively. Biocontrol efficacies of the strains were evaluated as 18.7 %, 33.5 %, 37.2 % and 60.35 % for K-19B, RK-92, TV-91C and KBA-10, respectively. Results were presented in Table 2.

According to the field experiment results, all four strains led to increases in potato tuber weight compared to control. The increases provided by TV-91C, KBA-10, RK-92 and K-19B were defined as 41.1%, 40.0%, 25.5% and 20.42%, respectively. The increases provided by TV-91C, KBA-10 and RK-92 were statistically important. While Strain K-19B increased the tuber weight, this strain led to decrease in tuber number but this was not statistically important. TV-91C, KBA-10 and RK-92 applications also increased tuber number. KBA-10 application brought about an extensive and statistically important increase (71.7%). Results were presented in Table 2.

Table 1. Some properties of candidate strains, and inhibition zones caused by the strains in Petri plates.

Strain	MIS identification	SIM (%)	16S rDNA Sequencing	idnt. (%)	N	P	inhibition zone (mm)
KBA-10	*Bacillus megaterium*	49.0	*Bacillus zhangzhouensis*	99	+	+	50
K-19B	*Pseudomonas putida*	80.3	*Pseudomonas cedrina*	99	+	+	48
TV- 91C	*Bacillus megaterium*	47.4	*Bacillus zhangzhouensis*	99	+	+	47
RK-92	*Panteoa agglomerans*	88.9	*Panteoa agglomerans*	99	+	+	46
TV-6F	*Bacillus subtilis*	83.1	NP	NP	+	-	45
TV-17C	*Bacillus subtilis*	67.7	NP	NP	+	+	45
FDG-37	*Pseudomonas fluorescens*	22.2	NP	NP	+	+	45
TV-13B	*Bacillus subtilis*	68.7	NP	NP	+	+	38
TV-20E	*Bacillus megaterium*	51.9	NP	NP	+	-	35
TV-12E	*Peanibacillus polymyxa*	55.1	NP	NP	+	+	30
TV-42A	*Pseudomonas putida*	11.3	NP	NP	+	+	25
TV- 90E	*Bacillus megaterium*	59.1	NP	NP	+	-	22
TV-95A	*Bacillus megaterium*	50.2	NP	NP	+	+	22
TV-3D	*Bacillus megaterium*	56.3	NP	NP	+	+	21
TV-22B	*Bacillus megaterium*	43.1	NP	NP	+	+	20
TV-12H	*Bacillus subtilis*	74.4	NP	NP	+	-	20
TV-73A	*Bacillus pumilus*	65.0	NP	NP	+	+	20
TV-67C	*Bacillus pumilus*	63.0	NP	NP	-	-	17
TV-83A	*Bacillus pumilus*	56.8	NP	NP	+	-	14
RK-103	*Bacillus pumilus*	62.6	NP	NP	+	+	14
TV-91D	*Panteoa agglomerans*	19.3	NP	NP	+	-	14
TV-131D	*Panteoa agglomerans*	42.2	NP	NP	+	+	12

SIM: similarity index, idnt: identification N: nitrogen fixing, P: Phosphate solubilizing, +: positive, -: negative, NP: not performed,

Table 2. Effects of the candidate strains on yield and disease development in field.

Application	Tuber weight (gr/seed bed)	Increase (%)	Tuber number (Number / seed bed)	Increase (%)	Infection rate (%)	Biocontrol efficacy (%)
K-19B	1145.70±210.35	20.4	13.80±4.78	-	28.0	18.7
KBA-10	1332.00±183.15*	40.0	23.70±3.71*	71.7	13.6	60.3
RK-92	1194.20±359.31*	25.5	16.30±4.78	18.1	22.8	33.5
TV-91C	1342.50±207.46*	41.1	16.20±3.70	17.3	21.5	37.2
Control	951.40±107.34	-	13.80±4.18	-	34.3	-

* **;** mean statistically important application according to Duncan's multiple range test at $p<0.05$

DISCUSSION

For sustainable agriculture, protection of environment is as important as improving crop yields and reducing plant disease. Chemicals are widely used for control of plant disease , but this cause environmental pollution and ecological destruction. So as to deal with these problems, researchers have been working extensively on effective biocontrol strategies, which support plant growth at the same time.

Obviously, potato common scab disease widely exists in the world and available options to control the disease are inadequate (Al-Mughrabi *et al*., 2016). The current study was conducted for developing effective biocontrol agents having capability of improving potato yield. A sum of 1048 bacteria were tested against *S. scabiei*, and 22 out of these showed antagonistic effects in-vitro. Most effective four strains were chosen for field trials. These four strain can fix nitrogen and can solubilize phospathe (Karagoz *et al*.,2012; Karagoz and Kotan, 2010; Cakmakci *et al.*, 2010; Erman *et al*., 2009). Our result showed that strains used reduced the severity of common scab disease. Especially KBA-10 contributed to substantially reducing disease severity. While the infection rate of the control application was estimated as 34.3%, KBA-10 application reduced the infection rate to 13.6% and biocontrol efficacy of the strain was recorded as 60.35%. In a former research, *Bacillus* sp. reduced the common scab disease by 40 % in-vitro (Han *et al*., 2005). Iturin A was evaluated as major component of *Bacillus* sp. and reserachers mentioned that this component is main inhibition factor effective on *S. scabiei* sporulation. Production of Macrolactin A, which have antibacterial and antiviral activity (Gustafson *et al*., 1989), by this strain was also reported. In the current study, any component produced by KBA-10 was not characterised. However, similar components can be produced by KBA-10.

In other research, *Bacillus amyloliquefaciens* BAC03 caused disease reduction with the efficacy ranging from 17% to 57% in field trial over two years. Additionally, chesnut tissues, ground horseradish, canola and clove essential oils were tested against the disease. All applications caused significant reduction of disease severity but the other applications were found to be less effective compared to the BAC03 (Meng *et al*., 2013). This can be considered as circumstantial evidence about *Bacillus* strains are very useful for reducing scab disease. *Bacillus* species can produce many biologically active metabolites. Additionally, they are resistant to heat, UV irradiation, organic solvents and can form endospore (Han *et al*., 2005). For these reasons, *Bacillus* species are preferable biocontrol agents. Schmiedeknecht and co-vorkers reported that some *Bacillus* applications reduced disease severity up to 70% in greenhouse and up to 67 % in addition to 16% potato yield increasing in field conditions (Schmiedeknecht *et al*., 1998).

In a research conducted in Canada, researchers aimed reducing scab disease and enhancing crop yield. Mustard meal, fuludioxonil and *Bacillus subtilis* treatments reduced disease severity 63.1%, 57.8% and 56.1%, respectively, in field conditions. In the same research, it was reported that high rate of chloropicrin (80 lb $acre^{-1}$) reduced common scab severity by 66%. Additionally, mustard meal increased marketable yields of potato by 24.6%, *Bacillus subtilis* 32.5% and fludioxonil 24.6% (Al-Mughrabi *et al*., 2016). It is clear that high dose chemical applications is only as effective as biocontrol agent applications. Thus, harmless applications for the environment are more preferable.

When all data and literature knowledge is considered, it can be seen that KBA-10 has great potantial for potato cultivation. This strain strongly inhibited the disease development and improved crop yields by 40.0%. In addition, KBA-10 increased tuber number by 71.7%. This is especially important for the production of high quality tuber seed with enhanced yield.

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