ISSN 1644-0692
www.acta.media.pl

ACTA SCIENTIARUM POLONORUM *Acta Sci. Pol. Hortorum Cultus, 14(5) 2015, 29-40*

BIOLOGICAL CONTROL OF ROOT ROT DISEASE CAUSED BY *Rhizoctonia solani* Kühn ON POTATO AND BEAN USING ANTAGONIST BACTERIA

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Abstract. In this study the use of 73 bacteria, isolated from rhizosphere area of tea (*Camellia sinensis*) plants in Black Sea Region in Turkey, as potential biocontrol agent against root rot disease caused by *Rhizoctonia solani* that was known as an important disease on potato and bean plants in Turkey and in the world were investigated. In *in vitro* conditions, 73 bacteria used for antagonistic tests, and 15 out of 74 were found to be effective against *R. solani* and formed between 0.20–2.30 cm inhibition zone and inhibited mycelium development of the pathogen. *In vivo* pot experiment also showed that antagonist bacteria strains had various level inhibition effect (between 12% and 83%) on *R. solani* compared to control. The study revealed that biocontrol agents might play an essential role in management of root rot diseases in potato and bean.

Key words: bean, potato, *Rhizoctonia solani*, biological control

INTRODUCTION

Rhizoctonia solani Kühn is one of the most important pathogen caused yield and quality losses in many plant species in particular on potato and bean throughout world, and *R. solani* causes damping off, root rot, root crown, leaf and stem blight particularly on agricultural crops [Jehtonen 2009, Kilicoglu and Ozkoc 2010].

The pathogen originated both in soil and seeds is a strong pathogen because it develop quickly on foods, colonized easily on host plant's root surfaces and spreading over on all epidermal cells in infected areas. *R. solani* is found in all natural soils. It can

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survive indefinitely in the soil through saprophytic mycelial growth or as small, hard, round to egg-shaped, brown to black bodies (1 to 10 mm diameter) called sclerotia and continuous cropping with susceptible crops such as beans, sugar beets or potatoes can increase pathogen population levels in soils [Aydın et al. 2011]. Irrigation water and soil movement spreads propagules of *Rhizoctonia*, and moderate to high soil moisture and low soil temperatures favors seedling disease. Damage is primarily limited to seedlings, but older plants can be affected if stressed by extremes in temperature generated by warm soil and cold irrigation water [Naeimi et al. 2010].

On worldwide basis different anastomosis groups (AGs) of *R. solani* that have different virulence level, has been identified [Jehtonen 2009]. Previous studies showed that AG-3 groups of *R. solani* is responsible disease in potato however the other anastomosis groups such as AG-2 type 1, AG-2 type 2, AG-4 and AG-5 are also included in infection both in tuber and stems [Demirci and Doken 1993]. The *R. solani* anastomosis groups AG-4 and AG-5 are mainly responsible disease in bean [Eken and Demirci 2004].

Control of the pathogen is difficult because of its ecological behavior, extremely broad host range and the high survival rate of sclerotia under various environmental conditions [Groth and Bond 2006]. In agriculture, crop protection relies heavily on chemical pesticides. However, there is a growing concern for negative health and environmental effects of such pesticides. Application of epiphytic bacteria as biological control agents is considered a nonpolluting approach for alternative plant protection. This biological control relies on a reduction of the pathogen population size by the control organism, thus keeping pathogen density below the threshold necessary for disease formation. There is now unequivocal evidence that antibiotics play a key role in the suppression of various soilborne plant pathogens by antagonistic microorganisms [Braun et al. 2010, Wensing et al. 2010].

Previous studies carried out in both field and greenhouse conditions showed that some fungal antagonists such as *Trichoderma* spp. and *Gliocladium* spp. have been used successfully against pathogen as biocontrol agent [Jehtonen 2009, Aydın et al. 2011].

Understanding the mechanisms of biological control of plant diseases through the interactions between antagonists and pathogens may allow us to select and construct the more effective biocontrol agents and to manipulate the soil environment to create a conducive condition for successful biocontrol. The mechanisms of biocontrol may involve and be divided into (i) antibiosis, (ii) competition, (iii) mycoparasitism, (iv) cell wall degrading enzymes, and (v) induced resistance. However, these mechanisms of biological control areprobably never mutually exclusive. They may include one and more processes [Adams 1990].

Previously it was shown that *Pseudomonas* spp., *Bacillus* spp. and *Streptomyces* spp. could be used effectively as biocontrol agent for control of *R. solani* infections [Tariq et al. 2010, Huang et al. 2011].

At present, there is limited knowledge and experience regarding the biological control of damping off and root rot disease caused by *R. solani* in Turkey, particularly in Eastern part of the country. Therefore, the main objective of the current study was to screen rhizospheric bacteria for *in vitro* antagonism against *R. solani* in order to select potential biological control agents, which could be used in the field.

MATERIAL AND METHODS

Material

In order to determine antagonistic effect of bacteria strains against soil-borne pathogen, *R. solani*, potato and bean cultivars are used. The potential biocontrol agent had been isolated from rhizosphere region of tea plants (*Camellia sinensis* L.) in both Trabzon and Rize provinces in Norteastern Turkey. A total of 73 potential biocontrol agent bacteria including the genera *Phyllobacterium, Arthrobacter*, *Brevibacillus*, *Bacillus*, *Burkholderia*, *Citrobacter*, *Enterobacter*, *Paenibacillus*, *Rhizobium*, *Stenotrophomonas*, *Rhodococcus*, *Pantoea*, *Chryseobacterium*, *Photobacterium*, *Lysobacter*, *Kurthia*, *Microbacterium*, *Lysobacter*, *Achromobacte*, *Acinetobacter* and *Kocuria* has determined and tested on pathogen. The selected bacteria have nitrogen fixing and phosphorus solubilizing capacity as well. In the study, a total 3 *R. solani* isolates (Rs-pat, B-227 and B-1) used, and Rs-pat isolated from potato, the B-227 and B-1 isolated from root and root crown of bean plants. The anastomosis groups of Rs-pat, B-277 and B-1 are AG-3, AG-4 and AG-5, respectively.

Method

Preparation of antagonistic suspensions. The collected bacterial isolates were stored -80ºC and grown on nutrient agar (NA) medium and incubated for 48 hour at 28ºC. After incubation a single colony was transferred to 500 ml flask containing nutrient broth (NB) medium. Sucrose has been added to strength adherence of antagonists to suspension. The contaminated liquid nutrient medium incubated at 28ºC in a rotary shaker at 140 rpm for 24 h. The bacterial suspension was diluted in sterile distilled water to a final concentration of 1×10^8 CFU/ml and resulting suspensions were used to treat potato tubers and bean seeds.

Preparation of *Rhizoctonia solani* inoculum. The pathogen has been developed on potato dextrose agar (PDA) for 1 week at 25°C. The wheat grain used as inoculum medium moisturized with pure water boiled for a period placed in bottles and sealed and autoclaved 2 day intervals at 121°C for 1 hour. Five pieces (4 mm – diameter) mycelial agar disc from the developed *R. solani* cultures on PDA medium inoculated on sterile wheat grain and bottles incubated for 3 weeks at 25°C [Demirer Durak 2011].

***In vitro* screening of potential antagonists.** The antagonist activity of the 73 preliminarily selected bacterial isolates was previously evaluated in Petri dishes by dual-culture pathogen mycelia-bacterial cells [Tian et al. 2003]. PDA plates were inoculated by a streak of the antagonistic bacterial strains. A disc (4mm in diameter) of the fungi was punched out with a sterilized corkborer from advancing zones of the fresh culture and placed on either side of bacteria inoculated place. To assess bacterial inhibitory activity, the size (cm) of a distinct inhibition zone, likely produced by the antagonistic bacteria toward the pathogen, was recorded after 7 days of incubation at 26°C. The experiment was repeated three times.

In *in vitro* tests, measurement of inhibition zone done when control mycelium filled with Petri dishes completely. Inhibition zone was measured from edge side of mycelium to bacteria colony. Percent inhibition is determined according to Cubukcu [2007].

$$\text{Percentage Inhibition} = C - T/C \times 100$$

where:

C = Colony diameter (cm) of the control

T = Colony diameter (cm) of the test plate

***In vivo* experiment.** After determining efficiency of isolates, the potential antagonists were tested against to *R. solani* isolates in *in vivo* conditions. Pots were filled with soil mix of soil, manure and perlite medium (1:1:1). The soil mix was autoclaved at 121°C for 1 hour in 2 day intervals. The prepared soil mix contaminated with pathogen-inoculated 15 wheat grains and the 15 grains spread on each pot. Meanwhile 15 non-pathogen inoculated wheat grains spread another pot accepted as control. Potato tubers and bean seeds cleaned with top water than waited 2 minutes in 70% ethyl alcohol. After that they waited in pure water ten minutes and dried, twice [Kotan et al. 2009]. Tubers and seeds waited in antagonistic suspension 2 hours and three seeds sowed each pot. Non-treated seeds and tubers is only waited in pure water and sowed. The experiment including positive control (clean soil + contaminated tuber/seed), negative control (clean soil + clean tuber/seed) and 15 potential antagonists and was designed with completely randomized with 4 replicates.

For greenhouse experiment evaluation, the plants on pots were rooted out, washed free of soil, and lesions on roots and crown of potato plants were evaluated on a scale of 0 to 3 in which 0 = no lesions, 1 = damage on 1/3 parts of crown above soil, 2 = damage on 1/3–2/3 parts of crown above soil, 3 = damage on more than 1/3 parts of crown above soil. For bean plants, symptoms on roots were evaluated on a scale of 1 to 5 in which 1 = no symptom, normal roots, 2 = local tissue colorization without necrosis, root development close to normal, 3 = intensive color change on tissues, root development close to normal, 4 = necrosis almost all roots, partial root length limitation, 5=root rot, serious limitation of root length [Eken and Demirci 2004]. The disease incidence index were evaluated according to Townsend-Heuberger [1943], as follows:

$$\text{Disease incidence index} = [\Sigma(SD \times BS)] \times 100/ESD \times TB$$

SD: Scala value

BS – the number of plants at same scale

ESD – the highest scale value

TB – total number of plants

The obtained disease incidence (%) results were used to determine effect value (%) according to control [Abbott 1925]:

$$\%\ \text{Effect} = X - Y/Y \times 100$$

X – disease incidence in control

Y – disease incidence in treatments

Data Analysis. The data were analyzed by ANOVA. Mean separation was accomplished using the Duncan Multiple Range Test ($P < 0.05$).

RESULTS

In the study, a total 73 potential candidate antagonist bacteria were tested against *R. solani* as biocontrol agent. 58 strain of *Phyllobacterium*, *Arthrobacter*, *Brevibacillus*, *Paenibacillus*, *Rhizobium*, *Stenotrophomonas*, *Rhodococcus*, *Pseudomonas*, *Chryseobacterium*, *Photobacterium*, *Kurthia*, *Microbacterium*, *Acinetobacter*, *Bacillus*, *Lysobacter* and *Kocuria* genera were found to be ineffective on mycelium development of 3 isolates of *R. solani* (tab. 1). Table 2 shows phosphorus solubilizing and nitrogen fixation capacity as well percent inhibition and inhibition zone of 15 effective bacteria against 3 isolates of *R solani* (Rs-pat, B-227 and B-1). According to *in vitro* test results; 15 bacteria strains (*Bacillus subtilis* (3), *Bacillus cereus* GC subgroup A (1), *Bacillus cereus* GC subgroup B (2), *Citrobacter freundii* (1), *Enterobacter intermedius* (1), *Lysobacter enzymogenes* (2), *Pseudomonas putida* biotype B (1), *Acinetobacter calcoaceticus* (1), *Burkholderia pyrrocinia* (2), *Pantoea agglomerans* GC subgroup C (1)) had inhibition zone between 0.20–2.30 cm. Among the bacteria strains 6/3 formed the highest inhibition zone (2.30 cm) and successfully inhibited colonial development of *R. solani* B-1 isolate. The bacteria 2/8 inhibited considerable mycelium development of Rs-pat and B-227 isolates with 2.10 cm and 2.00 cm inhibition zone, respectively. In dual culture tests, strain 2/8 inhibited mycelium development of Rs-pat and B-227 isolates with 63 ve 57% inhibition ratio. 6/3 strain have been found to inhibit mycelium development of B-1 isolate by 68%. Among the bacterial antagonists strain 3/7 was ineffective *in vitro* mycelium inhibition for B-1 and B-227, 13/3 and 17/2 were ineffective for Rs-pat and B-1, 64/4 for Rs-pat and B-227 and 5/4, 9/2 and 52/1 in effective for B-1 *in vitro* mycelium inhibition, respectively (tab. 2).

The disease incidence ratio as % for all treatments is determined and disease inhibition ratio (%) of root-bacteria treatments according to positive control, K (+) are given in Table 3. *In vivo* pot experiments are conducted on seed and tuber treatments and results showed that antagonists prevented disease caused by Rs-pat, B-227 and B-1 *R. solani* strains between 12–83, 21–77 and 18–78%, respectively (tab. 3). The most effective strains to control disease were 2/8, 5/6, 6/3 (83% control of disease) for Rs-pat, 6/3 (77% control of disease) for B-227 and 2/8 (78% control of disease) for B-1 strains (tab. 3). These results indicated that 2/8, 5/6 and 6/3 antagonist bacteria strains successfully controlled infection caused by three isolates of *R. solani* compared to control. The disease severity in control treatment were 83, 89 and 91% for Rs-pat, B-227 and B-1isolates while average disease severity after antagonists treatments on tubers were between 16–81% for Rs-pat and it was between 22–77 and 20–80% in seed bacteria treatments for B-227 and B-1 (tab. 3). We did not see disease symptom on negative control. The root bacteria (13/3, 17/2 and 64/4) that tested for *in vitro* antagonist effects on pathogen, did not form inhibition zone for mycelium development of Rs-pat but in pot experiment when it applied on tubers it prevented disease 39, 12 and 79%, respectively. Similarly, the strains 3/7 and 64/4 did not inhibit mycelium development of *R. solani* B-227 in petri dishes but in *in vivo* tests, they prevented disease development between 21 and 48%, respectively. The bacteria, 3/7, 5/4, 9/2, 13/3, 17/2 and 52/1 also did not inhibit mycelium development of B-1 isolate but in pot experiments it reduced disease severity between 18–66% (tab. 3).

Table 1. The ineffective bacteria strains on *Rhizoctonia solani* isolates in *in vitro* tests

Strain number	MIS Results	Strain Number	MIS Results
2/2	*Arthrobacter globiformis GC subgroupA*	14/2	*Bacillus simplex*
2/3	*Arthrobacter agilis*	20/1	*Bacillus megaterium GC subgroup B*
17/4	*Arthrobacter viscosus*	16/6	*Bacillus thuringiensis israelensis*
23/5	*Arthrobacter mysorens*	2/5	*Brevibacillus choshinensis*
16/4	*Achromobacter xylosoxidans denitrificans*	12/1	*Brevibacillus parabrevis GC subgroup A*
17/3	*Achromobacter xylosoxidans xylosoxidans*	7/6	*Chryseobacterium balustinum*
2/6	*Bacillus cereus GC subgroup A*	21/5	*Chryseobacterium indologenes*
5/3	*Bacillus cereus GC subgroup A*	11/3	*Kurthia sibirica*
7/2	*Bacillus cereus GC subgroup A*	23/2	*Kocuria erythromyxa*
7/4	*Bacillus cereus GC subgroup A*	9/8	*Lysobacter enzymogenes enzymogenes*
11/5	*Bacillus cereus GC subgroup A*	12/5	*Lysobacter enzymogenes enzymogenes*
3/4	*Bacillus cereus GC subgroup B*	16/2	*Lysobacter antibiticus*
5/1	*Bacillus cereus GC subgroup B*	16/1	*Microbacterium arabinogalactanolyticum*
15/2	*Bacillus cereus GC subgroup B*	22/1	*Paenibacillus validus (Bacillus gordonae)*
6/5	*Bacillus cereus GC subgroup B*	11/4	*Paenibacillus polymyxa*
12/2	*Bacillus cereus GC subgroup B*	5/8	*Pantoea agglomerans GC subgroup B*
15/1	*Bacillus cereus GC subgroup B*	8/8	*Photobacterium angustum*
19/4	*Bacillus cereus GC subgroup B*	1/1	*Phyllobacterium rubiacearum*
6/2	*Bacillus cereus GC subgroup B*	53/2	*Pseudomonas putida biotype A*
7/3	*Bacillus sphaericus GC subgroup B*	53/5	*Pseudomonas putida biotype A*
5/5	*Bacillus sphaericus GC subgroup D*	29/2	*Pseudomonas putida biotype B*
14/1	*Bacillus sphaericus GC subgroup D*	9/7	*Pseudomonas fluorescens biotype C*
3/3	*Bacillus sphaericus GC subgroup E*	21/4	*Pseudomonas fluorescens biotype C*
3/9	*Bacillus mycoides GC subgroup A*	10/1	*Pseudomonas fluorescens biotype G*
7/1	*Bacillus mycoides GC subgroup A*	2/7	*Rhizobium radiobacter*
13/2	*Bacillus mycoides GC subgroup A*	4/8	*Rhodococcus erythropolis*
17/2	*Bacillus mycoides GC subgroup A*	4/7	*Stenotrophomonas acidaminiphila*
11/6	*Bacillus mycoides GC subgroup B*	9/6	*Stenotrophomonas maltophilia*
15/3	*Bacillus mycoides GC subgroup B*	17/6	*Stenotrophomonas maltophilia*

MIS – Microorganism Identification System

On the other hand, we made observation on *in vivo* experiments and we noticed that antagonist bacteria treatments not only prevented disease incidence but also improved plant growth even we did not make measurements. In *in vivo* pot experiments we used the most effective bacteria that formed the highest inhibition zone against disease as well we used some moderate effective and even ineffective bacteria because there is inconsistence between the results of *in vitro* and *in vivo* tests. In present experiment also showed that some bacteria that found ineffective *in vitro* conditions were effective *in vivo* conditions as biocontrol agent.

Table 2. The *in vitro* effectiveness of potential antagonist bacteria on *Rhizoctonia solani* isolates and their nitrogen fixing and phosphor solubilizing capacity

Strain No	MIS results	*Rhizoctonia solani* strains						Development on N-free media	Development on NBRIP-BPB media
		Rs-pat		B-227		B-1			
		inhibition zone (cm)	inhibition ratio (%)	inhibition zone (cm)	inhibition ratio (%)	inhibition zone (cm)	inhibition ratio (%)		
2/8	*Bacillus subtilis*	2.10 k	63 k	2.00 j	57 i	1.91 g	52 g	+	+
3/1	*Bacillus cereus GC subgroup A*	0.40 d	28 e	0.30 c	26 c	0.60 d	35 e	K+	K+
3/7	*Citrobacter freundii*	0.30 c	28 e	0.0 a	0 a	0.0 a	0 a	+	K+
5/4	*Enterobacter intermedius*	0.30 c	28 e	0.30 c	26 c	0.0 a	0 a	Z+	K+
5/6	*Lysobacter enzymogenes enzymogenes*	1.80 i	31 g	1.80 h	33 f	2.10 h	63 h	+	Z+
6/3	*Bacillus subtilis*	2.00 j	36 h	1.90 i	33 f	2.30 i	68 i	K+	Z+
6/6	*Pseudomonas putida biotype B*	0.70 e	30 f	0.50 e	31 e	0.30 b	26 b	K+	+
9/2	*Lysobacter enzymogenes enzymogenes*	0.20 b	24 c	0.30 c	29 d	0.0 a	0 a	+	Z+
13/3	*Acinetobacter calcoaceticus*	0.0 a	0 a	0.20 b	25 b	0.0 a	0 a	+	K+
13/4	*Burkholderia pyrrocinia*	1.30 g	41 i	0.90 f	38 g	0.90 e	40 f	K+	K+
17/2	*Bacillus cereus GC subgroup B*	0.0 a	0 a	0.40 d	31 e	0.0 a	0 a	K+	Z+
19/5	*Bacillus cereus GC subgroup B*	0.80 f	18 b	0.50 e	25 b	0.40 c	31 c	+	-
24/2	*Pantoea agglomerans GC subgroup C*	1.60 h	46 j	1.40 g	48 h	1.70 f	40 f	+	+
52/1	*Bacillus subtilis*	0.30 c	27 d	0.30 c	31 e	0.0 a	0 a	K+	-
64/4	*Burkholderia pyrrocinia*	0.0 a	0 a	0.0 a	0 a	0.60 d	33 d	K+	K+

* – different letters indicate the statistical difference within same column among bacteria at 5% level

Table 3. The effects of antagonist bacteria on root rot disease severity in greenhouse conditions

Strain No	MIS result	*R. solani* isolates					
		Rs-pat		B-227		B-1	
		disease severity (%)	effect (%)	disease severity (%)	effect (%)	disease severity (%)	effect (%)
2/8	*Bacillus subtilis*	16 b	83 l	25 c	74 m	20 b	78 o
3/1	*Bacillus cereus GC subgroup A*	50 h	46 g	48 i	51 g	47 h	52 i
3/7	*Citrobacter freundii*	64 k	30 d	77 n	21 b	80 o	18 b
5/4	*Enterobacter intermedius*	44 g	52 h	53 l	45 d	55 l	43 e
5/6	*Lysobacter enzymogenes enzymogenes*	16 b	83 l	28 d	71 l	25 d	74 m
6/3	*Bacillus subtilis*	16 b	83 l	22 b	77 n	23 c	76 n
6/6	*Pseudomonas putida biotype B*	44 g	52 h	45 g	54 i	42 g	57 j
9/2	*Lysobacter enzymogenes enzymogenes*	69 l	25 c	53 l	45 d	57 m	41 d
13/3	*Acinetobacter calcoaceticus*	56 j	39 e	68 m	30 c	68 n	30 c
13/4	*Burkholderia pyrrocinia*	33 e	66 j	40 f	59 j	42 g	57 j
17/2	*Bacillus cereus GC subgroup B*	81 m	12 b	47 h	52 h	50 j	49 g
19/5	*Bacillus cereus GC subgroup B*	39 f	58 i	52 k	46 e	53 k	45 f
24/2	*Pantoea agglomerans GC subgroup C*	31 d	66 j	40 f	59 j	40 f	59 k
52/1	*Bacillus subtilis*	53 i	42 f	32 e	67 k	33 e	66 l
64/4	*Burkholderi apyrrocinia*	19 c	79 k	50 j	48 f	48 i	51 h
K1	*Pozitive Control*	83 n	0 a	89 o	0 a	91 p	0 a
K2	*Negative Control*	0 a	0 a	0 a	0 a	0 a	0 a

* – different letters indicate the statistical difference within same column among bacteria at 5% level

Rhizoctonia solani has a wide range of host plant lists and causes yield and quality losses via damp off, root rot and crown rot in plants and the control of disease is difficult because it is originated both in soil and plant material [Jehtonen 2009]. Some agricultural practices, to slow down of development of the disease and to reduce disease in following year, such as destruction of contaminated plants and plant wastes on the field, use of certificated planting material, use of solarization, selecting resistance cultivars, crop rotation with wheat and corn have been suggested [Aydın et al. 2011]. However the disease has a wide number of host plants thus the number of plants for crop rotation are limited. Solarization application can reduce pathogen population in soil but in same time it effect soil microflora and finally destroy beneficial soil microflora [Porras et al. 2007]. The agricultural practices applied to control disease are ineffective because the pathogen to develop fast in soil, survive a long period on organic wastes and to develop vegetative mycelium on relevant hosts presence [Turhan 2010]. More recently some control programs against the disease both on soil and tubers are carried out. It was reported that treatments of tubers/seed and soil with some chemicals suppress disease severity [Errampalli and Johnston 2001]. However soil fumigation with chemicals is not relevant method in field conditions. It also has high cost when applied in protected cultivation. Moreover, if the inoculums are intense in soil, the chemical fumigants can not enough pressure on the disease [Aydın et al. 2011]. Fungicide treatments are found effective on seed-borne infections while it was ineffective on contamination of soil origin [Wilson et al. 2008]. In addition, the use of fungicide has environmental and human health problems destroy nature and develop resistance against pathogen. Biological control by using antagonist bacteria is now accepted an alternative treatments or solution to control the disease.

This study showed that the use of antagonist bacteria is an alternative way to control *R. solani* and the study also showed that the antagonist bacteria treatments improved plant growth due to several mechanisms including atmospheric and soil free nitrogen fixing capacity, induced systemic resistance, mineralization of soil organic matter, solubilizing soil phosphorus, enzyme and hormone production [Ongena et al. 2004, Ryu et al. 2004, Jehtonen 2009].

A lot of studies have been conducted on biological control of *R. solani*. The studies conducted on last decade showed that the most effective bacteria to control soil-borne disease are belongs to *Pseudomonas* and *Bacillus* genera [Tariq et al. 2010, Huang et al. 2011]. Our present study also indicate that in greenhouse conditions the strains belongs to *Bacillus* reduced disease severity by 12–83% and strains belongs to *Pseudomonas* reduced disease severity by 52–57%. Previous studies also showed that antagonists belongs to *Bacillus* and *Pseudomonas* genera has successfully used to control *R. solani* [Bautista et al. 2007, Scherwinski et al. 2008]. Studies showed that *Pseudomonas* adapted well to rhizosphere and to develop fast and encourage systemic resistance. The inhibition of *Pseudomonas* is carried out by the production of siderophores like pseudobactin, pyochelin, pyoverdine, ferribactin, ferrichrome, phytosiderphores, etc., antibiotics like phenazines, pyoluteorin, tropolone, pyocyanine, 2,4-diacyetyl phloglucinol (DAPG), pyrrolnitrin and secondary metabolites like hydrogencyanide, phenazine-1--carbosylic acid (PCA), oomycin A, indole-3-acetic acid, chitinsae, β-1,3 glucanase, laminariase. So by the production of many antibiotics, siderophores and other toxins

Pseudomonas acts as an effective and broad-spectrum antagonistic spectrum [Anitha and Das 2011]. *Lysobacter enzymogenes* strain C3 used in field conditions and found to be effective antagonist against *R. solani* infections on turf grass [Kilic-Ekici and Yuen 2003]. In our study we used 2 *Lysobacter enzymogenes* strains and disease severity caused by *R. solani* was 83–91% in control plants while *Lysobacter enzymogenes* treatments reduced disease severity to 16–69%. Previously3 strains of *Enterobacter agglomerans* reported effective biocontrol agent and reduced disease severity between 64–86% on *R. solani* infections in cotton plants due to enzyme production [Chernin et al. 1995]. In present study, *Enterobacter intermedius* reduced disease severity caused by *R. solani* by 43–52%. Some rhizosphere bacteria such as *Acinetobacter* and *Citrobacter* are well known improving plant growth. *Acinetobacter baumannii* LCH001 strains had bioactive compound production (iturin A2, iturin A3, iturin A6) and inhibited development of *Cryphonectria parasitica*, *Glomerella glycines*, *Phytophthora capsici*, *Fusarium graminearum*, *Botrytis cinerea* and *R. solani* [Liu et al. 2007]. In our study, *Acinetobacter calcoaceticus* and *Citrobacter freundii* inhibited *R. solani* infections by 25 and 28% respectively.

As a conclusion, the study showed important interactions between host-pathogen-antagonist and results varied according to plant species, pathogen and bacteria strain. The results also indicated that the most effective bacteria species for control of disease has nitrogen fixing capacity and phosphorus solubilizing capacity. Thus these bacteria can be use both as biopesticide and biofertilizer. However, the resistance promote mechanism of bacteria against disease should be worked more detailed.

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BIOLOGICZNA WALKA ZE ZGNILIZNĄ KORZENI SPOWODOWANĄ PRZEZ *Rhizoctonia solani* Kühn NA ZIEMNIAKACH I FASOLI PRZY UŻYCIU BAKTERII ANTAGONISTYCZNYCH

Streszczenie. W niniejszym badaniu badano użycie 73 bakterii wyizolowanych z ryzosfery roślin herbaty (*Camellia sinensis*) w rejonie Morza Czarnego w Turcji jako potencjalnego środka w walce przeciwko zgniliźnie korzeni spowodowanej przez *Rhizoctonia solani* i znanej jako ważna choroba roślin ziemniaka i fasoli w Turcji i na całym świecie. W warunkach *in vitro* użyto 73 bakterie i stwierdzono, że 15 spośród 74 działało przeciwko *R. solani* i tworzyło 0,20–2,30-centymetrową strefę inhibicji, a także hamowało rozwój grzybni patogenu. Doświadczenie *in vivo* także wykazało, że szczepy bakterii antagonistycznych miały różny poziom efektu inhibicji (między 12% a 83%) na *R. solani* w porównaniu z kontrolą. Badanie wykazało, że środki ochrony biologicznej mogą odgrywać zasadniczą rolę w zwalczaniu zgnilizny korzeni ziemniaka i fasoli.

Słowa kluczowe: fasola, ziemniak, *Rhizoctonia solani*, walka biologiczna

Accepted for print: 12.05.2015

For citation: Donmez, M.F., Uysal, B., Demırcı, E., Ercıslı, S., Cakmakcı R. (2015). Biological control of root rot disease caused by *Rhizoctonia solani* Kühn on potato and bean using antagonist bacteria. Acta Sci. Pol. Hortorum Cultus, 14(5), 29–40.