J. Turk. Phytopath., Vol. 35 No. 1-3, 1-13, 2006 ISSN 0378 - 8024

Biological Control of *Pseudomonas syringae* pv. *syringae* and Nutritional Similarity in Carbon Source Utilization of Pathogen and its Potential Biocontrol Agents

Recep KOTAN* **Fikrettin SAHİN****

* Atatürk University, Faculty of Agriculture, Department of Plant Protection, TR-25240 Erzurum-Turkey

** Yeditepe University, Faculty of Engineering and Architecture, Department of Genetic and Bioengineering, TR-34755 Istanbul-Turkey

Accepted date: November 8, 2009

ABSTRACT

In this study, a total of 206 bacterial strains belonged to 62 different species in 35 genera were isolated from aerial parts of pome fruits. All bacterial strains were tested for antagonistic activity in *in-vitro* and *in-vivo* assays against *Pseudomonas syringae* pv. *syringae* causing leaf or tip necrosis on pome fruits. Only 71 of the strains tested were determined with antagonistic activity against the pathogen in *in-vitro* test. Twenty two of these strains were selected as potential antagonist due to their hyperparasitic activity or size of inhibition zone (50 mm or above) calculated *in-vitro* Petri assay. The pre-selected strains were further tested against the pathogen alone and/or in six different combinations using one year old Golden delicious apples shoots *in-vivo* conditions. The results showed that antagonistic bacterial strains tested significantly reduced diseases development caused by the pathogen. However, the best results were obtained from 4 strains of *Pantoea agglomerans* (RK 84, 85, 113 and 154), and a strain of *Leclercia adecarboxylata* (RK 164), *Pseudomonas putida* (RK 142), *Curtobacterium flaccumfaciens* (RK 114), *Erwinia rhapontici* (RK 135), *Alcaligenes piechaudii* (RK 137), *Serratia liquefaciens* (RK 102) and their combinations. All bacterial strains were also characterized based on sole carbon source utilization profiles using Microplate assay (BIOLOG, Hayward, CA). The data suggested that there was a correlation in the nutritional profile of pathogen and its biocontrol strains. Therefore, this is the first study providing evidence that nutritional similarity between *Pseudomonas syringae* pv. *syringae* and its biocontrol agents may be one of the important factors need to be determined for effective disease management programs.

Key words: Antibiosis, biocontrol, BIOLOG, competition, leaf necrosis, pomes fruit, *Pseudomonas syringae* pv. *syringae*

BIOLOGICAL CONTROL OF *PSEUDOMONAS SYRINGAE* PV. *SYRINGAE* AND NUTRITIONAL SIMILARITY IN CARBON SOURCE UTILIZATION OF PATHOGEN AND ITS POTENTIAL BIOCONTROL AGENTS

INTRODUCTION

Pseudomonas syringae pv. *syringae* van Hall, causes leaf or tip necrosis, infects a wide range of decidious fruit trees such as pear, peach, cherry and plum as well as other woody plant species (Canfield et al. 1986). This is one of the most widespread diseases resulting in significant economic losses in fruit tree-growing areas around the world (Canfield et al. 1986; Mansvelt and Hattingh 1986; Montesinos and Vilardell 1990; Whitesides and Spotts 1991; Balestra and Varvaro 1997). It is also seen that this pathogen is an important problem on stone (Kotan and Sahin 2002) and pome fruits (Kotan et al. 2006) in Turkey.

Disease severity is dependent on susceptibility of the host, aggressiveness of the pathogen and favorable environment of conditions (Endert and Ritchie 1984; Montesinos and Vilardell 1990; Moragrega et al. 2003). The pathogen over winters on dormant bud during the growing seasons. Large epiphytic populations of them occur on apparently healthy flowers, leaves and fruits. These epiphytic populations initiate subsequent disease outbreaks (Jones and Aldwinckle 1991). Severe outbreaks may reduce yields significantly.

Although this disease is economically important in some regions of the world, chemical control methods, based on treatments with copper compounds and antibiotics, are often unsuccessful (Burr et al. 1988; Scheck et al. 1996). Biological control offers a powerful and environmentally friendly alternative to the use of synthetic pesticides. In biological control, various mechanisms have been described, including antibiosis, production of hydrolytic enzymes, parasitism, induced resistance and competition for nutrients and space. Biocontrol agents in their native habitat may compete with other microorganisms for space and food, produce toxic substances, parasitize and/or kill other plant pathogens (Lorito et al. 1996). The competition is one of the most effective mechanisms in biological control. Many studies have demonstrated that bacteria are actively engaged in carbon consumption in the phyllosphere shortly after inoculation (McClure et al. 1998; Leveau and Lindow 2001). Although competition between microorganisms for nutrients and space has often been implied, but there has been no attempt to provide scientific data about the importance of nutritional relationship between pathogen and biocontrol agent during biological control (Janisiewicz et al. 2000, Ji and Wilson 2002; Dianese et al. 2003).

There are many studies concerning the epidemiology and chemical control of *P. s.* pv. *syringae.* However, there has been no attempt to study biological control of *P. s.* pv. *syringae* strains cause leaf and or tip necrosis on apple yet. The objectives of this study were; 1) To isolate and identify bacterial organisms from natural inhabitant of pome fruits. 2) To determine potential antagonistic bacterial strains against *P. s.* pv. *syringae* causing economically important disease on pome fruits. 3) To investigate nutritional relationship between *P. s.* pv. *syringae* and potential biocontrol agents, which may be important for effective biological control of disease.

MATERIALS and METHODS

Isolation, identification and maintenance of pathogenic and antagonistic bacterial strains

The pathogenic bacteria *P. s.* pv. *syringae* strain RK 257, and a collection of 206 non pathogenic bacterial strains isolated from phyllosphere of pome fruits (Kotan 2002; Kotan et. al. 2005) from different locations in eastern Anatolia region of Turkey were included in this study (Table 1).

All bacterial strains had been identified by gas chromatography using the MIDI system (Microbial Identification System, Inc., Newark, DE, version 5.0) (Roy 1988; Paisley 1995). Identification results showed that 206 non-pathogenic bacterial strains were belonged to 62 species in 35 genera. These identification results were confirmed by some of biochemical tests including Gram reaction, the presence of catalase, oxidase, potassium hydroxide (KOH), nitrate reduction, production of yellow fluorescence pigment on ***King's B Medium*** (KB), levan production on ***Nutrient Agar Sucrose*** medium (NAS) and arginine dihydrolase investigated according to the methods described by Fahy and Hayward (1983) (Kotan 2002).

Pathogenicity of all bacterial strains has been determined in our previous study on the basis of hypersensitivity test on tobacco and inoculation on apple twigs (cv Golden delicious). It was determined that pathogenicity and hypersensitivity test results of all antagonistic bacterial strains were negative (Kotan 2002). All bacterial strains were stored at – 80˚C in %15 glycerol and Luria Broth (LB) for using further studies.

In-vitro assays

All non-pathogenic bacterial strains were tested for their antagonistic activity against *P. s.* pv. *syringae* on Sensitive Agar (SA) plates in Lab conditions. The well diffusion assay described by Schilinger and Lucke (1989) was used in this study with a minor modification. Each non-pathogenic bacterial strains was streaked by using a sterile swap as a single line on the middle of SA plates which were streaked on the whole surface of SA plates with 100 µl of *P. s.* pv. *syringae* suspension (10^8 cfu/ml^{-1} in sterile water) previously. The inoculated plates were incubated at 26-27 ˚C for 7 days. The antagonistic activity was determined by measuring inhibitory zones (mm) around single line of the antagonistic bacteria. Some of the test strains were able to grow on *P. s.* pv. *syringae* inoculated SA plates by covering whole surface, which were evaluated as hyperparasitic organism. The antagonistic strains with inhibition zone of 50 mm or above and hyperparasitic strains were selected as candidate biocontrol agents and used in further test. All tests were applied three times per week to three replicate for each bacterial strains.

In-vivo assays

Twenty two antagonistic strains selected in *in-vitro* assay were tested for *in-vivo* assay alone or six different combinations listed in Table 2. An *in-vivo* test described by Moragrega et al. (2003) was used with a minor modification. The pathogenic and antagonistic bacterial strains were grown on nutrient broth (NB) for 24-48 h on rotary shakers under 130 rpm at 26-27 ˚C. Absorbance of bacterial suspension at 600 nm was adjusted to 10^8 cfu/ml^{-1} in sterilized water. For each treatment, 9 young apple shoots cv. Golden delicious (one year old) were taken from apple trees in the size of 15-20 cm length during growing seasons of 2001, and then sprayed by simultaneously 200 ml of the pathogen and biocontrol bacterial suspensions (1/1 ratio). The suspension of pathogen and sterile distillated water were used as positive and negative control, respectively. The inoculated 3 twigs were separately put in flasks with water and covered by polyethylene bags at room temperature for 3 days. After polyethylene bags removed, the flask with inoculated twigs were maintained on the greenhouse bench for 20 days and evaluated for symptom development of typical bacterial leaf or tip necrosis by using 1-5 scales (1 = no symptom on leaves, 2 = 25 % disease of leaves, 3 = % 50 disease of leaves, 4 = % 75 disease of leaves and 5 = % 100 disease of leaves) (Braun-Kiewnick et al. 1997). This test was repeated at least three times for each biocontrol strains.

Statistical analyses

In-vitro and *in-vivo* test results were analyzed by using SPSS (Statistical Package for Social Sciences, Windows 9.9) Statistical Analysis System. Analysis of variance (Duncan Multiple Range Test at p = 0.05) was used for data analysis.

Carbon utilization profiles of *P. s.* pv. *syringae* and biocontrol bacterial strains

Carbon utilization profiles of test organisms were determined based on Biolog GN2 or GN2 micro titer plate (Biolog, Inc., Hayward, CA) assay in this study. Microorganisms including pathogenic bacteria *P. s.* pv. *syringae* and 23 pre-selected biocontrol bacterial strains were streaked on ***Tryptic Soy Agar (***TSA) or Biolog Universal Growth Agar (BUG-M) agar plates and incubated for 48 hr at 27 ˚C. GN2 micro titer plates (Biolog, Inc.) was inoculated with 125 µl of the bacterial suspension into each well (10^8 cfu/ml^{-1}) and incubated at 27 ˚C for 24 or 48 h. The development of color was automatically recorded using a micro plate reader with a 590-nm wavelength filter. This system relies on the potential utilization of 95 substrates in a micro titer plate by bacteria. Color development in each well reflected the ability of the bacteria to utilize that specific carbon source. Similarity in carbon source utilization between bioagents and pathogen was estimated by using carbon utilization data of the Biolog GN micro titer plates according to the formulae NSI_{Biolog} = the number of compounds used by both the biocontrol bacteria and pathogen/the number of compounds used by pathogen (Wilson and Lindow 1994). All tests were applied one times for each bacterial strains.

RESULTS

Total 206 candidate antagonistic bacterial strains were tested for antagonistic activity against pathogen *P. s.* pv. *syringae* on Petri dishes assay. The minimum-maximum inhibition zones of the 62 different species were given in Table 1. The majority of tested strains belonged to *Pantoea agglomerans* (15.53 %), *Enterobacter agglomerans* (8.73 %), *Alcaligenes piechaudii* (7.76 %) and *Bacillus pumilus* (7.28 %). The data of *in-vitro* test studies showed that 61 of 206 strains tested in this study had antagonistic activity against *P. s.* pv. *syringae.*

Average inhibition zone for each bacterial strains tested were given in Table 2. The potential antagonistic bacterial strains showed inhibition zone in the size of 50 mm or above were identified as *P. agglomerans* (8 strains); *A. piechaudii* (5 strains); *Leclercia adecarboxylata* (2 strains); *Pseudomonas putida*, *B. pumilus*, *Curtobacterium flaccumfaciens*, *Erwinia rhapontici*, *Enterobacter intermedius*, *Serratia liquefaciens* and *Chromobacterium violaceum* (1 strain). The pre-selected 22 antagonistic strains belonged to 10 different species and their six different combinations were tested on shoot assays *in-vivo* condition. Disease severity data and nutritional similarities of test organisms were also summarized in Table 2. The pre-selected biocontrol strains and the combinations significantly reduced disease severity compared with the positive control $P \leq 0.05$. The most successful results were obtained from ten bacterial strains (*P. agglomerans* strain RK 84, 85, 113, 154; *L. adecarboxylata* strain RK 164; *Pseudomonas putida* strain RK 142; *C. flaccumfaciens* strain RK 114; *E. rhapontici* strain RK 135; *A. piechaudii* strain RK 137 and *S. liquefaciens* strain RK 102) and all combinations. There was no significant difference in the disease severity on apple shoots treated by between ten bacterial strains and/or six combinations of bacterial strains tested and the negative control (treated with sterilized water). Treatment with the remaining 12 strains had also reduced disease severity relatively comparing with positive control treatment but not complete control obtained with other 10 strains or their combinations (Table 2). Relationship between antagonistic activity based on disease severity and inhibition zone data and nutritional similarity demonstrated that there was strong similarity (0.60 % - 1.00 %) between carbon utilization profile and pre-selected biocontrol bacterial strains produced inhibition zone 50 mm or above, or covered whole surface on petri dish assay.

The utilization percentages of carbon sources by both pathogen and potential biocontrol agents with different antagonistic activity were given in Table 3. The result indicated that nutritional similarity of *P. s.* pv. *syringae* and its strong potential biocontrol strains were higher (83 %) than poor biocontrol strains (66 %) selected according to *in-vitro* and *in-vivo* antagonistic tests.

Table 1. Antagonistic activity of biocontrol bacterial strains against *P. s.* pv. *syringae* in *in-vitro* condition

Bacterial species	TS	ES	Zone	Bacterial species	TS	ES	Zone
Pantoea agglomerans	32	17	12 - HP	*Brevibacterium casei*	1	0	00 - 00
Alcaligenes piechaudi	16	13	15 - 71	*Brevundimonas diminuta*	1	0	00 - 00
Enterobacter agglomerans	18	7	17 - 44	*Burkholderia cepacia*	1	0	00 - 00
Bacillus pumilus	15	4	17 - 85	*Burkholderia pyriocinia*	7	0	00 - 00
Leclercia adecarboxylata	11	3	35 - HP	*Chryseobacterium indolegenes*	2	0	00 - 00
Enterobacter intermedius	4	2	16 - 53	*Citrobacter amalonaticus*	1	0	00 - 00
Erwinia chrysanthemi	6	2	17 - 45	*Enterobacter cloacae*	1	0	00 - 00
Serratia liquefaciens	8	2	28 - 64	*Erwinia rhapontici*	1	0	46 - 70
Acinetobacter radioresistens	1	1	26 - 36	*Escherichia coli*	1	0	00 - 00
Actinomadura yumaensis	3	1	25 - 35	*Klebsiella terrigena*	1	0	00 - 00
Bacillus mycoides	1	1	17 - 27	*Klebsiella trevisanii*	1	0	00 - 00
Bacillus subtilis	2	1	10 - 13	*Kocuria rosea*	1	0	00 - 00
Chromobacterium violaceum	1	1	46 - 55	*Kocuria varians*	1	0	00 - 00
Citrobacter freundii	1	1	12 - 31	*Methylobacterium mesophilicum*	1	0	00 - 00
Curtobacterium flaccumfaciens	4	1	50 - 75	*Micrococcus lylae*	1	0	00 - 00
Klebsiella pneumoniae	6	1	24 - 31	*Neisseria mucosa*	1	0	00 - 00
Pseudomonas putida	1	1	78 - 85	*Pediococcus pentosaceus*	1	0	00 - 00
Serratia fonticola	3	1	32 - 35	*Photobacterium damsela*	1	0	00 - 00
Vibrio hollisae	1	1	14 - 16	*Plesiomonas shigelloides*	2	0	00 - 00
Acinetobacter calcoaceticus	3	0	00 - 00	*Proteus vulgaris*	1	0	00 - 00
Acinetobacter johnsonii	3	0	00 - 00	*Pseudomonas balearica*	1	0	00 - 00
Aerococcus vridans	1	0	00 - 00	*Pseudomonas doudoroffii*	1	0	00 - 00
Agrobacterium radiobacter	7	0	00 - 00	*Pseudomonas huttiensis*	3	0	00 - 00
Agrobacterium rubi	2	0	00 - 00	*Pseudomonas viridiflava*	1	0	00 - 00
Alcaligenes xylosoxydans	1	0	00 - 00	*Ralstonia pickettii*	2	0	00 - 00
Bacillus GC group 22	1	0	00 - 00	*Salmonella typhimurium*	1	0	00 - 00
Bacillus cereus	3	0	00 - 00	*Serratia grimesii*	1	0	00 - 00
Bacillus lentimorbus	2	0	00 - 00	*Sphingomonas capsulata*	1	0	00 - 00
Bacillus licheniformis	3	0	00 - 00	*Vibrio alginolyticus*	1	0	00 - 00
Bacillus megaterium	4	0	00 - 00	*Yersinia enterocolitica*	1	0	00 - 00
Bacillus simplex	1	0	00 - 00				
Brevibacillus brevis	1	0	00 - 00	Total	206	61	

TS: The number of tested strains; ES: The number of effective strains; Zone: minimum- maximum inhibition zone (mm); HP: Some of the test strains were able to grow on *P. s.* pv. *syringae* inoculated SA plates by covering whole surface, which were evaluated as hyperparasitic organism

Table 2. Antagonistic activity of pre-selected bacterial strains in *in-vitro* assay tested on one year old apple shoots (cv Golden delicious) in *in-vivo* condition

Used bacterial strains	Strains	MZ (mm)*	NSI (%)	DS*
Pantoea agglomerans	RK 79	HP	80	2.00 ^{bcd}
Pantoea agglomerans	RK 84	HP	84	1.00 ^{a}
Alcaligenes piechaudii	RK 105	HP	80	3.66 ^{gh}
Pantoea agglomerans	*RK 113*	HP	78	1.33 ^{ab}
Alcaligenes piechaudii	*RK 143*	HP	80	2.00 ^{bcd}
Alcaligenes piechaudii	RK 156	HP	67	3.00 ^{efg}
Alcaligenes piechaudii	RK 158	HP	80	3.00 ^{efg}
Leclercia adecarboxylata	RK 164	HP	60	1.00 ^{a}
Pseudomonas putida	RK 142	81.00 ^{a}	84	1.00 ^{a}
Bacillus pumilus	RK 103	77.00 ^{a}	ND	3.33 ^{fgh}
Curtobacterium flaccumfaciens	RK 114	66.66 ^{ab}	ND	1.33 ^{ab}
Pantoea agglomerans	RK 154	55.33 ^{b}	78	1.33 ^{ab}
Erwinia rhapontici	RK 135	55.00 ^{b}	90	1.33 ^{ab}
Alcaligenes piechaudii	RK 137	53.66 ^{b}	68	1.66 ^{abc}
Pantoea agglomerans	*RK 86*	53.33 ^{b}	78	3.66 ^{gh}
Alcaligenes piechaudii	RK 157	50.33 ^{b}	84	2.33 ^{cde}
Pantoea agglomerans	RK 85	50.00 ^{b}	78	1.33 ^{ab}
Enterobacter intermedius	RK 91	50.00 ^{b}	60	2.00 ^{bcd}
Pantoea agglomerans	RK 92	50.00 ^{b}	72	2.33 ^{cde}
Serratia liquefaciens	RK 102	50.00 ^{b}	78	1.33 ^{ab}
Leclercia adecarboxylata	RK 163	50.00 ^{b}	70	2.66 ^{def}
Chromobacterium violaceum	RK 165	50.00 ^{b}	70	2.66 ^{def}
Pantoea agglomerans	RK 169	50.00 ^{b}	68	2.00 ^{bcd}
Combination A (RK 105, 156)			ND	1.66 ^{abc}
Combination B (RK 79, 164)			ND	1.33 ^{ab}
Combination C (RK 156, 103)			ND	1.00 ^{a}
Combination D (RK 80, 84)			ND	1.66 ^{abc}
Combination E (RK 160, 142)			ND	1.33 ^{ab}
Combination F (RK 105, 103, 114, 164, 179, 142)			ND	1.00 ^{a}
Positive control (Pathogen)			-	5.00 ^{ı}
Negative control (sd H_2O)			-	1.00^{a}

MZ: Mean inhibition zone; NSI: Nutritional similarity index; DS: disease severity as 1 - 5 scales; ND: Not determined; HP: Some of the test strains were able to grow on *P. s.* pv. *syringae* inoculated SA plates by covering whole surface, which were evaluated as hyperparasitic organism

*: Treatments followed by the same letter are not significantly different according to the least significant difference test at the 0.05 level

Table 3. Relationship in carbon utilization of pathogen *P. s.* pv. *syringae* and biocontrol bacterial strains based on GN2 or GP2 Microplate assay

Carbon sources utilized by both bioagents and pathogen							
Carbon sources	A	B	C	Carbon sources	A	B	C
D-Galactose	0.90	0.94	0.82	Succinic Acid	0.79	0.83	0.64
D-Mannitol	0.76	0.72	0.82	Glycyl-L-Glutamic Acid	0.79	0.83	0.64
Sucrose	0.86	0.94	0.73	D-Glucuronic Acid	0.76	0.83	0.64
D,L-Lactic Acid	0.86	0.94	0.73	D-Saccharic Acid	0.76	0.83	0.64
L-Serine	0.86	0.94	0.73	Glycerol	0.76	0.83	0.64
Inosine	0.83	0.94	0.73	Uridine	0.72	0.78	0.64
L-Arabinose	0.83	0.89	0.73	L-Histidine	0.72	0.72	0.64
m-Inositol	0.83	0.89	0.73	D-Sorbitol	0.69	0.72	0.64
D-Galacturonic acid	0.83	0.89	0.73	D-Fructose	0.66	0.67	0.64
D-Alanine	0.79	0.83	0.73	D-Arabitol	0.62	0.61	0.64
L-Alanine	0.90	1.00	0.73	α-D-Glucose	0.62	0.61	0.64
L-Aspartic Acid	0.90	1.00	0.73	Citric Acid	0.52	0.44	0.64
D-Gluconic Acid	0.83	1.00	0.73	Pyruvic Acid Methil Ester	0.76	0.89	0.55
Formic Acid	0.83	0.94	0.64	Cis-Aconitic Acid	0.72	0.83	0.55
L-Alanyl-Glycine	0.83	0.94	0.64	D-Mannose	0.69	0.78	0.55
L-Glutamic Acid	0.83	0.94	0.64	Quinic Acid	0.62	0.67	0.55
Bromosuccinic Acid	0.79	0.89	0.64	D,L,α-Glycerol Phosphate	0.72	0.83	0.45
L-Asparagine	0.79	0.89	0.64	α-Ketoglutaric Acid	0.55	0.61	0.45
The average of percentage carbon sources utilized					76 %	83 %	66 %

A: The percentage of carbon source utilized by both pathogen and all bioagents, B: Both pathogen and the most effective bioagents, C: Both pathogen and the less effective bioagents reduced disease severity on shoot assays

DISCUSSION

One of the most important alternative control methods of plant disease is the use of biological control agents. There are a lot of studies related to biological control of plant bacterial diseases (Cody et al. 1987; Lindemann and Suslow 1987; Ozaktan and Türküsay 1994; Zeller and Wolf 1996; Braun-Kiewnick et al. 1997; Wright et al. 2001; Kotan 2002). The number of studies related to biocontrol of leaf pathogens is relatively less. Furthermore, this is the first study providing evidence about biological control of *Pseudomonas syringae* pv. *syringae* causes the leaf or tip necrosis on pome fruits. Biological control of plant diseases involves interactions between a biological control agent and a plant pathogen; hence, an understanding of the ecology of the pathogen and the biological control agent is essential to the development of a biological control strategy (Dianesi et al. 2003).

The antagonistic activity test *in-vitro* condition is well established method and used a number of studies in order to monitor antibiosis capacity of biocontrol agents

(Lindemann and Suslow 1987; Ozaktan and Türküsay 1994; Zeller and Wolf, 1996). The antagonistic activity test was also used in this study to determine inhibition zone between pathogen and non-pathogen bacterial organism naturally habitant of pome fruits. The data in this study confirmed literature suggesting *in-vitro* assay can successfully be used for selection of antagonistic and hypersensitive organism. The pre-selected antagonistic bacterial strains with inhibition zone (50 mm or above) in *in-vitro* test were found more effective *in-vivo* test for reduction of disease severity on apple shoots tested. Similar results were reported by many other scientists in other biological control studies (Ozaktan and Türküsay 1994; Zeller and Wolf 1996; Braun-Kiewnick et al. 1997; Wright et al. 2001; Kotan 2002). The results of this study also demonstrated that total 22 bacterial strains and their combinations had great potential to control leaf or tip necrosis on apple caused by *P. s.* pv. *syringae*. Total 17 strains of the 32 *P. agglomerans* strains tested had more or less antagonistic activity on petri plates against the pathogen. 47.05 % of them reduced completely disease severity on shoot assays in comparison to positive control. Similarly 22.72 % of the pre-selected strains were *A. piechaudi* found to be effective biocontrol agent in *in-vivo* assay. There are a few studies in literature reporting *P. agglomerans*, *S. liquefaciens*, and *Pseudomonas* sp. were determined as successful biocontrol agents against some rhisosphere and phyllosphere plant pathogens (Colyer and Mount 1984, Cody et al. 1987, Lindow et al. 1996). It's known that *P. agglomerans* produces bacteriocin which inhibited *E. amylovora* by competing for nutrients (Zeller and Wolf 1996). Wright et al. (2001), states that pantocin A and pantocin B antibiotics produced by, *P. agglomerans* strain Eh318 inhibit *E. amylovora in-vitro* condition. However, there is no many studies providing evidence of using *A. piechaudii* strains in biological control of plant diseases. In this study, it was observed that mixtures of bacterial strains which have antibiosis and hyperparasitic activities were more effective against the pathogen than those have just antagonistic effect. Several mechanisms could be operated on these apple shoots, including antibiosis, hyperparasitism, induced resistance, and competition for space and limited nutrients.

The Biolog Microplate system is quite useful for identification and characterization of plant pathogenic bacteria based on determination of carbon utilization profile (Jay and Aaron 1991; Michael et al. 1993; Kotan et al. 2006). In this study, Biolog microplate system was utilized to determine the nutritional relationship between pathogen and biocontrol agents tested. The results indicated that there is a week relation in nutritional profile of *P. s.* pv. *syringae* and its potential biocontrol strains selected, which have significant disease reductions *in-vivo* assay. However, the other strains with relatively low similarity in terms of carbon source utilization were not effective in biological control of disease on apple *in vivo* assay. Effective biological control of disease may require higher nutritional relationship between plant pathogens and their biocontrol agents, which share and compete for space and nutrients. These findings are expected because pathogen and its biocontrol agents were sharing the same environmental niche and compete for nutrients. There are many previous works demonstrating competition between pathogen and other organism on the same host plants (Dianesi et al. 2003; Janisiewicz et al. 2000; Ji and Wilson 2002). However, there was no study to investigate the importance of nutritional similarity between *P. s.* pv.

syringae and its biocontrol agents. Therefore, it may be very important to determine nutritional relationship between pathogen and potential biocontrol agent under investigation for effective disease management. *P. s.* pv. *syringae* pathosystem on apple shoots has been used as a model of biological control in the present study. Further studies are necessary to find out role of nutritional similarity for effective disease management in natural conditions with this and many other pathosystems.

In conclusion, our results showed that many strains of *P. agglomerans*, *A. piechaudii*, *L. adecarboxylata*, *C. flaccumfaciens*, *E. agglomerans*, *E. chrysanthemi*, *E. rhapontici*, *P. putida* and *S. liquefaciens* have antagonistic activity against *P. s.* pv. *syringae*. These strains may have a great potential to be used as biocontrol agents.

Acknowledgement

The research was supported by Atatürk University of Turkey with the project number 2002/97.

ÖZET

PSEUDOMONAS SYRINGAE PV. *SYRİNGAE*'NİN BİYOLOJİK KONTROLÜ VE PATOJEN İLE POTANSİYEL BİYOAJANLARI ARASINDA KARBON KAYNAKLARININ KULLANIMINDAKİ BENZERLİKLER

Bu çalışmada; yumuşak çekirdekli meyvelerin toprak üstü kısımlarından 35 farklı cins ve 62 türe ait toplam 206 bakteriyel izolat elde edilmiştir. Bütün bakteriyel izolatlar yumuşak çekirdekli meyvelerde yaprak ve sürgünlerde nekrozlara sebep olan *Pseudomonas syringae* pv. *syringae*'ye karşı *in-vitro* ve *in-vivo* denemelerde antagonistik aktiviteleri için test edilmiştir. Test edilen izolatların sadece 71'inin *in-vitro* testlerde patojene karşı antagonistik aktiviteye sahip olduğu belirlenmiştir. Bu izolatlardan 22'si hiperparazitik aktiviteleri ya da *in-vitro*'daki petri denemelerinde 50 mm'nin üzerindeki inhibasyon zonlarından dolayı potansiyel antagonist olarak seçilmiştir. Seçilen bu izolatlar tek tek ya da 6 farklı kombinasyonlar halinde *in-vivo* şartlarda Golden delicious elma sürgünleri kullanılarak test edilmiştir. Elde edilen sonuçlar; bütün antagonistik bakterilerin patojenin sebep olduğu hastalığı önemli ölçüde azalttığını göstermiştir. Ancak en iyi sonuçlar dört adet *Pantoea agglomerans* (RK 84, 85, 113 ve 154), birer adet *Leclercia adecarboxylata* (RK 164), *Pseudomonas putida* (RK 142), *Curtobacterium flaccumfaciens* (RK 114), *Erwinia rhapontici* (RK 135), *Alcaligenes piechaudii* (RK 137), *Serratia liquefaciens* (RK 102) ve onların kombinasyonlarından elde edilmiştir. Bütün bakteri izolatları Biolog Microplateleri (BIOLOG, Hayward, CA) kullanılarak karbon kaynaklarını kullanımı esas alınarak karakterize edilmiştir. Sonuçlar bu patojen ile biyokontrol ajanlarının besin profillerinde bir korelasyonun olduğunu göstermiştir. Bu yüzden bu çalışma *Pseudomonas syringae* pv. *syringae* ile biyokontrol ajanları arasında gıda benzerliğinin etkili hastalık yönetimi proğramlarının belirlenmesinde ihtiyaç duyulan önemli bir faktör olduğunun ilk kanıtıdır.

Anahtar Kelimeler: Antibiyosis, biyokontrol, BIOLOG, rekabet, *Pseudomonas syringae* pv. *syringae*, yaprak nekrozu, yumuşak çekirdekli meyve

LITERATURE CITED

AYSAN, Y., A. KARATAS, O. CINAR, 2003: Biological control of bacterial stem rot caused by *Erwinia chrysanthemi* on tomato. **Crop Protection 22 (6)**, 807-811.

BALESTRA, GM., L. VARVARO, 1997. *Pseudomonas syringae* pv. *syringae* causal agent of disease on floral buds of *Actinidia deliciosa* (A. Chev) Liang et Ferguson in Italy. **Journal of Phytopathology** 145, 375-378.

BRAUN-KIEWNICK, A., BJ. JACOBSEN, DC. SANDS, 1997. Possible biocontrol mechanisms in the *Pseudomonas syringae-Pantoea agglomerans* interaction. **Phytopathology** 87, 11.

BURR, TJ., JL. NORELLI, B. KATZ, WF. WILCOX, SA. HOYING, 1988. Steptomycin -resistance of *Pseudomonas syringae* pv. *syringae* in apple orchards and its association with a conjugative plasmid. **Phytopathology** 78, 410-413.

CANFIELD, ML., S. BACA, LW. MOORE, 1986. Isolation of *Pseudomonas syringae* from 40 cultivars of diseased woody plants with tip dieback in Pacific Northwest nurseries. **Plant Disease** 70, 647-650.

CODY, YS., DC. GROSS, EL. JR. PROEBSTING, RA. SPOTTS, 1987. Suppression of Ice nucleation-active *Pseudomonas syringae* by antagonistic bacteria in fruit tree orchards and evaluations of frost control. **Phytopathology** 77, 1036-1044.

COLYER, PD., MS. MOUNT, 1984. Bacterization of potatoes with *Pseudomonas putida* and its influence on postharvest soft rot diseases. **Plant Disease** 68, 703-706.

DIANESE, AC., P. JI, M. WILSON, 2003. Nutritional similarity between leaf-associated nonpathogenic bacteria and the pathogen is not predictive of efficacy in biological control of bacterial spot of tomato. **Applied and Environmental Microbiology** 69(6), 3484-3491.

ENDERT, E., DF. RITCHIE, 1984. Detection of pathogenicity, measurement of virulence and determination of strain variation in *Pseudomonas syringae* pv. *syringae*. **Plant Disease** 68, 677-680.

FAHY, P.C., C. HAYWARD, 1983. Media and methods for isolation and diagnostic tests. In: Fahy, P.C., Persley, G.J. (eds.): Plant Bacterial Diseases: A Diagnostic Guide, 337-374. Academic Press, New York.

JANISIEWICZ, WJ., J. ROITMAN, 1988. Biological control of blue mold and gray mold on apple and pear with *Pseudomonas cepacia.* **Phytopathology** 78, 1697-1700.

JANISIEWICZ, WJ., TJ. TWORKOSKI, C. SHARER, 2000. Characterizing the mechanism of biological control of postharvest diseases on fruits with a simple method to study competition for nutrients. **Phytopathology** 90, 1196-1200.

JAY, LG., LM. AARON, 1991. Classification and characterization of heterotrophic microbial communities on the basis of patterns of community-level sole-carbon-source utilization. **Applied and Environmental Microbiology** 57(8), 2351-2359.

JI, P., M. WILSON, 2002. Assessment of the importance of similarity in carbon source utilization profiles between the biological control agent and the pathogen in biological control of bacterial speck of tomato. **Applied and Environmental Microbiology** 68, 4383-4389.

JONES, AL., HS. ALDWINCKLE, 1991. Compendium of apple and pear diseases. APS PRESS. **The American Phytopathological Society** 61-66.

KOTAN, R., 2002. Isolation And Identification Of Pathogenic And Saprophytic Bacterial Organisms From Pome Fruits Grown In Eastern Anatolia Region Of Turkey By Commercial And Molecular Techniques, And Researches On The Biological Control Strategies. Atatürk Üniversity, Graduate School of Natural and Applied Sciences, Department of Plant Protection, Ph. D. Thesis, p. 217.

KOTAN, R., F. SAHIN, 2002. First record of bacterial cancer by *Pseudomonas syringae* pv. *syringae*, on apricot trees in Turkey. **Plant Pathology** 51, 798.

KOTAN, R., F. SAHIN, A. ALA, 2005. Identification and pathogenicity of bacteria isolated from pome fruits trees in eastern Anatolia region of Turkey. **Journal of Plant Disease and Protection** 113 (1), 8-13.

LEVEAU, JH., SE. LINDOW, 2001. Appetite of an epiphyte: Quantitative monitoring of bacterial sugar consumption in the phyllosphere. Proc. Natl. Acad. Sci. USA. 98, 3446-3453.

LINDEMANN, J., TV. SUSLOW, 1987. Competition between ice nucleation-active wilt type and ice nucleation-deficient deletion mutant strains of *Pseudomonas syringae* and *Pseudomonas fluorescens* biovar I and biological control of frost injury on strawberry blossoms. **Phytopathology** 77, 882-886.

LINDOW, SE., G. MCGOURTY, R. ELKINS, 1996. Interactions of antibiotics with *Pseudomonas fluorescens* Strain A506 in the control of fire blight and frost injury to pear. **Phytopathology** 86, 841-848.

LORITO, M., V. FARKAS, S. REBUFF, BY. BODO, C. KUBIECK, 1996. Cell wall synthesis is a major target of mycoparasitic antagonism by *Trichoderma harzianum.* **Journal of Bacteriology** 178, 6382-6385.

MANSVELT, EL., MJ. HATTINGH, 1986. Pear blossom blast in South Africa caused by *Pseudomonas syringae* pv. *syringae*. **Plant Pathology** 35, 337-343.

MCCLURE, NC., AR. AHMADI, BG. CLARE, 1998. Construction of arange of derivatives of the biological control strain *Agrobacterium rhizogenes* K84: a study of factors involved in biological control of crown gall disease. **Applied and Environmental Microbiology** 64, 3977-3982.

MICHAEL, M., WB. JAMES, KQ. VIRGINIA, CM. JAMES, 1993. Evaluation of biology for identification of members of the family Micrococcaceae. **Journal of Clinic Microbiology** 67, 3170-3173.

MONTESINOS, E., P. VILARDELL, 1990. Relationships among population levels of *Pseudomonas syringae*, amount of ice nuclei and incidence of blast of dormant

flower buds in commercial pear orchards in Catalunya, Spain. **Phytopathology** 81, 113-119.

MORAGREGA, C., I. LLORENTA, C. MANCEAU, E. MONTESINOS, 2003. Susceptibility of Europen pear cultivars to *Pseudomonas syringae* pv. *syringae* using immature fruit and detached leaf assays. **European Journal of Plant Pathology** 109, 319-326.

OZAKTAN, H., H. TÜRKÜSAY, 1994. Investigation on the antagonistic effects of some epiphytic bacteria against *Erwinia amylovora* (Burr.) Winslow *et al.* The causal agent of fire bilight. 3 th Congress of Biological Control, 25-28 January, Izmir, Turkey, pp. 231-238.

PAISLEY, R., 1995. MIS whole cell fatty acid analysis by gas chromatography. MIDI, Inc., Newark, DE, 5.

ROY, MA., 1988. Use of fatty acids for the identification of phytopathogenic bacteria, **Plant Disease** 72, 460.

SCHECK, HJ., JW. PSCHEIDT, LW. MOORE, 1996. Copper and streptomicyn resistance in Strains *Pseudomonas syringae* from Pacific Northwest Nurseries. **Plant Disease** 80, 1034-1039.

SCHILINGER, U., F. LUCKE, 1989. Antibacterial activity of *Lactobacillus* strain isolated from meat. **Applied and Environmental Microbiology** 55, 1901-1906.

WHITESIDES, SK., RA. SPOTTS, 1991. Frequency, distribution and characteristics of endophytic *Pseudomonas syringae* in pear trees. **Phytopathology** 81, 453-457.

WILSON, M., SE. LINDOW, 1994. Ecological similarity and coexistence of epiphytic ice-nucleating (Ice^+) *Pseudomonas syringae* strains and a non-ice-nucleating (Ice^-) biological control agent. **Applied and Environmental Microbiology** 60, 3128-3137.

WRIGHT, SA., CH. ZUMOFF, L. SCHNEIDER, SV. BEER, 2001. *Pantoea agglomerans* Strain EH318 produces two antibiotics that inhibit *Erwinia amylovora in-vitro*. **Applied and Environmental Microbiology** 67(1), 284-292.

ZELLER, W., B. WOLF B, 1996. Studies on biological control of fire blight. **Acta Horticulture** 411, 341-345.