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Nutritional Similarity in Carbon Source Utilization of *Erwinia amylovora* and Its Potential Biocontrol Agents

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ABSTRACT

Total 206 bacterial strains belonged to 35 different genera, were isolated from pome fruits, and tested for antagonistic activity against *Erwinia amylovora* strains causing fire blight on pome fruits under *in-vitro* and *in-vivo* conditions. Sixty two of the test strains were selected as potential biocontrol agents, which displayed antagonistic activity against *Erwinia amylovora* strains in *in-vitro* condition. The antagonistic activities of the pre-selected strains alone and in combinations were further tested in *in-vivo* tests using one year-old Golden delicious apples twigs. The results suggested that 21 strains and their six different combinations had potential to be used as biocontrol agents for management of fire blight. The most successful results obtained from four *Pantoea agglomerans*, one *Enterobacter intermedius*, one *Pseudomonas putida* strains and six different combinations of the bacterial strains tested. Nutritional similarity was determined by using sole carbon source utilization profiles (BIOLOG, Hayward, CA) between biocontrol agents and pathogen. This study indicated that there is a strong correlation in nutritional profile of pathogen of fire blight and potential biocontrol agents selected. Therefore, this is the first study providing evidence that nutritional similarity between pathogen and biocontrol agents may be one of the important factors and needs to be used for effective disease management programs.

Key words: *A*ntibiosis, BIOLOG, biological control, *Erwinia amylovora*, fire blight, MIS, *Pantoea agglomerans*

INTRODUCTION

Fire blight, caused by *Erwinia amylovora* (Burrill, Winslow et al.), is a common and very serious bacterial disease limiting the production of pear, apple, and other pome fruits and other members of the family of Rosaceae in many parts of the world (Van der Zwet and Keil 1979). Characteristic symptoms of the disease are wilting and water soaking and eventual necrosis of affected tissues. Disease severity is dependent on the susceptibility of the host, the aggressiveness of the pathogen, and the environment (Demir and Gündogdu 1993). Fire blight was the first recorded Turkey in some pear orchards of central and western part of Anatolia in 1985 (Oktem and Benlioglu 1988) and also in Denizli, Aydın and İzmir provinces in 1986 (Demir and Gündogdu 1991). It

is now distributed practically in most of the pear, quince and apple growing regions of Turkey (Momol et al. 1991; Momol and Yegen 1993; Kotan 2002; Kotan et al. 2006).

Cultural management of the host, removing and destroying infected tissue from trees and pruning infected branches plays an important role in the severity of disease (Momol et al. 1991). Chemical control programs have focused on the suppression of *E. amylovora* on floral parts through the use of antibiotics (Pusey 1997). However, the high costs of chemical control, failures in chemical control due to resistance development, and lack of other effective control measures have, therefore, generated considerable interest in biological control of fire blight (Loper et al. 1991; Saygılı and Ustün 1996). Previous studies have reported the presence of some bacterial species releasing antimicrobial compounds which inhibite growth of *E. amylovora* (Wodzinski and Paulin 1994; Lindow et al. 1996; Pusey 1997; Nuclo et al. 1998; Pusey 1999; Johnson et al. 2000; Mercier and Lindow 2001; Wright et al. 2001; Jock et al. 2002; Pusey 2002; Stockwell et al. 2002; Ozaktan and Bora 2003; Johnson et al. 2004; Broggini et al. 2005). The first commercial biological control product for fire blight, Blight Ban (Plant Health Technologies, Boise, Idaho), went on the market Pf-A506, which competes with the fire blight bacterium for nutrients or blossoms, keeping the numbers of *Erwinia* low enough to avoid severe infection (Lindow et al. 1996).

Biological control of phytopathogens is known to be achieved by four different mechanisms including antibiosis, competition, hyperparatism and induced resistance (Janisiewicz and Korsten 2002). Most biocontrol agents apply only one of these four mechanisms; however, some may employ more than one. Those mechanisms are direct competition with the target organism, antibiosis, predation or parasitism of the target organism and induced resistance of the host plant (Cook and Baker 1983). Competition for nutrients and space can be an effective mechanism of biological control. However, these mechanisms of biological control have been difficult to study because no method has been available to determine the significance of each of the components of competition (Dianese et al. 2003).

It is well known that there is a great deal of specificity among pathogen, host, and biological control agents (Poppe et al. 2003). The specificity may be explained many factors, one of which is nutritional similarity between pathogen and biocontrol agents. However, there have been no attempts to study nutritional similarity between *E. amylovora* and its biocontrol agents so far.

Therefore, the aims of this study were to evaluate the nonpathogenic bacterial strains isolated from pome fruits for biological control of *E. amylovora*, and to determine nutritional similarity between *E. amylovora* and potential biocontrol agents by using sole carbon source utilization profiles.

MATERIALS and METHODS

Candidate antagonistic bacterial Strains used for testing

Total 206 nonpathogenic bacterial strains belonged to 35 different genera were used in this study (Table 1). The bacteria had been isolated originally from aerial part of

pome fruits from different locations in eastern Anatolia region of Turkey. All bacterial strains had been identified according to fatty acid methyl ester profile by using the MIDI system (Microbial Identification System, Inc., Newark, DE, version 5.0) (Roy 1988; Paisley 1995). Pathogenicity of all potential biocontrol bacterial strains has been determined in a previous study on the basis of hypersensitivity test on tobacco plants and inoculation on apple twigs cv. Golden delicious. All of them were not pathogen. But, the bacterium *E. amylovora* Strain RK-228 was determined to be pathogen on apple and pear (Kotan 2002). All strains were stored at – 80˚C in 15% glycerol and Luria Broth (LB) for using further studies.

Table 1. Antagonistic activity of biocontrol bacterial strains against *E. amylovora* in *in-vitro* condition

Bacterial genus[a]	NTS	*In-vitro* test results[b]			
		-	+	++	+++
Pantoea spp.	32	15	7	4	6
Bacillus spp.	31	22	7	2	0
Enterobacter spp.	23	13	8	2	0
Alcaligenes spp.	17	7	5	2	3
Serratia spp.	12	9	3	0	0
Leclercia spp.	11	8	3	0	0
Agrobacterium spp	9	9	0	0	0
Burkholderia spp.	8	8	0	0	0
Klebsiella spp.	8	6	2	0	0
Acinetobacter spp.	7	5	2	0	0
Erwinia spp.	7	5	0	2	0
Pseudomonas spp.	7	6	1	0	0
Curtobacterium spp.	4	4	0	0	0
Actinomadura spp.	3	3	0	0	0
Chryseobacterium spp.	2	2	0	0	0
Citrobacter spp.	2	2	0	0	0
Kocuria spp.	2	2	0	0	0
Plesiomonas spp.	2	2	0	0	0
Ralstonia spp.	2	2	0	0	0
Vibrio spp.	2	2	0	0	0
Aerococcus spp.	1	1	0	0	0
Brevibacillus spp.	1	1	0	0	0
Brevibacterium spp.	1	1	0	0	0
Brevundimonas spp.	1	1	0	0	0
Chromobacterium spp.	1	0	1	0	0
Escherichia spp.	1	1	0	0	0
Methylobacterium spp.	1	1	0	0	0
Micrococcus spp.	1	1	0	0	0
Neisseria spp.	1	1	0	0	0
Pediococcus spp.	1	1	0	0	0
Photobacterium spp.	1	1	0	0	0
Proteus spp.	1	1	0	0	0
Salmonella spp.	1	0	1	0	0
Sphingomonas spp.	1	1	0	0	0
Yersinia spp.	1	0	1	0	0
Number of total strains	206	144	43	11	9

[a] Source is Kotan (2002) and Kotan et al. (2005)

[b] -: no inhibition zone, +: 5-29 mm (weak inhibition zone), ++: 30-49 mm (moderate inhibition zone) and +++ :> 50 mm (strong inhibition zone), and all tests were repeated three times per week to three replicate for each bacterial strains

NTS: Number of tested strains

In-vitro antagonistic activity on Petri dishes

The nonpathogenic bacteria were tested for antagonistic activity against *E. amylovora* Strain RK-228 that was determined to be pathogen on apple and pear (Kotan et al. 2005). Standard medium Sensitive Agar (SA) was used all *in-vitro* Petri assays. The well diffusion assay described by Schilinger and Lucke (1989) was used with a minor modification that included by spreading 100 µl of suspension of 10^8 cfu/ml^{-1} of the pathogen on whole SA plates (95 mm diameter) surface and streaked 20 µl of suspension of 10^8 cfu/ml^{-1} of antagonistic bacteria with a sterile swap as single line the middle of the plates. 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. Data were the combined means from three separate experiments which were repeated for each strain. The ratings used were modified from Lee and Hwang (2002). The mean of inhibition zones was given in Table 1 as; -: no inhibition zone, +: 5-29 mm (weak inhibition zone), ++: 30-49 mm (moderate inhibition zone) and +++: > 50 mm (strong inhibition zone). Total 62 strains were selected as potential biocontrol agents based on the result of *in-vitro* tests and further tested *in-vivo* assay.

In-vivo assays on shoots

In-vivo assay, sixty-three antagonistic strains selected in in-vitro assay was tested for in-vivo assay alone or six different combinations of 10 strains (*Pantoea agglomerans* Strain RK-79, 80, 84 and 160, *Alcaligenes piechaudii* Strain RK-105 and 156, *Leclercia adecarboxylata* Strain RK-164, *Bacillus pumilus* Strain RK-103, *Curtobacterium flaccumfaciens* Strain RK-114 and *Pseudomonas putida* Strain RK-142). The combinations consisted of A (*A. piechaudii* strain RK-105 and RK-156), B (*P. agglomerans* strain RK-79 and *L. adecarboxylata* RK-164), C (*A. piechaudii* strain RK-156 and *B. pumilus* RK-103), D (*P. agglomerans* strain RK-80 and RK-84), E (*P. agglomerans* strain RK-160 and *P. putida* RK-142) and F (*A. piechaudii* strain RK-105, *B. pumilus* RK-103, *C. flaccumfaciens* RK-114, *L. adecarboxylata* RK-164, RK- *P. agglomerans* 79 and *P. putida* RK-142). An *in-vivo* test described by Moragrega et al. (2003) was used with a minor modification. One year-old Golden delicious apples twigs (15-20 cm length) were inoculated with 10^8 cfu/ml^{-1} suspensions of pathogen and antagonistic bacterial cultures grown on nutrient broth (NB) for 24-48 h on rotary shakers under 130 rpm at 26-27 ˚C. Absorbance of bacterial suspension was measured spectrophotometrically at 600 nm and appropriately diluted to 10^8 cfu/ml^{-1} with sterilized water. Each of apple twigs was sprayed by 200 ml of the pathogen and biocontrol bacterial suspensions, which were diluted 1/1 ratio. It was used the only pathogen suspension and sterile distillated water as positive and negative control, respectively. The inoculated twigs separately put in flasks were full with water and incubated in polyethylene bags at room temperature for 3 days and maintained on the greenhouse bench after which the bags were removed. Development of typical bacterial fire blight symptoms were observed on leaves of inoculated twigs after incubation 20 days. Results were evaluated by based on 1-5 scales (1 = no symptom on leaves, 2 =1-25% disease of leaves, 3=26-50% disease of leaves, 4=51-75% disease of leaves and 5=76-100% disease of

leaves) (Braun-Kiewnick et al. 1997). This test was repeated at least three times for each biocontrol strain.

Statistical analyses

In-vivo data were analyzed 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.

Determination of similarity in carbon source utilization profiles between the potential biocontrol agents and *E. amylovora*

Biolog GN and GP micro titer plates (Biolog, Inc., Hayward, CA) were used in this study. One or two days before the inoculation of Biolog plates, pathogen and potential biocontrol bacterial strains were streaked on TSA or BUG agar plates. Each well of biolog GN2 or GP2 micro titer plates (Biolog, Inc. Hayward, CA) was inoculated with 125 µl of the bacterial suspension respectively adjusted to the appropriate density (10^8 cfu/ml) and incubated at 27˚C for 24 and 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 on the Biolog GN and GP profiles with 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). The results were given in Table 3, 4.

RESULTS

According to *in-vitro* test results, only 63 of the 206 strains (30.58%) were found with an inhibitory activity against *E amylovora* (Table 1). Of which was 27.41% *Pantoea* sp., 16.12% *Alcaligenes* sp. and *Enterobacter* sp., 14.51% *Bacillus* sp. and 24.19% others belong to 9 different genus (*Acinetobacter* sp., *Chromobacterium* sp., *Erwinia* sp., *Klepsiella* sp., *Leclercia* sp., *Pseudomonas* sp., *Serratia* sp., *Salmonella* sp. and *Yersinia* sp.). The other 144 strains significantly did not have significant inhibitory activity against *E. amylovora.*

The *in-vivo* tests results, given in Table 2, demonstrated that twenty one bacterial strains belonging to 10 different species (*Alcaligenes* sp., *Bacillus* sp., *Chromobacterium* sp., *Curtobacterium* sp., *Enterobacter* sp., *Erwinia* sp., *Leclercia* sp., *Pantoea* sp., *Pseudomonas* sp.) and in six different combinations of some strains were found effective antagonist under *in-vivo* conditions. All of them significantly reduced disease severity compared with the positive control (treated with pathogen only). The remaining forty two strains belonging to 10 different species (*Acinetobacter* sp., *Alcaligenes* sp., *Bacillus* sp., *Enterobacter* sp., *Klepsiella* sp., *Leclercia* sp., *Pantoea* sp., *Salmonella* sp., *Serratia* sp. and *Yersinia* sp.) were not found effective antagonist under *in-vivo* conditions.

Table 2. Disease severity results on one year old apple shoots in *in-vivo* condition, and nutritional similarity in carbon source utilization between biocontol bacterial strains and pathogen

Treatments	Strains	Disease severity[a]	Nutritional similarity (%)[b]
Positive control (only pathogen)	RK-228	5.00 a	ND
The other 41 different strains		5.00 a	NT
Bacillus pumilus	RK-103	3.66 b	0.54
Chromobacterium violaceum	RK-165	3.66 b	1.00
Pantoea agglomerans	RK-113	3.66 b	0.97
Erwinia rhapontici	RK-135[b]	3.00 bc	1.00
Alcaligenes piechaudii	RK-136	2.66 cd	0.91
Alcaligenes piechaudii	RK-157	2.33 cd	1.00
Alcaligenes piechaudii	RK-158	2.33 cd	1.00
Erwinia chrysanthemi	RK-67	2.33 cd	0.97
Laclercia adecarboxylata	RK-163	2.33 cd	0.77
Laclercia adecarboxylata	RK-164	2.66 cd	0.74
Pantoea agglomerans	RK-92	2.33 cd	0.88
Alcaligenes piechaudii	RK-137[b]	2.33 cd	0.82
Curtobacterium flaccumfaciens	RK-114[b]	2.66 cd	0.85
Pantoea agglomerans	RK-154	2.00 de	1.00
Pantoea agglomerans	RK-169	2.00 de	0.82
Enterobacter intermedius	RK-91	1.33 ef	0.77
Pantoea agglomerans	RK-80	1.33 ef	0.94
Pantoea agglomerans	RK-123	1.33 ef	1.00
Pseudomonas putida	RK-142	1.33 ef	0.97
Pantoea agglomerans	RK-79	1.00 f	1.00
Pantoea agglomerans	RK-84	1.00 f	1.00
Negative control (water)		1.00 f	ND
Combination E		1.33 ef	0.97
Combination A		1.00 f	0.96
Combination B		1.00 f	0.87
Combination D		1.00 f	0.97
Combination C		1.00 f	0.76
Combination F		1.00 f	0.77

[a]: Disease severity tests were applied three times per week to three replicate for each bacterial strains. Based on 1-5 scales. Means with the same letter are not significantly different by Duncan multiple range test at $p<0.05$

[b]: Nutritional similarity was estimated by using carbon utilization data on the Biolog GN2 or GP2 profiles with the formulate NSI_{Biolog}= the number of compounds used by both the biocontrol bacteria and pathogen/the number of compounds used by pathogen.

The potential biocontrol strains were as follows; *Pantoea agglomerans* (8 strains), *Alcaligenes piechaudii* (5 strains), *Laclercia adecarboxylata* (2 strains), *Erwinia chrysanthemi*, *Bacillus pumilus*, *Enterobacter intermedius*, *Enterobacter agglomerans*, *Curtobacterium flaccumfaciens*, *Chromobacterium violaceum* and *Pseudomonas putida* (1 strain). The most successful results were obtained from six

bacterial strains (*P. agglomerans* RK-79, 80, 84 and 123; *E. intermedius* RK-91 and *P. putida* RK-142) in six different combinations *in-vivo* assays (Table 2) at 10^8 cfu/ml^{-1} concentrations. There was no significant difference in disease severity between these six successful combinations and/or the negative control (treated with sterilized water). The remaining 15 strains also reduced disease severity, but they were not as effective as the six bacterial strains fully controlled disease development on shoots.

Table 3. Relationship in carbon utilization of pathogen *E. amylovora* and biocontrol bacterial strains based on GN2 or GP2 Microplate assay

Carbon sources	A	B	C	Carbon sources	A	B	C
L-Arabinose	0.95	1.00	0.93	D-Sorbitol	0.76	0.83	0.73
Formic Acid	0.90	1.00	0.87	á-Ketoglutaric Acid	0.71	0.83	0.67
D,L-Lactic Acid	0.90	1.00	0.87	Glycogen	0.67	1.00	0.53
Bromosuccinic Acid	0.90	1.00	0.87	Succinic Acid Mono- Methil Ester	0.67	0.83	0.60
L-Alanine	0.90	1.00	0.87	L-Alaninamide	0.67	0.83	0.60
L-Alanyl-Glycine	0.90	1.00	0.87	D-Raffinose	0.48	0.50	0.47
L-Aspartic Acid	0.90	1.00	0.87	N-Acetyl-D-Glucosamine	1.00	1.00	1.00
Glycyl-L-Aspartic Acid	0.90	1.00	0.87	D-Galactose	1.00	1.00	1.00
L-Proline	0.90	1.00	0.87	â-Methyl-D-Glucoside	1.00	1.00	1.00
á-D-Glucose-1 Phosphate	0.90	1.00	0.87	Sucrose	1.00	1.00	0.93
D-Fructose	0.86	0.83	0.87	D-Trehalose	1.00	1.00	1.00
D-Psicose	0.86	1.00	0.80	Pyruvic Acid Methil Ester	1.00	1.00	0.93
Glycyl-L-Glutamic Acid	0.86	0.83	0.87	D-Gluconic Acid	1.00	1.00	0.93
Gentiobiose	0.81	0.50	0.93	L-Glutamic Acid	1.00	1.00	1.00
m-Inositol	0.81	1.00	0.73	L-Serine	1.00	1.00	0.93
D-Mannitol	0.81	0.83	0.80	Inosine	1.00	1.00	0.93
Succinic Acid	0.81	1.00	0.73	Glycerol	1.00	0.83	1.00
á-D-Glucose	0.76	0.83	0.73	D-Glucose-6 Phosphate	1.00	1.00	1.00
The average of percentage carbon source					0.85	0.93	0.85

A: % Carbon sources utilized by whole bioagents reduced disease severity *in-vivo* assays and *E. amylovora*, B: The most effective bioagents and *E. amylovora*, C: The less effective bioagents and *E. amylovora*

Nutritional similarity between bioagents which reduced disease severity in *in-vivo* assays, and *E. amylovora* (according to carbon source profiles, carbon sources, and the percentage of them utilized by both bioagents and *E. amylovora*, and utilized by only bioagents not *E. amylovora*) were given in Table 2, 3, 4 respectively. The nutritional similarity between the bioagents and *E. amylovora* was among 0.54% - 1.00% (Table 2). The number of the carbon sources utilized by the bioagents and *E. amylovora*, and utilized by only bioagents but not *E. amylovora* were 36, 29 respectively (Table 3, 4). The percentage of the carbon sources utilized by the whole bioagents and *E. amylovora*, the most effective bioagents and *E. amylovora*, the less effective bioagents and *E. amylovora* were 85%, 93% and 85% respectively (Table 3).

The percentage of carbon sources utilized by whole bioagents but not utilized by *E. amylovora*, the most effective bioagents but not *E. amylovora*, the less effective bioagents but not *E. amylovora* were 83%, 87% and 82% respectively (Table 4). This study indicated that there is a strong correlation in nutritional profile of pathogen of fire blight and potential biocontrol agents selected, and carbon sources utilized by the most active bioagents have important role in biocontrol.

Table 4. Relationship in carbon utilization of pathogen *E. amylovora* and biocontrol bacterial strains based on GN2 or GP2 Microplate assay

Carbon sources	A	B	C	Carbon sources	A	B	C
Dextrin	1.00	1.00	1.00	Uridine	0.90	1.00	0.87
Maltose	1.00	1.00	1.00	Thymidine	0.86	1.00	0.80
D-Mannose	1.00	1.00	1.00	Malonic Acid	0.81	0.67	0.87
L-Rhamnose	1.00	1.00	1.00	Quinic Acid	0.81	0.83	0.80
Acetic Acid	1.00	1.00	1.00	Urocanic Acid	0.81	0.83	0.80
Cis-Aconitic Acid	1.00	1.00	1.00	D-Arabitol	0.70	0.50	0.87
D-Galacturonic Acid	1.00	1.00	1.00	L-Omithine	0.67	0.67	0.67
L-Asparagine	1.00	1.00	0.93	ã-Aminobutyric Acid	0.67	0.83	0.60
D-Glucuronic Acid	0.95	1.00	0.93	D-Melibiose	0.62	0.67	0.60
D-Saccharic Acid	0.95	1.00	0.93	Citric Acid	0.62	0.67	0.60
D,L,á-Glycerol Phosphate	0.95	1.00	0.93	Tween 40	0.57	0.67	0.53
Glucuronamide	0.90	0.83	0.93	á-Hydroxybutyric Acid	0.57	0.67	0.53
D-Alanine	0.90	0.83	0.93	Tween 80	0.52	0.83	0.40
L-Histidine	0.90	0.83	0.93	Turanose	0.52	0.83	0.40
D-Serine	0.90	1.00	0.87				
The average of percentage carbon source					0.83	0.87	0.82

A: % Carbon sources utilized by whole bioagents reduced disease severity *in-vivo* assays but not *E. amylovora*, B: Utilized by the most effective bioagents but not *E. amylovora*, C: Utilized by the less effective bioagents but not *E. amylovora*

DISCUSSION

There are many microbial pesticides which are formulated for commercial uses, and have been used as a safe and alternative method in controlling of plant diseases (Broggini et al. 2005). But the number of microbial pesticides used against foliar pathogens is fairly less. Many of them have been used against post harvest pathogens or soil borne plant disease. The development of the biocontrol agents will help to decrease the negative effects (residues, resistance and environmental pollution) of chemical pesticides which are commonly and extensively used for plant disease management in agriculture. Fire blight is a major limiting factor in the production of pome fruit. It is most often initiated by epiphytic populations of *Erwinia amylovora* that develop on blossoms (Van der Zwet and Keil 1979). Research on biological control of fire blight has focused mainly on the flower stigma (Pusey 1999). So far, a lot of studies have successfully employed to determine antagonistic bacteria to control fire blight disease

(Johnson et al. 2000; Mercier and Lindow 2001; Wright et al. 2001; Jock et al. 2002; Pusey 2002; Stockwell et al. 2002; Ozaktan and Bora 2003; Broggini et al. 2005). These studies have been generally focused on *P. agglomerans* that is generally known as saprophytic bacteria and are useful for biological struggle. It's known that *P. agglomerans* produced bacteriocin, inhibited *E. amylovora* even it effective by competing with pathogenic bacteria place and nutriment (Zeller and Wolf 1996). There are also many studies in literature which supports the suggestion of utilizing the feature of secreting antibiotics of this type of bio agents against leaf spot disease and many after harvest diseases (Colyer and Mount 1984; Janisiewicz and Roitman 1988; Aysan et al. 2003). Wright et al. (2001), states that pantocin A and pantocin B antibiotics which are produced by *P. agglomerans* strain Eh318 inhibit *E. amylovora in-vitro*. But, it was a little known that *A. piechaudii* may be used against rhisosphere and philosopher plant pathogens (Colyer and Mount 1984; Cody et al. 1987; Lindow et al. 1996; Whiteman and Stewart 1998). The whole 8 different combinations showed the high antagonistic activity under *in-vivo* condition because bacterial formulations which are formed of mixtures of some bacterial strains should have different effect mechanisms. Therefore, applications of mixtures of biocontrol agents should be useful in reducing diseases.

In this study, total 206 bacterial strains which were isolated originally from aerial part of pome fruits from different locations in eastern Anatolia region of Turkey had been tested against *E. amylovora* in *in-vitro* assays, and it was found that the 62 strains had an inhibitory activity. But, only twenty-one strains of them and six different combinations of some strains significantly reduced disease *in-vivo* assays. *In-vitro* and *in-vivo* assays showed that there was a not correlation between strain activity, but *in-vitro* and *in-vivo* assays provide some useful information about the applicability of potential bioagents. The majority of effected strains belonged to *P. agglomerans* (38.09%). But this study is the first report that determined to be effective biocontrol agents such as *A. piechaudii*, *L. adecarboxylata*, *E. chrysanthemi*, *B. pumilus*, *E. intermedius*, *E. agglomerans*, *C. flaccumfaciens*, *C. violaceum* and *P. putida*. For this reason, this study is very important.

In *in-vivo* assays, effective bacterial strains with a high degree of similarity in utilization of carbon sources with the pathogen exhibited a higher degree of suppression of the disease. A significant correlation between similarity in carbon source utilization between antagonistic bacterial strains and the pathogen indicated that similarity in carbon source utilization profiles is a significant characteristic contributing to the biological control efficacy of these bacteria. These results agree with several studies (Janisiewicz et al. 2000; Ji and Wilson 2002; Dianes et al. 2003). Some bacterial strains provided significant disease reductions although the similarity in carbon source utilization was fairly low, and vice versa, indicating that some other biological control phenotypes may have been involved in biological control for some of these strains. In summary, similarity in carbon source utilization profiles between the biocontrol bacteria and *E. amylovora* was correlated with efficacy in control of fire blight *in-vivo* conditions. All the same, I am of the opinion that nutritional similarity index between pathogen and candidate antagonists could be used in an initial *in-vitro* screening for biological control of plant diseases. Furthermore; this is first

study determined to nutritional similarity between effected biocontrol agents and *Erwinia amylovora*. In conclusion, our results show that some bacterial strains have antagonistic activity against *E. amylovora*. These strains can be used as potential biocontrol agents. Future research will be aimed at testing the effect of this isolate under field conditions on naturally infected trees.

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ÖZET

ERWİNİA AMYLOVORA İLE POTANSİYEL BİYOAJANLAR ARASINDA KARBON KAYNAKLARININ KULLANIMINDAKİ BENZERLİKLER

Yumuşak çekirdekli meyvelerin toprak üstü kısımlarından 35 farklı cinse ait toplam 206 bakteriyel strain izole edilmiştir. Bu strainlerin antagonistik aktiviteleri, yumuşak çekirdekli meyvelerde ateş yanıklığına sebep olan bakteriyel etmen *Erwinia amylovora*'ya karşı *in-vitro* ve *in-vivo*'da test edilmiştir. *İn-vitro* şartlarda patojene karşı antagonistik aktivite gösteren strainlerin 62'si potansiyel biyoajan olarak seçilmiştir. Bu strainler ve bazı kombinasyonları bir yıllık Golden delicious elma sürgünleri kulanılarak *in-vivo*'da test edilmiştir. Sonuçlar test edilen strainlerin 21'i ve bazılarının 6 farklı kombinasyonları ateş yanıklığının kontrolünde potansiyel biyoajan olarak kullanılabileceğini göstermiştir. En başarılı sonuçlar; dört *Pantoea agglomerans*, bir *Enterobacter intermedius*, bir *Pseudomonas putida* straini ve altı farklı kombinasyondan elde edilmiştir. Patojen ve biyoajanlar arasındaki kullandıkları karbon kaynaklarının (BIOLOG, Hayward, CA) benzerlikleri değerlendirilmiştir. Bu çalışma; ateş yanıklığı etmeni ile etkili biyoajanları arasında karbonhidrat profilleri açısından güçlü bir ilişkinin olduğunu göstermesi yanısıra patojen ve biyoajanları arasındaki besin tercihlerinin benzerliğinin etkili hastalık kontrol programlarında önemli faktörlerden birisi olabileceğinin de kanıtıdır.

Anahtar Kelimeler: *A*ntibiyosis, ateş yanıklığı, BIOLOG, biyolojik kontrol, *Erwinia amylovora*, MIS, *Pantoea agglomerans*, rekabet

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