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Identification and pathogenicity of bacteria isolated from pome fruit trees in the Eastern Anatolia region of Turkey

Identifizierung und Pathogenität von Bakterien aus ostanatolischen Kernobstbäumen

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Summary

Bacteria including possible pathogenic and non-pathogenic species were isolated from pome fruits grown in five different provinces of the Eastern Anatolia region of Turkey during the springs 1999, 2000 and 2001. A total of 324 bacterial strains belonging to 76 species in 36 genera were identified by using Sherlock Microbial Identification System (MIS). The most abundant bacterial species was *Erwinia amylovora* (16.9%), followed by *Pseudomonas syringae* pv. *syringae* (16.6%), *P. agglomerans* (11.7%), *Bacillus pumilus* (10.8%), *Enterobacter intermedius* (7.7%), *Alcaligenes piechaudii* (5.8%), *Leclercia adecarboxylata* (4%) and 69 other species (26.5%). Eighty-two of the strains from 12 different species are potential pathogens because they were hypersensitive response (HR) positive and infected Golden Delicious apple leaves. This is the first study to show that *A. piechaudii*, *B. pumilus*, *Chromobacterium violaceum*, *E. intermedius*, *Erwinia rhapontici*, *Pantoea agglomerans*, *Pseudomonas aeruginosa*, *Pseudomonas huttiensis*, *Pseudomonas cichorii* and *Pseudomonas putida* are possible foliar pathogens of pome fruits but, further in-depth testing will be needed to confirm these preliminary results. Possible pathogenic strains were characterized by fatty acid methyl ester profiles, metabolic fingerprints, serological and biochemical tests. However, discrepancies were found among identification results for some species. Our data therefore suggest that a single approach can not be adequate for the identification of plant pathogenic bacteria.

Key words: Biolog, ELISA, *Erwinia amylovora*, MIS, pome fruits, *Pseudomonas syringae* pv. *syringae*

Zusammenfassung

Pflanzenpathogene und nicht pathogene Bakterien wurden im Frühjahr 1999, 2000 und 2001 in fünf ostanatolischen Provinzen aus Kernobstbäumen isoliert. Mit Hilfe des Sherlock-Mikrobenidentifikationssystems (MIS) konnten insgesamt 324 Bakterienstämme aus 76 Arten in 36 Gattungen identifiziert werden. Die am weitesten verbreitete Bakterienart war *Erwinia amylovora* (16,9%), gefolgt von *Pseudomonas syringae* pv. *syringae* (16,6%), *P. agglomerans* (11,7%), *Bacillus pumilus* (10,8%), *Enterobacter intermedius* (7,7%), *Alcaligenes piechaudii* (5,8%), *Leclercia adecarboxylata* (4%) und 69 weiteren Arten (26,5%). Bei 82 Stämmen aus 12 Arten handelt es sich um potenzielle Pflanzenpathogene, da sie eine Hypersensitivitätsreaktion auslösten und Blätter der Apfelsorte Golden Delicios infizierten. Bisher waren *A. piechaudii*, *B. pumilus*, *Chromobacterium violaceum*, *E. intermedius*, *Erwinia rhapontici*, *Pantoea agglomerans*, *Pseudomonas aeruginosa*, *Pseudomonas huttiensis*, *Pseudomonas cichorii* und *Pseudomonas putida* als Blattpathogene des Kernobstes unbekannt, aber weitere Untersuchungen sind zur Bestätigung dieses Befundes erforderlich. Potenziell pathogene Bakterienstämme wurden durch ihre Fettsäuremethylester-Profile, metabolische Fingerabdrücke sowie durch serologische und biochemische Tests charakterisiert. Diskrepanzen zwischen den Ergebnissen der verschiedenen Verfahren weisen darauf hin, dass ein einzelner Test keine sichere Identifizierung pflanzenpathogener Bakterien gewährleisten kann.

Stichwörter: Biolog, ELISA, *Erwinia amylovora*, Kernobst, MIS, *Pseudomonas syringae* pv. *syringae*

1 Introduction

Pome fruit trees (apple, pear, mountain pear, quince, medlar and loquat) and related trees in the pome fruit section of the rose family are widely grown in Turkey for home fruit or commercial purposes. However, several infectious disease agents (biotic pathogens such as fungi, bacteria, viruses, nematodes, and mycoplasms) and non-infectious factors (abiotic factors such as temperature, moisture, nutrients, soil conditions, and chemicals) can seriously reduce fruit quality, aesthetic appearance and tree health.

Fire blight (*Erwinia amylovora* (Burrill) Bergey et al.), blister spot (*Pseudomonas syringae* pv. *papulans* (Rose) Dhanvantari), bacterial blossom blast and blister bark (*Pseudomonas syringae* pv. *syringae* van Hall.), crown gall (*Agrobacterium tumefaciens* (E.F. Smith & Townsend) Conn) and hairy root (*Agrobacterium rhizogenes* (Riker et al.)) are well-known bacterial diseases on pome fruits (Jones and Aldwinckle 1991). Fire blight is a destructive disease of rosaceous plant species (Zhang and Geŷder 1997). The disease is currently present in 44 countries, including most parts of North and Central America, the Pacific Rim and most countries of the Middle East, the Balkans and the Mediterranean area (Donat et al. 2005). It was the first recorded in Turkey in some pear orchards of the central and western part of Anatolia in 1985 (Öktem and Benlioğlu 1988). It is now distributed practically in most of the pear, quince and apple growing regions of Turkey (Momol and Yegen 1993; Demir and Gündogdu 1993; Kotan 2002). *P. syringae* pv. *syringae* van Hall infects a wide range of deciduous fruit trees such as pear, peach, cherry and plum as well as other woody plant species (Canfield et al. 1986; Roos and Hattingh 1987). In 1999 and 2000, it was reported that *P. syringae* pv. *syringae* caused bacterial canker on nearly 80% of apricot (*Prunus armeniaca*) trees grown in commercial orchards and home gardens in some provinces in Turkey (Kotan and Sahin 2002). Crown gall is a plant disease that is widespread all over the world, and is particularly serious in nurseries. The disease has been recorded in Turkey and it is stated that this is probably the most serious disease affecting apple more than others pome fruits (Turan and Tokgönül 1993). There is not any report about another bacterial disease on pome fruits. Furthermore, there have been no attempts to determine the epiphytic and pathogenic bacteria causing disease on pome fruits in the Eastern Anatolia Region of Turkey up to now.

Effective bacterial disease management strategies depend on rapid and reliable identification of pathogens at an early stage of disease development (Miller and Martin 1988). There are many conventional and molecular techniques that have been developed and used for identification of microorganisms. Conventional techniques including morphological, physiological and biochemical tests are commonly used for microbial identification. There are a lot of their disadvantages

compared with new molecular methods (Fahy and Hayward 1983; Miller and Martin 1988; Roy 1988; Sasser 1990; Bereswill et al. 1997; Bereswill et al. 1998; Jeng et al. 2001) but these tests are still used alongside new molecular tests because molecular methods also have disadvantages but overcome some of the problems of the older tests. For example, a semi-selective media was suggested to detect *E. amylovora*, but occasionally, levan-deficient strains have been isolated from plant tissue with fire blight symptoms (Bereswill et al. 1998). Recently, whole-cell fatty acid analysis, SDS-PAGE, carbon pattern utilization, genomic profiling and microarray techniques are commonly used molecular identification techniques. Fatty acid methyl ester (FAMEs) analysis using the Sherlock Microbial Identification System (MIDI, Inc., Newark, DE, USA) (MIS) is a reliable, fast, cost-effective and sensitive method for identification of bacteria from various sources (Roy 1988; Sasser 1990). Whole-cell fatty acid (FA) profiles have been used in bacterial classification for over 35 years and have become increasingly important in bacterial identification (Roy 1988; Sasser 1990). The Biolog system was developed by Biolog, Inc. (Hayward, CA, USA) to identify Gram-negative and -positive bacteria on the basis of metabolic fingerprinting. This technique is quite useful for the identification of some plant-pathogenic bacteria (Verniere et al. 1993; Holmes et al. 1994), but it is stated that the Biolog system is not yet accurate enough to serve as a primary method for identifying some bacteria (Miller et al. 1993). The reliability of serological assays, the other important molecular technique, depends on the quality of the antiserum. Previously described monoclonal antibodies were too specific to identify all *E. amylovora* strains (Lin et al. 1987). Polyclonal antibodies can also cross-react with many bacteria due to common surface antigens (Roberts 1980).

One of the aims of this study was to isolate and identify pathogenic and non-pathogenic bacterial strains from diseased pome fruits grown in the Eastern Anatolia region of Turkey using MIS, and to characterize pathogenic strains by metabolic fingerprints, biochemical and serological tests. The other aim was to evaluate isolated non-pathogenic bacteria as potential biocontrol agents of pome fruit diseases in following studies as the identification of antagonistic bacteria is an important advantage for biocontrol.

2 Materials and methods

2.1 Isolation of bacterial strains

Bacteria were isolated from aerial part (fruits, leaves, flowers, branches, shoots) of pome fruits (apple, pear, quince, mountain pear, medlar and loquat) exhibiting characteristic symptoms of bacterial diseases from different locations (Artvin, Erzincan, Erzurum, Igdir and Kars) in the Eastern Anatolia region of Turkey during springs of the years 1999, 2000 and 2001. Isolation was performed by cutting surface-disinfested plant materials into small pieces from the margins of spots with a sterile razor blade. The cut plant tissue was inserted into and soaked in a test tube containing 2 ml sterile water. Loopfulls of the resulting aqueous suspension were streaked onto standard medium nutrient agar (NA), semi-selective medium King's medium (KB) (King et al. 1954) and nutrient agar plus 5% sucrose (NAS) (Miller and Schroth 1972) simultaneously. Plates were incubated at 27 °C for 2-5 days. Representative colonies were selected and purified by repeated re-streaking on NA, and were than stored at −80 °C in 15% glycerol and LB broth for further studies.

2.2 Identification of bacterial species by MIS

Preparation and analysis of FAME from whole cell fatty acids of bacterial strains were performed according to the method described by the manufacturer`s manual (Sherlock Microbial Identification System version 4.0, MIDI, Inc., Newark, DE, USA) (Miller and Berger 1985; Roy 1988). FAMEs were separated by gas chromatography (HP6890, Hewlett Packard, Palo Alto, CA, USA) with a fused-silica capillary column (25 m x 0.2 mm) with cross-linked 5% phenyl methyl silicone. FAME profiles of each bacterial strain were identified by comparing the commercial databases (TSBA 40) with the MIS software package. The identity of bacterial strains was revealed by computer comparison of FAME profiles of the unknown test strains with those in the library. For MIS, Biolog, ELISA, hypersensitivity, pathogenicity and biochemical tests *E. amylovora* strain PD-761 was used as a positive control.

2.3 Hypersensitivity and pathogenicity tests

All of the bacterial strains identified by MIS were tested for hypersensitivity on tobacco plants (*Nicotina tabacum* L. var. Samsun) as described by Klement et al. (1964). The bacterial suspension (10^8 cfu/ml) prepared in sterile distilled water and infiltrated into the intercostal area of the leaves of tobacco plants by using a 3-cc syringe (Becton Dickinson, Franklin Lakes, NJ, USA). The inoculated plants were incubated in a completely randomized design on the greenhouse bench for 24–48 h at 20–28 °C. The presence of rapid tissue necrosis at the inoculation site was recorded within 24–48 h after infiltration. This test was repeated at least three times for each strain. For HR and pathogenicity tests sterilized distilled water (sdH_2O) was used as a negative control.

The bacterial strains that induced HR on tobacco plants were selected and tested for pathogenicity on young shoots of Golden Delicious apples as described previously (Kotan and Sahin 2002). Pathogenicity tests were performed by using bacterial cultures grown for 48 h at 27 °C on NA. Each strain was suspended in sdH_2O, bacterial concentration was adjusted to 10^8 cfu/ml, and the suspension was the sprayed on the leaves by using a bottle sprayer. Shoots put in glass flasks separately were filled up with water. They were covered with polyethylene bags and incubated in a moisture chamber (> 90% relative humidity) for 5-6 weeks at 25 ±°C. Then they were observed for bacterial spots on leaves. For each strain three shoots were used. If there was a bacterial spot on any leave, the pathogenicity of the specific bacterial strain was evaluated as positive, or not negative. Koch`s postulates for each pathogen bacterial strains exhibiting leaf symptom were fulfilled by repeating re-isolation and re-inoculation at least three times. The pathogen strains were re-isolated from lesions on inoculated leaves. Recovered strains were identical to initial strains, based on MIS, Biolog, ELISA, and biochemical tests.

2.4 Biolog GN and GP identification of bacteria

One or two days before the inoculation of Biolog GN2 and GP2 plates (Biolog), potential pathogenic bacterial strains were streaked on TSA or BUG agar plates. Each well of Biolog GN2 or GP2 microtiter plates was inoculated with 125 µl of the Gram-negative or -positive 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 colour was automatically recorded using a microplate reader with a 590-nm wavelength filter. Identification (Biolog Microlog 34.20 database) and ASCII file output of test results, applying the automatic threshold option, were performed using BIOLOG420/Databases/GN601 and GP601 KID software (Bochner 1989).

2.5 Detection of Erwinia amylovora with ELISA

Classical ELISA double antibody sandwich technique was used for screening *E. amylovora*. During the first step the surface of the microtiter plate was coated with the antigen-specific IgG antibody (Loewe, Sauerlach, Germany, catalog no. 07069). After the incubation step, the antigen was bound to the fixed antibody forming the antibody-antigen complex. Then the antibody-antigen complex reacts with the alkaline phosphatase-labelled antibody forming the double antibody sandwich. This was followed by an enzymatic assay whereby the presence of the specific antigen was indicated by the positive reaction of alkaline phosphates with 4-nitrophenyl-phosphate yielding free 4-nitrophenol. The enzymatic reaction was monitored at 405 nm after 1 and 2 hours. Reactions were generally considered positive if the absorbance value was greater than 0.1 because non-specific background reading of normal serum (negative control) were fourfold less than 0.1.

2.6 Biochemical tests

Gram reaction, the presence of catalase, oxidase, potassium hydroxide (KOH), nitrate reduction and arginine dihydrolase were investigated according to the methods described by FAHY and HAYWARD (1983). Production of yellow fluorescence pigment was tested on KB medium and was checked under UV light after 1-3 days. Levan production was tested on NA (Difco) with 50 g l^{-1} sucrose and was recorded after 1 and 2 days.

3 Results

Bacteria were isolated from pome fruits grown in five different locations in the Eastern Anatolia region of Turkey. Location and host distribution of the bacterial strains are given in Table 1. A total of 324 bacterial strains was identified by MIS. Identification (MIS), hypersensitivity and pathogenicity test results of the bacterial strains are summarized in Table 2. According to the MIS results, a total of 118 bacterial strains belonging to 29 different species induced a HR on tobacco leaves. Eighty-two of them caused necrotic lesions on sprayed Golden Delicious apple leaves. The potential pathogenic bacterial strains were as follows: 40 *E. amylovora*, 20 *P. syringae* pv. s*yringae*, 6 *E. rhapontici*, 4 *P. putida*, 3 *P. huttiensis*, 2 *C. violaceum* and *P. agglomerans*, 1 *A. piechaudii*, *B. pumilus* GC subgroup B, *E. intermedius*, *P. aeruginosa* and *P. cichorii*. It is well known that *E. amylovora* and *P. syringae* pv. s*yringae* are pathogens of pome fruits, but other bacterial species have not been known before as causing bacterial diseases on pome fruits. MIS identification results of potential pathogenic bacteria were compared with the Biolog, ELISA and biochemical test results (Table 3). MIS results of whole *E. amylovora* and *P. syringae* pv. s*yringae* strains were confirmed by Biolog and/or ELISA and biochemical tests, but MIS results of the others possible pathogenic speecies were not confirmed.

4 Discussion

In our study we isolated from pome fruit trees a total of 324 bacterial strains belonging to 76 different bacterial species and 36 genera. Most of the strains (57.1%) were isolated from pear since bacterial symptoms were more intensive on pear trees than on the other host trees. Of the total of 324 bacterial strains, 16.9% were *E. amylovora*, 16.6% *P. syringae* pv. s*yringae*, 11.7% *P. agglomerans*, 10.8% *B. pumilus*, 7.7% *E. intermedius*, 5.8% *A. piechaudii*, 4.0% *Leclercia adecarboxylata* and 26.5% the other 69 species. The most abundant bacterial species was *E. amylovora* (16.9%), followed by *P. syringae* pv. s*yringae* (16.6%), *P. agglomerans* (11.7%), *B. pumilus* (10.8%), *E. intermedius* (7.7%), *A. piechaudii* (5.8%), *Leclercia adecarboxylata* (4.0%) and 69 other species (26.5%). The occurrence of bacterial species belonging to the genera *Arthrobacter*, *Bacillus*, *Clavibacter*, *Curtobacterium*, *Erwinia*, *Klebsiella*, *Micrococcus*, *Pantoea* and *Pseudomonas* as epiphytic and endophytic populations on pome fruit trees has been reported previously (JOHNSON and STOCKWELL 1998). However, the remaining bacterial species are reported for the first time from pome fruit trees in this study. These differences in bacterial flora could be explained by a number of complex and changing environmental factors such as temperature, moisture, nutrients, soil conditions, and agrochemicals (JOHNSON and STOCKWELL 1998; JENG et al. 2001). In the present study, 36% of the 324 bacterial strains were found to be HR positive on tobacco and only 25% were potentially pathogenic on Golden Delicious apple. In other words, 75% of the strains were present on pome fruits as epiphytic (saprophytic) populations. This finding may be explained by the consideration that some of the bacterial strains inducing HR on tobacco may not be a pathogen on the original host. Namely pome fruits may be non-hosts of several bacterial plant pathogens.

E. amylovora was the most abundant pathogen isolated from pear (31), apple (6) and mountain pear (3), which are known to be hosts of this pathogen (VAN DER ZWET and KEIL 1979). Quince, medlar and loquat are also well-known host of *E. amylovora*, however none of these pathogen strains were isolated from them in this study. This result may be explained by host specifity of pathogen or host resistance in quince, medlar and loquat grown in the Eastern Anatolia region of Turkey. Therefore, a future study is needed to test this hypothesis. The second predominant pathogen was *P. syringae* pv. s*yringae* isolated from pear (15), mountain pear (4) and quince (1). This result is not surprising since *P. syringae* pv. s*yringae* occurs on apricot in different locations in the Eastern Anatolia region of Turkey (KOTAN and SAHIN 2002), but it was not known previously that *P. syringae* pv. s*yringae* causes disease on pome fruits in Turkey. The data in this study provide further evidence for the previous report that *B. pumilus* may be pathogen of pome fruits (SALEH et al. 1997). *E. rhapontici*, *P. putida*, *P. huttiensis*, *C. violaceum*, *P. agglomerans*, *A. piechaudii*, *E. intermedius*, *P. aeruginosa* and *P. cichorii* were identified as possible pathogens of pome fruits in this study. However, further studies are required to test the pathogenicity of these bacteria on a wide range of pome trees. *A. tumefaciens* could not be isolated in this study, but *A. radiobacter* (seven strains) and *A. rubi* (two strains) have been obtained from pome fruits; however, they have not been inoculated on pome tree root for pathogenicity tests. In this study, a several bacterial strains were isolated and identified that are human pathogens and some of the isolated strains are potential biocontrol agents against plant pathogens.

All *E. amylovora* and *P. syringae* pv. s*yringae* strains identified with the MIS and Biolog methods gave the same identification results, but a majority of the other potential bacterial pathogens did not give similar results. All potential pathogen strains with the exception of *P. syringae* pv. s*yringae* were additionally tested with ELISA. All strains identified as *E. amylovora* according to MIS and Biolog gave a positive reaction with the antigen-specific IgG antibody, however, a total of three strains identified as *E. rhapontici* according to MIS or as *E. amylovora*, *S. odorifera* and *E. cloacae* according to Biolog gave also a positive reaction with the antigen. In conclusion, the classical ELISA double antibody sandwich technique clearly distinguished *E. amylovora* from other pathogenic bacteria on pome fruits. Some strains identified by MIS were not confirmed by Biolog and/or ELISA assays. There were important differences among MIS, Biolog and/or ELISA results for some strains. The results show that MIS and Biolog identification systems alone are not yet accurate enough to serve as a primary method for identifying some of the plant pathogenic bacterial species, but especially ELISA could be used as an alternative to the classical, physiological and biochemical tests

Table 1: Location and host distribution of bacterial strains isolated from pome fruits

Locations	Hosts												Total	
	Pear		Apple		Mountain pear		Quince		Loquat		Medlar			
Erzurum	105*	17**	65*	7**	12*	7**	13*	0**	0*	0**	4*	0**	199*	31**
Igdir	28	10	6	1	0	0	1	0	0	0	0	0	35	11
Erzincan	21	12	3	2	0	0	0	0	0	0	0	0	24	14
Artvin	19	14	4	0	0	0	8	4	8	1	2	0	41	19
Kars	12	6	12	1	0	0	1	0	0	0	0	0	25	7
Total	185	59	90	11	12	7	23	4	8	1	6	0	324	82

* Number of total bacterial strains; ** number of pathogenic bacterial strains.

Table 2: Sherlock Microbial Identification System (MIS), hypersensitivity and pathogenicity test results of bacterial strains isolated from pome fruits in Eastern Anatolia region of Turkey

MIS results	Strain numbers			MIS results	Strain numbers		
	Total	HR +	PAT+		Total	HR +	PAT+
Erwinia amylovora	40	40	40	*Bacillus megaterium* GC-B	1	–	–
Pseudomonas syringae pv. *syringae*	20	20	20	*Bacillus mycoides* GC-A	1	–	–
Erwinia rhapontici	7	6	6	*Bacillus simplex*	1	–	–
Pseudomonas huttiensis	11	8	3	*Bacillus subtilus*	2	–	–
Pseudomonas putida biotype A	4	3	3	*Brevibacillus brevis*	1	–	–
Pantoea agglomerans	38	6	2	*Brevibacterium casei*	1	–	–
Chromobacterium violaceum	3	2	2	*Brevundimonas diminuta*	1	–	–
Pseudomonas cichorii	3	3	1	*Burkholderia cepacia*	1	–	–
Alcaligenes piechaudii	17	1	1	*Chryseobacterium indologenes*	2	–	–
Bacillus pumilus GC-B	13	1	1	*Citrobacter freundii*	1	–	–
Enterobacter intermedius	5	1	1	*Curtobacterium flaccumfaciens*	4	–	–
Pseudomonas aeruginosa	1	1	1	*Enterobacter agglomerans* GC-III	2	–	–
Pseudomonas putida biotype B	1	1	1	*Enterobacter cloacae*	1	–	–
Pseudomonas savastonoi pv. *fraxinus*	3	3	–	*Erwinia chrysanthemi* biotype II	1	–	–
Erwinia carotovora	2	2	–	*Erwinia chrysanthemi* biotype IV	2	–	–
Leclercia adecarboxylata	13	2	–	*Erwinia chrysanthemi* biotype V	3	–	–
Pseudomonas doudoroffii	3	2	–	*Escherichia coli*	1	–	–
Pseudomonas fluorescens biotype B	2	2	–	*Klebsiella pneumoniae*	6	–	–
Aerococcus vridans	1	1	–	*Klebsiella terrigena*	1	–	–
Alcaligenes xylosoxydans	2	1	–	*Klebsiella trevisanii*	1	–	–
Bacillus marinus	1	1	–	*Kocuria rosea*	1	–	–
Bacillus megaterium GC-A	4	1	–	*Kocuria varians*	1	–	–
Bacillus pumilus GC-A	4	1	–	*Methylobacterium mesophilicum*	1	–	–
Brevibacillus laterosporus	1	1	–	*Micrococcus lylae*	1	–	–
Burkholderia pyrrocinia	8	1	–	*Neisseria mucosa*	1	–	–
Citrobacter amalonaticus	2	1	–	*Pediococcus pentosaceus*	1	–	–
Clavibacter michiganense	1	1	–	*Photobacterium damsela*	1	–	–
Enterobacter agglomerans GC-I	17	1	–	*Plesiomonas shigelloides*	2	–	–
Neisseria subflava	1	1	–	*Proteus vulgaris* GC-A	1	–	–
Pseudomonas fluorescens biotype A	1	1	–	*Pseudomonas balearica*	1	–	–
Pseudomonas syringae pv. *populans*	1	1	–	*Pseudomonas chlororaphis*	1	–	–
Pseudomonas syringae pv. *tabaci*	1	1	–	*Pseudomonas viridiflava*	1	–	–
Acinetobacter calcoaceticus	3	–	–	*Ralstonia pickettii*	2	–	–
Acinetobacter johnsonii	3	–	–	*Salmonella typhimurium*	1	–	–
Acinetobacter radioresistens	1	–	–	*Serratia fonticola*	3	–	–
Actinomadura yumaensis	3	–	–	*Serratia grimesii*	1	–	–
Agrobacterium radiobacter	7	NT	NT	*Serratia liquefaciens*	8	–	–
Agrobacterium rubi	2	NT	NT	*Sphingomonas capsulata*	1	–	–
Bacillus GC-22	1	–	–	*Vibrio alginolyticus*	1	–	–
Bacillus cereus GC-A	2	–	–	*Vibrio hollisae*	1	–	–
Bacillus lentimorbus	2	–	–	*Yersinia enterocolitica*	1	–	–
Bacillus licheniformis	3	–	–	Total strain numbers	324	118	82
				Reference strain *Erwinia amylovora* Ea-PD 761		+	+

HR: number of hypersensitivity positive strains on tobacco pants (*Nicotina tabacum* L. var. Samsun); PAT: number of pathogenicity-positive strains on young shoots of Golden Delicious apples leaves; NT: not tested.

to increase the speed of detection and discrimination of *E. amylovora*. Therefore, our data suggest that a single approach may not be sufficient for a reliable identification of plant-pathogenic bacteria.

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Table 3: Sherlock Microbial Identification System (MIS), Biolog, ELISA and some biochemical test results of possible pathogen bacterial strains

Total no. of strains	MIS results	BIOLOG results	ELISA *	Oxi-dase	Cata-lase	KOH	Nitrate reduc-tion	Arginine dihydro-lase	Levan	Pig-ment on KB
1	*Erwinia amylovora* (Ea-PD 761)	*Erwinia amylovora*	+	–	+	+	–	–	+	–
40	*Erwinia amylovora* (All strain)	*Erwinia amylovora* (All strain)	+	–	+	+	–	–	+	–
20	*Pseudomonas syringae pv. syringae* (All strain)	*Pseudomonas syringae pv. syringae*	NT	–	+/w+	+	–	–	+/w+	+/w+
2	*Pseudomonas huttiensis* RK-261 and 262	*Pseudomonas huttiensis*	–	–	+	+	–	+	w+	w+
1	*Bacillus pumilus* RK-106	*Bacillus pumilus*	–	–	–	–	–	–	–	–
1	*Pseudomonas putida* biotype A RK-28	*Pseudomonas putida*	–	+	+	+	–	+	–	+
1	*Pseudomonas huttiensis* RK-260	*Pseudomonas floridana*	–	–	+	+	–	+	w+	w+
3	*Erwinia rhapontici* RK-208, 227 and 219	*Erwinia amylovora*	+	–	+	+	–	–	+	–
1	*Erwinia rhapontici* RK-234	*Serratia odorifera*	+	–	+	+	–	+	+	–
1	*Erwinia rhapontici* RK-243	*Enterobacter cloacae*	+	–	+	+	+	–	+	–
1	*Pantoea agglomerans* RK-10	*Aeromonas encheleia*	+	–	+	+	–	–	–	–
1	*Erwinia rhapontici* RK-189	*Pantoea agglomerans*	–	–	+	+	–	–	–	–
1	*Enterobacter intermedius* RK-90	*Serratia odorifera*	–	–	+	+	–	–	+	–
1	*Pseudomonas cichorii* RK-166	*Enterobacter agglomerans*	–	+	+	+	+	+	–	w+
1	*Pseudomonas putida* biotype A RK-238	*Aeromonas enteropelogenes*	–	+	+	+	–	w+	–	+
1	*Pseudomonas putida* biotype A RK -249	*Pseudomonas marginalis*	–	w+	+	+	+	+	–	+
1	*Pseudomonas putida* biotype B RK -239	*Serratia plymuthica*	–	+	+	+	–	+	–	+
1	*Alcaligenes piechaudii* RK-155	*Pantoea agglomerans*	–	+	+	+	–	–	–	–
1	*Chromobacterium violaceum* RK-231	*Photobacterium angustum*	–	–	+	+	–	+	+	+
1	*Chromobacterium violaceum* RK-259	*Brevundimonas vesicularis*	–	–	+	+	–	+	+	+
1	*Pseudomonas aeruginosa* RK-168	*Serratia plymuthica*	–	+	+	+	w+	+	–	w+
1	*Pantoea agglomerans* RK-87	*Enterobacter asburiae*	–	–	+	+	–	–	–	–

* Polyclonal antibody (Loewe cat. no. 07069) specific to *E. amylovora*; NT: not tested; +: positive reaction; –: negative reaction; w+: weak positive reaction; s+: strong positive reaction; +/w+: positive or weak positive reaction.

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