

## Bacterial Spot and Blight Diseases of Ornamental Plants caused by Different *Xanthomonas* Species in Turkey

MUSTAFA MIRIK<sup>1</sup>, YESIM AYSAN<sup>2</sup> and FULYA BAYSAL-GUREL<sup>3\*</sup>

<sup>1</sup>Department of Plant Protection, Agriculture Faculty, Namik Kemal University, Tekirdag, Turkey; <sup>2</sup>Department of Plant Protection, Agriculture Faculty, Cukurova University, Adana, Turkey; <sup>3</sup>Otis Floyd Nursery Research Center, Tennessee State University, McMinnville, USA

\*Corresponding author: [fbaysalg@tnstate.edu](mailto:fbaysalg@tnstate.edu)

### Abstract

Mirik M., Aysan Y., Baysal-Gurel F. (2018): Bacterial spot and blight diseases of ornamental plants caused by different *Xanthomonas* species in Turkey. Plant Protect. Sci., 54: 240–247.

Putative strains belonging to *Xanthomonas* spp. causing leaf spot and blight diseases on geranium (*Pelargonium peltatum* and *P. hortorum*), begonia (*Begonia × tuberhybrida*), anthurium (*Anthurium andraeanum*), Chinese hibiscus (*Hibiscus rosa-sinensis*), and English ivy (*Hedera helix*) growing in Turkey were isolated. All bacterial strains were classified as Gram-negative, oxidase negative, catalase, levan and starch hydrolysis positive, with hypersensitive reaction positive on tobacco and pathogenic to host plants. Identification of these strains was further confirmed by serological method using ELISA kits, conventional PCR, carbon utilisation, and FAME. Results of the identification showed that 28, 24, 10, 2, and 1 strains were identified as *X. axonopodis* pv. *begoniae*, *X. hortorum* pv. *pelargonii*, *X. axonopodis* pv. *dieffenbachiae*, *X. hortorum* pv. *hederae*, and *Xanthomonas* sp., respectively. This is the first report of *X. hortorum* pv. *hederae* on English ivy in Turkey.

**Keywords:** bacteria; detection; ornamentals; *Xanthomonas*

Production of ornamental plants in Turkey has been progressively increased in the last years (325% increase in the production area between 2011 and 2016), specifically in Antalya, Izmir, Adana, Mersin, Sakarya, and Yalova provinces (KAZAZ *et al.* 2015; TurkStat 2017; <http://www.turkstat.gov.tr/>). The annual export value of the ornamental industry in Turkey was \$78 million in 2015 (TOPPEA 2016).

Occurrence of bacterial blight caused by *Xanthomonas axonopodis* pv. *dieffenbachiae* on tail flower (AYSAN & SAHIN 2003), bacterial leaf spot caused by *X. axonopodis* pv. *begoniae* on begonia (ORNEK *et al.* 2007) and bacterial blight caused by *X. hortorum* pv. *pelargonii* on geranium (MIRIK *et al.* 2009) has been reported in previous studies in Turkey. Geranium is the host of *X. hortorum* pv. *pelargonii* which

causes leaf spot, stem rot and blight (DAUGHTREY *et al.* 1995). Begonia has been identified as the only host of *X. axonopodis* pv. *begoniae* (DAUGHTREY *et al.* 1995). Specific symptoms associated with this pathogen are leaf spots with irregular necrotic lesion, leaf blight and stem canker on begonia. Bacterial blight caused by *X. axonopodis* pv. *dieffenbachiae* may result in foliar blight and vascular discoloration on anthurium. Characteristic early foliar symptoms are water-soaked spots on the lower surface of leaves and along leaf margins and advanced foliar symptoms are large, irregular necrotic areas surrounded by a bright yellow margin. As a result of the vascular infection, infected plant stems, petioles, appear as black, wet and slimy. Bacterial exudates may appear on the infected plant parts as well.

Supported by the Scientific and Technical Research Council of Turkey (TUBITAK), Grant No. TOVAG 106 O 333, and by the Cukurova University, Turkey, Project No. ZF2005BAP9.

The purpose of this study was to identify the species of *Xanthomonas* causing leaf spot and blight on different ornamental plants using culturing, biochemical tests, serological method, carbon utilisation and fatty acid methyl ester analysis, and molecular techniques. Reports related with other bacterial pathogens identified during this survey have been published (MIRIK *et al.* 2011a, b).

## MATERIAL AND METHODS

**Survey.** During the surveys, small- and large-scale ornamental greenhouses were visited in Adana (Yüreğir, Seyhan, Karaisali, Ceyhan districts), Antalya, Istanbul, Izmir (Tire, Odemiş, Bayındır districts), Mersin (Yenice, Huzurkent, Mezitli, Tece, Erdemli, Silifke, Aydıncik districts), Sakarya, Tekirdag, and Yalova provinces (Figure 1). Disease surveys were carried out at three-month intervals for two consecutive years for the presence of the bacterial leaf spot and blight on ornamental plants including geranium (*Pelargonium peltatum* and *P. hortorum*), begonia (*Begonia × tuberhybrida*), anthurium (*Anthurium andraeanum*), Chinese hibiscus (*Hibiscus rosa-sinensis*), and English ivy (*Hedera helix*). In each greenhouse, five to ten blocks were selected along a zigzag pattern with at least 2 m between blocks according to the production size of the ornamental greenhouses. At each block, plants were assessed for bacterial diseases and initial identification of the various bacterial diseases observed was made based on symptoms as described in the Compendium of Ornamental Foliage Plant Diseases (CHASE 1987) and Diseases of Woody Ornamentals and Trees in Nurseries (JONES & BENSON 2001).

**Bacterial isolation.** Bacterial strains were isolated from 80 plant samples exhibiting disease symptoms during the survey period. The plant samples (including

leaves and stem tissues) were placed in a paper bag and transferred to the laboratory on ice in a cooler for isolation and identification of the causal organism. Surface-sterilised small pieces of leaf and stem tissues were macerated in sterilised distilled water. A loopful of suspension was streaked onto yeast extract dextrose calcium carbonate agar medium (YDC) (LELLIOTT & STEAD 1987) and incubated at 25°C for 3–14 days. Single colonies on YDC were selected, and purified isolates from single colonies were stored in 15% glycerol at –80°C in Dr. Yesim Aysan's bacterial culture collection and used for further studies.

**Pathogenicity tests.** *Xanthomonas* strains isolated during surveys were tested on their original host plants for pathogenicity test. The test plants were obtained as liners from commercial producers. Liners were transplanted in a potting medium of peat and pine bark (1 : 1) amended with recommended rates of micronutrients and fertiliser for each plant species and maintained in a greenhouse for 2 months. *Begonia × tuberhybrida* cv. Nonstop® Mocca White, *Pelargonium peltatum* (Ivy leaf), *P. hortorum* cv. Moulin Rouge, *Anthurium andraeanum* cv. Arizona, *Hibiscus rosa-sinensis* cv. Rainbow, and *Hedera helix* cv. Bulgaria plants (10 plants/each host) were inoculated with bacterial suspension ( $10^7$  CFU/ml) of each strain using a sterilized needle. After inoculations, plants were covered with clear polyethylene bags for 24 h at 25°C. Then the bags were removed and plants were maintained in a controlled climate room, at 25°C, 70% RH and 16/8-h day/night light conditions. Disease development was evaluated 5–14 days after inoculation and re-isolations were made from the diseased plants. Sterilised distilled water was applied as a control treatment for each plant species.

The reference strains of *X. hortorum* pv. *pelargonii* GSPB 1955 (provided by Dr. Batur-Michaelis, Göttingen, Germany), *X. axonopodis* pv. *begoniae* BPIC



Figure 1. Map of the Turkish provinces where surveys were conducted in the present study

<https://doi.org/10.17221/10/2017-PPS>

2013/94 (provided by Dr. Alivizatos, Benaki Enstitu, Greece), and *X. axonopodis* pv. *dieffenbachiae* JS990 and JV589 (provided by Dr. Jouen, Reunion Island, France) were used as positive controls in this study.

**Identification of the strains.** The identification of each strain was initially confirmed based on morphological, biochemical, physiological, and hypersensitivity tests such as potassium hydroxide (KOH) solubility for gram reaction, oxidative/fermentative metabolism, catalase reaction, levan production, starch hydrolysis, and hypersensitivity on tobacco leaves as described by LELLIOTT and STEAD (1987) and SCHAAD *et al.* (2001). All tests were repeated three times. Colony morphology of *Xanthomonas* strains was observed on different semi-selective media such as Tween B (McGUIRE *et al.* 1986), CKTM (SIJAM *et al.* 1992), SX agar (SHAAD & WHITE 1974), SM agar (CHUN & ALVAREZ 1983) and modified D-5 agar (MD-5) (KUAN & MINSVAGE 1985). The reference strains of *X. hortorum* pv. *pelargonii* GSPB 1955 (provided by Dr. Batur-Michaelis), *X. axonopodis* pv. *begoniae* BPIC 2013/94 (provided by Dr. Alivizatos), and *X. axonopodis* pv. *dieffenbachiae*, JS990 and JV589 (provided by Dr. Jouen) were used as positive controls.

**Identification of the strains with ELISA.** The serological identification of the bacterial strains was performed according to the previously described indirect ELISA method (COLIGAN *et al.* 1991) by using specific monoclonal antiserum for *X. axonopodis* pv. *begoniae* (BRA 23700) and *X. axonopodis* pv. *dieffenbachiae* (BRA 47600; both Agdia, Elkhart, USA); and according to the previously described ELISA method (McLAUGHLIN & CHEN 1990) by using enzyme conjugate (Loewe, Sauerlach, Germany) and antiserum BRA 32502; Agdia, USA) for *X. hortorum* pv. *pelargonii*. Absorbance values were recorded in an ELISA reader (Titertek Multiskan microplate reader; Titertek-Berthold, Pforzheim, Germany) at 405 nm. Phosphate buffered saline (PBS) and healthy leaf extract of ornamental plants were used as negative control and reference strain was used as positive control.

**Identification of the strains by PCR.** Genomic DNA isolation of *Xanthomonas* strains from different host plants along with reference strains (*X. hortorum* pv. *pelargonii* GSPB 1955, *X. axonopodis* pv. *begoniae* BPIC 2013/94, *X. axonopodis* pv. *dieffenbachiae* JS990 and JV589) was done according to the method described by DE BOER and WARD (1995).

PCR tests of *Xanthomonas* strains were conducted as described by MANULIS *et al.* (1994), LEITE *et al.* (1995), and SULZINSKI *et al.* (1996), and the primers that are complementary to each bacterial species are listed in Table 1.

The final volume of 25 µl of the reaction mix PCR Master Mix (Promega, Madison, USA) consisted of 12.5 µl, 20 pmol forward primer 2.0 µl, 20 pmol reverse primer 2.0 µl, H<sub>2</sub>O 6.5 µl, and genomic DNA 2.0 µl.

Agarose gel electrophoresis of PCR products was conducted as described by SAMBROOK *et al.* (1989). 100-bp DNA marker (Thermo Fisher Scientific, Waltham, USA) was used as the molecular weight marker.

**Carbon utilisation and fatty acid methyl ester (FAME) analysis.** Pure cultures of 12 isolates (4 strains of *X. hortorum* pv. *pelargonii* Sar1-1/a r, Sar5-1b/a r, İzmir-1, Sardunya-3), 3 strains of *X. axonopodis* pv. *begoniae* (Xab1/r, Xcb13/r, İzmir 11/1-1), 3 strains of *X. axonopodis* pv. *dieffenbachiae* (Ant-1a, Ant-1b, Ant-2a), 2 strains of *X. hortorum* pv. *hederae* (Sarmasik 1/4 and Sarmasik 2/1), and all reference strains were grown and tested for utilisation of the carbon sources available on GN2 Microplate (Biolog Inc., Hayward, USA). The carbon utilisation patterns were read with a microplate reader after 24 h of incubation and analysed by a cluster analysis program provided by BIOLOG.

FAME profiles were generated for 18 selected strains (6 strains of *X. hortorum* pv. *pelargonii* Sar1-1/a r, Sar2-3/c r, Sar4-1a/a r, Sar5-1b/a r, İzmir-1, Sardunya-3), 6 strains of *X. axonopodis* pv. *begoniae* (Xab1/r, Xcb7/r, Xcb13/r, İzmir 11/1-1, Begonya Ekim, Kırmızı Begonya), 4 strains of *X. axonopodis* pv. *dieffenbachiae* (Ant-1a, Ant-1b, Ant-2a, Ant-2d),

Table 1. DNA amplification conditions and primers used for PCR

Pathogen	Size	Sequence 5'→3'	Amplification conditions	Reference
<i>X. hortorum</i> pv. <i>pelargonii</i>	1.2 kb	GAGTGTCCAGTGGCAAGC GTTGCTGCCTCTTCCTGC	40 cycles 94°C 1 min/58°C 1 min/72°C 5 min	MANULIS <i>et al.</i> (1994)
<i>X. hortorum</i> pv. <i>pelargonii</i>	197 bp	ACGCGCTACCAAAAGGCAAAGAG GATCTGCGGTTGTCCTGAAGATTGG	30 cycles 94°C 1 min/64°C 1 min/72°C 2 min	SULZUNSKI <i>et al.</i> (1996)
<i>X. axonopodis</i> pv. <i>begoniae</i>	619 bp	GCACGCTCCAGATCAGCATCGAGG GGCATCTGCATGCGTTGCTCTCCG	30 cycles 94°C 30 s/61°C 40 s/72°C 45 s	LEITE <i>et al.</i> (1995)

Table 2. DNA amplification conditions and primers used for BOX-PCR

Primer	Sequence 5'→3'	Amplification conditions
BOXA-1R	CTACGGCAAGGCGACGCTGACG	30 cycles 94°C 1 min/53°C 1 min/65°C 8 min

and 2 strains *X. hortorum* pv. *hederae* (Sarmasik 1/4 and Sarmasik 2/1) as well as for reference strains. FAMEs were prepared and extracted from the bacterial cells by using the procedure described by CHASE *et al.* (1992). Following the extraction, FAME samples were analysed on an HP6890 gas chromatograph (Hewlett Packard, Palo Alto, USA). FAME profiles were identified using the commercial trypticase soy broth agar database with the Microbial Identification System software package (Sherlock MIS v4.5; Microbial ID, Inc., Newark, Denmark).

**Determination of genotypic relationship.** The primers for BOX-PCR (Louws *et al.* 1994) and the conditions are shown in Table 2. PCR amplification was carried in 25 µl final volume containing 3 µl of DNA template, 1.5 µl 20 pmol of each respective primer, 12.5 µl Master Mix (Promega, Madison, USA), and 6.5 H<sub>2</sub>O. All the amplifications were performed in a Techne TC-412 Thermal Cycler (Bibby Scientific, Staffordshire, UK).

All data were subjected to the statistical analysis program SPSS (Statistical Package for Social Sciences), and the genetic relationship rates were determined by a dendrogram using Cluster Analysis.

## RESULTS

**Survey.** A total of 116 greenhouses were visited during surveys. The suspected plant samples consisting of 5 host genera originated from 36 greenhouses in Adana, 21 greenhouses in Mersin, 9 greenhouses in Izmir, 8 greenhouses in Istanbul, and 6 greenhouses in Tekirdag provinces.

**Geranium:** In these surveys, *Pelargonium peltatum* and *P. hortorum* plants exhibited characteristic symptoms of bacterial disease in Adana, Istanbul, Izmir, and Tekirdag provinces. These characteristic symptoms were small, round water-soaked spots, irregular necrotic lesions with yellow border and large angular, yellow or dead areas bounded by the veins on infected geranium leaves.

**Begonia:** Leaf spot symptoms on *Begonia × tuberhybrida* were observed in Adana, Istanbul, Izmir, and Mersin provinces. The main symptom observed in the collected samples was water-soaked spots on the leaf margin. In rare cases, wilting of the leaves

and petioles followed by V-shaped yellowing and as a result of systemic infections, bacterial exudates and blight symptoms were observed.

**Anthurium:** Leaf spot symptoms were observed on *Anthurium andraeanum* in Adana and Istanbul provinces. Marginal or interveinal water-soaked spots surrounded by chlorotic or necrotic areas were observed on infected foliage. In some cases, wilting also occurred as a result of systemic infection in plants. Bacterial exudates were also observed in warm and humid conditions.

**English ivy:** Leaf spots were identified on *Hedera helix* in Mersin. Initial symptoms were irregular brown leaf spots surrounded by a yellow halo on infected leaves.

**Chinese hibiscus:** Small yellow-brown spots were observed on *Hibiscus rosa-sinensis* in Adana province.

**Bacterial isolation.** In total, 65 *Xanthomonas* strains were obtained from 80 plant samples consisting of 5 host genera. Ten bacterial strains from anthurium, 28 bacterial strains from *Begonia × tuberhybrida*, 24 bacterial strains from geranium, 2 bacterial strains from English ivy, and 1 bacterial strain from Chinese hibiscus were isolated from the infected plants collected during the surveys. *X. axonopodis* pv. *begoniae* was observed as dark yellow, opaque, swollen and non-mucoid colonies on YDC, and *X. hortorum* pv. *pelargonii*, *X. axonopodis* pv. *dieffenbachiae*, *X. hortorum* pv. *hederae* were observed as light yellow, circular, smooth and non-mucoid colonies on YDC.

**Pathogenicity tests.** Each bacterial strain of *Xanthomonas* (total 65) was tested on the original host from where they were isolated. 7–10 days after inoculation leaf spot, blight, and water-soaked areas were observed and typical yellow spots turned into brown spots, on their original host plants (Table 3). Re-isolation was completed from each symptomatic host plant. Re-isolated bacteria were identified by colony characteristics and fatty acid methyl ester analysis. All sterilised water-treated control plants remained disease-free and no bacteria were re-isolated.

**Identification of the strains.** Morphological differences between *X. hortorum* pv. *pelargonii*, *X. axonopodis* pvs *begonia* and *dieffenbachiae* were determined on five different semi-selective media.

**Tween B:** Strains of begonia, geranium, and anthurium were circular and surrounded by an area



<https://doi.org/10.17221/10/2017-PPS>

Table 3. Diagnosis of *Xanthomonas* strains from different plant species

<i>Xanthomonas</i> spp.	Strain	Host	Location	KOH	O	Starch	C	L	O/F	M	HR	P
<i>X. hortorum</i> pv. <i>pelargonii</i>	GSPB 1955	reference strain	Germany	+	–	+	+	+	O	+	+	+
<i>X. hortorum</i> pv. <i>pelargonii</i> (24 strains)	Sar1-1/a r, Sar1-1/b r, Sar1-3/b r, Sar2-3/a r, Sar2-3/c r, Sar2-6/a r, Sar2-6/d r, Sar2-7/a r, Sar2-7/b r, Sar3-1/a r, Sar3-2/c r, Sar4-1a/a r, Sar4-1b/b r, Sar4-2/a r	<i>Pelargonium peltatum</i>	Adana	+	–	+	+	+	O	+	+	+
	Sar5-1b/a r, Sar5-3/a r, Sar5-4a/a r, Sar5-4b/c r, Sar5-5/a r	<i>P. peltatum</i>	Istanbul	+	–	+	+	+	O	+	+	+
	İzmir-1, İzmir-2	<i>P. peltatum</i>	İzmir	+	–	+	+	+	O	+	+	+
	Sardunya-1, Sardunya-2, Sardunya-3	<i>P. hortorum</i>	Tekirdağ	+	–	+	+	+	O	+	+	+
<i>X. axonopodis</i> pv. <i>begoniae</i>	BPIC 2013/94	reference strain	Greece	+	–	+	+	+	O	+	+	+
<i>X. axonopodis</i> pv. <i>begoniae</i> (28 strains)	Xab1/r, Xab2/r, Xab3/r, Xab4/r, Xab5/r, Xab6/r, Xcb7/r, Xcb8/r, Xcb9/r, Xcb14/r, Xcb15/r, Xcb16/r, Xcb17/r	<i>Begonia × tuberhybrida</i>	Adana	+	–	+	+	+	O	+	+	+
	Xcb10/r, Xcb11/r, Xcb12/r, Xcb13/r, Silivri-beg-1, Silivri-beg-2, Silivri-beg-3	<i>Begonia × tuberhybrida</i>	Istanbul	+	–	+	+	+	O	+	+	+
	Begonya Ekim, Kırmızı Begonya	<i>Begonia × tuberhybrida</i>	Mersin	+	–	+	+	+	O	+	+	+
	İzmir 11/1-1, İzmir 11/1-2, İzmir 11/1-3, İzmir 11/2-1, İzmir 11/2-2, İzmir 11/2-3	<i>Begonia × tuberhybrida</i>	İzmir	+	–	+	+	+	O	+	+	+
<i>X. axonopodis</i> pv. <i>dieffenbachiae</i>	JS 990	reference strains	France	+	–	+	+	+	O	+	+	+
	JV 589			+	–	+	+	+	O	+	+	+
<i>X. axonopodis</i> pv. <i>dieffenbachiae</i> (10 strains)	Ant-1a, Ant-1b, Ant-1c, Ant-1d, Ant-1e	<i>Anthurium andraeanum</i>	Adana	+	–	+	+	+	O	+	+	+
	Ant-2a, Ant-2b, Ant-2c, Ant-2d, Ant-2e	<i>A. andraeanum</i>	Istanbul	+	–	+	+	+	O	+	+	+
<i>X. hortorum</i> pv. <i>hederae</i> (2 strains)	Sarmasik 1/4, Sarmasik 2/1	<i>Hedera helix</i>	Mersin	+	–	+	+	+	O	+	+	+
<i>Xanthomonas</i> sp. (1 strain)	Hibus-1	<i>Hibiscus rosa-sinensis</i>	Adana	+	–	+	+	+	O	+	+	+

KOH – potassium hydroxide; O – oxidase; starch – starch hydrolysis; C – catalase; L – levan formation; O/F – oxidative-fermentative; M – motility; HR – hypersensitive reaction on tobacco; P – pathogenicity

of white crystalline light-yellow colonies. All three species consisted of the white crystalline field as reported by MCGUIRE *et al.* (1986). These zones became more pronounced in geranium strains.

**CKTM:** Strains of begonia, geranium, and anthurium were circular with pale yellow appearance as reported previously by SIJAM *et al.* (1992).

**SM:** Strains of begonia and anthurium produced mucoid colonies where the central area was green, and had the purplish-blue background. Geranium strains developed purple colonies as reported by SCHAAD and WHITE (1974).

**SX:** Geranium, begonia, and anthurium strains developed slowly on the SX medium. Colonies were

observed as lighter colour than those on SM medium as reported by CHUN and ALVAREZ (1984).

**MD-5:** Bacterial colonies of geranium, begonia, and anthurium strains were 3–5 mm in diameter, circular, yellow, and convex shapes at 30°C as reported by KUAN and MINSVAGE (1985).

Bacterial strains were identified according to diagnostic tests (Table 3). All *Xanthomonas* and reference strains were oxidative and negative for oxidase, but positive for potassium hydroxide, starch hydrolysis, catalase, and levan formation and all strains caused a positive hypersensitive reaction on tobacco (*Nicotiana tabacum* var. *Samsun*) (LELLIOTT & STEAD 1987; SCHAAD *et al.* 2001).

According to the test results, 24 strains were identified as *X. hortorum* pv. *pelargonii*, 28 strains as *X. axonopodis* pv. *begoniae*, 10 strains as *X. axonopodis* pv. *dieffenbachiae*, and 2 strains as *X. hortorum* pv. *hederae*. Bacterial strains obtained from Chinese hibiscus were not identified into the species level. Molecular, serological tests, carbon utilisation, and FAME analysis were conducted to support results of classical diagnosis.

**Diagnosis of bacterial strains with ELISA.** In order to support classical and pathogenicity tests, a serological test was conducted using commercial ELISA kits (Agdia BRA 23700, BRA 32502, and BRA 47600). In ELISA test, 28 strains were positive for *X. axonopodis* pv. *begoniae*, 24 strains were positive for *X. hortorum* pv. *pelargonii*, and 10 strains were positive for *X. axonopodis* pv. *dieffenbachiae*.

**Identification of *Xanthomonas* strains with PCR test.** In PCR test, nine regional strains isolated from geranium were confirmed as *X. hortorum* pv. *pelargonii* using two specific primers. All *X. hortorum* pv. *pelargonii* strains and reference isolate (GSPB 1955) produced a single band with the expected sizes of 197 and 1.2 kb, as previously reported by MANULIS *et al.* (1994) and SULZINSKI *et al.* (1996).

The identity of the 28 *X. axonopodis* pv. *begoniae* strains was confirmed by species-specific primers that yielded amplicons of the expected size of 619 bp as previously reported by LEITE *et al.* (1995).

**Identification of strains with BIOLOG diagnosis system.** According to results of the use of carbon sources (BIOLOG tests), selected four strains (Sar1-1/a r, Sar5-1b/a r, Izmir-1, Sardunya-3) were identified as *X. hortorum* pv. *pelargonii*, selected three strains (Xab1/r, Xcb13/r, Izmir 11/1-1) as *X. axonopodis* pv. *begoniae*, selected three strains (Ant-1a, Ant-1b, Ant-2a) as *X. axonopodis* pv. *dieffenbachiae* and selected two strains (Sarmasik 1/4 and Sarmasik 2/1) as *X. hortorum* pv. *hederae*.

**Identification of strains with fatty acid methyl ester analysis.** Selected 18 [6 strains of *X. hortorum* pv. *pelargonii* (Sar1-1/a r, Sar2-3/c r, Sar4-1a/a r, Sar5-1b/a r, Izmir-1, Sardunya-3), 6 strains *X. axonopodis* pv. *begoniae* (Xab1/r, Xcb7/r, Xcb13/r, Izmir 11/1-1, Begonya Ekim, Kirmızı Begonya), 4 strains *X. axonopodis* pv. *dieffenbachiae* (Ant-1a, Ant-1b, Ant-2a, Ant-2d), and 2 strains *X. hortorum* pv. *hederae* (Sarmasik 1/4 and Sarmasik 2/1)] strains were also identified according to FAME analysis. A total of 37 different fatty acids belonging to *Xanthomonas* species strains were identified. Bacterial strains were determined as *X. axonopodis* pv. *begoniae*, *X. hortorum* pv. *pelargonii*, *X. axonopodis* pv. *dieffenbachiae*, and *X. hortorum* pv. *hederae* with 25–71% of similarity based on FAME analysis. Rate of relative relations is shown with cluster analysis in Figure 2.

**Genotype characterisation of strains with BOX-PCR.** Genetic dissimilarities of *X. hortorum* pv. *pelargonii*, *X. axonopodis* pv. *begoniae*, *X. axonopodis* pv. *dieffenbachiae*, and *X. hortorum* pv. *hederae* strains were determined by using BOXA1R primer as described by LOUWS *et al.* (1994). The result of the BOX-PCR study clearly showed differences between the geranium and begonia strains. All data

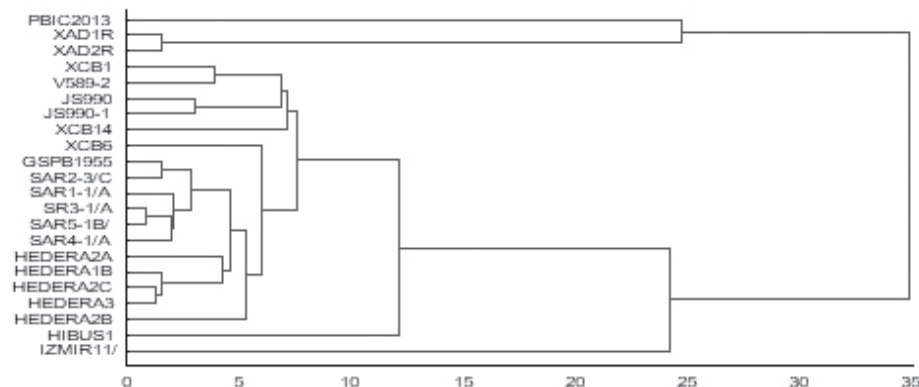


Figure 2. Phenotype relatives of *Xanthomonas* strains causing leaf spot and leaf blight according to the FAME results

<https://doi.org/10.17221/10/2017-PPS>

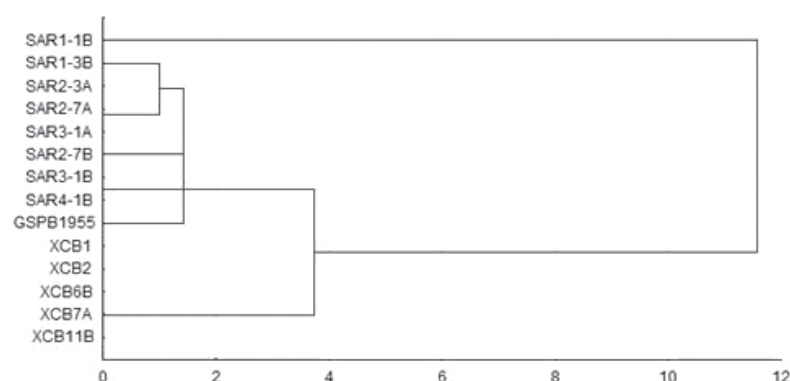


Figure 3. Genotypic relationships of *X. hortorum* pv. *pelargonii* and *X. axonopodis* pv. *begoniae* strains with BOX-PCR

were cluster analysed with SPSS (Statistical Package for Social Sciences). Differences between bacterial strains obtained from different plant species were established using cluster analyses (Figure 3).

## DISCUSSION

In this study, ornamental plant production areas were surveyed to determine possible bacterial diseases causing organisms on geranium, begonia, anthurium, Chinese hibiscus and English ivy in Adana, Antalya, Istanbul, Izmir, Mersin, Sakarya, Tekirdağ, and Yalova provinces. Diagnosis studies revealed that *X. axonopodis* pv. *dieffenbachiae* was identified as a causal disease agent on *Anthurium andraeanum*; *X. axonopodis* pv. *begoniae* on *Begonia × tuberhybrida*; *X. hortorum* pv. *pelargonii* on *Pelargonium peltatum* and *P. hortorum*, *X. hortorum* pv. *hederae* on *Hedera helix*, and *Xanthomonas* sp. on *Hibiscus rosa-sinensis*.

*X. axonopodis* pv. *dieffenbachiae*, *X. axonopodis* pv. *begoniae*, and *X. hortorum* pv. *pelargonii* were previously reported to occur on different plant species by AYSAN and SAHİN (2003), ORNEK *et al.* (2007), and MIRİK *et al.* (2009), respectively. This is the first report of *X. hortorum* pv. *hederae* on *H. helix* in Turkey.

Identification methods such as starch hydrolysis, catalase and levan formation were not sufficient to differentiate the species from each other. Semi-selective media were identified as a useful method to differentiate the species. On SM media, overall it was possible to differentiate geranium strains from begonia and anthurium strains. ELISA test was determined as the most successful identification method for *X. axonopodis* pv. *begoniae*, *X. hortorum* pv. *pelargonii*, and *X. axonopodis* pv. *dieffenbachiae*. The results also revealed that primers designed by

MANULIS *et al.* (1994) and SULZINSKI *et al.* (1996) can be successfully used for *X. hortorum* pv. *pelargonii*. BOX-PCR was used for genotypic characterisation of the isolates. Besides, FAME analysis was also found to be a useful method for phenotypic characterisation.

In conclusion, we found a variety of *Xanthomonas* spp. present in greenhouse production of ornamental plants in Turkey. This study provides a baseline of *Xanthomonas* spp. present in the ornamental plant industry of the particular provinces, with which future surveys may detect changes in the frequency of *Xanthomonas* spp. in these areas over time. After *X. hortorum* pv. *hederae* was confirmed on *H. helix* in Turkey, control measures were adopted, consisting in the eradication of the infected plants and quarantining the contaminated greenhouses to prevent spread, as well as surveillance intensification at the ports of entry to prevent new introductions. Since some of ornamental plant seedlings, seeds or rootstocks were exported from different countries, special precautions must be taken for ornamental plant bacterial diseases with detailed tests at the entrance of the seedlings into the host country.

## References

- Aysan Y., Sahin F. (2003): First report of bacterial blight of anthurium caused by *Xanthomonas axonopodis* pv. *dieffenbachiae* in Turkey. *Plant Pathology*, 52: 783.
- Chase A.R. (1987): *Compendium of Ornamental Foliage Plant Diseases*. St. Paul, APS Press.
- Chase A.R., Stall R.E., Hodge N.C., Jones J.B. (1992): Characterization of *Xanthomonas campestris* strains from aroids using physiological, pathological, and fatty acid analysis. *Phytopathology*, 82: 754–759.
- Chun W.W.C., Alvarez A.M. (1983): A starch-methionine medium for isolation of *Xanthomonas campestris* pv. *campestris* from plant debris in soil. *Plant Disease*, 67: 632–635.

- Coligan J.E., Kruesbeek A.M., Margulies D.H., Shevache E.M., Strober W. (1991): Current Protocols in Immunology. New York, John Wiley & Sons.
- Daughtrey M.L., Wick R.L., Peterson J.L. (1995): Compendium of Flowering Potted Plant Diseases. St. Paul, APS Press.
- De Boer S.H., Ward J. (1995): PCR detection of *Erwinia carotovora* subsp. *atroceptica* associated with potato tissue. *Phytopathology*, 85: 854–858.
- Jones R.K., Benson D.M. (2001): Diseases of Woody Ornamentals and Trees in Nurseries. St. Paul, APS Press.
- Kazaz S., Erken K., Karaguzel O., Alp S., Ozturk M., Kaya A.S., Gulbag F., Temel M., Erken S., Sarac Y.I., Elinc Z., Salman A., Hocagil M. (2015): Sus bitkileri uretiminde degisimler ve yeni arayislar. TMMOB Ziraat Muhendisleri Odasi Ziraat Muhendisligi VIII. Teknik Kongresi.
- Kuan T.L., Minsavage G.V. (1985): Detection of *Xanthomonas campestris* pv. *carotae* in carrot seed. *Plant Disease*, 69: 758–760.
- Leite R.P.J.R., Jones J.B., Somodi G.C., Minsavage G.V., Stall R.E. (1995): Detection of *Xanthomonas campestris* pv. *vesicatoria* associated with pepper and tomato seed by DNA amplification. *Plant Disease*, 79: 917–922.
- Lelliott R.A., Stead D.E. (1987): Diagnostic procedures for bacterial plant diseases. In: Methods for the Diagnosis of Bacterial Diseases of Plants. Blackwell Scientific Publications: 58–59.
- Louws F.J., Fulbright D.W., Stephens C.T., Debruiji F.J. (1994): Specific genomic fingerprints of phytopathogenic *Xanthomonas* and *Pseudomonas* pathovars and strains generated with repetitive sequences and PCR. *Applied and Environmental Microbiology*, 60: 2286–2295.
- Manulis S., Valinsky L., Lichter A., Gabriel D.W. (1994): Sensitive and specific detection of *Xanthomonas campestris* pv. *pelargonii* with DNA primers and probes identified by random amplified polymorphic DNA analysis. *Applied and Environmental Microbiology*, 60: 4090–4094.
- McGuire R.G., Jones J.B., Sasser M. (1986): Tween media for semi-selective isolation of *Xanthomonas campestris* pv. *vesicatoria* from soil and plant material. *Plant Disease* 70: 887–891.
- McLaughlin R.J., Chen T.A. (1990): ELISA methods for plant pathogenic prokaryotes. In: Hampton R., Ball E., De Boer S. (eds): Serological Methods for Detection and Identification of Viral and Bacterial Plant Pathogens, A Laboratory Manual. St. Paul, APS Press: 197–204.
- Mirik M., Unlu S., Aysan Y. (2009): First report of *Xanthomonas hortorum* pv. *pelargonii* causing bacterial blight of geranium in Turkey. *Plant Pathology*, 59: 403–404.
- Mirik M., Aysan Y., Sahin F. (2011a): Characterization of *Pseudomonas cichorii* isolated from different hosts in Turkey. *International Journal of Agriculture and Biology*, 13: 203–209.
- Mirik M., Aysan Y., Sahin F. (2011b): Characterization of *Pseudomonas savastanoi* pv. *savastanoi* strains isolated from several host plants in Turkey and report of *Fontanisia* as a new host. *Journal of Plant Pathology*, 93: 263–270.
- Ornek H., Aysan Y., Mirik M., Sahin F. (2007): First report of bacterial leaf spot disease, caused by *Xanthomonas axonopodis* pv. *begoniae*, on begonia in Turkey. *Plant Pathology*, 56: 347.
- Sambrook J., Fritsch E.F., Maniatis T. (1989): Molecular Cloning – A Laboratory Manual. Cold Spring Harbor, Cold Spring Harbor Laboratory Press.
- Schaad N.W., White W.C. (1974): A selective medium for soil isolation and enumeration of *Xanthomonas campestris*. *Phytopathology*, 64: 876–880.
- Schaad N.W., Jones J.B., Lacy G.H. (2001): Gram negative bacteria, *Xanthomonas*. In: Schaad N.W., Jones J.B., Chun W. (eds): Laboratory Guide for Identification of Plant Pathogenic Bacteria. St. Paul, APS Press: 175–193.
- Sijam K., Chang C.J., Gitaitis R.D. (1992): A medium for differentiating tomato and pepper strains of *Xanthomonas campestris* pv. *vesicatoria*. *Canadian Journal of Plant Pathology*, 14: 162–164.
- Sulzurski M.A., Moorman G.W., Schlandhauser B., Romaine C.P. (1996): Characteristics of a PCR-based assay for in planta detection of *Xanthomonas campestris* pv. *pelargonii*. *Journal of Phytopathology*, 144: 393–398.
- TOPPEA (2016): Turkish Ornamental Plants Industry Report 2016. Available at [www.susbitkileri.org.tr/content/docs/2016-ornamental-plant-sector-in-turkey.pdf](http://www.susbitkileri.org.tr/content/docs/2016-ornamental-plant-sector-in-turkey.pdf)

Received: 2017–01–12

Accepted after corrections: 2018–01–29

Published online: 2018–02–19