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

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Abstract


Putative strains belonging to Xanthomonas spp. causing leaf spot and blight diseases on geranium (Pelargonium peltatum and P. hortorum), begonia (Begonia x 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.

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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 (Yuregir, Seyhan, Karaisali, Ceyhan districts), Antalya, Istanbul, Izmir (Tire, Odemis, Bayindir districts), Mersin (Yenice, Huzurkent, Mezitli, Tece, Erdemli, Siliﬁke, Aydincik 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
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, 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 and hypersensitivity on tobacco leaves as described by McGuire (1986). All tests were repeated three times. Colony morphology of Xanthomonas strains was observed on different semi-selective media such as Tween B agar (Shaad & White 1974), SM agar (Chun & Alvarez 1983) and modified D-5 agar (MD-5) agar (Kuan & Minsavage 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 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, H₂O 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, Sar5-1/b/a r, Izmir-1, Sardunya-3), 3 strains of X. axonopodis pv. begoniae (Xab1/r, Xcb13/r, Izmir 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 Sarmsik 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, Sar5-1/b/a r, Izmir-1, Sardunya-3), 6 strains of X. axonopodis pv. begoniae (Xab1/r, Xcb7/r, Xcb13/r, Izmir 11/1-1, Begonya Ekim, Kirmizi 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

<table>
<thead>
<tr>
<th>Pathogen</th>
<th>Size</th>
<th>Sequence 5’→3’</th>
<th>Amplification conditions</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>X. hortorum pv. pelargonii</td>
<td>1.2 kb</td>
<td>GAGTGTCCAGTGGCCAGGC</td>
<td>40 cycles 94°C 1 min/58°C</td>
<td>Manulis et al. (1994)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>GTTGCTGCTCCTTCCTGC</td>
<td>1 min/72°C 5 min</td>
<td></td>
</tr>
<tr>
<td>X. hortorum pv. pelargonii</td>
<td>197 bp</td>
<td>ACCGCCCTACCAAAAGGCAAGAG</td>
<td>30 cycles 94°C 1 min/64°C</td>
<td>Sulzinski et al. (1996)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>GATCTGGGTGTCTGCAAGATTGG</td>
<td>1 min/72°C 2 min</td>
<td></td>
</tr>
<tr>
<td>X. axonopodis pv. begoniae</td>
<td>619 bp</td>
<td>GACGCCCTCAGATCGACGTCGAGG</td>
<td>30 cycles 94°C 30 s/61°C</td>
<td>Leite et al. (1995)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>GGCATCTGATCGGTGTCTCCTGC</td>
<td>40 s/72°C 45 s</td>
<td></td>
</tr>
</tbody>
</table>

https://doi.org/10.17221/10/2017-PPS
and 2 strains of *P. 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 analyzed 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 H₂O. 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 sterilized 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 pv. 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

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

<table>
<thead>
<tr>
<th>Primer</th>
<th>Sequence 5′→3′</th>
<th>Amplification conditions</th>
</tr>
</thead>
<tbody>
<tr>
<td>BOXA-1R</td>
<td>CTACGGCAAGGCAGCTGACG</td>
<td>30 cycles 94°C 1 min/53°C 1 min/65°C 8 min</td>
</tr>
</tbody>
</table>

https://doi.org/10.17221/10/2017-PPS
Table 3. Diagnosis of *Xanthomonas* strains from different plant species

<table>
<thead>
<tr>
<th><em>Xanthomonas</em> spp.</th>
<th>Strain</th>
<th>Host</th>
<th>Location</th>
<th>KOH</th>
<th>O</th>
<th>Starch</th>
<th>C</th>
<th>L</th>
<th>O/F</th>
<th>M</th>
<th>HR</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>X. hortorum</em> pv. <em>pelargonii</em></td>
<td>GSPB 1955</td>
<td><em>Pelargonium</em></td>
<td>Germany</td>
<td>+</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>O</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>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, Sar4-1/a r, Sar4-1b/b r, Sar4-2/a r</td>
<td>Pelargonium <em>peltatum</em></td>
<td>Adana</td>
<td>+</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>O</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Sar5-1/a r, Sar5-3/a r, Sar5-4a/a r, Sar5-4b/c r, Sar5-5/a r, Sar5-7/a r</td>
<td></td>
<td></td>
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<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>İzmir-1, İzmir-2</td>
<td>P. <em>peltatum</em></td>
<td>İzmir</td>
<td>+</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>O</td>
<td>+</td>
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</tr>
<tr>
<td>Sardunya-1, Sardunya-2, Sardunya-3</td>
<td>P. <em>hortorum</em></td>
<td>Tekirdağ</td>
<td>+</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>O</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td><em>X. axonopodis</em> pv. <em>begoniae</em></td>
<td>BPIC 2013/94</td>
<td>Begonia × <em>tuberhybrida</em></td>
<td>Greece</td>
<td>+</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>O</td>
<td>+</td>
<td>+</td>
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</tr>
<tr>
<td>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</td>
<td>Begonia × <em>tuberhybrida</em></td>
<td>Adana</td>
<td>+</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>O</td>
<td>+</td>
<td>+</td>
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<tr>
<td>Xcb10/r, Xcb11/r, Xcb12/r, Xcb13/r, Silivri-beg-1, Silivri-beg-2, Silivri-beg-3</td>
<td>Begonia × <em>tuberhybrida</em></td>
<td>Istanbul</td>
<td>+</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>O</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Begonya Ekim, Kurmuzi Begonya</td>
<td>Begonia × <em>tuberhybrida</em></td>
<td>Mersin</td>
<td>+</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>O</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>İzmir 11/1-1, İzmir 11/1-2, İzmir 11/2-1, İzmir 11/2-2, İzmir 11/2-3</td>
<td>Begonia × <em>tuberhybrida</em></td>
<td>İzmir</td>
<td>+</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>O</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td><em>X. axonopodis</em> pv. <em>dieffenbachiae</em></td>
<td>JS 990</td>
<td>Anthurium</td>
<td>France</td>
<td>+</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>O</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>JRV 589</td>
<td>Anthurium <em>andraeanum</em></td>
<td>Adana</td>
<td>+</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>O</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Ant-1a, Ant-1b, Ant-1c, Ant-1d, Ant-1e</td>
<td>Anthurium <em>andraeanum</em></td>
<td>Adana</td>
<td>+</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>O</td>
<td>+</td>
<td>+</td>
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</tr>
<tr>
<td>Ant-2a, Ant-2b, Ant-2c, Ant-2d, Ant-2e</td>
<td>A. <em>andraeanum</em></td>
<td>Istanbul</td>
<td>+</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>O</td>
<td>+</td>
<td>+</td>
<td>+</td>
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</tr>
<tr>
<td><em>X. hortorum</em> pv. <em>hederae</em> (2 strains)</td>
<td>Sarmasik 1/4, Sarmasik 2/1</td>
<td>Hedera <em>helix</em></td>
<td>Mersin</td>
<td>+</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>O</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Xanthomonas sp. (1 strain)</td>
<td>Hibiscus-1</td>
<td>Hibiscus <em>rosa-sinensis</em></td>
<td>Adana</td>
<td>+</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>O</td>
<td>+</td>
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<td>+</td>
</tr>
</tbody>
</table>

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 Minsavage (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 levann 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, Sar2-3/c, Sar4-1a/a, Sar5-1b/a, 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, 10 strains as X. axonopodis pv. begoniae, 4 strains X. axonopodis pv. dieffenbachiae, and 2 strains X. hortorum pv. hederae) 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
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 Sahin (2003), Ornek et al. (2007), and Mirik 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**


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