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Full Length Article

Characterization of *Pseudomonas cichorii* Isolated from Different Hosts in Turkey

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ABSTRACT

Stem and pith necrosis symptoms on tomato, varnish spot symptoms on lettuce and leaf spot symptoms on dwarf umbrella trees were observed in late winter and spring of 2002 and 2008 in the Eastern Mediterranean region of Turkey. Fluorescent bacteria were isolated from typical diseased plants on King's medium B as nearly pure cultures. All of the bacterial strains isolated in the study were identified as *Pseudomonas cichorii* compared with reference strains GSPB 2097, CFPB 2102 and NCPPB 3802, based on conventional methods, ELISA and fatty acid methyl ester analysis. The strains were divided into five different groups according to cluster analysis of fatty acid compositions in phenotypic characterization of the strains. In sensitive assays, tomato, lettuce and dwarf umbrella trees strains are sensitive to cupric sulfate and streptomycin. The study also demonstrated that *P. cichorii* strains isolated from tomato, lettuce and dwarf umbrella trees can be distinguished based on BOX-PCR experiments in genotypic characterization of the strains. In pathogenicity tests, tomato and lettuce strains were more aggressive on tomato, lettuce and dwarf umbrella trees, while dwarf umbrella trees strains were pathogenic only on their host. When strains were inoculated to their original host, they were more aggressive on their host than on other species. This is the first detail study of *P. cichorii* causing stem and pith necrosis on tomato, bacterial rot and varnish spot on lettuce and leaf spot on dwarf umbrella trees in Turkey.

Key Words: Tomato; Lettuce; Dwarf umbrella trees; FAME; PCR; BOX-PCR; Phenotypic & genotypic relationship

INTRODUCTION

Tomato (*Lycopersicum esculentum*) was one of the most important greenhouse and field-grown vegetables in Turkey, with a production of 10 985 400 tons in 2008 (FAO, 2010). Fresh market tomatoes are grown in greenhouses in the Mediterranean region of Turkey. Stem and pith necrosis is a serious disease of greenhouse-grown tomatoes in the region (Aysan, 2001; Sahin *et al*., 2005). Several *Pseudomonas* species (e.g., *P. corrugata, P. cichorii, P. viridiflava* & unidentified *Pseudomonas* species) have been reported to be pathogens of this disease (Sahin *et al*., 2005). Spots on stem, lesions on petiole and fruit stalk, hollowing and browning of pith and discoloration of vessels have been defined as typical symptoms of the disease on tomato. Disease incidence was about 20-25% on tomato in 2002-2004 (Aysan *et al.,* 2006).

Lettuce (*Lactuca sativa*) is an important field-grown vegetable in Turkey. Bacterial rot and varnish spot is a destructive disease of lettuce in many countries including Turkey. The disease is characterized by shiny, dark brown spots scattered on the blades, petioles and particularly along the veins of leaves, underneath the second or third head leaves, with no soft rot. Disease incidence was recorded as 10% on lettuce in 2002 (Aysan *et al.,* 2003).

Dwarf umbrella trees (*Schefflera arboricola*) are native to the tropical and subtropical regions of Asia. Because of climate conditions of the Eastern Mediterranean region, dwarf umbrella trees are grown in the region as foliage potted ornamental plants. Leaf spot symptoms on dwarf umbrella tree were first observed in late winter and spring of 2006 and 2008 in two commercial ornamental greenhouses in the region. Disease incidence was estimated at about 10% on dwarf umbrella trees in 2008 (Aysan *et al.,* 2009).

The purpose of this study was to isolate and identify of the causal agents of leaf spot disease of tomato, lettuce and dwarf umbrella tree grown in the eastern Mediterranean region of Turkey and to characterize them by morphological, physiological and biochemical tests, ELISA, whole cell fatty acid analysis and pathogenicity tests compared with reference lettuce, tomato and dwarf umbrella tree strains. *Pseudomonas cichorii* strains isolated from different host plants (lettuce, tomato & dwarf umbrella tree) were determined based on BOX-PCR experiments in genotypic characterization of the strains. Copper (Cu)

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and streptomycin sensitivity was also determined in the study.

MATERIALS AND METHODS

Bacterial strains: In 2002 and 2008, isolations of bacteria were made from typical stem and pith necrosis symptoms on tomato, varnish spot symptoms on lettuce and leaf spot symptoms on dwarf umbrella tree in Adana and Mersin provinces, located in the Eastern Mediterranean region of Turkey. The surface of the infected plant tissue was disinfected by wiping with 70% ethanol and tissue was macerated in 0.85% (w/v) NaCl saline for about 20 min. A loopful of the suspension of infected tissue in sterile saline was streaked on the surface of plates containing King's medium B (KB) (King *et al.*, 1954) and incubated at 25°C for 48 h. Dominant colonies were sub-cultured on KB for pure cultures. Isolated strains were stored in 40% glycerol at -80°C. GSPB 2097 (Gottinger Sammlung phytopathogener Bakterien, Georg-August University Gottingen, Germany), NCPPB 3802 (National Collection of Plant Pathogenic Bacteria, Harpenden, England) and CFBP 2101 (Collection Francaise de Bacteries Phytopathogenes, Angers, France), were used as reference strains of *P. cichorii* for comparison with the test bacterial strains (Table I).

Characterization: Twelve strains isolated from tomato, lettuce and dwarf umbrella tree in Turkey were characterized by the following tests as described by Lelliot and Stead (1987) and Klement *et al.* (1990): potassium hydroxide solubility for gram reaction, fluorescent pigmentation on KB, levan formation on nutrient agar plus 5% sucrose, oxidase reaction, induction of soft rot on potato tubers, arginine dihydrolase activity, hypersensitive reaction on tobacco leaves (*Nicotiana tabacum* cv. Samsun), catalase production, oxidative/fermentative metabolism of glucose, ability to reduce nitrates to nitrites, gelatin liquefaction, colony appearance on yeast dextrose chalk agar medium (YDC), KB, and on nutrient agar, acid from L (+) arabinose, D-arabinose, glucose, inulin, lactose, mannitol, mannose, maltose, melibiose, raffinose, sorbitol, sucrose and trehalose as carbon compounds.

Resistance to copper and streptomycin: Sensitivity was tested on SPA medium (20.0 g of sucrose, 5.0 g of peptone, 0.5 g of dibasic potassium phosphate, 0.25 g of magnesium sulfate & 15.0 g of agar in 1000 mL of distilled water) amended with copper sulphate or streptomycin as described by Ritchie and Dittapongpitch (1991). Fresh solutions of copper or streptomycin were prepared in sterile distilled water and filter-sterilized. Different concentrations of copper (30, 100 & 200 mg/mL) and streptomycin (20, 100 & 150 mg/mL) were added to SPA before pouring into the petri dishes. *P. cichorii* strains, isolated from tomato, lettuce and dwarf umbrella tree, were streaked on the amended SPA plates and incubated for 48 h at 25°C and the presence or absence of growth was recorded.

Pathogenicity tests: For the pathogenicity tests, three replicates of tomato (*Lycopersicum esculentum* Mill cv. H-2274), lettuce (*Lactuca sativa* cv. Yedikule) and dwarf umbrella tree (*Schefflera arboricola* cv. Gold Capella) were inoculated with suspensions (10^8 cfu/mL) of tomato, lettuce and dwarf umbrella tree strains by injection of stems with a sterile needle and spraying onto leaves by a hand sprayer. After inoculations, plants were covered with clear polyethylene bags for 24 h at 25°C. Bags were removed one day later and plants were maintained in a controlled climate room at 25°C, 70% RH and 16 h/8 h day/night. Disease development on tomato and lettuce plants was evaluated 7-10 days after inoculation. Symptoms on dwarf umbrella tree plants were recorded 30 days after inoculation. Re-isolations were made from the diseased plants. Sterile distilled water and reference strains of *P. cichorii* were used as negative and positive controls.

Serological techniques: For serological tests, *Pseudomonas cichorii*-specific polyclonal antibody (Tomato 1) was used to confirm the identity of *Pseudomonas cichorii* strains at species level. The antibody was developed at Genetic Engineering and Biotechnology Research Institute of Turkish Scientific and Technical Research Society (Gebze-Kocaeli, Turkey). Serological identification of the bacterial strains was performed according to the previously described indirect-ELISA method (McLaughlin & Chen, 1990).

Fatty acid methyl ester analysis: Selected strains (three reference strains and 10 regional strains) were also identified according to fatty acid methyl ester (FAME) analysis. This part of the study was carried out in Biotechnology Application and Research Center of Ataturk University (Erzurum, Turkey). Whole-cell fatty acids were extracted and methylated as described by Miller and Berger (1985), Stead (1988, 1989) and Sasser (1990). FAMEs were separated by gas chromatography (HP6890, Hewlett Packard, Palo Alto, CA). FAME profiles were identified using the commercial trypticase soy broth agar database with the Microbial Identification System software package (Sherlock MIS version 4.5, Microbial ID, Inc., Newark, DE).

Determination of Genotypic Relationship by PCR Tests

Genomic DNA isolation: Bacterial isolates were grown on 9 mL liquid nutrient broth for 24 h. A one milliliter bacterial suspension was put in a sterile Eppendorf tube and then centrifuged it at 14 000 xg for 20 min. Pellets were retained and the supernatant were discarded. To ensure cell lysis, 100 µL 1% SDS+TAE buffer were added to pellets and vortexed. Tubes were incubated for 3 h in a water bath at 50°C. A half volume of 7.5 M ammonium acetate was added and samples were centrifuged at 14 000 xg. Supernatants with the remaining DNA (approximately 130 µL) were transferred into new Eppendorf tubes. An equal volume of freezer-chilled isopopanol (2-propanol) was added and tubes were placed in a freezer for 45 min. Samples were centrifuged for 10 min at 10 000 g. Pellets were washed with 100 µL cold 70% ethanol and centrifuged for 10 min at 10000 × *g*. Pellets were combined with 50 µL

sterile bi-distilled water and samples were vortexed. DNA was separated on a 1% agarose gel at 70 volts for 30 min then stained with ethidium bromide.

Determination of genotypic relationship: The primers and the conditions that are complementary to BOX, REP and ERIC (Louws *et al.*, 1994; Norman *et al.,* 2003) are shown in Table II. BOX-PCR, ERIC-PCR and REP–PCR amplifications were carried out in 25.0 µL final volume containing 3.0 µL of DNA template, 1.5 µL of each respective primer, 12.5 µL Master Mix (Promega M7502), and 6.5 µL H_2O. All the amplifications were performed in a Techne Thermocycler (TC-412). PCR amplification products were run on a 1.5% agarose gel using 1kb and 100 bp DNA ladders as markers. Bands formed by isolates were recorded. All data were analyzed by the comprehensive statistical analysis program Statistical Package for Social Sciences (SPSS). A dendogram was created to analyze genetic relationships.

RESULTS

Bacterial strains: Surveys were conducted in lettuce producing areas in Adana and Mersin provinces to detect leaf spot and vein rot symptoms on lettuce. One lettuce producer used the same seeds and transplants in infected fields in different locations, Misis (Adana) and Tarsus (Mersin) provinces. Symptoms of the disease on dwarf umbrella tree plants are characterized by small margins of brown with wide yellow halos on leaves. The disease was detected only in the production area in Adana. *Pseudomonas cichorii* is characterized by general chlorosis of tomato plant, enlarged brown-black spots on the stem nodes and branches coming from them, initial yellow and then dark brown discoloration of the pith and vascular discoloration. These disease symptoms were detected in six different greenhouses in Erdemli province in Mersin. A total of 12 bacterial strains were isolated and purified from lettuce (3 strains), dwarf umbrella tree (3 strains) and tomato (6 strains) from samples collected in different locations in Adana and Mersin in the eastern Mediterranean region of Turkey.

Characterization: All strains were gram negative, had fluorescent pigmentation on KB, did not produce levan type colonies on sucrose nutrient agar, were oxidase positive, did not cause soft rot on potato slices, were arginine dihydrolase activity negative, had positive hypersensitive reaction on tobacco leaves (Table III), were catalase positive, oxidative. No strains grew at 5% NaCl and did not reduce nitrates to nitrites. Negative results were obtained for gelatin liquefaction, using citrate starch and casein hydrolysis for all strains (Table IV). Negative results were obtained for acid production from L (+) arabinose D-arabinose, glucose, inulin, lactose, mannitol, mannose, maltose, melibiose, raffinose, sorbitol, sucrose and trehalose as carbon copounds. All of the strains were grown on KB, which produced non-mucoid, cream and flourescent colonies. All strains also grew on YDC and Nutrient Agar medium, producing flat, non-mucoid and cream colonies. Based on these test results, the bacterial strains were identified as *P. cichorii.*

Resistance to Cu and streptomycin: No strains grew on SPA amended with cupric sulfate (30, 100 & 200 mg/mL) and streptomycin (20, 100 & 150 mg/mL). All strains were not resistant Cu and streptomycin.

Table I: *Pseudomonas cichorii* strains used in this study

Design. No.	Host	Origin	Source
Lettuce-1	Lettuce	Adana, Turkey	This study
Lettuce-2	Lettuce	Mersin, Turkey	This study
Lettuce-3	Lettuce	Adana, Turkey	This study
Tomato-1	Tomato	Adana, Turkey	This study
Tomato-2	Tomato	Adana, Turkey	This study
Tomato-3	Tomato	Adana, Turkey	This study
Tomato-4	Tomato	Mersin, Turkey	This study
Tomato-5	Tomato	Mersin, Turkey	This study
Tomato-6	Tomato	Mersin, Turkey	This study
Schefflera-1	Dwarf umbrella tree	Adana, Turkey	This study
Schefflera-2	Dwarf umbrella tree	Adana, Turkey	This study
Schefflera-3	Dwarf umbrella tree	Adana, Turkey	This study
GSPB 2097	Lettuce	-	Dr. K. Rudolph
CFPB 2101	Lettuce	France	Dr. L. Sutra
NCPPB 3802	Tomato	Mersin, Turkey	Dr. S. Tokgonul

Table II: DNA amplification conditions and primers used for BOX-PCR, ERIC-PCR and REP–PCR

Method	primer	Sequence 5'-3'	Amplification conditions
BOX-PCR	BOXA-1R	CTACGGCAAGGCGACGCTGACG	30 cycles 94 °C 1 min/53 °C 1 min/65°C 8 min
ERIC-PCR	ERIC 1R	ATGTAAGCTCCTGGGGATTCAAC	30 cycles 94 °C 1 min/52 °C 1 min/65°C 8 min
	ERIC 2	AAGTAAGTGACTGGGGTGAGCG	
REP-PCR	REPIR-1	IIIICGICGICATCIGGC	30 cycles 94 °C 1 min/44 °C 1 min/65°C 8 min
	REP2-1	ICGICTTATCIGGCCTAC	

Table III: LOPAT characters of *Pseudomonas cichorii* strains

Design. No.	Host	Tests				
		L*	O	P	A	T
Lettuce -1	Lettuce	-	+	-	-	+
Lettuce -2	Lettuce	-	+	-	-	+
Lettuce -3	Lettuce	-	+	-	-	+
Tomato -1	Tomato	-	+	-	-	+
Tomato -2	Tomato	-	+	-	-	+
Tomato -3	Tomato	-	+	-	-	+
Tomato -4	Tomato	-	+	-	-	+
Tomato -5	Tomato	-	+	-	-	+
Tomato -6	Tomato	-	+	-	-	+
Schefflera -1	Dwarf umbrella tree	-	+	-	-	+
Schefflera -2	Dwarf umbrella tree	-	+	-	-	+
Schefflera -3	Dwarf umbrella tree	-	+	-	-	+
GSPB 2097	Lettuce, reference strains	-	+	-	-	+
CFPB 2101	Lettuce, reference strains	-	+	-	-	+
NCPPB 3802	Tomato, reference strains	-	+	-	-	+

*L:Levan production from sucrose, O: Oxidase reaction, P:Patato rot, A:Arginine dihydrolase, T:Tobacco hypersensitivity

Table IV: Characterization of *Pseudomonas cichorii* strains

Isolates	KOH	Growth on 5% NaCl	Using Citrate	Liquefaction of gelatin	Reduction of nitrate nitrite	Starch	Cas	C
Lettuce-1	-	-	-	-	-	-	-	+
Lettuce-2	-	-	-	-	-	-	-	+
Lettuce-3	-	-	-	-	-	-	-	+
Tomato-1	-	-	-	-	-	-	-	+
Tomato-2	-	-	-	-	-	-	-	+
Tomato-3	-	-	-	-	-	-	-	+
Tomato-4	-	-	-	-	-	-	-	+
Tomato-5	-	-	-	-	-	-	-	+
Tomato-6	-	-	-	-	-	-	-	+
Schefflera -1	-	-	-	-	-	-	-	+
Schefflera -2	-	-	-	-	-	-	-	+
Schefflera -3	-	-	-	-	-	-	-	+
GSPB 2097	-	-	-	-	-	-	-	+
CFPB 2101	-	-	-	-	-	-	-	+
NCPPB 3802	-	-	-	-	-	-	-	+

KOH: Potassium hydroxide, Starch: Starch hydrolysis, Cas: Casein hydrolysis, C: Catalase, reduction

Pathogenicity tests: Disease development on tomato and lettuce plants was evaluated 7-10 days after inoculation. Symptoms on dwarf umbrella tree plants were recorded 30 days after inoculation. No symptoms appeared on control plants. Isolation from artificially infected plants recovered the bacterium originally inoculated. All re-isolated bacteria were stored at 4°C for identified and then used for further studies. All strains produced symptoms on all plants (tomato, lettuce & dwarf umbrella tree), but when the strains were inoculated to the original host, they were more aggressive than on the other species (Table V). Tomato strains inoculated to the original host were more aggressive than on lettuce and dwarf umbrella tree plants. Also with lettuce strains, when inoculated to lettuce and tomato plants, they were more aggressive than dwarf umbrella tree plants. But dwarf umbrella tree strains were less aggressive on all plants. Reference strains (GSPB 2097 & CFPB 2101) were less aggressive on all plants, while reference strain NCPBB 3802 was more aggressive on tomato and lettuce plants but less aggressive on dwarf umbrella tree plants.

Table V: Pathogenicity tests of *Pseudomonas cichorii* strains on different hosts

Designation Nr.	Host	Patogenicity Tests		
		Tomato	Lettuce	Schefflera
Lettuce -1	Lettuce	+++	+++	++
Lettuce -2	Lettuce	+++	+++	++
Lettuce -3	Lettuce	+++	+++	++
Tomato -1	Tomato	+++	+++	++
Tomato -2	Tomato	+++	+++	++
Tomato -3	Tomato	+++	+++	++
Tomato -4	Tomato	+++	+++	++
Tomato -5	Tomato	+++	+++	++
Tomato -6	Tomato	+++	+++	++
Schefflera -1	Dwarf umbrella tree	+	+	++
Schefflera -2	Dwarf umbrella tree	+	+	++
Schefflera -3	Dwarf umbrella tree	+	+	++
GSPB 2097	Lettuce, reference strains	+	+	++
CFPB 2101	Lettuce, reference strains	+	+	++
NCPPB 3802	Tomato, reference strains	+++	+++	++

+: less aggressive; ++: more aggressive; +++: most aggressive

Serological techniques: All of the *P. cichorii* strains in this study reacted with polyclonal antiserum (Tomato 1) developed and used with indirect ELISA. The mean absorbance values of three replications in indirect ELISA tests were between 0.930 and 1.263 in lettuce strains, 0.975 and 1.662 in tomato strains and 0.950 and 0.988 in dwarf umbrella tree strains at A_{405} wavelength. The mean absorbance values of three replications in indirect ELISA tests were between 0.102 and 0.195 in the negative control and 1.002 and 2.170 in the positive control at A_{405} wavelength (Table VI). Tomato, lettuce and dwarf umbrella tree isolates gave results close to the values of the positive control and more than twice the value of the negative control. Therefore all isolates were considered to have a positive ELISA test, confirming the traditional diagnostic methods.

Table VI: Mean absorbance values of indirect ELISA tests

Designation Nr.	Mean	Result
Negative Control (PBS)	0.195	-
Negative Control (Healthy tomato leaf)	0.102	-
Positive Control, GSPB 2097	1.002	+
Positive Control, CFPB 2101	2.170	+
Positive Control, NCPPB 3802	1.045	+
Lettuce-1	0.930	+
Lettuce-2	1.045	+
Lettuce-3	1.263	+
Tomato-1	1.031	+
Tomato-2	1.002	+
Tomato-3	1.066	+
Tomato-4	1.059	+
Tomato-5	1.662	+
Tomato-6	0.975	+
Schefflera-1	0.988	+
Schefflera-2	0.950	+
Schefflera-3	0.957	+

Fatty acid methyl ester analysis: The identity of the strains was confirmed to be *P. cichorii* on the basis of FAME analysis with similarity indices ranging from 84-97%. All *P. cichorii* strains isolated from tomato, lettuce and dwarf umbrella tree had 11 different fatty acids that included saturated (12:0, 16:0 & 18:0), unsaturated (11 methyl 18:1 w7c & 18:1 w7c), hydroxyl (10:0 3-OH, 12:0 2OH & 12:0 3OH), cyclopropane (17: CYCLO) and two Sum in Features

(3, 7) (Table VII). The strains were divided into four different groups according to cluster analysis of fatty acids (Fig. 1). Group A included only dwarf umbrella trees strain, Scheflera 1; group B included only lettuce strain, Lettuce 1; group C was included four tomato strains, one lettuce strain and two reference strains (Tomato 1, 2 4 & 5; Lettuce 3; CFBP 2101 & NCPPB 3802): while group D was included two tomato strains, one lettuce and reference strain (Tomato 3, 6, Lettuce 2 & GSPB 2097). There was no correlation between strain and location. There was correlation between host and pathovar of the strain isolated. In light of these results, different pathogenesis may be due to availability of resources in the host plants or to the fact that they are different pathovars.

Determination of genotypic relationship by PCR tests: DNA obtained from the regional (lettuce, tomato & dwarf umbrella tree) and reference isolates (GSPB 2097, CFBP 2101 & NCPPB 3802) was used for BOX-PCR, ERIC-PCR and REP-PCR. BOX-PCR yielded many polymorphic bands and was useful in differentiating isolates. As shown in Fig. 2, DNAs isolated from the same host gave similar band patterns, but changed with sequence differences of types from different hosts. For this reason, the lettuce, tomato and dwarf umbrella tree bacterial strains are thought to be of different origin (Fig. 3).

As seen in PCR studies of tomato pith and stem necrosis, lettuce bacterial rot and varnish spot, and dwarf umbrella tree bacterial leaf spot caused by *Pseudomonas cichorii*, isolates can be differentiated from each other by different PCR band patterns. Our studies have shown that BOX-PCR is successful in identifying differences in isolates from different hosts.

DISCUSSION

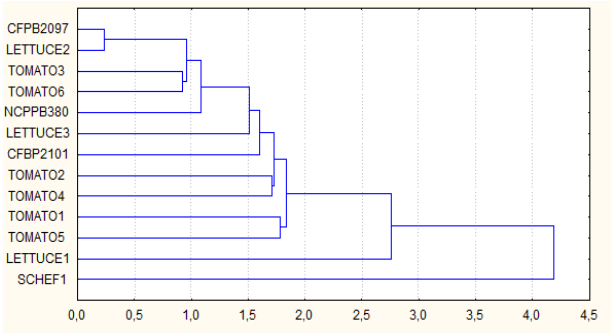
A total of 12 bacteria were isolated from leaf spot and midrib rot symptoms on lettuce (3 strains), dwarf umbrella tree (3 strains) and tomato (6 strains) in Adana and Mersin provinces. The 12 isolates consisted of fluorescent bacteria as determined on King's medium B under UV_{366nm} radiation. All isolates were HR positive on tobacco plants and non-pectolytic on potato tuber slices (Table IV). The isolates represented a morphologically diverse set of bacteria belonging to LOPAT (Table III) group III fluorescent pseudomonads (Lelliot & Stead, 1987). In the LOPAT tests they belonged to a group that was indicative for *P. cichorii*. However, it should be noted that a negative reaction in the potato rot test is typical for *P. cichorii* (Lelliot & Stead, 1987).

In the pathogenicity tests all isolates were screened for the ability to produce symptoms on lettuce, dwarf umbrella tree and tomato. Each of three isolates tested was pathogenic on dwarf umbrella tree, causing symptoms similar to those seen on naturally infected plants within 30 days. Lesions were initially wide water-soaked areas on leaves. All tomato isolates tested were pathogenic on

Table VII: The results of fatty acid analysis of *Pseudomonas cichorii* strains

Fatty Acids	No. Of Strains	Range	Mean	Standard Derivation
10:0 3OH	13	3.00-4.18	3.27	0.32
12:0	13	4.26-6.01	4.76	0.44
12:0 2OH	13	2.38-3.58	2.83	0.32
12:0 3OH	13	3.41-4.46	4.11	0.34
Sum in feature3	13	35.70-39.90	37.46	1.14
16:0	13	24.13-26.51	24.92	1.01
17: CYCLO	1	1.23	1.23	-
18:1 w7c	13	18.10-23.16	21.39	1.60
18:0	11	0.83-1.44	1.08	0.21
11 methyl 18:1 w7c	3	0.56-1.08	0.90	0.29
Sum in feature7	1	0.30	0.30	-
Summed Feature 3		16:1 w7c /15 ISO 2OH		
Summed Feature7		Unknown 18.846/19:1 w6c		

Fig. 1: Phenotypic relationship of *Pseudomonas cichorii* isolates with whole cell fatty acid analysis



tomato, causing symptoms similar to naturally infected plants. At 7 days after inoculation, lesions were shiny, dark brown spots scattered on the petioles and particularly along the veins of leaves. Spots or stem lesions on petioles, hollowing of fruit stalks and discoloration of vessels in pith have been defined as typical symptoms of the disease on tomato. A difference in symptom development on each host plant has occurred in pathogenicity tests (Chase & Brunk, 1984). *Pseudomonas cichorii* has been a serious pathogen of numerous plant genera including lettuce (Grogan *et al*., 1977), chrysanthemum (Jones *et al*., 1983; Mc Fadden, 1961), celery (Thayer & Wehlburg, 1965), cabbage (Wehlburg, 1963), geranium (Engelhard *et al.*, 1983) and many members of the important foliage plant family Araceae (Wehlburg *et al.*, 1966). This is the first report of *Pseudomonas cichorii* causing serious leaf blight on dwarf umbrella tree.

All strains were not detected resistant to Cu and streptomycin. Cooksey *et al.* (1990) isolated several Cu-resistant bacteria species from tomato plants and seeds and each bacterium contained either plasmid or chromosomal DNAs.

Repetitive sequenced-based PCR genomic fingerprinting with the BOXA1R primer (Martin *et al.*, 1992) was used to assess the genetic diversity of *P. cichorii* isolated from different host plants. The generated genomic

Fig. 2: Box-PCR bands. Line 1: Marker (100 bp); Line 2: Positive control (CFBP 2101); Line 3: Lettuce isolate; Line 4: Tomato isolate; Line 5: Dwarf umbrella tree isolate; Line 6-7: Tomato isolates; Line 8: Negative control; Line 9: Dwarf umbrella tree isolate; Line 10: Lettuce isolate; Line 11: Negative control; Line 12-13: Tomato isolates; Line 14: Dwarf umbrella tree isolate; Line 15: Lettuce isolate

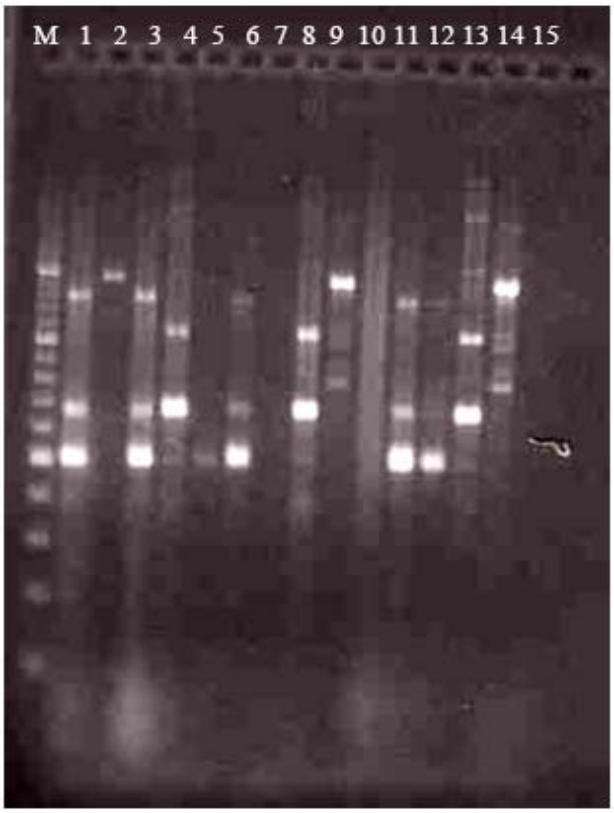
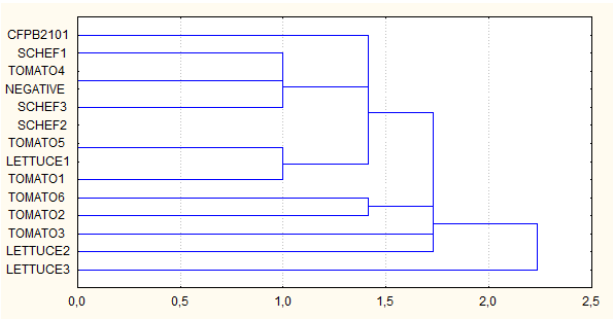


Fig. 3: Genotypic relationship of *Pseudomonas cichorii* isolates with BOX-PCR



patterns consisted of 13 or more DNA fragments ranging in size from approximately 0.5-2.0 kb. Cluster analysis of the BOX-PCR patterns of the 12 *P. cichorii* strains not important genetic heterogenicity. At a cut-off value of 98% similarity, a clustering wave was obtained that did not contradict that the isolates were from different host plants. Cluster analysis of the BOX-PCR patterns revealed two distinct but genetically homogenous groups when delineated at a 98% similarity level. These findings agree with the result of Cottyn *et al.* (2009), who also found a similarly low genetic relatedness between the 53 strains of *P. cichorii* tested. As a result, as seen in the PCR studies, isolates of *Pseudomonas cichorii* causing pith necrosis on tomato, vascular rot in lettuce and leaf spot on dwarf umbrella trees were separated from each other by forming different bands. Strains were isolated different hosts, especially Box-PCR to determine the differences are more successful in our work has been revealed.

Table VII shows the fatty acids used in numerical analysis of the FAME profile, revealing that most (about 92%) of the *P. cichorii* strains studied were distributed into two clusters, cluster I and cluster II. Cluster I included tomato and lettuce strains, but cluster II included only schfflera strains. The *P. cichorii* strains had a characteristic fatty acid profile (10:0 3OH, 12:0 2 OH, 12:3OH) as previously described (Stead *et al.,* 1992), but the dwarf umbrella tree strains had two different fatty acids (sum in feature & 18:1w7c).

In this study *P. cichorii* strains isolated from tomato, lettuce and dwarf umbrella tree plants were identified by classical morphological and biochemical tests. Also, all strains were identified as *P. cichorii* based on fatty acid methyl ester analysis and ELISA tests, and phenotypic and genotypic relationships have been revealed.

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