ETIOLOGY AND HOST RANGE OF BACTERIAL LEAF BLIGHT AND NECROSIS OF SQUASH AND MUSKMELON IN IRAN

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SUMMARY

In Summer and Autumn of 2010, blighted cucurbits suspected of bacterial infection were observed in the East Azarbayejan province of Iran. Infected plants exhibited spots on the leaves which, in the early stages of the disease, appeared as small, yellow and water-soaked with chlorotic haloes. As time went by, the spots enlarged, necrotized and coalesced so as to cover a large area of the blade, while the leaves rolled along their longitudinal axis. Such symptoms had not previously been observed in cucurbit*s* in East Azarbayejan. To investigate the etiology of this disorder, symptomatic leaves were collected from affected plants and the putative agent was isolated and identified as *Pseudomonas syringae* pv. *aptata* based on morphological, physiological, biochemical, molecular and pathogenicity tests. In BOX-PCR, four bacterial isolates from cucurbits formed a coherent group and their sequences showed a high similarity (99%) with *P. syringae* pv. *aptata* GSPB1067, as confirmed by phylogenetic analysis based on *gyrB* and *rpoD.* To the best of our knowledge, this is the first report of leaf blight and necrosis of Cucurbitaceae caused by *P. syringae* pv. *aptata* in Iran.

Key words: Pseudomonas syringae pv. *aptata*, *Cucurbitaceae*, *rpoD*, *gyrB*, *BOX-PCR*

INTRODUCTION

Members of Cucurbitaceae (960 species in 125 genera), a family a great agricultural relevance (Jeffrey, 2005), are subjected to infections by fungal, bacterial, viral, and phytoplasmal pathogens and to nematode attacks (Robinson and Walters, 1997), which can affect the plants at all stages of growth till harvest (Babadoost and Zitter, 2009). As to of bacterial pathogens, their occurrence is reported from different regions of the world, e.g. *Pseudomonas syringae* pv. *lachrymans* (*Psl*), the causal agent of cucumber angular leaf spot, and *Pseudomonas marginalis* pv. *marginalis* (*Pmm*), *Pseudomonas viridiflava*, *Pseudomonas syringae* pv. *syringae* (*Pss*) and *Pseudomonas syringae* pv. *aptata* (*Psa*), the agents of bacterial blight (Ohta *et al*., 1976; El-Sadek *et al*., 1992; Sharrock *et al*., 1997; Goumans and Chatzaki, 1998; Morris *et al*., 2000; Fatmi *et al*., 2008; Liu *et al*., 2010). This list further includes *Erwinia tracheiphila* (Bacterial wilt) (Sanogo *et al*., 2011), *Acidovorax avenae* subsp. *citrulli* (Bacterial fruit blotch) (Somodi *et al*., 1991; Mirik *et al*., 2006), *Xanthomonas cucurbitae* (Bacterial leaf spot) (Bryan, 1926; Lamichhane *et al*., 2010) and *Serratia marcescens* (Yellow vine disease) (Bruton *et al*., 1995; Bost *et al*., 1999) that have been reported from many regions in the world, whereas *Pantoea ananatis*, the agent of internal fruit rot of melons and *Sphingomonas melonis* that causes brown spot on the fruits of *Cucumis melo* var. *inodorus* have been recorded only from Japan and Spain, respectively (Buonaurio *et al*., 2002; Kido *et al*., 2008).

FAO statistics (FAOSTAT, 2013), indicate that, in 2011, Iran came second in the world for production of watermelons and cucumbers and fourth for the production of pumpkins, squash and gourd. These crops are infected by *Psl* and *Pmm* in Iranian some provinces (Ghobakhloo *et al*., 2002; Harighi, 2007), while *P. viridiflava*, *Pss* and *Psa* were observed in hosts other than cucurbits (Bahar *et al*., 1982; Shams-Bakhsh and Rahimian, 1997; Arabi *et al*., 2006).

In September and October of 2010, diseased plants exhibiting necrotic spots confined to the leaves (Fig. 1a), were observed in field-grown cucurbits in the East Azarbayejan province of Iran. During the early stages of the disease, the spots appeared as small, yellow, water-soaked with chlorotic haloes. As time went by, the spots enlarged, necrotized and coalesced so as to cover a large area of the blade, while the leaves rolled along their longitudinal axis. Such symptoms had not previously been observed in cucurbit*s* in East Azarbayejan. Considering the importance of these crops, the alleged existence of several bacteria damaging them, and since only *Psl* and *Pmm* had previously been reported in Iran, a study was undertaken for the identification of the putative responsible of the disease and determine its taxonomic position through biochemical, host range, and genetic characterization.

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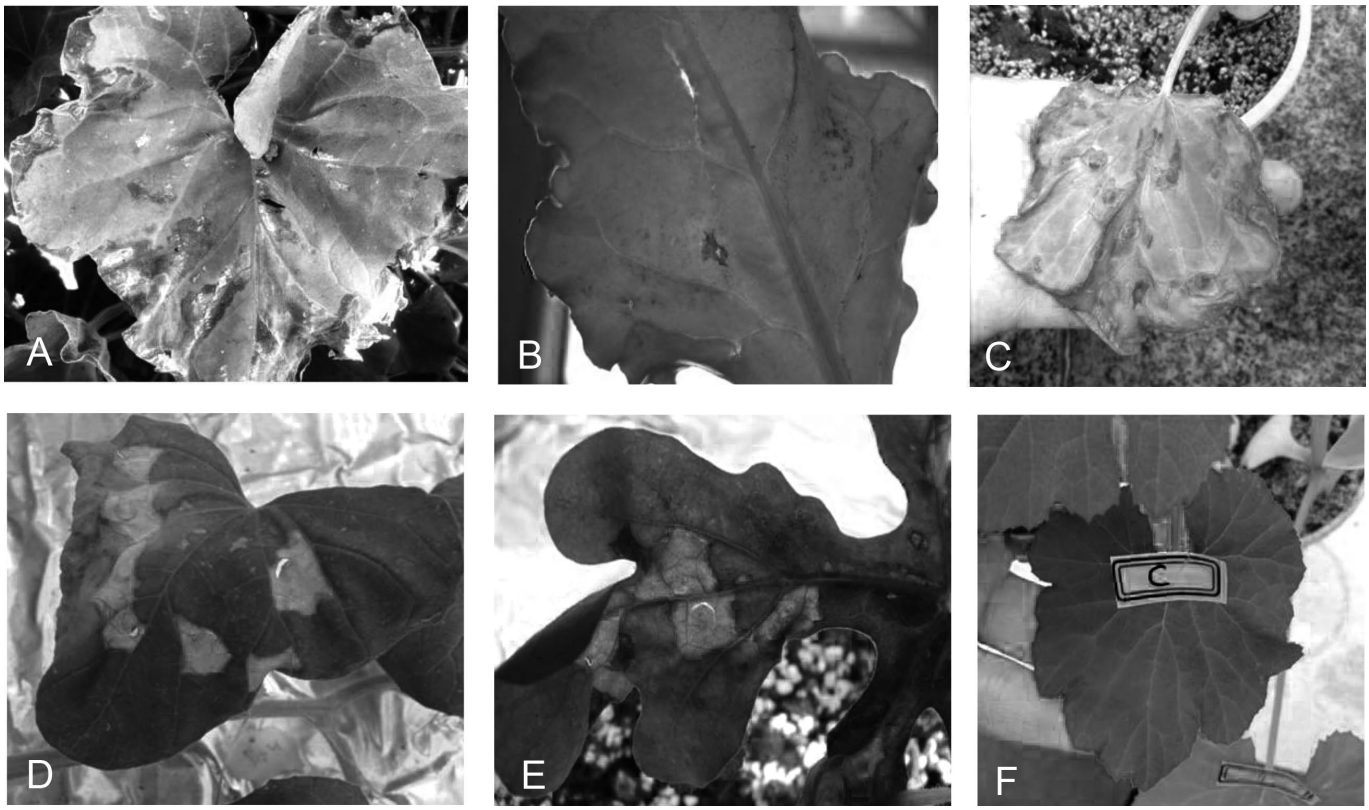

Fig. 1. Symptoms on the leaves: **A**. Leaf collected in the field, **B-E.** Symptoms obtained in pathogenicity test after eight days on the leaves of sugar beet**,** squash, muskmelon and watermelon, respectively. Within three or four days, these lesions expanded and often coalesced into large, grayish-brown, desiccated areas. **F.** control.

MATERIALS AND METHODS

Pathogen isolation. During summer and autumn 2010, sample of cucumber (*Cucumis sativus* L.), squash (*Cucurbita moschata*, (Duch. ex Lamk) Duch. ex Poiret), watermelon (*Citrullus lanatus* (Thunb.) Matsum et Nakai), muskmelon (*Cucumis melo* L.) and melon (*Cucumis melo* L. var. *cantalupensis*) leaves with small yellow-brown angular spots, coalescing to form irregular necrotic spots were collected in East Azarbayejan province in Iran. Samples were washed using tap water to remove soil particles, were surface sterilized in 0.5% sodium hypochlorite for 30 sec and rinsed twice using sterile distilled water. Small pieces of tissues between necrotic and chlorotic areas were then excised and macerated in sterile distilled water. The resulting suspension was streaked on nutrient agar medium (NA) (Schaad *et al*., 2001) and, after 48 h, representative bacterial colonies that had developed were purified by repetitive streaking onto NA medium.

Host range and pathogenicity test. To study host range and pathogenicity of bacterial strains, seeds of plants including cucumber (*C. sativus* L.), Armenian cucumber (*Cucumis melo* L. var. *flexousus*), muskmelon (*C. melo* L.), melon (*C. melo* L. var. *cantalupensis*), watermelon (*C. lanatus* (Thunb.) Matsum. et Nakai), pumpkin [*Cucurbita maxima* Duch. ex Lam and *Cucurbita moschata* (Duch. ex Lamk) Duch. ex Poiret], wheat (*Triticum aestivum* L.), barley (*Hordeum vulgare* L.), common bean (*Phaseolus vulgaris* L.), pea (*Pisum sativum* L.) and sugar beet (*Beta vulgaris* L.) were sown in 30 cm diameter plastic pots filled with autoclaved potting mixture consisting of sand, garden soil and bark shavings (1:1:1, v/v) and were kept for three weeks under greenhouse condition. Suspension of bacterial isolates in sterile saline phosphate buffer (PBS; 40 mM Na_2HPO_4, 25 mM KH_2PO_4), containing 10^8 CFU/ml, based on turbidity measurement (600 nm wavelength) were prepared. Petioles of each seedling were slashed with a sterile razor blade and plants were sprayed to run-off with the bacterial suspension. Alternatively, leaves were infiltrated with a dilute bacterial suspension using a hypodermic syringe fitted with a 26-gauge needle. Inoculated plants were kept in a greenhouse at 15-24°C. Plants inoculated with the razor slash method were covered with polyethylene bags for the first couple of days post inoculation. Three plants were used in each inoculation method. Controls for each set were treated similarly except that PBS buffer was used instead of inocula.

Biochemical and physiological tests. Gram staining, biochemical and physiological tests were performed according to Schaad *et al.* (2001). These tests included levan production, oxidase reaction, potato rot, arginine dihydrolase production, tobacco hypersensitive reaction (LOPAT),

Table 1. Primer names, sequences and size of PCR product (bp) used in this study

Primer names	Sequence from 5' to 3'	Size of band	Annealing temperature	References
BOXAlR	CTACGGCAAGGCGACGCTGACG	–	53 °C	Versalovic *et al.*, 1991
syrB	B1-CTTTCCGTGGTCTTGATGAGG B2-TCGATTTTGCCGTAATGAGTC	752-bp	60°C	Sorensen *et al.*, 1998
rpoD	PsrpoDF-TGAAGGCGARATCGAAATCGCCAA PsrpoDR-YGCMGWCAGCTTYTGCTGGCA	700-bp	55°C	Parkinson *et al.*, 2011
gyrB	UP-1-GAAGTCATCATGACCGTTCTGCAYGCNGGNGGNAARTTYGA UP-2r-AGCAGGGTACGGATGTGCGAGCCRTCNACRTCNGCRTCNGTCAT	1220-bp	60°C	Yamamoto and Harayama, 1995

production of fluorescent pigment on KB medium, glucose fermentation/oxidation (O/F), nitrate reduction, catalase reaction, urease, H_2S from cysteine, starch and esculin hydrolysis, gelatin liquefaction, and growth at 37°C, NaCl tolerance and utilization of some carbon substrates. *Pss* (IBSBF451) was used as a reference strain. Hypersensitive response on tobacco leaves was performed as described (Klement *et al*., 1964).

DNA preparation. DNA was extracted and purified according to Ausubel *et al.* (1992) with slight modification, i.e. extraction with a single phenol-chloroform step, precipitation in 2-propanol and suspension in 100 µl sterile distilled water. Concentration and quality of DNA were determined using a NanoDrop 2000 spectrophotometer (Thermo Scientific, USA) and by gel electrophoresis on agarose.

Polymerase chain reaction (PCR). The specific primer pair B1 and B2 were used for amplification of the *syrB* gene which yields a 752 bp DNA fragment (Sorensen *et al*., 1998). Whereas the *gyrB* gene was amplified using universal UP-1 and UP-2r primers which yield a 1220 bp fragment (Yamamoto and Harayama, 1995). The primers PsrpoDf and PsrpoDr which produce a 700 bp product were used for amplifying the *rpoD* gene (Parkinson *et al*., 2011). Details of all the primers used in this study are provided in Table 1.

PCR runs were performed in a 20 µl reaction mixture in an Eppendorf thermocycler and the amplified products were electrophoresed on 1% TAE agarose gel at room temperature and 90V cm^{-1} for 1 h. Gels were stained with ethidium bromide then viewed and photographed under UV illumination.

BOX-PCR analysis. BOX-PCR analysis was conducted for all strains isolated from cucurbits and some other reference strains. The 22-mer BOXA1R oligonucleotide (MWG, Germany) was used to generate fingerprinting profiles and PCR conditions were those described by Versalovic *et al.* (1991). Amplification reactions were performed in volumes of 25 µl, containing 2 µM of the single BOX primer, 200 µM each of dNTPs, (CinnaGen, Iran), PCR buffer (10 mM, Tris-HCl, pH 9.0, 50 mM KCl, 1.5 mM $MgCl_2$), 1.5 U of *Taq* DNA polymerase (CinnaGen, Iran) and 100 ng of template DNA. Amplification was performed with a Bio-Rad Thermal Cycler programmed for an initial denaturation step of 5 min at 95°C, followed by 38 cycles of 1 min at 94°C 1 min at 53°C and 2 min at 72°C with a final elongation step of 10 min at 72°C. PCR products were separated by 1.5% agarose gel electrophoresis in TAE buffer (Ausubel *et al*., 1992) at 10 Vcm^{-1} over 2 h, stained with ethidium bromide and visualized under a UV transilluminator. Bands sizes were assigned by direct comparison with current DNA standards (100 bp Plus, Fermentas, MBI, Germany). For each strain, the presence and the absence of a band was scored as 1 and 0, respectively. A distance matrix between strains was calculated using the Jaccard coefficient and a dendrogram was constructed by the Unweighted Pair Group Method with Arithmetic Mean (UPGMA) method using MVSP software.

Sequence alignment and phylogenetic analysis. The PCR products were resolved by gel electrophoresis and purified using an AccuPrep PCR purification kit (Bioneer Corporation, South Korea) according to the manufacturer's instructions and were custom sequenced (Bioneer Corporation). The sequence products were blasted in NCBI (www://ncbi.nlm.nih.gov). Sequences were aligned, using the program Clustal W, with the sequences of some type and pathotype strains of *Pseudomonas syringae*. Phylogenetic trees were constructed with bootstrapping (1000 replications) and phylogenetic analysis was performed using the neighbour-joining method (Tajima and Nei, 1984) with Mega 4.0 software (Tamura *et al*., 2007). Published sequences of *Pseudomonas cichorii* isolates including TEIC555, PPST50936, CFBP2101, BC3309 and BC3287 were used as outgroups for this analysis.

RESULTS

Pathogen isolation, host range and pathogenicity tests. Three bacterial strains (M24, M25, and M29) from squash and another strain (M35) from muskmelon were isolated in East Azarbayajan. In pathogenicity tests, all strains caused leaf blight and necrosis on the leaves of *C. sativus*, *C. lanatus*, *C. maxima*, *C. moschata*, *C. melo*, *C. melo* var.

Table 2. Pathogenicity of the bacterial isolates in vestigated in this study.

	Isolates			
	M24	**M25**	**M29**	**M35**
Cucumis sativus	+[a]	+	+	+
Cucumis melo var. *flexousus*	–[b]	–	–	–
Cucumis melo	+	+	+	+
Cucumis melo var. *cantalupensis*	+	+	+	+
Citrullus lanatus	+	+	+	+
Cucurbita maxima	+	+	+	+
Cucurbita moschata	+	+	+	+
Triticum aestivum	–	–	–	–
Hordeum vulgare	–	–	–	–
Phasaeolous vulgaris	–	–	–	–
Pisum sativum	–	–	–	–
Beta vulgaris	+	+	+	+

[a] (+): Positive, [b] (–): Negative

cantalupensis and *Beta vulgaris* one or two weeks post inoculation. Initial symptoms appeared as water-soaked spots on the leaves which, within three or four days, expanded and often coalesced into large, grayish-brown, desiccated areas (Fig. 1B-E). Re-isolation was done and high populations of bacteria were isolated from these lesions after 10 days. *C. melo* var. *flexousus*, *T. aestivum*, *H. vulgare*, *P. vulgaris*, *P. sativum* and control plants (Fig. 1F) did not develop any symptoms (Table 2).

Identification of *P. syringae*. Morphological, physiological and biochemical tests (LOPAT), indicated that the bacterial isolates belonged to *P. syringae* pathovars (Table 3). All of the four isolates were tested for the presence of the *syrB* gene which is required for syringomycin production in *Pseudomonas syrigae* pathovars. Results showed that all of them amplified a 752 bp fragment with *syrB* primers.

BOX analysis. The DNA fingerprints of the four isolates form cucurbits and some reference strains of *P. syringae* pathovars from different hosts (Table 4) were determined by BOX-PCR. The resulting dendrogram (Fig. 2) suggests high similarity between the genomic fingerprints of strains from squash and muskmelon which formed a separated group as a cucurbits group. Between this group and each of the other reference strains of *P. syringae* pathovars which were used in this study, the highest

Table 3. Determinative tests for identification of bacterial isolates.

M35	M29	M25	M24	*Pss* (IBSBF451)	Test
–[a]	–	–	–	–	Gram staining
cream	cream	cream	cream	cream	Color
+[b]	+	+	+	+	Levan
–	–	–	–	–	Oxidase
–	–	–	–	–	Pot rot
–	–	–	–	–	Arginine dihydrolase
+	+	+	+	+	HR[c]
+	+	+	+	+	Catalase
+	+	+	+	+	Fluorescent in King's B
O	O	O	O	O	O/F[d]
–	–	–	–	–	Hydrolysis of starch & gelatin
+	+	+	+	–	Hydrolysis of esculin
–	–	–	–	–	Urease
–	–	–	–	–	Nitrate reduction
–	–	–	–	–	H2S from cysteine
–	–	–	–	–	Growth at 37 °C
+	+	+	+	+	Growth at 2 & 3% NaCl
–	–	–	–	–	Growth at 5% NaCl
					Utilization of:
+	+	+	+	+	Sucrose
+	+	+	+	+	Mannitol
–	–	–	–	–	Cellobiose
+	+	+	+	+	D-Sorbitol
–	–	–	–	–	L-Rhamnose
–	–	–	–	–	L-Arabinose
+	+	+	+	+	Citrate
–	–	–	–	–	L-Tartrate
–	–	–	–	–	D-Tartrate
+	+	+	+	+	L-Lactate

[a] (–): Negative, [b] (+): Positive, [c] Hypersensitive reaction, [d] Oxidative fermentative test

Table 4. Reference strains of some pathovars of *Pseudomonas syringae* used in BOX-PCR analysis

Bacteria	**Host**	**Location**	**Reference or source**
Pseudomonas syringae pv. *syringae* ATCC19310	*Syringa vulgaris*	Great Britain	ATCC[1]
P. syringae pv. *syringae* B301D	*Pyrus communis*	England	CFBP[2]
P. syringae pv. *syringae* B728a	Snap bean	USA	CFBP
P. syringae pv. *aptata* GSPB1067	*Beta vulgaris*	Germany	GSPB[3]
P. syringae pv. lachrymans GSPB82a	*Cucumis sativus*	Germany	GSPB
P. syringae pv. *syringae* ICMP3189	*Phaseolus vulgaris*	New Zealand	ICMP[4]
P. syringae pv. *syringae* ICMP4917	*Citrus lemon*	France	ICMP
P. syringae pv. *syringae* ICMP19246	Wheat	Iran	ICMP
P. syringae pv. *syringae*	Barley	Iran	VRU19[5]
P. syringae pv. *syringae* IBSBF445	Stone fruit	Brazil	IBSBF[6]

[1] ATCC: American Type Culture Collection.
[2] CFBP: Collection Francaise Bacteria Phytopathogenique.
[3] GSPB: Gottinger Sammlung Phytopathogener Bakterien.
[4] ICMP: International Collection of Microorganism from Plants.
[5] VRU: Vali-E-Asr University of Rafsanjan.
[6] IBSBF: Instituto Biologico Secao Bacteriologia Fitopatologica.

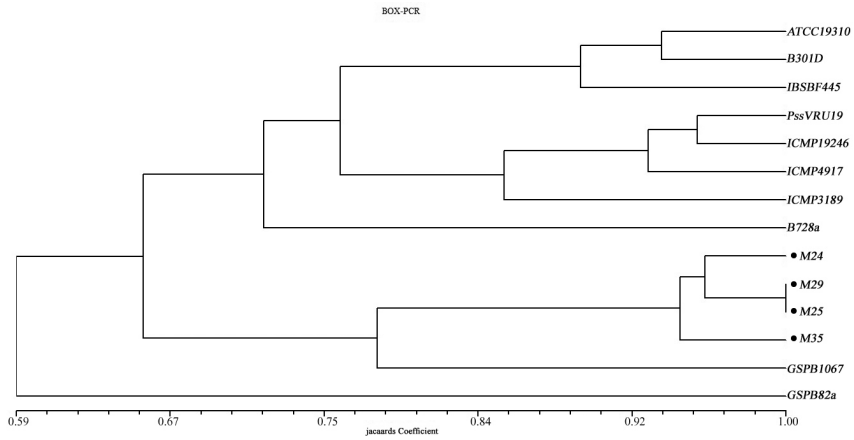

Fig. 2. Dendrogram illustrating the relationship between four bacterial isolates from squash and muskmelon described in Table 2 and reference strains of some pathovars of *P. syringae* described in Table 4 by UPGMA. The genomic fingerprints of strains from squash and muskmelon form a separated group denoted "cucurbit" group, and have a high similarity with *P. syringae* pv. *aptata* GSPB1067.

similarity exists with *P. syringae* pv. *aptata* GSPB1067 isolated from *B. vulgaris.*

Sequence alignment and phylogenetic analysis. *gyrB* and *rpoD* are protein-encoding housekeeping genes that have evolved much faster than rRNAs and thus *Pseudomonas* phylogeny based on these sequences is expected to provide higher resolution compared to 16S rDNA sequences (Yamamoto *et al.*, 2000; Sarris *et al.*, 2012). Therefore, the sequences of *gyrB* and *rpoD* genes were employed for identification of the recovered isolates. The sequence of the *gyrB* gene of isolate M24, selected as a representative, was deposited in GenBank under the accession No. of KC915023. Blast search of GenBank revealed that this isolate has high identity (99%) with *Psa.* For confirmation of the results, the *rpoD* gene of all four strains was sequenced and analyzed. The results showed that all of them belonged to *Psa* with 99% identity. The Sequencence of *rpoD* genes were deposited in GenBank under the Nos. KC984306 (M24), KF723405 (M35), KF723406 (M25) and KF723407 (M29). Phylogenetic trees constructed with sequences of the recovered isolates and some type and pathotype strains of *Pseudomonas syringae* pathovars are presented in Fig. 3a and 3b.

DISCUSSION

On the basis of biochemical and physiological tests, the bacterial strains isolated from squash and muskmelon in East Azarbayejan belonged to *P. syringae* pathovars. In addition to Another survey carried out in East Azarbayejan in summer of 2013 yielded confirmatory results of the 2010 investigation for the same bacteria were isolated.

P. syringae is a heterogeneous phytopathogenic species that includes approximately 50 pathovars (Young, 1991). In many cases, biochemical and nutritional tests cannot differentiate strains at the pathovar level (Little *et al.*, 1998). Nine genomospecies were described within *P. syringae* based on DNA studies. Strains belonging to the several pathovars of *P. syringae* viz *Pss*, *Psa*, *P. syringae* pv. *pisi* and *P. syringae* pv. *papulans*, were clustered in genomospecies I (Gardan *et al.*, 1999). With pathogenicity tests, all strains caused necrotic spots on the leaves of cucurbits and sugar beet but *Cucumis melo* var. *flexousus*, *T. aestivum*, *H. vulgare*, *P. vulgaris*, *P. sativum* did not show any symptoms. However, because of pathogenicity to sugar beet, it can be concluded, according to Morris *et al.* (2000), that these strains belong to *Psa.*

In PCR with B1 and B2 primers, all strains amplified the expected 752 bp fragment. Furthermore, sequencing of *rpoD* and *gryB* genes showed that our strains had high identity (99%) with *Psa.* The resulting dendrograms (Figs. 3a and b) showed that *rpoD* gene sequencing is better than *gyrB* and provides a better resolution for *Pseudomonas* spp. classification as reported by Parkinson *et al.* (2011).

The dendrogram of BOX-PCR suggests that genomic fingerprints of strains from squash and muskmelon had high similarity to each other and formed a separated group denoted "cucurbit group". Among this group and other reference strains of *P. syringae* pathovars used in this study, the highest similarity was found with *P. syringae* pv. *aptata* GSPB1067.

Taking all the above into account these bacterial isolates appear to belong to *Psa* according to the *rpoD* and *gryB* genes sequencing, although BOX fingerprint showed some difference. These results are in accordance with those of Mosivand *et al.* (2009), who have shown that fingerprint of the *Pss* strains isolated from sugarcane using ERIC- and BOX-PCR primers are distinct from those of the strains isolated from stone fruits and wheat. In another study, the results of analysis of the BOX fingerprints from *Pss* strains have shown that the strains isolated from stone fruits, graminous hosts and pome fruits form a relatively distinct cluster, which sepates them from the strains isolated from other hosts (Pour and Taghavi, 2011).

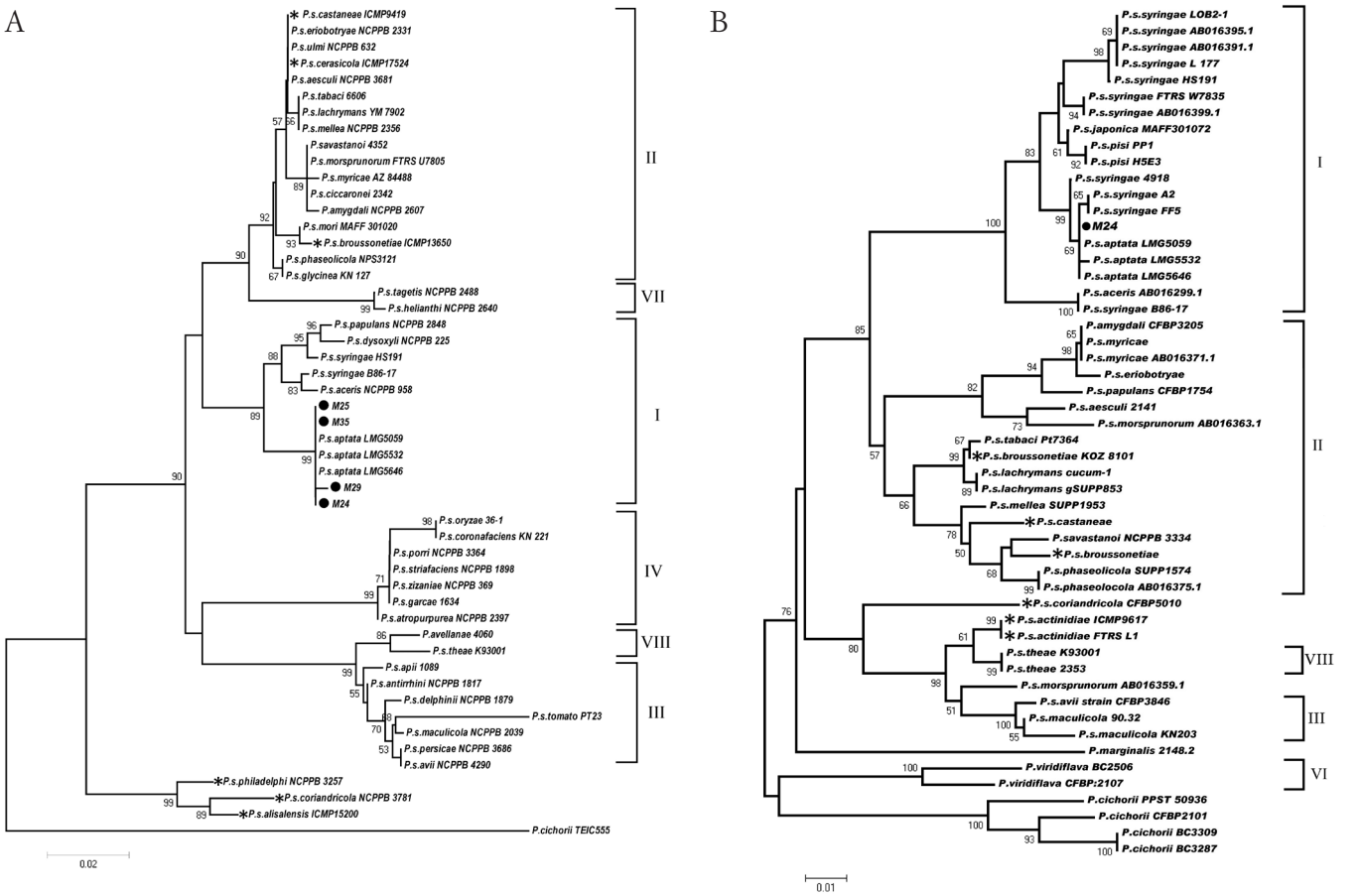

Fig. 3. Phylogenetic trees of the *rpoD* (A) and *gyrB* (B) genes sequences of the recovered bacterial isolates and some type and pathotype strains of *Pseudomonas syringae* pathovars. Phylogenetic analysis was carried out by the neighbor-joining algorithm implemented with Mega 4.0. *Pseudomonas cichorii* strain TEIC555 and *P. cichorii* strains PPST50936, CFBP2101, BC3309 and BC3287 were used as outgroup in trees A and B, respectively. Clustering of poudomonas species are in agreement with Genomospecies groups described by Gardan *et al.* (1999).
*: Not investigated by Gardan *et al.* (1999). Black dots indicate the four bacterial isolates investigated in this study.

Studies in which *P. syringae* pathovars including *phaseolicola*, *glycinea*, *tabaci*, *lachrymans* and *mori* were investigated by ERIC- and REP-PCR showed that although differences among strains within a pathovar can be trivial, these methods could be used to identify and classify strains of *P. syringae* pathovars (Weingart and Volksch, 1997).

Moreover, it was found that genomic groups proposed by Gardan *et al.* (1999) could be distinguished using BOX-PCR. The results corresponded with those by Gardan *et al.* (1999) that grouped them into two extra clusters. Two *P. syringae* strains of unknown pathovars recovered from beans and three *P. syringae* pv. *actinidiae* strains have been grouped in two extra clusters and might constitute two new species (Marques *et al.*, 2008).

Based on the results of biochemical tests, serotyping and syringomycin production assays, the strains of *P. syringae* causing cantaloupe blight in France closely resemble strains of *P. syringae* pathovars *aptata*, *atrofaciens*, *japonica*, *lapsa* and *syringae* (Morris *et al.*, 2000). In BOX-PCR profiles, the cantaloupe strains have many bands in common with profiles of strains of these five pathovars, but they are not identical to any of them. Host range studies showed that virulence to sugar beet is a common feature of strains virulent on cantaloupe, but not to strains avirulent on cantaloupe. So that they proposed the strains attacking cantaloupe in France could be considered as *Psa* Riffaud and Morris (2002) isolated a pathogen of cantaloupe, *Psa*, from retention basins used for irrigation of cantaloupe in south-western France using the immunofluorescence colony-staining (IFC) method. These results showed that *Psa* can survive in water used to irrigate cantaloupe crops and could be a source of inoculums for epidemics of bacterial blight of cantaloupe. *Psa* has also been reported as a pathogen of sugar beet in New South Wales and Queensland (Moffett, 1983; O' Brien and Sparshott, 1999). In other study in Belgium, strains of the pathovars *aptata* and *atrofaciens* producing toxic lipodepsipeptides (TLP+), were tested on fruit tree leaves and on their hosts, sugar beet and wheat to determine their ability to induce disease. The strains of the pathovar *aptata* were pathogenic to pear (50%), sweet and sour cherry (25%), plum (12.5%) and wheat (25%) and also to sugar beet hypocotyls. Conversely, strains from Belgian orchards were pathogenic on sugar beet and on wheat. One of two strains

of *P. syringae* pv. *atrofaciens* was non-pathogenic and the other was only pathogenic to wheat. Indeed, the genetic differences observed between real *aptata* and *atrofaciens* strains from sugar beet and wheat and the strains from Belgian orchards (Gilbert *et al*., 2009) resulted in keeping the Belgian TLP+ strains in the pv. *syringae* (Gilbert *et al*., 2010). In Iran *Psa* has only been reported from the suburbs of Babol (near Caspian Sea) as an agent of bacterial leaf spot and blight of garden beet (*B. vulgaris*) in 2001-2002 (Arabi *et al*., 2006).

In cucurbits, several pathovars of *P. syringae* have been reported from many different parts of the world such as *Psl* from Israel (Volcani, 1964), Egypt (El-Sadek *et al*., 1992); Puerto Rico (Cortes-Monllor and Rodriguez-Marcano, 1991); Japan (Watanabe and Ohucffi, 1983; Fatmi *et al*., 2008), *Pss* from New Zealand (Sharrock *et al*., 1997) where it causes wart-like eruptions on the fruits of buttercup squash (*C. maxima*), and *Psa* from France (Morris *et al*., 2000; Riffaud *et al*., 2003). In Iran, only *Psl* (Harighi, 2007) and *Pmm* (Ghobakhloo *et al*., 2002) have been reported as pathogens of cucurbits thus, to the best of our knowledge, this is the first report of *Psa* as the cause of cucurbit diseases in Iran.

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