J. Crop Prot. 2014, 3 (3): 345-355 _____

Research Article

Association of *Pantoea agglomerans* with the citrus bacterial canker disease in Iran

Faegheh Sherafati[1], Pejman Khodaygan[*1], Mahdi Azadvar[2], Ebrahim Sedaghati[1], Roholah Saberi-Riseh[1] and Sareh Baghaee-Ravari[3]

1. Department of Plant Protection, Faculty of Agriculture, Vali-E-Asr University of Rafsanjan, Iran.
2. Jiroft and Kahnouj Agricultural Research Center, Jiroft, Iran.
3. Department of Plant Protection, Faculty of Agriculture, Ferdowsi University of Mashhad, Iran.

Abstract: Samples of leaf, twig and fruit from 'Mexican' lime (*Citrus aurantifolia*) and grapefruit (*Citrus paradisi*) with symptoms of bacterial canker were collected from different provinces throughout Iran during spring and summers of 2010 and 2011. Yellow, gram-negative colonies were isolated from infected tissue samples. Results of pathogenicity assays indicated that some isolates incited tissue hyperplasia, hypertrophy and raised callus-like lesions typical of canker in hosts while other isolates stimulated flat necrotic and water-soaked lesions on leaves. Candidate samples of each group were identified according to morphological and physiological characteristics. Detections were also made using specific primers and partial sequencing of 16SrDNA for Pantoea group and *gyrB* for Xanthomonas group. Results showed that one group was characterized as the typical *Xanthomonas citri* subsp. *citri* strain while the other group containing most of the isolates was identified as *Pantoea agglomerans*. Samplings done frequently in different seasons revealed the presence of high populations of *P. agglomerans* with bacterial canker, especially in warmer and drier regions. These bacteria were able to incite canker-like symptoms on grapefruit seedlings and could be reisolated after two months.

Keywords: *Pantoea agglomerans*, Citrus canker-like disease, pathogenicity, Iran

Introduction

Citrus (*Citrus* spp.) is one of the most important crops in the world. Its production in Iran has reached an annual amount of 40000 tons (Anonymous. 2012). Citrus Bacterial Canker (CBC) is a destructive disease affecting citrus production worldwide; it is caused by *Xanthomonas* spp. The disease appears as erumpent lesions on the top and bottom of leaf surfaces, twigs and fruit. It can be a serious threat especially if there is frequent rainfall at the stage of fruit development (Das, 2003). Fawcett and Jenkins reported that CBC originated in India and Java and that canker lesions were detected on the oldest citrus specimens kept at the Herbaria of the Royal Botanic Gardens in Kew, England (Cook, 1988). In Iran, CBC disease was first reported on citrus trees in kahnouj, Kerman province (Alizadeh and Rahimian, 1990) and is now widely distributed across southern Iran. There are recognizably five different forms or types of CBC that are distinct from each other in terms of pathogenicity and geographical distribution. A canker (Asiatic

Handling Editor: Dr. Masoud Shams-Bakhsh

[*] **Corresponding author**, e-mail: Pkhodaygan@vru.ac.ir
Received: 13 September 2013, Accepted: 28 December 2013
Published online: 2 February 2014

[DOR: 20.1001.1.22519041.2014.3.3.10.9]

canker) caused by the bacterium *Xanthomonas citri* subsp. *citri* (*Xcc*), is the most severe and destructive form and is pathogenic to almost all citrus varieties. Types B (B cancrosis), C (Mexican lime cankrosis) and D (Mexican bacteriosis) are caused by the bacterium *Xanthomonas fuscans* subsp. *aurantifolii*. E type of canker or citrus bacterial spot (CBS) caused by *Xanthomonas alfalfae* subsp. *citrumelonis* has been reported from nurseries in Florida (Schaad *et al.*, 2006). Canker causes extreme loss and damage through fruit drop, reduced quality and quantity of fruit yield and defoliation (Hartung and Civerolo, 1989). Recently another type of CBC canker, Pathotype A*, has been characterized, which is phenotypically and genetically related to pathotype A. This type of disease has a narrow host range and causes citrus canker on Mexican lime (*Citrus aurantifolia*) it has a much less severe impact on citrus culture. Strains genetically related to pathotypes A and A* and that infect Mexican lime and *Citrus macrophylla* were recently detected in Florida and classified as a pathotype A^w (Ngoc *et al.*, 2009). Diversity and shifting host range have sometimes been related to modifications in terms of capability of virulence in genes by horizontal gene transfer or intragenomic recombination or mutation. The genus *Pantoea* is a gram negative facultative anaerobic bacterium that belongs to the family Enterobacteriaceae. This bacterium has several species and sub-species; it has been isolated from diverse ecological and geographical sources. *P. agglomerans* is commonly found in plants growing naturally, animals, humans, soil and water and as such is considered an epiphytic and commensally growing bacterium on surfaces of many different plants (Weinthal *et al.*, 2011). Phylogenetic studies and comparisons with other species of Enterobacteriaceae family revealed that the genus *Pantoea* is highly diverse. Biochemical characteristics such as Fosfomycin resistance and utilization of D-tartrate could distinguish *P. agglomerans* from other *Pantoea* species (Delétoile *et al.*, 2009). Several species of the genus *Pantoea* are known as plant pathogens. Stewart's vascular wilt is a disease affecting maize and corn caused by *P. stewartii* subsp. *stewartii* (Brady *et al.*, 2008). *P. ananatis* causes disease on many agricultural crops including sudangrass, onion, maize, and pineapple. Some isolates of this bacterium have evolved to become host-specific, gall-forming pathogens on various plants (Manulis and Barash, 2003). *P. agglomerans* causes root and crown gall disease in gypsophila and beet. Recently, *Pantoea* strains have been isolated from Eucalyptus trees in South America and they show disease symptoms similar to bacterial blight and dieback of Eucalyptus in South Africa (Brady *et al.*, 2008). *P. agglomerans* is also a plant pathogen that can cause disease in humans (Cruz *et al.*, 2007). Several strains of *P. agglomerans* are considered as commercial biological agents, for example: strain C9-1 could be used against the causal agent of fire blight (*Erwinia amylovora*) (Rezzonico *et al.*, 2009). There is wide diversity in terms of ability of *P. agglomerans* strains that indicates flexibility in the genome caused by horizontal gene transfer. Plasmids and other mobile elements are known to interfere in horizontal gene transfer (Manulis and Barash., 2003). Since there are some reports of pathogenicity of *P. agglomerans* on plants such as rice, beet and cotton, this study discusses the possible influence of *P. agglomerans* in terms of pathogenicity or severity of symptoms of citrus bacterial canker.

Materials and Methods

Bacterial isolates

Bacterial strains were isolated from infected leaves, twigs and fruits of 'Mexican' lime (*C. aurantifolia*) and grapefruit (*C. paradisi*) during the period of spring to summer 2010 and 2011 from different provinces in Iran. Segments of tissue bearing lesions were cut and teased apart in a few drops of sterile double distilled water. Following a few minutes of incubation, loopfulls of the suspension were streaked onto plates of glucose yeast extract nutrient agar (GYNA) and incubated at 25^{o}C for 3-4 days. Single colonies were restreaked on GYNA and pure gram-negative isolates with similar,

[DOR: 20.1001.1.22519041.2014.3.3.10.9]

yellow colonies were kept at 4°C (Schaad *et al.*, 2001). The type strain *Xcc* (ICMP17123) was provided from New Zealand culture collection and included as the reference in all tests. Bacterial strains used for genotypic analysis in this study are shown in Table 1.

Pathogenicity test

Pathogenicity of purified isolates was evaluated using attached and detached leaf assays. Ten detached leaves of Grapefruit (*C. paradisi*) and Mexican lime (*C. aurantifolia*) were disinfected with 70% ethanol, rinsed three times in sterile water and placed on 1% agar plate. Bacterial isolates were suspended in sterile saline phosphate buffer (PBS; 40 mM Na_2HPO_4, 25 mM KH_2PO_4), adjusted to 0.1 OD at 620 nm (10^5 cfu/ml) and infiltrated to the leaves. Petri dishes were immediately sealed with parafilm and plates incubated in a growth chamber. For attached leaf assay, immature leaves (75% expanded) on greenhouse grown seedlings were punctured with a needle and sprayed with bacterial suspension and kept on separated quarantine greenhouses. Control leaves were treated with sterile buffer. Plates and seedlings were incubated at 26–29 °C until symptoms appeared. The assays were repeated four times.

Physiological and biochemical tests

Comparisons were made on all strains for the evaluations of morphological, biochemical and physiological properties (Schaad *et al.*, 2001). Xanthomonadin extraction was carried out according to the method cited by (Irey and Stall, 1981) and yellow pigment was scanned between 400 and 480 nm using a UV-spectrophotometer.

Total DNA extraction

Processes of extraction and purification of DNA from pure bacterial cultures were performed using the method cited in (Ausubel *et al.*, 1992) with slight modification. DNA was extracted in a single phenol-chloroform step, precipitated in 2-propanol and resuspended in 100 µl mili-Q water. Concentration and quality of DNA were determined using a NanoDrop (Thermo science2000) spectrophotometer and by gel electrophoresis on agarose.

Detection of *Xanthomonas* isolates by direct PCR

Two pairs of primers, XACF/XACR and J-pth1/J-pth2, which were designed for identification of *X. citri* subsp. *citri*, were used for amplification of DNA from isolates. The PCR assay was performed using the method described by (Suk Park *et al.*, 2006).All amplifications were carried out in a final volume of 25µl containing 10X reaction buffer (10mM Tris-HCl, 50mM KCl, 1.5mM, $MgCl_2$), 0.2mM of each dNTPs, 2.5 pmol/µl of each primer, 2 units of *Taq* polymerase and approximately 50 ng of genomic DNA. Reactions were run for 34 cycles, each consisting of 1 min. at 95°C, 45 sec. at 60°C, and 2 min. at 72 °C, with initial denaturation of 5 min. at 94°C and final extension of 10 min. at 72°C. After PCR amplification, five µl of the PCR products were run in 1% (w/v) agarose gel, stained with ethidium bromide and visualized under a UV transilluminator. Sterile water was used as negative and *Xcc* ICMP 17123 was used as the positive control for each PCR reaction. Primer names, sequences and size of PCR product (bp) used in this study are indicated in Table 2.

***gyrB* and 16s rDNA sequencing and phylogenetic analysis**

The 16s rDNA sequence was determined for some facultative aerobic strains, PCR and sequence analysis of the 16s rDNA were performed as described by (Marchesi *et al.*, 1998). PCR reactions were done in a total volume of 50 µl containing 2 U *Taq* DNA polymerase, 2.5 pmol/µl of each primers with forward 63F and reverse primer 1387r, 200 µM of each dNTPs, 10X reaction buffer, 1.5 mM $MgCl_2$ and 50 ng DNA template. Amplifications were performed as follows; initial denaturation at 95 °C for 3 min. was followed by 36 cycles of denaturation for 1 min. at 95 °C, annealing for 1 min. at 66 °C for *gyrB* (Ngoc *et al.*, 2010) (65 °C for 16s rDNA), extension for 1 min. at 72 °C and a final extension step at 72°C for 10 min. The

[DOR: 20.1001.1.22519041.2014.3.3.10.9]

PCR products were evaluated by gel electrophoresis and purified using the Bioneer DNA cleanup kit (AccuPrep Gel Purification Kit, Bionner, South Korea). Direct sequencing of PCR products was achieved using the same series of primers. Both forward and reverse strands were sequenced using ABI Prism Automatic Sequencer (Bionner, South Korea). Data on sequences were compared visually and aligned using the multiple alignment software CLUSTALW, and compared with sequences of some other type and pathotype strains. Phylogenetic trees were generated by neighbor-joining, using Jukes-Cantor corrected distances (Tamura *et al.*, 2007), evaluations for statistical confidence for sequence groups were determined by bootstrap test (1000 replicate test) using MEGA4 software.

Table 1 Bacterial strains used for genotypic analysis in this study.

Strain	Location	Date of Sampling	Host	16s rDNA	*gyrB*	Accession Number	Year of Isolation
302	Hormozgan Province (Haji abad)	November	*C. aurantifolia*	-	*Xcc*	KF137647	2010
322	Sisitan and Balochestan Province (Iran shahr)	July	*C. aurantifolia*	-	*Xcc*	KF137648	2010
330	Kerman Province (Sirjan)	January	*C. aurantifolia*	-	*Xcc*	KF137649	2010
333	Kerman Province (Jiroft)	September	*C. aurantifolia*	-	*Xcc*	KF137650	2011
348	Fars Province (Darab)	November	*C. aurantifolia*	-	*Xcc*	KF137651	2010
358	Ilam Provinces (Darreh majin)	July	*C. aurantifolia*	-	*Xcc*	KF137652	2011
335	Kerman Province (Jiroft)	March	*C. paradisi*	*P. agglomerans*	-	KF017591	2010
358	Kerman Province (Jiroft)	March	*C. paradisi*	*P. agglomerans*	-	KF114393	2010
359	Kerman Province (Jiroft)	April	*C. paradisi*	*P. agglomerans*	-	KF114394	2010
360	Kerman Province (Jiroft)	April	*C. paradisi*	*P. agglomerans*	-	KF114395	2011
361	Kerman Province (Jiroft)	April	*C. paradisi*	*P. agglomerans*	-	KF114396	2011
362	Kerman Province (Jiroft)	July	*C. paradisi*	*P. agglomerans*	-	KF114397	2011
Xcc ICMP17123	-	-	*C. aurantifolia*	-	-	-	-

Table 2 Primer names, sequences and size of PCR product (bp) used in this study.

Primer	Sequence	Size of band	Reference
16s rDNA	63f: CAG GCC TAA CAC ATG CAA GTC 1387r: GGG CGG WGT GTA CAA GGC	1300bp	(Marchesi *et al*. 1998)
gyrB	gyrB1: GCC GAG GTG ATC CTC ACC GT gyrB2: GGC CGA GCC ACC TGC CGA GT	1000bp	(Ngoc *et al*, 2010)
XACF/XACR	XacF: CGT CGC AAT ACG ATT GGA AC XacR: CGG AGG CATTGTCGA AGG AA	561bp	(Suk Park *et al*, 2006)
J-pth1/J-pth2	J-pth1: CTT CAA CTC AAA CGC CGG AC J-pth2: CAT CGC GCT GTT CGG GAG	197bp	(Cubero and Graham, 2002)

[DOR: 20.1001.1.22519041.2014.3.3.10.9]

Results

Bacterial isolation, physiological, biochemical and molecular detection tests

A total of 400 strains were isolated in this study. All strains formed circular, convex and yellow colonies on GYNA medium. They were gram, oxidase and arginine hydrolysis negative. Isolates gave a positive hypersensitivity reaction on pepper leaves and were unable to produce fluorescent pigment on King's B medium. Furthermore, they were able to tolerate 1-2 % NaCl. Based on results of the O/F test, one group (100 isolates) was aerobic and according to several tests (hydrolysis of starch, Tween 80 and gelatin, reducing substance from sucrose, production of hydrogen sulfide, tolerance to 3% NaCl and ability to utilize Glucose, Xylose, Fructose, Galactose, Sucrose, Mannitol and Trehalose), they belonged to the genus *Xanthomonas.* Furthermore Xcc-specific primers were used to identify strains of this group. Specific 561 bp bands were detected from those strains as well as the reference strain *Xcc* ICMP17123 Xanthomonadin pigment was also extracted from these strains and showed maximum absorption at 443 nm with two small shoulders at 418 and 466 nm. All isolates of the second group that consisted of facultative anaerobic strains (300 isolates) recovered from diseased citrus were negative for hydrogen sulphide generation from cysteine, indol production, and positive for gelatin liquefaction and they were also able to reduce nitrate to nitrite. Members of this group could not produce acid from Lactose, Melibiose, Melezitose, Raffinose, meso-Erythritol, Dulcitol and D-Sorbitol. While they evaluated positive for D-Fructose, D-Mannose, D-Galactose, Maltose, Sucrose, Trehalose and D-Mannitol assays. Based on phenotypic tests, the facultative anaerobic strains were *P. agglomerans.*

Pathogenicity test

We performed separate inoculations by native strains and pathotype strains in two quarantine greenhouses. Inoculation of detached grapefruit and Mexican lime leaves with obligate aerobic strains resulted in slight water soaked lesions that appeared two or three days post inoculation (dpi). This was followed by the formation of raised callus like tissue 6 or 7 dpi. The detached leaves inoculated with facultative anaerobic strains induced extensive water-soaking and flat necrotic lesions after 4 days (Fig. 1). Based on the symptoms formed on lime seedlings, obligate aerobic strains caused typical bacterial canker symptoms on leaves in 5-14 dpi whereas facultative anaerobic strains induced water soaking areas in 5-14 dpi. In contrast, facultative anaerobic strains created tiny raised, water soaked and corky lesions on leaves of Mexican lime and grapefruit in 60-70 dpi and small, light-brown lesions developed around the point of inoculation. The symptoms progressed in subsequent days (Fig. 2). Reisolation was done with high bacterial population. Control leaves inoculated with PBS buffer did not develop any symptoms.

gyrB and 16S rDNA sequencing

Six isolates from each group of strains were used for sequencing. With some exceptions partial sequence analysis of *gyrB* (1000 bp) revealed high levels of similarity among the obligate aerobic strains and included them in the clade formed by the *Xcc* (Fig. 3). BLAST searches of the 1.2-kb sequences showed that 16S rDNA of facultative anaerobic strains had high similarity (99 to 100%) to the 16S rDNA of *P. agglomerans* (Fig. 4).

[DOR: 20.1001.1.22519041.2014.3.3.10.9]

Figure 1 Symptoms on leaf surface of *C. paradisi* developed 5-14 days after inoculation by *Pantoea* (left) and *Xcc* (right) isolates.

Figure 2 Canker like symptoms (b) on adaxial (a) and abaxial (c) leaf of *C. paradisi.* Symptoms developed 60 days after inoculation by *Pantoea* isolate.

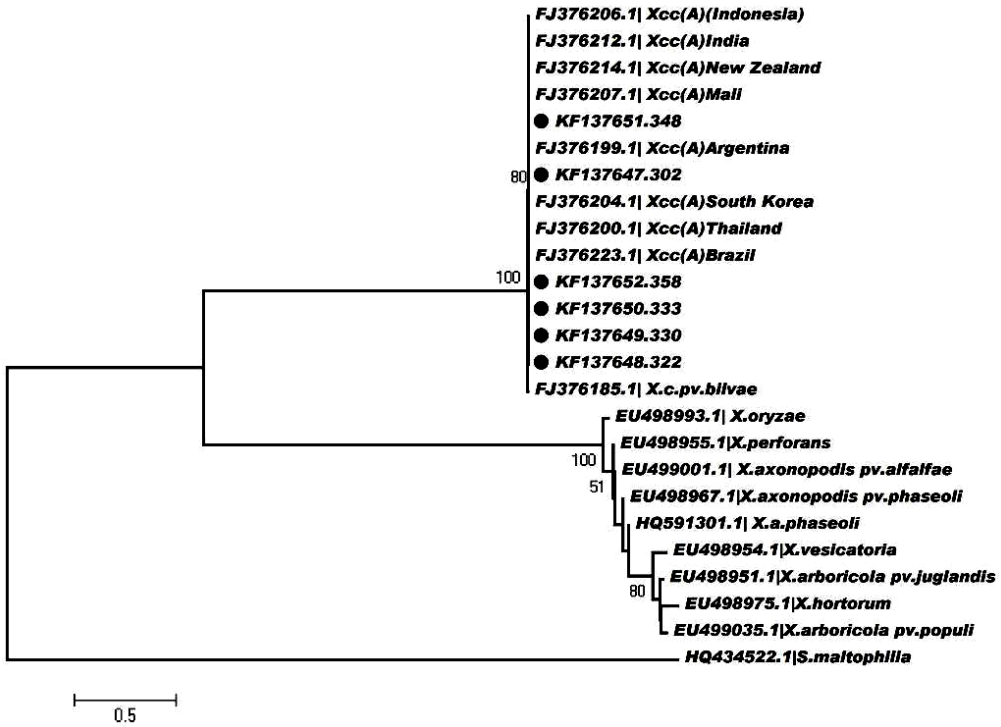

Figure 3 Phylogenetic tree derived from neighbor-joining analysis of the *gyrB* from *X. citri* strains infecting Citrus in Iran. Strains 302, 322, 330, 333, 348 and 358: Iranian *X. citri* strain; *X. oryza.* pv. *oryza* was used as outgroup for this analysis.

[DOR: 20.1001.1.22519041.2014.3.3.10.9]

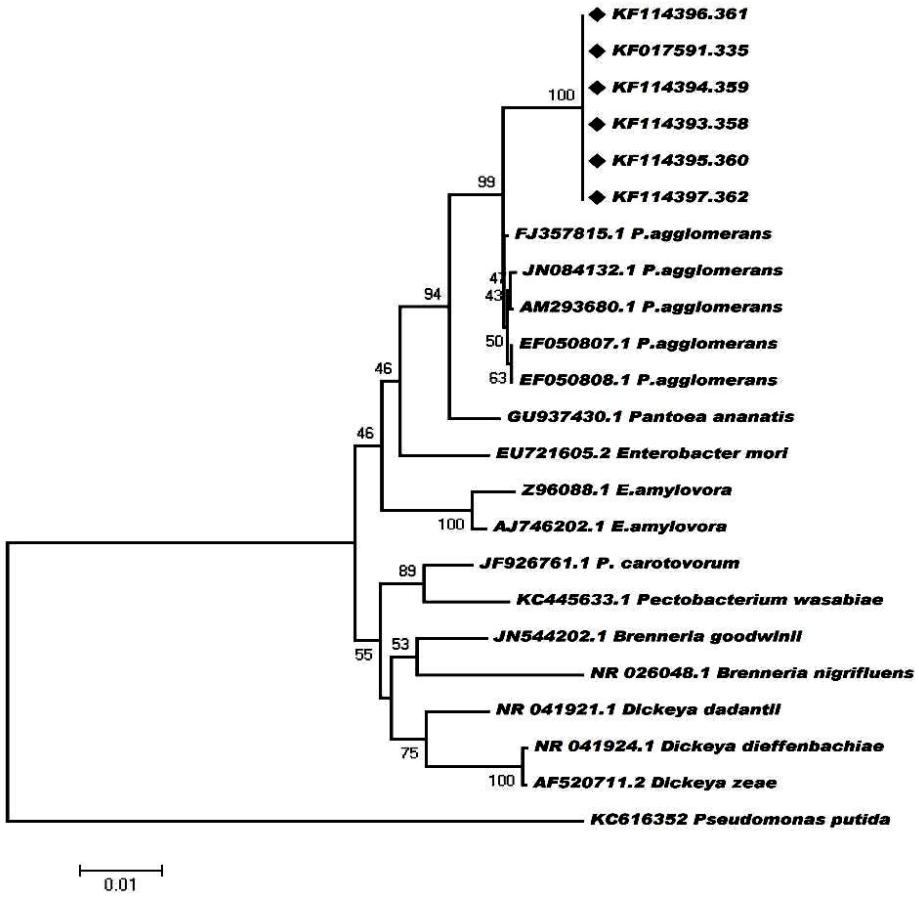

Figure 4 Phylogenetic tree derived from neighbor-joining analysis of the 16S rDNA genes from *P. agglomernce* strains infecting Citrus in Iran. Numbers: KF017591.335, KF114393.358, KF114394.359, KF114395.360, KF114396.361 and KF114397.362: Iranian *P. agglomerance* strain; *Pseudomonas putida* KC516352 was used as outgroup for this analysis.

Discussion

The yellow and facultative anaerobic bacterium isolated from citrus cankers in this study had the physiological and biochemical characteristics of genus *Pantoea*. This genus is ubiquitous, it colonizes humans and plants and is found in the general environment. Many species of *Pantoea* are known to be endophytic in plants while commonly found in diverse ecological niches including aquatic environments, sediment or soil (Rezzonico *et al.*, 2009). Endophytes are organisms that live inside plants without causing disease and include microbes that are benefit for their hosts by aiding the acquisition of nutrients and controlling pathogens. It was frequently found that some bacterial endophytic isolates from healthy plants inhibited the growth of some seedlings in reinoculation assays, possibly through the production of certain metabolites (van Peer *et al.*, 1990). Many studies have investigated the pathogenicity of *Pantoea* in several plant hosts, and identified possible factors contributing to pathogenicity during development of disease. It seems that pathenogenicity of *P. stewartii* is related to the *hrp/wts* gene cluster, which directs the synthesis of type three secretion systems (Frederick *et al.*, 2001). Reports on *Pantoea* have often associated it with pathogenic plant bacteria such as *Pseudomonas savastanoi* (Marchi *et al.*, 2006). It is also known to be the most common and dominant saprophytic bacterium found in citrus canker

[DOR: 20.1001.1.22519041.2014.3.3.10.9]

lesions. Interaction between *Xanthomonas* and *Pantoea* may reduce multiplication of *Xanthomonas* and suppress the development of canker (Goto *et al*., 1979). Plasmids can be mobilized between different bacteria by conjugation (Krishnapillai, 1996). Transferring its pathogenic property to a saprophytic bacterium is characterized as horizontal gene transfer, which is the process of acquiring DNA from an outside source and other than by vertical transmission from a parental cell. The complete mechanism of horizontal gene transfer is not fully described. It seems that environmental conditions may play roles in its activation. Genomic islands are considered as a mechanism related to horizontal gene transfer (Lawrence, 2004). Pathogenicity islands (PAI) are a specific group of genomic island that are variable in structure and source of evolution, it has been suggested that they contribute to genomic variability and virulence of bacterial pathogens (Hacker and Kaper, 2000). Several factors affecting virulence and secretion proteins are encoded by PAI (Hacker *et al.*, 1997). Similarity of factors affecting virulence among different bacteria indicates the possibility of horizontal gene transfer (Juhas *et al.*, 2009). It is clear that *P. agglomerans* has been altered from a saprophyte form to gall-forming bacterium and has been reported as a tumorigenic pathogen on some plants. This conversion is caused by acquisition of pPATH plasmid containing a pathogenicity island. It is nonconjugative but its presence has been confirmed in *Erwinia*, *Pseudomonas*, and *Xanthomonas* (Weinthal *et al.*, 2011). Results of this study demonstrate that *Pantoea* had the ability to cause development of lesions on citrus. Redundancy of *Pantoea* as an endophytic bacterium and its association with citrus bacterial canker may mean that *Pantoea* has gained the ability to induce canker on citrus. This event can occur by transmission of parts of PAI from *Xanthomonas* to *Pantoea*. Several reports concerning the pathogenicity of *Pantoea* on different plants, demonstrated that this bacterium may gain some genes related to pathogenicity from other main pathogens. The reason for occurrence of this phenomenon needs further investigation. *P. agglomerans* was previously confined to being an endophyte bacterium on citrus but it could now be introduced as a new threat to citrus with high population. *P. agglomerans* has high genetic flexibility (Manulis and Barash., 2003). This has been mentioned in other studies on this bacterium and other family members of Enterobacteriace, furthermore this bacterium is an important factor in biological control of other plant pathogens. Due to the potential of *P. agglomerans* for the acquisition of some specific genes involved in pathogenicity, its application in research for biological control should be done with caution. CBC is worldwide and one of the most important diseases afflicting citrus, so study of the disease is crucial. To our knowledge, this is the first report on presence of *Pantoea* and its pathogenicity on citrus in Iran. Future studies will be conducted on how to improve the significance of the interaction between *P. agglomerans* and *Xcc*.

References

Alizadeh, A, and Rahimian, H. 1990. Citrus canker in Kerman Province. Iranian Journal of Plant Pathology, 26: 1-4.

Anonymous. 2012. Annual Agricultural Statistics. Ministry of Jihad-e-Agriculture of Iran. <www.maj.ir>.

Ausubel, F., Brent, R., Kingston, R., Moore, D., Seidman, J., Smith, J. and Struhl, K. 1992. Current Protocols in Molecular Biology. Greene and Wiley. Brooklyn, NY.

Brady, C., Cleenwerck, I., Venter, S., Vancanneyt, M., Swings, J. and Coutinho, T. 2008. Phylogeny and identification of *Pantoea* species associated with plants, humans and the natural environment based on multilocus sequence analysis (MLSA). Systematic and Applied Microbiology, 31: 447-460.

Cook, A. 1988. Association of citrus canker pustules with leaf miner tunnels in North Yemen. Plant Disease, 72: 546.

Cruz, A. T., Cazacu, A. C and Allen, C. H. 2007. *Pantoea agglomerans*, a plant pathogen causing human disease. Journal of Clinical Microbiology, 45: 1989-1992.

Cubero, J. and Graham, J. H. 2002. Genetic relationship among worldwide strains of

[DOR: 20.1001.1.22519041.2014.3.3.10.9]

Xanthomonas cauing canker in citrus species and design of new primers for their identification by PCR. Applied and Environmental Microbiology, 68: 1257-1264.

Das, A. 2003. Citrus canker-A review. Journal of Applied Horticulture, 5: 52-60.

Delétoile, A., Decré, D., Courant, S., Passet, V., Audo, J., Grimont, P., Arlet, G. and Brisse, S. 2009. Phylogeny and identification of *Pantoea* species and typing of *Pantoea agglomerans* strains by multilocus gene sequencing. Journal of Clinical Microbiology, 47: 300-310.

Frederick, R. D., Ahmad, M., Majerczak, D. R., Arroyo-Rodríguez, A. S., Manulis, S. and Coplin, D. L. 2001. Genetic organization of the *Pantoea stewartii* subsp. *stewartii* hrp gene cluster and sequence analysis of the *hrpA*, *hrpC*, *hrpN*, and *wtsE* operons. Molecular Plant-Microbe Interactions, 14: 1213-1222.

Goto, M., Tadauchi, Y. and Okabe, N. 1979. Interaction between *Xanthomonas citri* and *Erwinia herbicola* in vitro and in vivo. Annals of the Phytopathological Society of Japan, 45: 618-624.

Hacker, J., Blum-Oehler, G., Mühldorfer, I. and Tschäpe, H. 1997. Pathogenicity islands of virulent bacteria: structure, function and impact on microbial evolution. Molecular Microbiology, 23: 1089-1097.

Hacker, J., Kaper, J. B. 2000. Pathogenicity islands and the evolution of microbes. Annual Reviews in Microbiology, 54: 641-679.

Hartung, J. and Civerolo, E. 1989. Restriction fragment length polymorphisms distinguish *Xanthomonas campestris* strains isolated from Florida citrus nurseries from *X. c.* pv. *citri*. Phytopathology, 79: 793-799.

Irey, M. and Stall, R., 1981. Value of xanthomonadins for identification of pigmented *Xanthomonas campestris* pathovars.Proceeding International Conference Plant Pathogenic Bacteria. 5th, pp. 85-95.

Juhas, M., Der Meer, V., Roelof, J., Gaillard, M., Harding, R. M., Hood, D. W. and Crook, D. W. 2009. Genomic islands: tools of bacterial horizontal gene transfer and evolution. FEMS Microbiology Reviews, 33: 376-393.

Krishnapillai, V. 1996. Horizontal gene transfer. Journal of Genetics, 75: 219-232.

Lawrence, J. G. 2005. Horizontal and vertical gene transfer: the life history of pathogens. Contribiotion of Microbiology 12: 255-271.

Manulis, S. and Barash, I. 2003. *Pantoea agglomerans* pvs. *gypsophilae* and *betae*, recently evolved pathogens? Molecular Plant Pathology, 4: 307-314.

Marchesi, J. R., Sato, T., Weightman, A. J., Martin, T. A. and Fry, J. C., Hiom, S. J., Wade, W. G. 1998. Design and evaluation of useful bacterium-specific PCR primers that amplify genes coding for bacterial 16S rRNA. Applied and Environmental Microbiology, 64: 795-799.

Marchi, G., Sisto, A., Cimmino, A., Andolfi, A., Cipriani, M., Evidente, A. and Surico, G. 2006. Interaction between *Pseudomonas savastanoi* pv. *savastanoi* and *Pantoea agglomerans* in olive knots. Plant Pathology, 55: 614-624.

Ngoc, L. B. T., Vernière, C., Boyer, C., Vital, K., Pruvost, O., Le Mai, N. and Le Thi Thu, H. 2009. Pathotype Identification of *Xanthomonas citri* pv. *citri* Strains Causing Citrus Canker in Vietnam. Plant Disease, 93: 671-671.

Ngoc, L. B. T., Vernière, C., Jouen, E., Ah-You, N., Lefeuvre, P., Chiroleu, F., Gagnevin, Land Pruvost, O. 2010. Amplified fragment length polymorphism and multilocus sequence analysis-based genotypic relatedness among pathogenic variants of *Xanthomonas citri* pv. *citri* and *Xanthomonas campestris* pv. *bilvae*. International Journal of Systematic and Evolutionary Microbiology, 60: 515-525.

Rezzonico, F., Smits, T. H. M., Montesinos, E., Frey, J. E. and Duffy, B. 2009. Genotypic comparison of *Pantoea agglomerans* plant and clinical strains. BMC Microbiology, 9: 204.

Schaad, N., Jones, J. and Chun, W. 2001. Plant Pathogenic Bacteria 3rd ed. The Bacteriology Committee of the American Phytopathological Society. APS Press, St. Paul, MN, USA.

[DOR: 20.1001.1.22519041.2014.3.3.10.9]

Schaad, N. W., Postnikova, E., Lacy, G., Sechler, A., Agarkova, I. V., Stromberg, P. E. and Stromberg, V. K. and Vidaver, A.M. 2006. Emended classification of xanthomonad pathogens on citrus. Papers in Plant Pathology, 96.

Suk Park, D., Wook Hyun, J., Jin Park, Y., Sun Kim, J., Wan Kang, H., Ho Hahn, J. and Joo Go, S. 2006. Sensitive and specific detection of *Xanthomonas axonopodis* pv. *citri* by PCR using pathovar specific primers based on *hrpW* gene sequences. Microbiological Research, 161: 145-149.

Tamura, K., Dudley, J., Nei, M. and Kumar, S. 2007. MEGA4: molecular evolutionary genetics analysis (MEGA) software version 4.0. Molecular Biology and Evolution, 24: 1596-1599.

van Peer, R., Punte, H. L., de Weger, L. A. and Schippers, B. 1990. Characterization of root surface and endorhizosphere pseudomonads in relation to their colonization of roots. Applied and Environmental Microbiology, 56: 2462-2470.

Weinthal, D. M., Barash, I., Tzfira, T., Gaba, V., Teper, D., Sessa, G. and Manulis-Sasson, S. 2011. Characterization of nuclear localization signals in the type III effectors *HsvG* and *HsvB* of the gall-forming bacterium *Pantoea agglomerans*. Microbiology, 157: 1500-1508.

[DOR: 20.1001.1.22519041.2014.3.3.10.9]

همراهی *Pantoea agglomerans* با بیماری شانکر باکتریایی مرکبات در ایران

فائقه شرافتی[1]، پژمان خدایگان[1*]، مهدی آزادوار[2]، ابراهیم صداقتی[1]، روح‌الله صابری ریسه[1] و ساره بقایی راوری[3]

۱- گروه گیاهپزشکی دانشگاه ولی‌عصر رفسنجان، رفسنجان، ایران.
۲- مرکز تحقیقات کشاورزی جیرفت و کهنوج، جیرفت، ایران.
۳- گروه گیاهپزشکی دانشگاه فردوسی مشهد، مشهد، ایران.
* پست الکترونیکی نویسنده مسئول مکاتبه: Pkhodaygan@vru.ac.ir
دریافت: ۲۲ شهریور ۱۳۹۲؛ پذیرش: ۷ دی ۱۳۹۲

چکیده: در بهار و تابستان ۱۳۸۹ و ۱۳۹۰ نمونه‌هایی از برگ، سرشاخه و میوه‌های لیموترش (*Citrus aurantifolia*) و گریپ‌فروت (*C. paradisi*) با علائم شانکر باکتریایی از استان‌های مختلفی در ایران جمع‌آوری گردید. از بافت‌های آلوده، باکتری‌های گرم منفی جداسازی گردید. نتایج آزمون بیماریزایی پس از مایه‌زنی به برگ گیاهان لیموترش و گریپ‌فروت نشان داد که تعدادی از جدایه‌ها باعث هایپرپلازی و هایپرتروفی بافت شده و زخم‌های شبه‌شانکر، برجسته مشابه با علائم عمومی بیماری تولید نمودند. درحالی‌که سایر ایزوله‌ها زخم‌های نکروتیک و آبسوخته روی برگ ایجاد نمودند. برخی از جدایه‌ها لکه‌های برجسته مشابه با علائم عمومی بیماری و برخی دیگر باعث بروز لکه‌های آبسوخته و نکروزه تخت روی برگ میزبان شدند. نمایندگانی از هر دو گروه براساس ویژگی‌های مورفولوژیکی و فیزیولوژیکی مورد بررسی قرار گرفت. ردیابی با استفاده از آغازگر اختصاصی انجام شد و بخشی از ژن 16s rDNA و *gyrB* تعیین ترادف شد. براساس نتایج، گروهی از جدایه‌ها به‌عنوان استرین‌های *Xanthomonas citri* subsp. *citri* مشخص و گروه دیگر شامل اغلب جدایه‌ها، به‌عنوان *Pantoea agglomerans* شناسایی شدند. نمونه‌برداری در فصول مختلف تکرار شده و وجود جمعیت بالای *P. agglomerans* در زخم شانکر باکتریایی، به‌ویژه در مناطق گرم و خشک‌تر محرز گردید. این باکتری توانایی القای علائمی مشابه با علائم شانکر روی نهال‌های گریپ‌فروت را دارد و پس از دو ماه، مجدداً از بافت آلوده جداسازی گردید.

واژگان کلیدی: *Pantoea agglomerans*، بیماری شبه شانکر مرکبات، بیماری‌زایی، ایران

[DOR: 20.1001.1.22519041.2014.3.3.10.9]