Plant Pathol. J. 25(4) : 429-432 (2009)

The Plant Pathology Journal

Note

Bacterial Fruit Rot of Apricot Caused by *Burkholderia cepacia* in China

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(Received on September 8, 2009; Accepted on October 16, 2009)

An unreported disease of apricot was observed in orchards in Zhejiang province, China. Symptoms started as water soaked lesions on the fruit surface. Later, water-soaked areas developed and spread to the entire fruit, resulting in soft rot of the whole fruit. The causal organism isolated from symptomatic fruits was identified as *Burkholderia cepacia* based on its biochemical and physiological characteristics and confirmed by the cellular fatty acid composition and Biolog data as well as 16S rRNA gene sequence analysis. The bacterial isolates caused similar symptoms when inoculated onto fruits of apricot. In addition, European plum, Japanese plum, nectarine and kiwifruit were susceptible to the *B. cepacia* pathogen. However, the *B. cepacia* pathogen failed to cause any visible symptoms when it was inoculated onto 16 other fruits. This is the first report of a bacterial disease of apricot caused by *B. cepacia* in China.

Keywords : apricot, fruit rot disease, host range, identification, *Burkholderia cepacia*

Apricot (*Prunus armeniaca*) is one of the most important fruits, which is regarded as a nutritious and tonic food and enjoyed by the world wide popularity. Most apricot production in the world is centered in Europe and Asia. In particular, the apricot is believed to have originated in China, where it has been cultivated for over 4,000 years. In addition, the total acreage of apricot orchards has been increasing by 20% annually. In 2008, over 660, 000 metric tons of fresh fruits were harvested in China (Li et al., 2008).

Throughout the centuries, the fruit, kernels, oil and flowers of the apricot have been used in medicine. In China, a famous medicine known as 'Apricot Gold' was made from the kernels of trees which grew in certain areas. This medicine was reputed for the powers to prolong life. The Chinese also believed that apricots reacted sympathetically to women's ailments. However, apricot trees has been reported to be a natural host of *Pseudomonas syringae* pv. *syringae*, which caused bacterial canker and blast (Kotan and Sahin, 2002). Furthermore, Aysan et al. (2003) reported that crown gall of apricot was caused by *Agrobacterium tumefaciens*. Nowadays, *Xanthomonas airboricola* pv. *pruni* (*X. campestris* pv. *pruni*) is a ubiquitous bacterium that causes bacterial spot of apricot (Boudon et al., 2005). In the present study, we report the occurrence of fruit rot disease of apricot, and the identification and characterization of the bacterial pathogen for the first time.

Disease occurrence and symptoms. Beginning in April 2009, a previously undescribed bacterial fruit rot disease of apricot was observed in an orchard in Zhejiang province, China. Initial symptoms were water-soaked lesions on the surface of preharvested fruits. As these lesions expanded and elongated, the diseased tissues became soft and watery (Fig. 1). In general, the disease is not very severe while the scattered diseased fruits were observed on about 10 apricot trees in one orchard. Severely affected apricot fruits resulted in soft rot of the entire fruit, which reduced the quality and marketability of apricot.

Bacterial isolation. Small sections of the surface-sterilized tissue were excised aseptically from the margins of diseased areas of fruit and macerated individually in 40 μl of sterile distilled water. The resulting suspensions were streaked onto nutrient agar (NA) (Schaad et al., 2001). Individual bacterial colonies formed after 2-3 days of incubation at 28°C were isolated from culture plates, and stored in 15% aqueous glycerol at −70°C for further studies. Totally ten *Burkholderia*-like isolates were obtained from diseased fruit tissues of apricot. In addition, infiltration of tobacco leaves with cell suspensions of the bacterial isolates all resulted in typical hypersensitivity reactions within 24 h.

Pathogenicity. Artificial inoculations on preharvested fruits of apricot (cultivar Jintaiyang) were made in an orchard. Bacterial colonies grown on NA for 48 h at 28°C were suspended in sterile distilled water. The suspensions were diluted to approximately 1.0×10^8 colony forming

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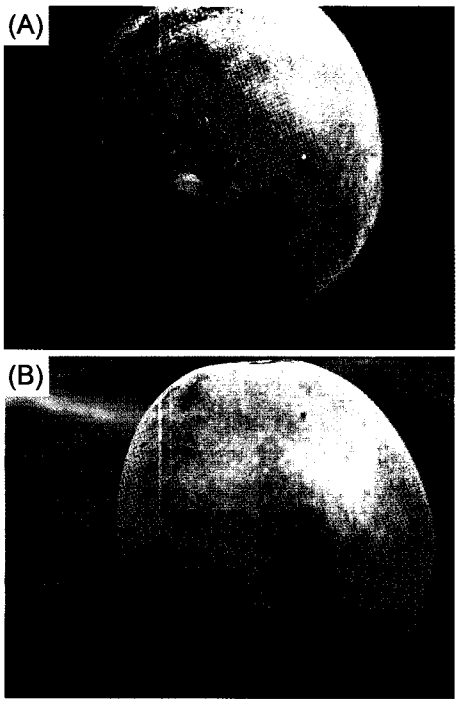

Fig. 1. Fruit rot symptoms caused by *Burkholderia cepacia* on apricot. (A) artificial inoculation; (B) uninoculated control.

units/ml. Suspension concentrations were confirmed by dilution plating on NA (Li et al., 2008a). Wounds with a diameter of 0.3 mm were made with a sterilized needle and then bacterial suspensions were sprayed onto fruits surfaces with an aerosol-propelled sprayer until runoff. Negative control plants were inoculated with sterile water in the same way. Five fruits per treatment were used as replicates and the inoculation experiments were conducted twice. All inoculated fruits were covered with plastic bags for 3 days to keep humidity high. After 1 week, apricot fruits artificially inoculated with the isolates developed symptoms similar to the symptoms commonly observed in the field. In addition, the same bacterium was reisolated from inoculated fruits. Control fruits remained symptomless.

Host range. To test pathogenicity of the bacterial isolates on hosts other than apricot, two representative isolates Bca 0901 and Bca 0902 were selected and inoculated onto the following plants: European plum (*Prunus domestica* cv. French), Japanese plum (*Prunus salicina* cv. Zhuli), banana (*Musa sapientum* cv. Gaobajiao), nectarine (*Prunus persica* cv. Batsch), kiwifruit (*Actinidia chinensis*), black plum (*Prunus nigra*), apple (*Malus pumila* cv. Domestica and cv. Jiali and cv. Taipingyangmeigui and cv. Tengmu), Asian pear (*Pyrus pyrifolia* cv. Huangguan), fragrant pear (*Pyrus ussuriensis*), lemon (*Citrus limonum* cv. Youlike), orange (*Citrus sinensis* cv. Tiancheng), red grapefruit (*Citrus paradisi*), orange (*Citrus reticulata*), peach (*Prunus persica* cv. Yuandongbaitao and cv. Pingguxiantao), mango (*Anthracothorax viridigula* cv. Datailong and cv. Xiaoxiangya). Each treatment had three replicates and the experiment was carried out twice. Harvested fresh fruits were bought from the local market and inoculated and incubated in a growth chamber at 28°C and 90% relative humidity with a photoperiod of 16 h. After one week, disease symptoms were not developed on the 16 selected fruits inoculated with the representative isolates except European plum, Japanese plum, nectarine and kiwifruit, on which water-soaked lesions were produced around the inoculation sites, expanding quickly. None of the control fruits treated with sterile distilled water developed symptoms.

Identification of the representative isolates. Bacterial characteristics of the two representative isolates Bca 0901 and Bca 0902 were investigated by the methods of Schaad et al. (2001). The reference strain LMG1222 of *Burkholderia cepacia* was provided by the Belgian Co-ordinated Collections of Microorganisms, BCCM, Gent, Belgium. Both isolates were morphologically similar to the reference strain when incubated at 30°C for 48 h. Colonies on nutrient agar were smooth, translucent, and convex with entire margins, and not pigmented either on NA or on King B agar. Classical bacteriological tests indicated that the isolates were Gram-negative and obligatorily aerobic. However, the two isolates were able to utilize melibiose and raffinose while the reference strain LMG1222 did not utilize them (Table 1).

The cellular fatty acid compositions of the two isolates were determined as described by Kim et al. (2007) and Li et al. (2008b). In general, the profiles of the two isolates are similar to that of strain LMG 1222^T. Predominant fatty acids of both bacterial isolates were C16:0, C-16:1, and C18:1 (Table 2). Profiles were compared with the MIDI identification database TSBA50, version 5.00 (MIDI Inc., Newark, DE, USA). The fatty acid profiles of the isolates Bca 0901 and Bca 0902 gave best matches with *B. cepacia* with similarity indices of 0.52 and 0.58, respectively. Also carbon source assimilation of the 2 isolates was examined by the Biolog GN test kit (Biolog Inc., Hayward, Co.) according to the manufacturer's specifications. Experiments were performed as described by Choi et al. (2006) and Li et al. (2009). Comparing the profile with the BIOLOG identification database GN4.01, the Biolog database identified the isolates Bca 0901 and Bca 0902 as *B. cepacia* with similarity indices of 0.65 and 0.68, respectively. Thus, based on the results of the above two analyses, the bacterial isolates were confirmed to be *B. cepacia.*

Amplification of the 16S rRNA genes was performed by PCR as described by Li et al. (2006) and Kim et al. (2009). The sequences obtained from the isolates Bca 0901 and Bca

Table 1. Morphological, physiological and biochemical characters of the bacterial isolates obtained from naturally infected *Prunus armeniaca*

Test	Bca 0901	Bca 0902	LMG1222[c]
Flagellation	Polar, >1	Polar, >1	Polar, >1
Grows aerobically	+[b]	+	+
Grows anaerobically	–	–	–
Oxidative/Fermentative	Oxidative	Oxidative	Oxidative
Oxidase	+	+	+
Catalase	+	+	+
Tobacco hypersensitivity	+	+	+
Utilization of[a]			
Adonitol	+	+	+
Arabinose	+	+	+
Arabitol	+	+	+
Cellobiose	+	+	+
Fucose	+	+	+
Lactose	–	–	–
Maltose	–	–	–
Melibiose	+	+	–
Raffinose	+	+	–
Rhamnose	–	–	–
Sorbitol	+	+	+
Sucrose	+	+	+
Trehalose	+	+	+
Xylitol	+	+	+

[a]BIOLOG data are presented only for the reactions, in which the strains of *Burkholderia cepacia* can be distinguished from other *Burkholderia* species.
[b]+, positive; –, negative
[c]The reference strain LMG1222 of *B. cepacia* was provided by the Belgian Co-ordinated Collections of Microorganisms, BCCM, Gent, Belgium.

0902 were used for sequence database searches in GenBank and sequence similarities to the known *B. cepacia* strains were measured using the BLAST program. The sequences of 16S rRNA obtained in this study were compared with previously published sequences of *Burkholderia*. Phylogenetic trees were generated using the genetic distance-based neighbor-joining algorithms within MEGA version 4.0 (http://www.megasoftware.net/). The 16S rRNA gene sequences of isolates Bca 0901 and Bca 0902 have been submitted into GenBank data under the accession numbers of GQ352647 and GQ352648, respectively.

BLAST analyses showed that the isolates Bca 0901 and Bca 0902 had 99% homology to those of other *B. cepacia* strains that had been deposited in the GenBank database. These data are consistent with the results of morphological, biochemical and physiological tests, as well as Biolog data and fatty acid analysis. In addition, the phylogenetic analysis revealed that the two isolates and *B. cepacia* LMG1222 clustered within a group and well separated from other

Table 2. Analysis of cellular fatty acids of *Burkholderia cepacia* isolates obtained from naturally infected fruits of apricot

Fatty acid	Cellular fatty acids (%)		
	Bca 0901	Bca 0902	LMG1222[a]
13:1	1.49	1.33	–
14:0	6.85	7.08	3.9
15:1 w6c	0.18	–	–
15:0	–	–	–
14:0 3OH	2.44	3.33	4.8
16:1 w7c	13.54	26.22	15.0
16:0	18.96	17.07	22
17:0 cyclo	13.35	8.92	9.1
16:1 2OH	0.82	1.77	1.1
16:0 2OH	0.90	1.37	1.0
16:0 3OH	1.23	2.14	4.5
18:1 w7c	29.29	26.22	29
18:0	0.50	–	1.1
19:0 cyclo w8c	6.33	3.00	5.6
18:1 2OH	2.05	3.16	2.3

[a]Data from Fain and Haddock (2001).

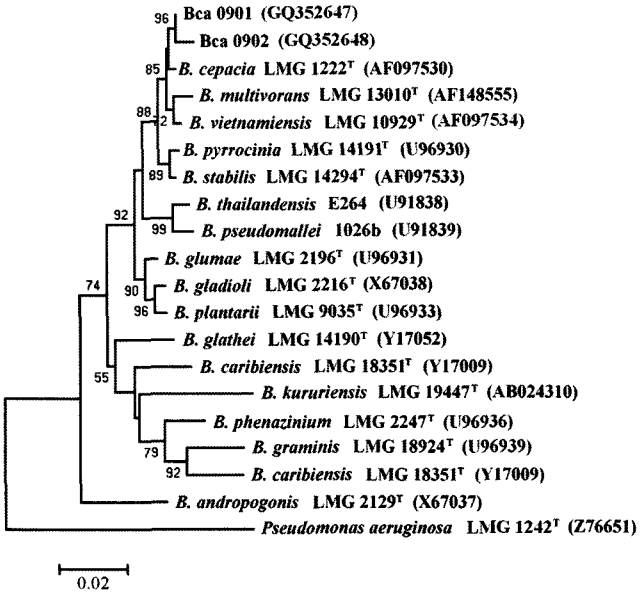

Fig. 2. Phylogenetic tree generated by the neighbor-joining method on the basis of partial 16S rDNA sequences showing the position of the bacterial isolates Bca 0901 and Bca 0902 among members of the genus *Burkholderia*. The tree was generated by the neighbor-joining method based on the two-parameter Kimura correction of evolutionary distances. Bootstrap analyses (1000 replicates) for node values from 50% are indicated. *Pseudomonas aeruginosa* was used as the outgroup. Bar, 0.05 substitutions per nucleotide position.

species of *Burkholderia* (Fig. 2). It is therefore considered that the two isolates should be identified as *B. cepacia*.

This is the first report in China that *B. cepacia* is the causal pathogen of bacterial fruit rot of apricot. The bacteria

only caused fruit rot when they were inoculated by needle puncture or infiltration but not by spraying without wounding, confirming that the bacteria infected fruit tissues via wounds. Indeed, *B. cepacia* is a gram-negative bacteria commonly found in such habitats as soil, water, plant rhizosphere, animal surfaces, humans and hospital environment (Zhang and Xie, 2007). In addition, it has also been described as a phytobacterium causing soft rot in onions (Burkholder, 1950; Campo and Zapata, 1996; Fang et al., 2007), bacterial blotch on oyster mushroom (Gill and Cole, 1992; Alameda and Mignucci, 1998) and finger-tip rot on banana (Lee et al., 2003). Therefore, further studies are required to evaluate the phenotypic and genetic differences between *B. cepacia* isolates from apricot and *B. cepacia* isolates from other sources.

Acknowledgements

This project was supported by Zhejiang Provincial Natural Science Foundation of China (Y3090150), National Natural Science Foundation of China (30600475) and the Agricultural Ministry of China (nyhyzx200803010).

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