Plant Pathol. J. 23(4) : 314-317 (2007)

The Plant Pathology Journal

Note

First Report on Bacterial Soft Rot of Graft-cactus *Chamaecereus silvestrii* Caused by *Pectobacterium carotovorum* subsp. *carotovorum* in Korea

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(Received on August 7, 2007; Accepted on October 24, 2007)

A soft stem rot disease was observed on *Chamaecereus silvestrii* (Korean name: Sanchui), a scion of graft-cactus, in major growing areas of Suwon (National Horticulture Research Institute), Anseong, Eumseong, Cheonan, Daegu, and Goyang, Korea during 2000 and 2001. Typical symptoms were soft rots characterized by moist and watery decay of the whole cactus stem, which initiated as small water-soaked lesions and enlarged rapidly to the entire stem. The causal organism isolated from the infected stems was identified as *Pectobacterium carotovorum* subsp. *carotovorum* (*Erwinia carotovora* subsp. *carotovora*) based on its physiological and biochemical characteristics and confirmed by the cellular fatty acid composition and Biolog analyses. Artificial inoculation of the bacterium produced the same soft rot symptoms on the cactus stems, from which the same bacterium was isolated and identified. This is the first report of the *P. carotovorum* subsp. *carotovorum* in the graft-cactus *C. silvestrii* in Korea.

Keywords : bacterial soft rot, *Chamaecereus silvestrii*, graft-cactus, *Pectobacterium carotovorum* subsp. *carotovorum*

A graft-cactus, which is composed of stock and scion cactus species, is a major commercial product in Korea. It is the most important exporting ornamental plant due to its high quality in the world market. The most widely cultivated stock cactus is a three-angled cactus (*Hylocereus trigonus*). Two scion cacti commonly used in Korea are plain cacti, *Gymnocalycium mihanovichii* and *Chamaecereus silvestrii*, of which the Korean common names are Bimoran and Sanchui, respectively.

One of the limiting factors for cultivating a graft-cactus in a greenhouse is stem rot disease. Various fungal diseases in cacti are known worldwide, of which the three major fungal stem rot diseases known in Korea are caused by *Fusarium oxysporum* (Chang et al., 1998), *Bipolaris cactivora* (Hyun et al., 1998; Kim et al., 2004), and *Glomerella cingulata* (Kim et al., 2000). However, no bacterial disease has been reported yet in Korea.

During 2000-2001 in the major cactus-growing areas of Korea, Suwon, Anseong, Eumseong, Cheonan, Daegu, and Goyang, soft rot symptoms were found on *C. silvestrii* stems. The causal organism was isolated and identified as a bacterium. Its pathogenicity was confirmed. Since bacterial pathogens are easily transmitted mechanically through wounds during grafting and multiply rapidly in warm greenhouse conditions, a bacterial disease may be a potential to outbreak without recognition. Therefore, we report the occurrence of bacterial soft rot of a graft-cactus, *C. silvestrii* (Korean name Sanchui cactus), for the first time in Korea, and the identification and characterization of the bacterial pathogen.

Disease occurrence and symptoms. Occurrence of a soft stem rot was observed infrequently on *C. silvestrii* in most of surveyed areas but often in major growing areas of Suwon, Anseong, Eumseong, Cheonan, Daegu, and Goyang, Korea during 2000-2001. Soft rot symptoms were characterized by moist and watery decay of the cactus stem (Fig. 1A). Initially one or several small water-soaked lesions were formed on a stem, and enlarged rapidly to finally rot the whole stem.

Bacterial isolation and pathogenicity. Pathogen isolation was conducted using infected *C. silvestrii* stems of National Horticulture Research Institute in Suwon. Small stem tissues were cut from the edge of the diseased area with flame-sterilized razor blade and surface-sterilized with 75% ethanol for 30 sec and 1% sodium hypochlorite for 60 sec, and rinsed with sterile water. The stem tissues were placed on a flamed slide glass and added with 1-2 drops of sterile water, allowing 10 min to stand, which were streaked on nutrient agar. Single bacterial colonies formed after 2-3 days of incubation at 28°C were isolated from the culture

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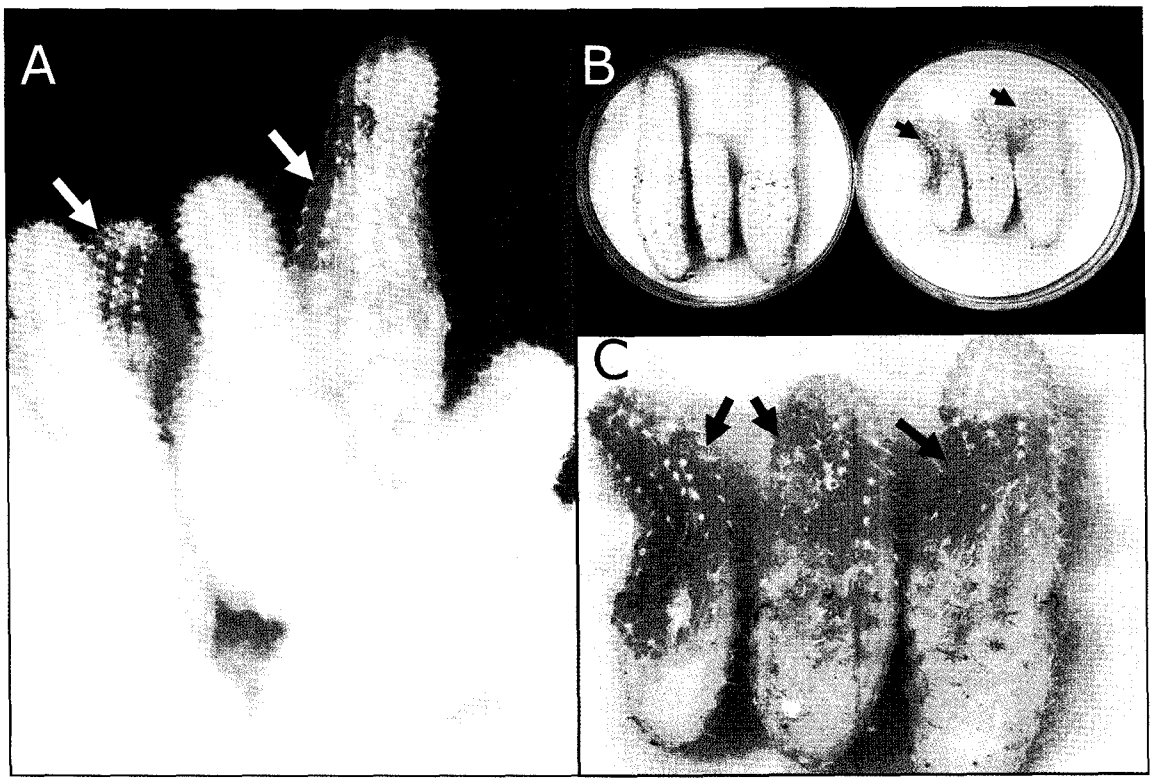

Fig. 1. Typical symptoms of soft rot on *C. silvestrii* naturally infected (A) and artificially induced at 12 hours after inoculation (B, C), showing moist and watery decay of the whole cactus stem (arrows). Cactus stems on left side of (B) are uninoculated controls with no soft rot symptoms.

plates, and stored in 15% sterile glycerol at −70°C for further use.

We selected one isolate (CBR1) from several colonies with the same colony morphology (forming grayish-white colonies on nutrient agar) as a type culture for further studies. The bacterial isolate grown on nutrient agar for 2-3 days was collected in sterile distilled water and the concentration was adjusted to 1.0×10^8 cells/ml as inoculum for pathogenicity test. One-year-old stems of *C. silvestrii* were inoculated with the bacterial suspension after wounding with a sterilized needle until run-off, and placed in glass Petri-dishes containing moistened paper towel, which were kept at 28°C in an incubator. Each Petri-dish contained three stems, and the experiment replicated three times. Sterile distilled water was used as control.

CBR1 caused typical soft rot symptoms on the stems of *C. silvestrii* at 12 hrs after artificial wound inoculation (Fig. 1B, 1C), showing watery decay of the whole stem within 12 hours after the bacterial inoculation. Water-inoculated control did not induce any soft rot symptoms (Fig. 1B).

Identification of the bacterial isolate. Bacterial characteristics of isolate CBR1 from *C. silvestrii* were investigated by the methods of Dicky and Kelman (1988). Gram staining, colony color on nutrient glucose agar (NGA), fluorescent pigment production on King's B agar, anaerobic growth, flagella type and formation of spores were investigated in order to identify the genus of the present isolate. The morphology of the bacterial cells was investigated with the aid of electron microscopy after negative staining with 2% phosphotungstic acid (pH 7.0).

Table 1. Comparison of characteristics of the present bacterial isolate CBR1 from the soft rot of *Chamaecereus silvestrii* with those of the genus *Erwinia*

Characteristics	CBR1	*Erwinia*[a]
Gram stain	−[b]	−
Yellow or orange colonies in NGA, YDC or NBY media	−	V
Fluorescent pigment on KB	−	−
Grows anaerobically	+	+
Grows aerobically	+	+
More than four peritrichous flagella	+	+
Growth on D-1 agar	−	−
Spore formation	−	−
Aerial mycelium	−	−

[a]Data from the Laboratory Guide for Identification of Plant Pathogenic Bacteria (Dicky and Kelman, 1988).
[b]+: positive, −: negative reaction, V: variable.

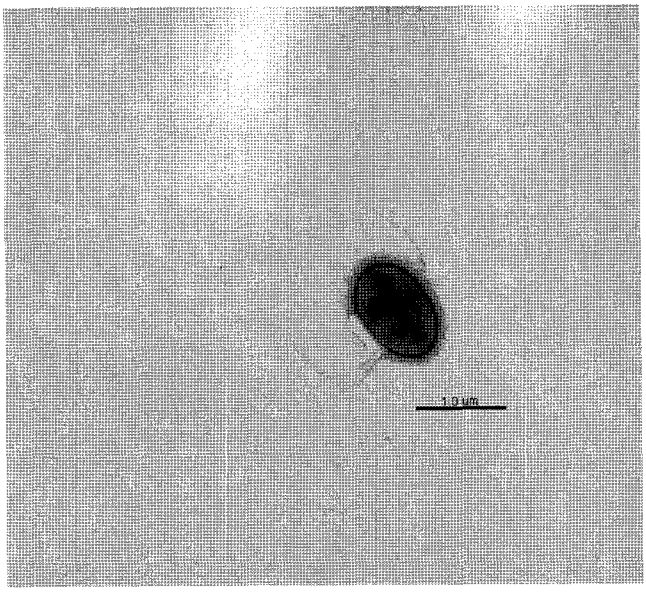

Fig. 2. Electron microscopy of a bacterium isolated from soft rot of *C. silvestrii*, showing a rod shape with peritrichous flagella. Bar represents 1 μm.

The isolate CBR1 was Gram-negative and formed grayish white colonies at 2 days after incubation on nutrient agar. This isolate did not produce fluorescent pigments on King's B agar and could grow aerobically or anaerobically. This isolate also did not form spores and aerial mycelium (Table 1). Electron microscopic observation exhibited that the bacterial cells were bacilli form with peritrichous flagella, one of the typical characteristics of *Erwinia* spp. (Fig. 2). These physiological and morphological characteristics of the present bacterial isolate CBR1 coincide with those of the genus *Erwinia* (Dicky and Kelman, 1988).

Several biochemical tests such as pectate degradation, gelatin liquefaction, acetoin production, phosphase activity, indole production, production of reducing substance from sucrose, growth at 36-37°C, and production of acids from D-lactose, palatinose, trehalose, α-methyl glucoside, dulcitol and melibiose were investigated for the identification of the bacterial isolate at the species level (Table 2).

The isolate CBR1 was identified as *Erwinia* sp. because it showed positive reactions on the tests of pectate degradation, potato soft rot, gelatin liquefaction, and acetoin production, and grew at 36-37°C, but did not show sensitivity to erythromycin, and did not produce phosphase, gas from glucose, and lecithinase. Also the bacterial isolate produced acids from D-lactose, trehalose, melibiose, and cellobiose, but not from methyl α-d glucoside. All of these characteristics match well to those of *E. carotovora* subsp. *carotovora* described by the methods of Dicky and Kelman (1988) and Lelliott and Dicky (1984).

Profiles of cellular fatty acid compositions of the bacterial isolate were analyzed by using a Hewlett-Packard model 5890A gas-liquid chromatography, which is called GC-FAME analysis. The results of GC-FAME analysis showed that the isolate CBR1 had 12 fatty acids (data not shown), among which hexadecanoic acid was the highest (28.96%) and others were in order of *cis*-11-octadecanoic acid (18.69%), dodecanoic acid (6.05%), tetradecanoic acid (2.92%) and heptadecanoic acid (1.29%). These cellular fatty acid compositions of CRB1 were in the similarity of 66.3% to those of *E. carotovora* subsp. *carotovora* in the library database [the Microbial Identification System Library for aerobes (ver. 3.90)] (Paisley, 1998). Also carbon source assimilation of the bacterial isolate was examined by the Biolog GN test kit (Biolog Inc., Hayward, Co.) according to the manufacturer's specifications. The isolate CBR1 utilized 42 carbon sources including D-cellobiose but not 54 carbon sources including D-arabitol in the GN 96 well microplate (Biolog Inc., USA) (data not shown). The Biolog database gave CBR1 strain a high similarity to *E. carotovora* subsp. *carotovora* (similarity of 74%) with a match probability of 100%. Thus, based on the results of the above two analyses, the bacterial isolate CRB1 was confirmed to be *E. carotovora* subsp. *carotovora.*

The bacterial pathogen isolated from soft rot of *C. silvestrii* was a bacterium identified as *E. carotovora* subsp. *carotovora.* In the pathogenicity test, this bacterium decayed the cactus stem very rapidly, indicating that it is

Table 2. Comparison of characteristics of the present bacterial isolate CBR1 from the soft rot of *C. silvestrii* with those of *E. carotovora* subspepcies

Characteristics	CBR1	*E. carotovora* subsp. *carotovora*[a]	*E. carotovora* subsp. *atroseptica*
Pectate degradation	+[b]	+	+
Potato soft rot	+	+	+
Gelatin liquefaction	+	+	+
Acetoin production	+	+	+
Sensitive to erythromycin	−	−	−
Phosphase	−	−	−
Gas from glucose	−	−	−
Lecithinase	−	−	−
Growth at 36-37°C	+	+	+
Acid production from			
D-lactose	+	+	+
Trehalose	+	+	+
Maltose	−	−	V
Methyl α-d glucoside	−	−	+
Melibiose	+	+	+
Cellobiose	+	+	+

[a]Data from Dickey and Kelman (1988) and Lelliott and Dicky (1984).
[b]+: positive, −: negative reaction. V: variable.

highly virulent and has a potential for rapid transmission as many other bacterial soft rots do. The bacterial pathogen of *E. carotovora* subsp. *carotovora* has been reported to cause soft rot disease on several plants such as ginseng (Yu et al., 1991), melon (Yi and Kim, 1996), and soybean sprout (Park et al., 1996). However, there have been no descriptions on the bacterial soft rot disease caused by *E. carotovora* subsp. *carotovora* on cactus in Korea. Recently, Hauben et al. (1998) suggested that the genus *Erwinia* would be reclassified as genus *Pectobacterium*. This suggestion was approved by International Journal of Systemic Bacteriology (validation list No. 68) in 1999, and *E. carotovora* subsp. *carotovora* was listed as *Pectobacterium carotovorum* subsp. *carotovorum*. As we follow the current bacterial nomenclature system, this is the first report in Korea that *Pectobacterium carotovorum* subsp. *carotovorum* is the causal pathogen of bacterial soft rot of *C. silvestrii*.

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