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Occurrence of Bacterial Soft Rot of Lily Bulb Caused by *Pectobacterium carotovorum* subsp. *carotovorum* and *Pseudomonas marginalis* in Korea

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Soft rot symptom was observed on lily bulb in the fields and at a low temperature storage house from 1999 to 2000 in Korea. The small dark-brown lesion appeared on the bulb, and enlarged and developed into the inner scales of the bulb. The bulb became water soaked and gave out unpleasant odor. Two different pathogenic bacteria were isolated from infected tissues. The causal bacteria were identified as *Pectobacterium carotovorum* subsp. *carotovorum* (*Erwinia carotovora* subsp. *carotovora*) and *Psudomonas marginalis* based on bacteriological characteristics. Pathogenicity of the bacteria was proven by Koch's postulations. This is the first report of bacterial soft rot of lily bulb in Korea caused by the two bacteria.

Keywords **:** lily, soft rot, *Pectobacterium carotovorum* subsp. *carotovorum*, *Pseudomonas marginalis*.

Lily (*Lilium longflorum* Thumb.) is a member of the family *Liliaceae*, which has about 130 species growing worldwide. In Korea, lily has been bred for cutting flowers and grown all over the country for export and domestic consumption (Jeong et al., 1991). The major producing districts of lily in Korea are Jeju-do, Gangwon-do, Chungcheongnam-do, and Gyeonggi-do, with the area under cultivation estimated at 245 ha producing 74 million lilies (MAF, 2001). Lily is mostly cultivated by using bulbs and tubers, and its production is constantly increasing in the country.

There are some reports of bacterial diseases on flowering plants such as bacterial spot on Ivy-aures (*Scindapsus aures*) (Choi and Han, 1994); bacterial brown rot on Scarlet kafir lily (*Clivia* spp.) (Han and Choi, 1994); bacterial spot on bejamina (Choi et al., 1989); bacterial soft rot on begonia, violet, and Scarlet kafir lily (Han and Choi, 1994); bacterial blight on chrysanthemum (Choi and Han, 1992); and bacterial rot on gladiolus (Choi and Han, 1992). However, there is no report of bacterial disease on lily yet.

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The ratio of lily bulb rot during storage averaged 40.9%, ranging from 10 to 90%. From 1999 to 2000, diseased lily bulbs were collected from low temperature storage houses or from the fields. This study reports the result of isolation and identification of the causal bacteria of diseased lily bulbs.

Rotten lily bulbs were collected and the ratio of rotten bulbs were surveyed from low temperature storages of lily farms and fields from 1999 to 2000 in Taean, Chungnam. Fifty (50) rotten bulbs were selected from a block. After a short disinfection with 0.35% NaOCl and 70% ethanol, diseased bulb tissue was placed in a micro-tube containing sterile water, and ground with a small pestle. Diluted solution was plated on nutrient agar medium (NA). The plates were incubated at 28°C for 48 hours. Single colony was isolated from the plate, diluted to 20% glycerol, and then stored at -80°C for further use.

For the pathogenicity test, the isolates were incubated for 48 hours on NA and adjusted to 10^8 cells/ml with sterile water. Pin-pricked lily bulbs were inoculated with the inoculum and placed at 28°C under humid condition. Occurrence of rotten bulb was examined at 2, 3, 5, and 7 days after inoculation. Water-soaked lesion developed 48-72 hours after inoculation on pin-pricked bulb. The lesion was similar to the natural symptom observed in the storages or in the fields (Fig. 1).

The isolates from lily bulbs were identified as similar to those described by Schaad (1988). The isolates, which were pathogenic to lily bulb, were divided into two groups based on bacteriological characteristics (Table 1). Twelve isolates belonging to Group I were Gram-negative, anaerobic, having more than four flagella, did not produce yellow or orange pigment on nutrient broth yeast-extract agar (NBY) medium, non-fluorescence on King's B (KB) medium, and did not produce mycelium or spore. Thus, the bacteria belonging to Group I were classified as *Erwinia* based on Schaad (1988). Two isolates belonging to Group II were Gram-negative, aerobic, did not produce yellow or orange pigments on NBY medium, fluorescence on KB medium, and did not produce mycelium or spore. Thus, the bacteria

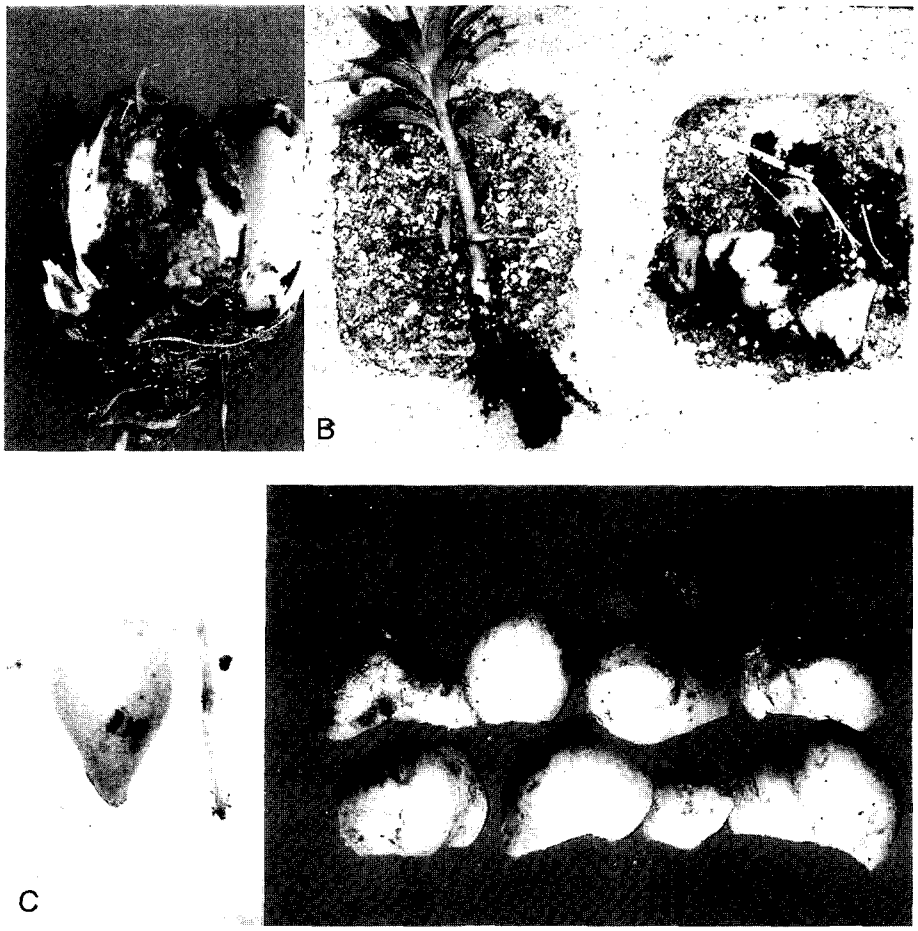

Fig. 1. Symptoms of soft rot on lily bulbs caused by *Pectobacterium carotovorum* subsp. *carotovorum* or *Pseudomonas marginalis*. Typical symptoms developed on the bulbs at low temperature storage (**A**) or in the field (**B**). Symptoms observed after artificial pin-pricking inoculation (**C** and **D**).

belonging to Group II were classified as *Pseudomonas* based on Schaad (1988).

The bacteria in Group I were positive for gelatin liquefaction, did not reduce nitrate, and did not produce indole and reducing sugar from sucrose. In addition, they produced acid from arabinose, glucose, lactose, mannitol, sorbitol, or sucrose, but did not produce acid from maltose (Table 2). These characteristics were the same as those of *Pectobacterium carotovorum* subsp. *carotovorum* (*Erwinia carotovora* subsp. *carotovora*) as described by Dickey and Kelman (1988), Lelloitt (1984), and Tsuchiya et al. (1979). Therefore, these bacteria were classified as *Pectobacterium*

Table 1. Comparison of the characteristics of the present isolates from rotten lily bulbs with those of genera *Pectobacterium* and *Pseudomonas*

Characteristics	Present isolates		*Pectobacterium*[a]	*Pseudomonas*
	Group I (n=12)	Group II (n=2)		
Gram stain	–[b]	–	–	–
Anaerobic growth	+	–	+	–
More than four peritrichous flagella	+	–	+	–
Yellow or orange colonies on NBY medium	–	–	V	–
Fluorescent pigment on KB	–	+	–	V
Growth on D-1 agar	–	–	–	–
Spores formed	–	–	–	–

[a] Details of the genera *Pectobacterium* and *Pseudomonas* are described by Schaad, N. W. (1988).
[b] Symbols: + = Positive; – = negative; V = variable.

Table 2. Species identification of the present isolates belonging to Group from rotten lily bulbs

Characteristics	Present isolates (n=12)	*P. c.* subsp. *carotovorum*[a]		
		D	L	T
Gelatin liquefaction	+	+	+	+
Nitrate reduction	–		–	–
Indole production	–	–	–	–
Reducing substances from sucrose	–	–		
Acid from:				
Arabinose	+		+	
Cellobiose	V	+	+	+
Glucose	+		+	+
Lactose	+	+	+	+
Maltose	–	–	V	V
Mannitol	+		+	+
Rhamnose	V		+	+
Sorbitol	+		+	V
Sucrose	+		+	+

[a]Details of the species *Pectobacterium carotovorum* subsp. *carotovorum* are described by Dickey and Kelman (1988), Lelliott (1984), and Tsuchiya et al. (1979).
[b]Symbols: + = Positive; – = negative; V = variable.

Table 3. Species identification of the present isolates belonging to Group from rotten lily bulbs

Characteristics	Present isolates (n=12)	*P. amarginalis*[a]
Levan formation	+[b]	+
Oxidase activity	+	+
Arginine dihydrolase	+	+
Nitrate to N_2	–	–
Growth at 41°C	–	–
Potato rot	+	+
Used for growth:		
Mannitol	+	+
Cellobiose	V	–
Sorbitol	+	+
Trehalose	+	+
Sucrose	+	+
D-Tartrate	–	V
D-Arabinose	–	–
L-Rhamnose	V	V

[a]Details of the species *Pseudomonas marginalis* are described by Hildebrand et al. (1988).
[b]Symbols: + = Positive; – = negative; V = variable.

carotovorum subsp. *carotovorum*.

Bacteria Group II produced levan, were positive to oxidase or arginine dihydrolase activity, did not reduce nitrate, caused potato rot, and did not grow at 41°C. Meanwhile, they grew using mannitol, sorbitol, trehalose or sucrose, but did not grow using D-tartrate or D-arabinose (Table 3). These characteristics were the same as those of *Pseudomonas marginalis* as described by Hildbrand et al. (1988). Thus, these bacteria were classified as *Pseudomonas marginalis*.

There was no previous report of bacterial disease caused by *Pectobacterium carotovorum* subsp. *carotovorum* or *Pseudomonas marginalis* on lily bulb in Korea. Therefore, this study proposed that the disease be named as bacterial soft rot of lily bulb.

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