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Bacterial Soft Rot of *Dendrobium phalaenopsis* and *Phalaenopsis* Species by *Erwinia chrysanthemi*

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Occurrence of soft rots was observed on *Dendrobium phalaenopsis* and *Phalaenopsis* sp. that were grown at the greenhouses in Sunchon and Kwangyang areas, Chonnam province of Korea in 1997 and 1998. Typical soft rot symptom appeared frequently on young plants of *D. phalaenopsis* and *Phalaenopsis* sp. Soft rot symptom usually appeared on old leaves of *D. phalaenopsis*, and extended into whole leaves, accompanying blighting of whole plants. Symptom began as a small water-soaked lesion on old leaves of *Phalaenopsis* sp., which enlarged rapidly on the leaves and eventually resulted in soft rots of whole plants. The causal organism isolated from the infected lesions was identified as *Erwinia chrysanthemi* based on its pathogenicity, physiological and biochemical characteristics, and the results of the BIOLOG™ program. The bacterial soft rot caused by *E. chrysanthemi* was firstly described in *D. phalaenopsis* and *Phalaenopsis* sp. in Korea.

Keywords : bacterial soft rot, *Dendrobium phalaenopsis*, *Erwinia chrysanthemi*, *Phalaenopsis* sp.

Orchid is one of the most popular ornamental plants all over the world. Recently, demand of orchid plants in Korea is rapidly growing due to its aesthetic relief. Orchid plants are cultivated by about 500 commercial farmers in Korea (Orchid Research Society, 1994).

At present time, mass production of orchid in Korea is mostly achieved in a greenhouse. Cultural practice of heating and heat conservation of a greenhouse in a cold season inevitably induces high humidity which promotes occurrence and development of several plant diseases (Ishii, 1973; Park et al., 1996; Uchida, 1994). Especially, young orchid plants grown under close spacing with poorly air-circulated condition in the greenhouse are extremely vulnerable to the infection of several plant pathogens and once infected, severe damage to whole plants in a greenhouse can occur.

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Bacterial soft rot on young plants of *Dendrobium phalaenopsis* and *Phalaenopsis* sp. was firstly observed in the greenhouses located at Kwangyang and Sunchon areas, Chonnma province of Korea in 1997. Here, we present results on identification of the bacterial pathogen isolated from the infected lesions of young plants of *D. phalaenopsis* and *Phalaenopsis* sp. and also sensitivity of the pathogen to several antibiotics used for bacterial disease control.

Materials and Methods

Isolation of bacterial strains. Small tissue pieces were cut from the edge of the diseased area on the leaves of *D. phalaenopsis* and *Phalaenopsis* sp. treated with 95% ethyl alcohol for 1 min and rinsed with sterile water, followed by placing on nutrient agar at 28°C for 2-3 days. Single colonies were isolated from the culture and used for further studies.

Pathogenicity test. Each strain isolated from *D. phalaenopsis* and *Phalaenopsis* sp. was diluted into 10^8 cells/ml to prepare inoculum for pathogenicity test. The leaves of *D. phalaenopsis* and *Phalaenopsis* sp. at 4-leaf stage were inoculated with a bacterial suspension after wounding with sterilized pins until run-off. The materials were wrapped with polyethylene film to give 100% relative humidity and kept at 28°C in a growth chamber. Three plants were inoculated in each experiment with three replicates. Disease severity was rated 7 days after inoculation.

Characterization of bacterial strains. Bacterial characteristics of strain DEN-C isolated from *D. phalaenopsis* and strain PAL-A isolated from *Phalaenopsis* sp. were investigated by the methods of Schaad (1988) and Dickey and Kelman (1988). Gram staining, colony color on nutrient glucose agar, fluorescent pigment production on King's B agar, anaerobical growth, flagella type and formation of spores were investigated in order to identify the genus of the strains. The morphology of the bacterial cells was also investigated with the aid of electron microscopy after staining with 2% phosphotunstic acid (PTA, pH 7.0). Several biochemical tests such as pectate degradation, gelatin liquefaction, acetoin production, sensitivity to erythromycin, phosphatase activity, indole production, production of reducing substances from sucrose, growth at 36-37°C, and production of acids from D-lactose, palatinose, trehalose, maltose, α-methyl glucoside, dulcitol and melezitose were also investigated for the identification of the strains at the species level.

Utilization of 95 carbon sources by strains DEN-C and PAL-A was also investigated in the GN 96 well microplate (BIOLOG, Inc., U.S.A.) in order to identify the species of the strains using BIOLOG™ program.

Investigation of antibiotic sensitivity. Several antibiotics generally used to control bacterial diseases were screen to determine the sensitivity of strains DEN-C and PAL-A to the antibiotics by paper disc method. Combined use of streptomycin sulfate and oxytetracycline or kasugamycin and copper oxychloride and single use of carbenicylin, streptomycin, validamycin-A or kasugamycin were employed in this study. The strains were streaked on the nutrient agar plate and paper disc containing 250 μg/ml or 500 μg/ml of antibiotics was placed on the center of the plate, respectively. Diameter of clear zones formed around the paper discs was measured 2-3 days after incubation.

Results

Symptom. Soft rot symptom appeared on old leaves of *D. phalaenopsis*, and extended into whole leaves, accompanying blighting of whole plants (Fig. 1A). Symptom began as a small water-soaked lesion on old leaves of *Phalaenopsis* sp. and then, enlarged rapidly on the leaves and eventually resulted in soft rots of whole plants (Fig. 1B). The diseases were transmitted easily and rapidly among seedlings which were cultivated in a small pot under moist greenhouse conditions.

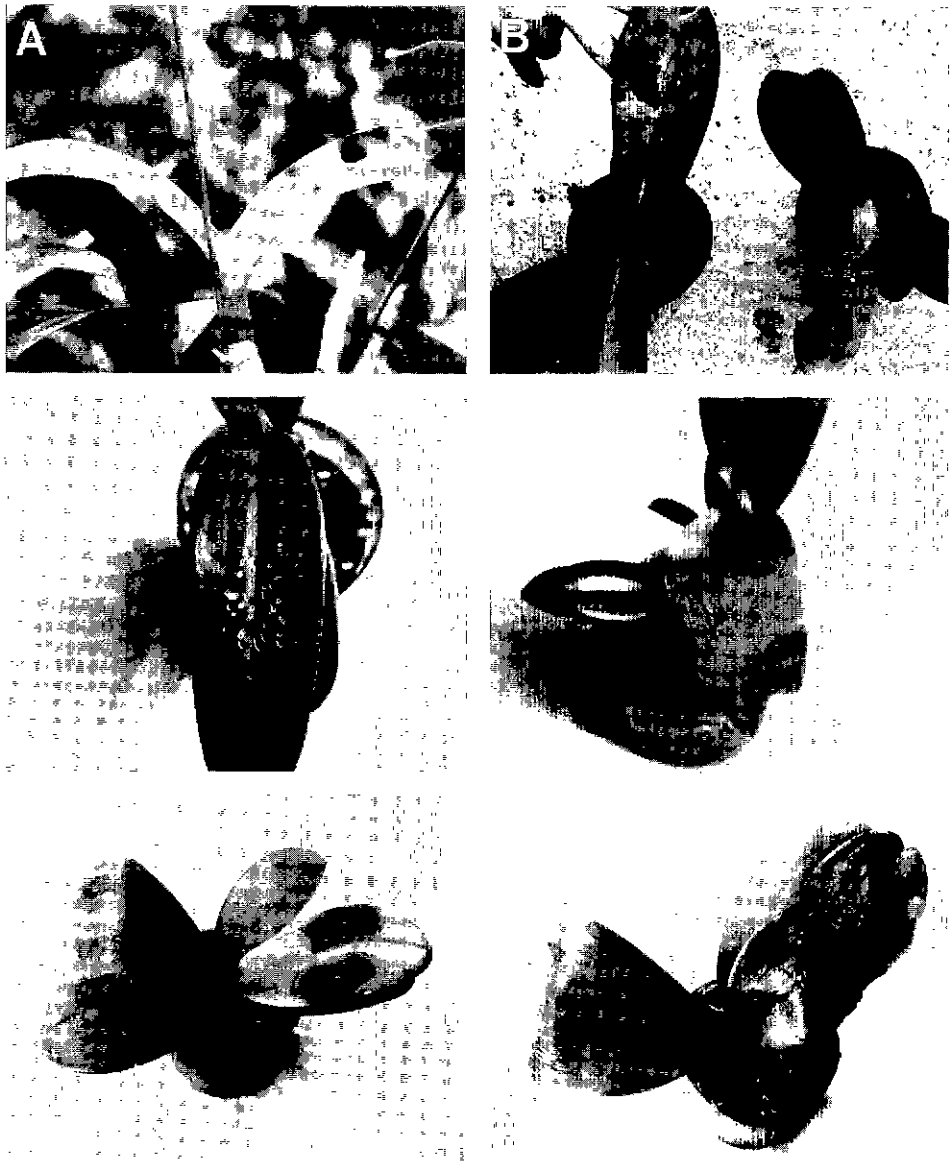

Fig. 1. Typical symptoms of soft rot on *Dendrobium phalaenopsis* (A) and *Phalaenopsis* sp. (B) and soft rot symptoms on *D. phalaenopsis* (C and D) and *Phalaenopsis* sp. (E and F) induced by artificial inoculation of *Erwinia chrysanthemi* strains DEN-C and PAL-A, respectively.

Table 1. Pathogenicity of *Erwinia chrysanthemi* strain DEN-C isolated from *Dendrobium phalaenopsis* and *E. chrysanthemi* strain PAL-A isolated from *Phalenopsis* sp. on leaves of *D. phalaenopsis* and *Phalaenopsis* sp., respectively

Stain	Pathogenicity[a]	
	Dendrobium phalenopsis	*Phalenopsis* sp.
DEN-C	+	++
PAL-A	++	++

[a] Pathogenicity was evaluated by severity of soft rot 3 days after inoculation on leaves. +: mild symptom, ++: severe symptom.

Pathogenicity of the strains isolated. Strain DEN-C isolated from *D. phalaenopsis* and strain PAL-A isolated from *Phalaenopsis* sp. caused typical soft rot symptoms on leaves of *D. phalaenopsis* and *Phalaenopsis* sp. 12-24 hours after artificial wound inoculation, respectively. Especially, the leaves of *Phalaenopsis* sp. began to be rotted 24 hours after inoculation of strains DEN-C and PAL-A, respectively. PAL-A induced large lesions on *D. phalaenopsis* and *Phalaenopsis* sp., respectively (Fig. 1E and 1F). DEN-C induced similar lesions on *Phalaenopsis* sp. (Fig. 1D) but it induced relatively small lesions on *D. phalaenopsis* until 3 days after inoculation (Fig. 1C). However, all the lesions developed rapidly thereafter and the whole plants resulted in death by wilting 1 week after inoculation in all treatments.

Identification of the pathogenic bacteria. Strains of DEN-C and PAL-A were gram-negative and formed grayish white colonies 2 days after incubation on nutrient glucose agar. They did not form fluorescent pigments on King's B agar and grew anaerobically. They also had more than four peritrichous flagella and did not form spores (Table 2). Electron microscopic observation exhibited that the bacterial cells were bacillus form with peritrichous flagella, which is one of the typical characteristics of *Erwinia* spp. (Fig. 2).

Strains DEN-C and PAL-A identified as *Erwinia* spp. showed positive reactions on the tests of pectate degradation, gelatin liquefaction, acetoin production, sensitivity to erythromycin, phosphatase activity and indole production, and they grew at 36-37°C but did not produce reducing substances from sucrose. They also produced acids from D-lactose and palatinose, but not from trehalose, maltose, α-methyl glucoside, dulcitol and melezitose (Table 3). These characteristics were in accordance with those of *E. chrysanthemi* which were described by Dickey and Kelman (1988). Therefore, the pathogenic bacteria isolated from *D. phalaenopsis* or *Phalaenopsis* sp. were finally identified as *E. chrysanthemi*.

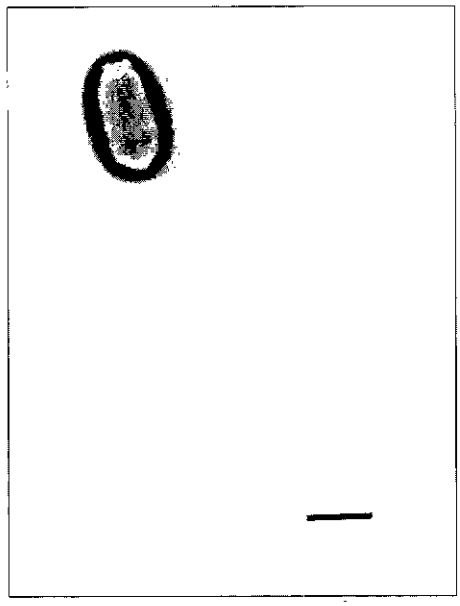

Fig. 2. Electron microscopic morphology of *Erwinia chrysanthemi* strain PAL-A isolated from *Phalaenopsis* sp. Scale bar represents 1 μm.

Strain DEN-C utilized 34 carbon sources including N-acetyl-D-galactosamine but not 61 carbon sources including α-cyclodextrin in the GN 96 well microplate (BIOLOG, Inc., U.S.A.). Strain PAL-A utilized 40 carbon sources but not 55 carbon sources. Therefore, strains DEN-C and PAL-A were also identified as *E. chrysanthemi* with similarities of 94% and 88% in the BIOLOG™ program,

Table 2. Genus identification of the bacterial strains DEN-C and PAL-A isolated from diseased *Dendrobium phalaenopsis* and *Phalaenopsis* sp., respectively

Characteristics[a]	DEN-C	PAL-C	*Erwinia*[b]	*Pseudomonas*	*Xanthomonas*
Gram stain	-	-[c]	-	-	-
Yellow or orange pigment on NGA	-	-	v	-	+
Fluorescent pigment on KB	-	-	-	v	-
Anaerobical growth	+	+	+	-	-
More than four peritrichous flagella	+	+	+	-	-
Spore formation	-	-	-	-	-

[a] NGA and KB are abbreviations of nutrient glucose agar and King's medium B agar, respectively.
[b] Data from Schaad(1988).
[c] +: positive reaction, −: negative reaction, V: variable.

Table 3. Comparison of bacteriological characteristics of strains DEN-C and PAL-A, determined to genus *Erwinia*, with those of *Erwinia chrysanthemi*

Characteristics	DEN-C	PAL-A	*E. chrysanthemi*[a]
Pectate degradation	+[b]	+	+
Gelatin liquefaction	+	+	V
Acetoin production	+	+	+
Sensitivity to erythromycin	+	+	+
Phosphatase	+	+	+
Indole production	+	+	V
Reducing substances from sucrose	−	−	V
Growth at 36°C	+	+	+
Acid production from			
D-Lactose	+	+	V
Trehalose	−	−	−
Maltose	−	−	−
α -Methyl glucoside	−	−	−
Palatinose	+	+	V
Dulcitol	−	−	−
Melezitose	−	−	−

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

respectively (Table 4).

Sensitivity to antibiotics. Strain DEN-C was sensitive to streptomycin sulfate + oxytetracycline and carbenicylin but insensitive to streptomycin, validamycin-A, kasugamycin and kasugamycin + copper oxychloride. Strain PAL-A was sensitive to streptomycin sulfate + oxytetracycline, carbenicylin and streptomycin but insensitive to validamycin-A, kasugamycin and kasugamycin + copper oxychloride (Table 5).

Discussion

Bacterial soft rot on *D. phalaenopsis* and *Phalaenopsis* sp. was firstly observed in the greenhouses located at Kwangyang and Sunchon areas in 1997. Bacterial pathogen isolated from the infected lesions was identified as *Erwinia chrysanthemi*. The bacterial pathogen of *Erwinia chrysanthemi.* has been reported to cause soft rot disease on several plants such as *Chrysanthemum morifolium* (Choi and Han, 1992), *Cymbidium* spp. (Jin et al., 1994), *Aloe* spp. (Jin et al., 1994), *Scindapsus aureus* (Choi and Han, 1994) and *Raphanus sativus* (Park et al., 1999). However, there have

Table 4. The results of GN microplate test of the present strains DEN-C and PAL-A isolated from *Dendrobium phalaenopsis* and *Phalaenopsis* sp., respectively

Characteristic	Result		Characteristic	Result	
	DEN-C	PAL-A		DEN-C	PAL-A
water	-	-	p-hydroxy phenylacetic acid	-	-
α-cyclodextrin	-	-	itaconic acid	-	-
dextrin	-	±	α-keto butyric acid	-	-
glycogen	-	-	α-keto glutaric acid	-	-
tween 40	-	-	α-keto valeric acid	-	-
tween 80	-	-	D,L-lactic acid	-	-
N-acetyl-D-galactosamine	-	-	malonic acid	-	±
N-acetyl-D-glucosamine	+	+	propionic acid	-	-
adonitol	-	-	quinic acid	-	-
L-arabinose	+	+	D-saccharic acid	+	+
D-arabitol	-	-	sebacic acid	-	-
cellobiose	-	±	succinic acid	+	+
i-erythritol	-	-	bromo succinic acid	+	+
D-fructose	+	+	succinamic acid	+	±
L-fucose	-	-	glucuronamide	-	-
D-galactose	+	+	alaninamide	-	-
gentiobiose	-	±	D-alanine	-	-
α-D-glucose	+	+	L-alanine	-	-
m-inositol	+	+	L-alanyl-glycine	-	-
α-lactose	-	-	L-asparagine	+	+
α-D-lactose lactulose	-	-	L-aspartic acid	+	+
maltose	±	±	L-glutamic acid	-	±
D-mannitol	+	+	glycyl-L-aspartic acid	-	-

Table 4. Continued

Characteristic	Result		Characteristic	Result	
	DEN-C	PAL-A		DEN-C	PAL-A
D-mannose	+	+	glycyl-L-glutamic acid	-	-
D-melibiose	+	+	L-histidine	-	-
β-methyl D-glucoside	+	+	hydroxy L-proline	-	-
psicose	+	+	L-leucine	-	-
D-raffinose	+	+	L-ornithine	-	-
L-rhamnose	-	-	L-phenyl alanine	-	-
D-sorbitol		-	L-proline	-	-
sucrose	+	+	L-pyro glutamic acid	-	-
D-trehalose	-	-	D-serine	-	-
turanose	-	-	L-serine	+	+
xylitol	-	-	L-threonine	-	-
methyl pyruvate	+	+	D,L-carnitine	-	-
mono-methyl succinate	+	+	γ-amino butyric acid	-	-
acetic acid	±	±	urocanic acid	-	-
cis-aconitic acid	±	±	inosine	-	-
citric acid	+	+	uridine	-	-
formic acid	+	+	thymidine	-	±
D-galactonic acid lactone	-	-	phenyl ethylamine	-	-
D-galacturonic acid	+	+	putrescine	-	-
D-gluconic acid	+	+	2-amino ethanol	-	-
D-glucosaminic acid	-	-	2,3-butanediol	-	-
D-glucuronic acid	-	-	glycerol	+	+
α-hydroxy butyric acid	-	-	D,L-α-glycerol phosphate	+	+
α-hydroxy butyric acid	-	+	glucose-1-phosphate	+	+
γ-hydroxy butyric acid	-	-	glucose-6-phosphate	+	+

[b] +: positive reaction, −: negative reaction, : weak reaction

Table 5. Sensitivity of *Erwinia chrysanthemi* PAL-A and DEN-C to several chemicals on potato dextrose agar

Antibiotics	Concentration (ppm)	Diameter of inhibition zone (mm)	
		DEN-C	PAL-A
Streptomycin sulfate + oxytetracycline Wp[a]	250	0	0
	500	12	17
Carbenicillin disodium Wp	250	18	35
	500	38	40
Streptomycin Wp	250	0	0
	500	0	17
Validamycin-A Sc	250	0	0
	500	0	0
Kasugamycin Sc	250	0	0
	500	0	0
Kasugamycin+ copper oxychloride Wp	250	0	0
	500	0	0

[a] Wp : wettable powder; Sc: suspension concentrate.

been no descriptions on the bacterial soft rot disease caused by *E. chrysanthemi* on *D. phalaenopsis* and *Phalaenopsis* sp. in Korea so far. This is the first report in Korea that *E. chrysanthemi* is the causal pathogen of bacterial soft rot on *D. phalaenopsis* and *Phalaenopsis* sp.

Supply of sufficient light and fresh air is required for the proper cultivation of *D. phalaenopsis* and *Phalaenopsis* sp. in a greenhouse. Because *D. phalaenopsis* and *Phalaenopsis* sp. are originated from tropical regions, farmers are usually tend to keep the temperature of greenhouse higher during a cold season in Korea. However, the management of high temperature in a greenhouse, escpecailly in winter, induces highly humid environment of a greenhouse on the condition of poor air circulation. Such a highly humid environmental condition in a greenhouse largely contributes to the occurrence and development of several plant diseases which are mainly responsible for the failure in the cultivation of *D. phalaenopsis* and *Phalaenopsis* sp. in Korea. Therefore, proper management of environmental conditions in a greenhouse is very crucial to prevent *D. phalaenopsis* and *Phalaenopsis* sp. from disease occurrence and development.

Several antibiotics were tested *in vitro* to screen the effec-

tive control agents against *E. chrysanthemi*. Combined use of streptomycin sulfate and oxytetracycline and single application of carbenicylin were found to be the most successful in controlling the bacterial growth. Interestingly, *E. chrysanthemi* (strain PAL-A) obtained from *Phalaenopsis* sp. in a greenhouse of Sunchon area was very sensitive to streptomycin but, the other strain of *E. chrysanthemi* (strain DEN-C) obtained from *D. phalaenopsis* in a greenhouse of Kwangyang area was resistant to the same antibiotics. This contradictive responses of the two stains of *E. chrysanthemi* to streptomycin can be explained by spraying episodes of the antibiotic in the two different areas. The bacterial strain of PAL-A has never been exposed to the antibiotics before. On the other hand, strain DEN-C has been controlled by the antibiotics in Kwangyang area for the last few years. Therefore, it is very likely that strain DEN-C already acquired resistant ability to the antibiotics. If it were true, the findings suggest that consecutive use of single antibiotics should be avoid to effectively control bacterial soft rot diseases and cross applications of several antibiotics can be one of alternatives by suppressing the emergence of resistant strain of bacterial pathogens to antibiotics.

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