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Comparative Analysis of Korean and Japanese Strains of *Pseudomonas syringae* pv. *actinidiae* Causing Bacterial Canker of Kiwifruit

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Genomic and phenotypic characteristics of the bacterial strains of *Pseudomonas syringae* pv. *actinidiae* and *P. syringae* pv. *syringae* collected from several kiwifruit orchards of Korea were investigated and compared with those from Japan to elucidate their phylogenic relationships. All the strains of *P. syringae* pv. *actinidiae* and pv. *syringae* tested were sensitive to copper sulfate but Korean and Japanese strains showed quite different responses to streptomycin. Korean strains were sensitive to streptomycin, but most of the Japanese strains of *P. syringae* pv. *actinidiae* were highly resistant to streptomycin. Japanese strains were also relatively more resistant to oxytetracycline than Korean strains. Plasmid profiles were not valuable to distinguish Korean strains of *P. syringae* pv. *actinidiae* from Japanese strains. One or more indigenous plasmids with more than 15 kb in size were detected in all strains of *P. syringae* pv. *actinidiae*, but the number and sizes of plasmids harbored in *P. syringae* pv. *actinidiae* were variable among the strains regardless of their geographic origins. There also observed no significant relationship among resistance levels of the strains of *P. syringae* pv. *actinidiae* to antibiotics, their pathogenicity and plasmid profiles. RAPD profiles were useful to analyze the strains of *P. syringae* pv. *actinidiae* and pv. *syringae*. All the strains of *P. syringae* pv. *actinidiae* fell into a wide cluster separated from the strains of *P. syringae* pv. *syringae*, but Korean strains of *P. syringae* pv. *actinidiae* were separated from Japanese strains. The results support that Korean and Japanese strains of *P. syringae* pv. *actinidiae* may have different phylogenic origins.

Keywords : bacterial canker, kiwifruit, plasmid, *Pseudomonas syringae* pv. *actinidiae*, RAPD

Bacterial canker of kiwifruit (*Actinidia deliciosa*) was first reported in Japan in early 1980s (Serizawa et al., 1989) and its occurrence has become one of the most serious limiting factors for cultivating the kiwifruits. The disease has been also reported in Korea (Koh et al., 1994), Iran (Mazarei and Mostofipour, 1994), and Italy (Scortichini, 1994). Since the disease has damaged kiwifruit production enough to destroy some of the orchards, it is very urgent to develop an efficient control strategy for the disease (Koh et al., 1994; Serizawa et al., 1989; Ushiyama, 1993).

The causal organism of bacterial canker of kiwifruit was identified as *Pseudomonas syringae* pv. *actinidiae*, which is a new pathovar of *P. syringae* by Takikawa et al. (1989). Ushiyama et al. (1992a, 1992b, 1993) inferred that the bacterium might be originated from wild *Actinidia* spp. distributed in northern areas of Japan, but its exact origin is still debatable. Bacterial canker was first observed in 1988 in Korea, 5 years after its first occurrence in Japan (Koh et al., 1994). The disease has spread rapidly throughout the cultivation areas of kiwifruit from Jeju Island where the disease was first observed in Korea. It is suspected that *P. syringae* pv. *actinidiae* might be introduced into Korea from Japan with the imported seedlings of kiwifruit in mid 1980s when bacterial canker had severely occurred in Japan. However, there has been no report on the characteristics and exact origin of the bacterium in Korea until now.

In this study, genomic and phenotypic characteristics of the bacterial strains collected from several kiwifruit orchards of Korea were investigated and compared with those from Japan to elucidate their phylogenic relationships.

Materials and Methods

Strains and media. Twenty-one strains of *P. syringae* pv. *actinidiae*, causal bacterium of canker and 2 strains of *P. syringae* pv. *syringae*, causal bacterium of blossom blight were isolated from diseased leaves or stems of kiwifruits in major cultivating areas of Korea in 1999, respectively. Ten strains of *P. syringae* pv. *actinidiae* including the pathotype

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Table 1. Strains of *Pseudomonas syringae* pv. *actinidiae* and *P. syringae* pv. *syringae* used in this study

Code	Strains	Pathovars	Geographic origins	Year of Source	Collection
1	PaS10	*P. syringae* pv. *actinidiae*	Shizuoka, Japan	1996	Dr. Serizawa
2	PaS11	*P. syringae* pv. *actinidiae*	Shizuoka, Japan	1996	Dr. Serizawa
3	PaS14	*P. syringae* pv. *actinidiae*	Shizuoka, Japan	1996	Dr. Serizawa
4	PaS28	*P. syringae* pv. *actinidiae*	Shizuoka, Japan	1996	Dr. Serizawa
5	PaS33	*P. syringae* pv. *actinidiae*	Shizuoka, Japan	1996	Dr. Serizawa
6	PaK2	*P. syringae* pv. *actinidiae*	Kanagawa, Japan	1995	Dr. Nakajima
7	PaI1	*P. syringae* pv. *actinidiae*	Kanagawa, Japan	1995	Dr. Nakajima
8	PaI2	*P. syringae* pv. *actinidiae*	Kanagawa, Japan	1995	Dr. Nakajima
9	PaB1	*P. syringae* pv. *actinidiae*	Kanagawa, Japan	1995	Dr. Nakajima
10	Kw11	*P. syringae* pv. *actinidiae*	Kanagawa, Japan	1995	Dr. Nakajima
11	CJW3	*P. syringae* pv. *actinidiae*	Bukcheju, Korea	1999	This study
12	CJW4	*P. syringae* pv. *actinidiae*	Bukcheju, Korea	1999	This study
13	CJW5	*P. syringae* pv. *actinidiae*	Bukcheju, Korea	1999	This study
14	CJW7	*P. syringae* pv. *actinidiae*	Bukcheju, Korea	1999	This study
15	CJW9	*P. syringae* pv. *actinidiae*	Bukcheju, Korea	1999	This study
16	WGD12	*P. syringae* pv. *actinidiae*	Wando, Korea	1999	This study
17	WGD15	*P. syringae* pv. *actinidiae*	Wando, Korea	1999	This study
18	WGD16	*P. syringae* pv. *actinidiae*	Wando, Korea	1999	This study
19	WGD17	*P. syringae* pv. *actinidiae*	Wando, Korea	1999	This study
20	JJY8	*P. syringae* pv. *actinidiae*	Jindo, Korea	1999	This study
21	JJG3	*P. syringae* pv. *actinidiae*	Jindo, Korea	1999	This study
22	JJG4	*P. syringae* pv. *actinidiae*	Jindo, Korea	1999	This study
23	JJG7	*P. syringae* pv. *actinidiae*	Jindo, Korea	1999	This study
24	JJG9	*P. syringae* pv. *actinidiae*	Jindo, Korea	1999	This study
25	JJG10	*P. syringae* pv. *actinidiae*	Jindo, Korea	1999	This study
26	JYG2	*P. syringae* pv. *actinidiae*	Jindo, Korea	1999	This study
27	JYG4	*P. syringae* pv. *actinidiae*	Jindo, Korea	1999	This study
28	JYG6	*P. syringae* pv. *actinidiae*	Jindo, Korea	1999	This study
29	JYG10	*P. syringae* pv. *actinidiae*	Jindo, Korea	1999	This study
30	JJJ3	*P. syringae* pv. *actinidiae*	Jindo, Korea	1999	This study
31	HMY1	*P. syringae* pv. *actinidiae*	Haenam, Korea	1999	This study
32	PSJA1	*P. syringae* pv. *syringae*	Chiba, Japan	1995	Dr. Matsuyama
33	PS5107	*P. syringae* pv. *syringae*	Chiba, Japan	1995	Dr. Matsuyama
34	PaB2	*P. syringae* pv. *syringae*	Kanagawa, Japan	1995	Dr. Nakajima
35	HMH5	*P. syringae* pv. *syringae*	Haenam, Korea	1999	This study
36	SSG2	*P. syringae* pv. *syringae*	Sunchon, Korea	1999	This study

strain Kw11 and 3 strains of *P. syringae* pv. *syringae* collected in Japan (Table 1).

Type strain Kw11 of *P. syringae* pv. *actinidiae* was kindly provided from Dr. Y. Takikawa, Shizuoka University and 5 strains of *P. syringae* pv. *actinidiae* were obtained from Dr. S. Serizawa, Shizuoka Citrus Experiment Station, Japan, respectively. The other four strains of *P. syringae* pv. *actinidiae* and one strain of *P. syringae* pv. *syringae* were obtained from Dr. M. Nakajima, Ibaraki University and the other two strains of *P. syringae* pv. *syringae*, were obtained from Dr. N. Matsuyama, Kyushu University, Japan, respectively. All the strains were kept at Department of Applied Biology, Sunchon National University, Korea. A single colony of each strain was isolated on the Peptone-Sucrose-Agar (PSA; peptone 2%, sucrose 2%, agar 1.5%, pH 6.8) medium. *P. syringae* pv. *actinidiae* and all the other strains were transferred onto fresh PSA, and cultivated at 18-20C.

Analysis of resistance to antibiotics. The isolates were tested for resistance to streptomycin and oxytetracycline by spreading 100 ul of suspensions of each strain (approximately 10^6 cfu/ml) onto PSA. After the bacterial suspension was absorbed on the surface of PSA, sterile blank Bacto concentration disks (6.35 mm diameter, Difco) were dipped

in suspensions of streptomycin sulfate or oxytetracycline containing 1, 10, 25, 50, 100, 250, 500, and 1000 ug a.i./ml, blotted to remove excess moisture, then placed equidistantly apart on the surface of PSA. Three replicate plates, each containing the eight antibiotic concentrations of streptomycin or oxytetracycline and a water control disk, were prepared for each strain. Inhibition zones, the distance from the edge of the disk to the bacterial lawn, were measured after 2 days of inoculation at 20 C. Whenever the inhibition zone was less than 1 mm, the strain was recorded as resistant to the antibiotic.

Copper sensitivity of the strain was determined by spotting 10 ul of bacterial suspension of each strain (approximately 10^6 cfu/ml) onto casitone-yeast extract-glycerol medium (CYE). Strains also were spotted onto CYE amended with cupric sulfate to obtain concentrations of 0.05, 0.1, 0.5, 1.0, 1.5, 2.0, and 4.0 mM. Plates were incubated for 2 days at 21 C, then the presence or absence of growth was determined. Three replicates of each copper concentration were included in one experiment for each isolate. Minimum inhibitory concentrations (MIC) of streptomycin, oxytetracycline, and copper of each strain were determined based on the growth results on the media tested.

Isolation and analysis of plasmid DNA. Indigenous plasmid DNAs of *P. syringae* pv. *actinidiae* and pv. *syringae* were isolated by the alkaline lysis extraction procedure (Maniatis et al., 1982; Sambrook et al., 1989). The single colony of each strain was inoculated in 5 ml nutrient broth (peptone 5%, beef extract 3%, pH 7.4) at 20 C for 24 hours, and then harvested by centrifugation for 15 min at 3000 × g. Lysis of bacterial cells was performed at room temperature for 5 min in a solution of 50 mM glucose, 25 mM Tris. HCl, 10 mM EDTA, and lysozyme, followed by incubation on ice for 5 min with 2 volumes of 0.2 N NaOH and 1% SDS. The plasmid DNA was separated from chromosomal DNA and cell wall debris by adjusting the solution from alkaline (pH 12.0-12.5) to a neutral condition (pH 7.0) by adding sodium acetate to a final concentration of 1 M followed by centrifugation for 15 min at 3000 × g. Plasmid DNA was purified by phenol/chloroform extraction and ethanol precipitation. They were visualized with ultraviolet light after staining in ethidium bromide. Sizes of DNA fragments were estimated using 5 kb DNA ladder as a molecular size standard. All plasmid DNA experiments were repeated at least twice.

Extraction and purification of genomic DNA. The genomic DNAs of *P. syringae* pv. *actinidiae* and pv. *syringae* were extracted by the alkaline lysis method for PCR and Southern blot analysis (Maniatis et al., 1982; Sambrook et al., 1989). The single colony of each strain was inoculated in nutrient broth (peptone 0.5%, beef extract 0.3%, pH 7.4) and cultivated in a shaking incubator at 20 C for 24 hours. The bacterial pellet was harvested in 50 ml of oak ridge tube after centrifugation in 3,500 rpm for 5 min, followed by suspension in a solution of 50 mM glucose, 25 mM Tris-HCl (pH 8.0), 10 mM EDTA and 2 mg/ml lysozyme. The suspension was kept at 50C for 10 min. after adding 10% of SDS and then treated with RNase, followed by incubation at 37 C for 6 hours after adding Pronase (10 mg/ml protease A, 10 mM Tris, pH 7.6) for protein decomposition. The suspension was extracted with TE-saturated phenol and TE-saturated phenol : chloroform : isoamyl alcohol (25 : 24 : 1, v/v/v) one time each, followed by extracting two times with chloroform : isoamyl alcohol (24 : 1, v/v) and precipitating with cold ethyl alcohol. The precipitated genomic DNA was washed with 70% of cold ethyl alcohol and dried. The genomic DNA was melted with TE-buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0) and kept at 4C for further studies.

Random Amplified Polymorphic DNA (RAPD) analysis. One hundred and sixty random primers including 40 commercial 10-mer oligonucleotide primers (Operon kit A, B; USA) and 120 commercial 12-mer oligonucleotide primers (Wako kit A, C; Japan) and 2 synthesized 20-mer oligonucleotide primers (Bioneer Co., Korea) were used to analyze the RAPD profiles of 31 strains of *P. syringae* pv. *actinidiae* and 5 strains of *P. syringae* pv. *syringae*. Amplification was performed in a total volume of 10 ul reaction mixture with 50 pmol of primer, 60 ng of genomic DNA, 0.2 Unit of *Taq* DNA polymerase, 1X reaction buffer (including 2 mM $MgCl_2$), and 1 mM of dNTPs (dCTP, dGTP, dATP, dTT), and extra sterilized distilled water. The PCR was carried out in a GeneAmp PCR system 9600 (Perkin-Elmer Cetus). The amplification profile was an initial denaturation step of 94 for 2 min followed by 45 cycles of 94 for 15 sec, 45 for 30 sec, 72 for 90 sec, and a final extension step of 72 for 5 min.

Cluster analysis. RAPD patterns of genomic DNAs with each of the random primers used were compared for the 31 strains of *P. syringae* pv. *actinidiae* and 5 strains of *P. syringae* pv. *syringae*. Presence (coded 1) or absence (coded 0) of each fragment was recorded. A dendrogram was constructed based on the binomial data matrix of RAPD analysis, using a unweighted pair group method with an arithmetic average (UPGMA) option in the Numerical Taxonomy System for Personal Computer (NYSYS-pc) (Rohlf, 1993).

A dendrogam was also constructed using the same binomial data matrix based on presence or absence of each

plasmid on the plasmid profiles for the 31 strains of *P. syringae* pv. *actinidiae* and 5 strains of *P. syringae* pv. *syringae*.

Table 2. Minimal inhibitory concentration of chemicals and pathogenicity on leaves of kiwifruit in strains of *Pseudomonas syringae* pv. *actinidiae* and *P. syringae* pv. *syringae*

Strain	Minimal inhibition concentration[a]			Patho-genicity[b]
	Streptomycin (ug/ml)	Oxytetracy-cline (ug/ml)	Copper sulfate (mM)	
PaS10	500	50	0.5	++
PaS11	10	10	0.5	+
PaS14	500	50	0.5	+
PaS28	10	50	0.5	+
PaS33	500 <	50	0.5	++
PaK2	500	10	0.5	+
PaI1	500	10	1	+
PaI2	500	50	0.5	++
PaB1	500	10	0.5	+
KW11	10	10	0.5	++
CJW3	10	1	0.5	++
CJW4	10	1	0.5	+
CJW5	10	1	0.5	++
CJW7	10	1	0.5	++
CJW9	10	1	0.5	++
WGD12	10	1	0.5	+
WGD15	10	1	0.5	++
WGD16	10	1	0.5	++
WGD17	10	10	0.5	+
JJY8	10	25	0.5	++
JJG3	10	1	0.5	++
JJG4	10	1	0.5	+
JJG7	10	1	0.5	++
JJG9	10	1	0.5	++
JJG10	10	10	0.5	++
JYG2	10	10	0.5	++
JYG4	10	1	0.5	+
JYG6	10	1	0.5	+
JYG10	10	25	0.5	+
JJJ3	10	10	0.5	++
HMY1	10	1	0.5	++
PSJA1	10	1	0.5	+
PS5107	100	10	0.5	±
PaB2	10	25	0.5	±
HMH5	10	25	0.5	−
SSG2	10	25	0.5	±

[a]Determined two days after incubation on peptone sucrose agar or caston yeast extract glucose agar at least three times.
[b]Tested by artificial wound inoculation on leaf of kiwifruit. ++, water-soaked necrotic lesion with halo; +, necrotic spot; ±, hypersensitive lesion; −, no lesion.

Results

Resistance of bacterial strains to antibiotics and pathogenicity. All of the Korean strains of *P. syringae* pv. *actinidiae* and pv. *syringae* were highly sensitive to streptomycin at 10 ug a.i./ml, whereas 7 out of the 10 Japanese strains of *P. syringae* pv. *actinidiae* and one strain of the 3 Japanese strains of *P. syringae* pv. *syringae* were highly resistant to streptomycin at 500 ug a.i./ml (Table 2). All of the Korean strains of *P. syringae* pv. *actinidiae*, except the strains JJY8 and JYG10, were also sensitive to oxytetracycline at 10 ug a.i./ml, whereas 5 strains of the 10 Japanese strains of *P. syringae* pv. *actinidiae* were resistant to oxytetracycline at 50 ug a.i./ml. In contrast, all of the Korean and Japanese strains of *P. syringae* pv. *actinidiae* were sensitive to copper sulfate. There was no significant relationship among resistance levels of the strains of *P. syringae* pv. *actinidiae* to antibiotics and their pathogenicity (Table 2).

Profiles of plasmids. One or more indigenous plasmids with more than 15 kb in size were detected in all strains of *P. syringae* pv. *actinidiae*, but the number and sizes of plasmids harbored in *P. syringae* pv. *actinidiae* were variable among the strains regardless of their geographic origins (Fig. 1 and 2). Most of the Korean strains had 4 plasmids but all of the 4 strains (WGD12, WGD15, WGD16, and WGD17) from Wando Island and one strain (JJY8) from the 8 strains from Jindo Island have 6 plasmids. Three Japanese strains (PaS10, PaS33, and PaI2) had 4 plasmids like most of the Korean strains but the pathotype strain KW11 had one plasmid with approximately 20 kb in size and the other 6 strains had 1-3 plasmids with different sizes. On the contrary, no plasmid

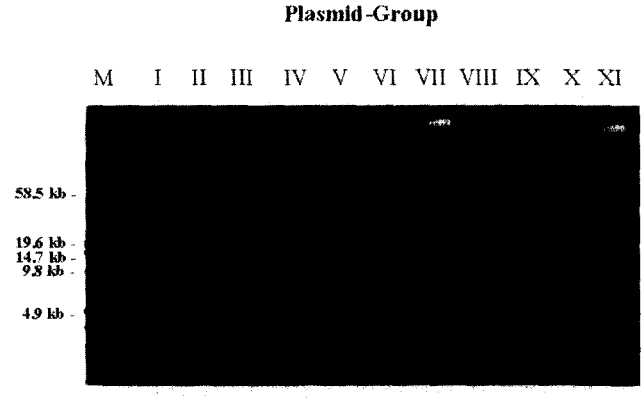

Fig. 1. Agarose gel electrophoresis of 11 different plasmid groups from 31 strains of *Pseudomonas syringae* pv. *actinidiae* and 5 strains of *P. syringae* pv. *syringae* collected from kiwifruit orchards in Korea and Japan. M indicates molecular marker of 5 kb DNA ladder. I to XI indicate 11 different plasmid groups.

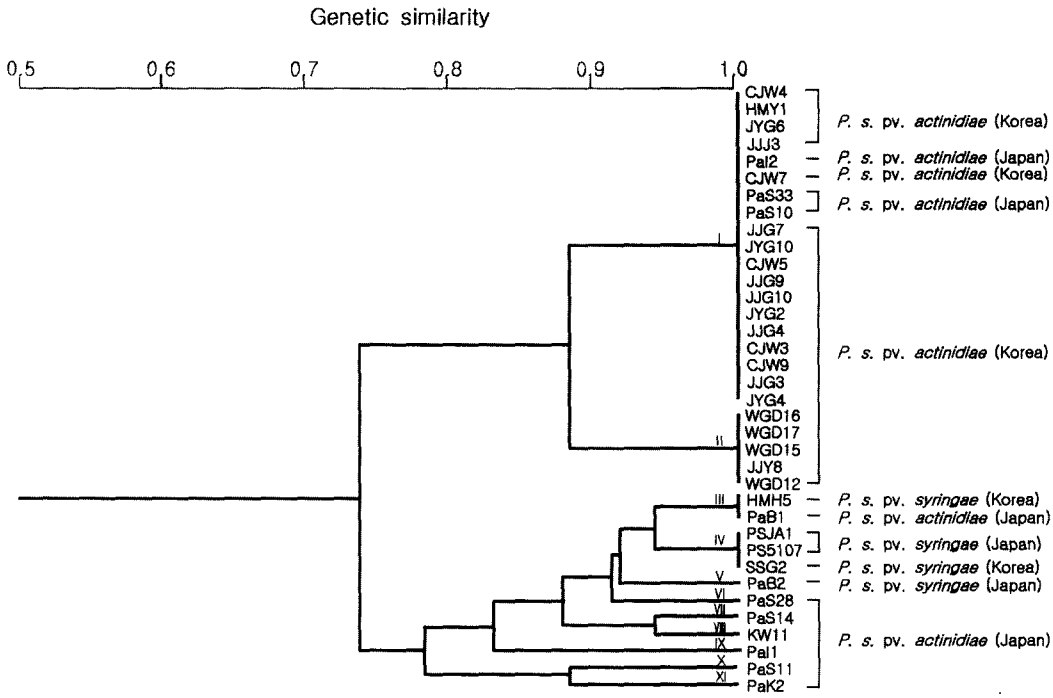

Fig. 2. Dendrogram derived from the profiles of plasmids in the 31 strains of *Pseudomonas syringae* pv. *actinidiae* and 5 strains of *P. syringae* pv. *syringae*. I to XI indicate 11 different plasmid groups in Fig. 1.

Table 3. Nucleotide sequences of the 8 random primers with total number of informative DNA amplimers obtained with each primer in RAPD experiments

Primer	Sequence (5' → 3')	Informative amplimers	Size range (kb)	Remark
OPA-13	CAG CAC CCA C	9	0.5 ~ 1.6	Operon Tech.
A-24	CTC CTG CTG TTG	11	0.5 ~ 3.0	Wako Co.
A-29	GGT TCG GGA ATG	10	0.7 ~ 3.0	Wako Co.
A-44	GAC GGT TCA AGC	12	0.4 ~ 3.0	Wako Co.
C-24	CCT TGG CAT CGG	15	0.1 ~ 1.5	Wako Co.
C-44	CGC AGC CGA GAT	11	0.5 ~ 3.0	Wako Co.
OLD-F	CAC GAT ACA TGG GCT TAT GC	9	0.2 ~ 3.0	Bioneer Co.
OLD-R	CTT TTC ATC CAC ACA CTC CG	6	0.3 ~ 1.4	Bioneer Co.

was detected in the Korean strain SSG2 and two Japanese strains PS5107 and PSJA1 of *P. syringae* pv. *syringae*, and a peculiar plasmid was detected from the Korean strain HMH5 and the Japanese strain PaB2, respectively.

Profiles of RAPDs. The selected 8 primers amplified 9-15 DNA fragments ranged from 100 to 3000 bp in size, respectively, and produced 83 amplified informative DNA fragments (Table 3) (Fig. 3). Cluster analysis of genetic distance based on the RAPDs generated a dendrogram of relationships between strains of *P. syringae* pv. *actinidiae* and *P. syringae* pv. *syringae* (Fig. 4). All strains of *P. syringae* pv. *actinidiae* fell into a wide cluster separated from the strains of *P. syringae* pv. *syringae*, but all Korean strains of *P. syringae* pv. *actinidiae* were separated from all the Japanese strains.

Discussion

Bacterial canker disease has become one of the most serious limiting factors for cultivating the kiwifruits in Korea (Koh et al., 1994). Population biological studies might be helpful to develop a reasonable control strategy against the disease. However, unfortunately, there is no basic data to characterize the populations of *P. syringae* pv. *actinidiae* distributed in Korea and, moreover, their origin is still unknown, because the disease has occurred on the kiwifruits newly introduced into Korea in recent years.

Control of bacterial canker has mainly depended on spray of bactericides (Koh et al., 1999; Serizawa, 1989;

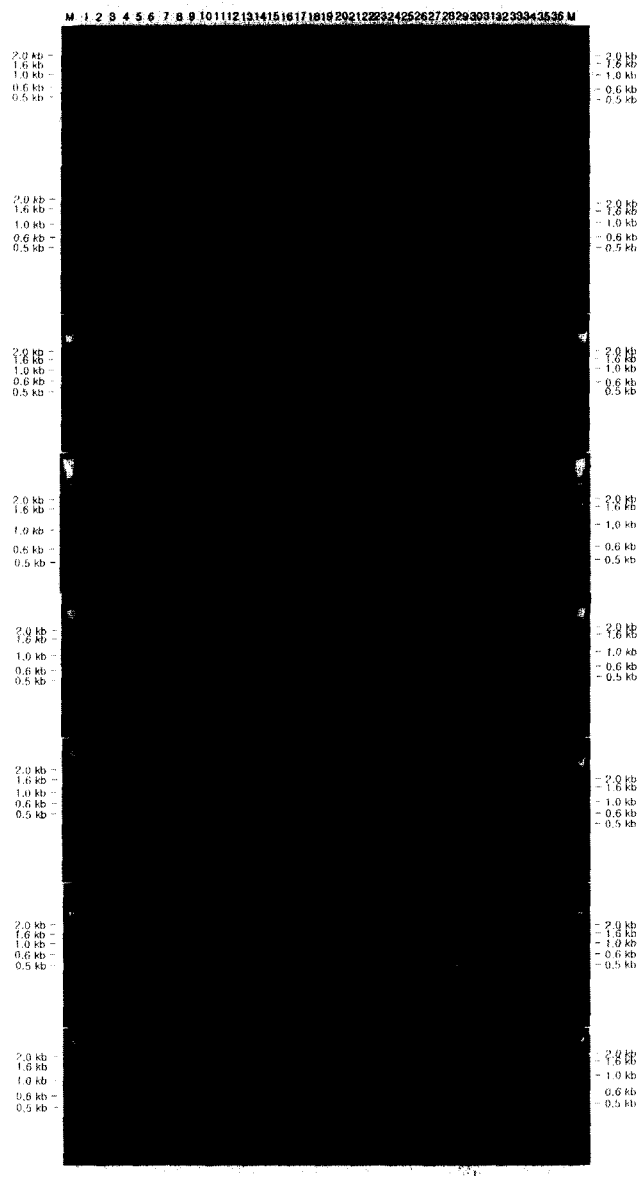

Fig. 3. RAPD patterns of the 31 strains of *Pseudomonas syringae* pv. *actinidiae* and 5 strains of *P. syringae* pv. *syringae*. Lanes marked 1 to 36 are the strains listed in Table 1. M indicates molecular marker of 1 kb DNA ladder.

Ushiyama, 1993). Routine use of bactericides for the control of bacterial canker inevitably results in resistant strains (Sundin and Bender, 1993). The occurrence of resistant strains to streptomycin and copper has been reported as a serious problem to control bacterial canker in kiwifruit in Japan (Goto et al., 1994; Nakajima et al., 1995; Ushiyama 1993), but not reported in Korea yet. Therefore, analysis of the occurrence of resistant strains to antibiotics might be useful to compare the population structure of the bacterial canker bacterium distributed in Korea and Japan.

In this study, all of the strains of *P. syringae* pv. *actinidiae* tested were sensitive to copper sulfate but Korean and Japanese strains showed quite different responses to streptomycin and oxytetracycline. Korean strains were sensitive to streptomycin, but most of the Japanese strains of *P. syringae* pv. *actinidiae* were highly resistant to streptomycin. Similarly, Japanese strains were also relatively more resistant to oxytetracycline than Korean strains. Considering that Japanese strains were collected three to four years earlier than Korean strains collected in 1999, Japanese strains were much more resistant to streptomycin and oxytetracycline than Korean strains. These results seem to be related to the history of use of antibiotics as well as epidemics of bacterial canker of kiwifruit in Japan and Korea. The most popular antibiotic, Agrimycin® WP (streptomycin sulfate + oxytetracycline), has been used to control the disease more than 15 years in Japan since mid 1980s, but the antibiotics began to use in kiwifruit orchards in Korea in recent years, because the disease occurred 5 years or more later in Korea than in Japan. Although we could not find a streptomycin-resistant strain of *P. syringae* pv. *actinidiae* in Korea, Han et al. (2003b) confirmed that strains of *P. marginalis* collected from phylloplanes of kiwifruit in 1998 had already had *strA-strB* genes on chromosomes. Since these commensal bacteria can serve as reservoirs of resistance genes, streptomycin-resistant strains may occur in the populations of *P. syringae* pv. *actinidiae* distributed in Korea sooner or later.

The number and sizes of plasmids harbored in *P. syringae* pv. *actinidiae* were variable among the strains regardless of their geographic origins. Therefore, plasmid profiles were not valuable to distinguish Korean strains of *P. syringae* pv. *actinidiae* from Japanese strains. There also observed no significant relationship between resistance levels of strains of *P. syringae* pv. *actinidiae* and their plasmid profiles. Four common plasmids were detected from 5 Korean strains (WGD12, WGD15, WGD16, WGD17, and JJY8) and 3 Japanese strains (PaS10, PaS33, and PaI2) but the Korean strains were highly sensitive to streptomycin but the Japanese strains were highly resistant. On the contrary, some pairs of strains of *P. syringae* pv. *actinidiae* had same levels of resistance to streptomycin even though they had different plasmids in number and size. This suggests that streptomycin-resistance genes are not linked to plasmids of *P. syringae* pv. *actinidiae*. However, we cannot exclude the possibility that genetic changes were not detected in the number or size of plasmids because of minor variations such as changes of base sequences, even though streptomycin-resistance genes are directly linked to plasmids.

RAPD experiments have been used for rapid detection and identification of plant pathogenic bacteria or for precise analysis of their structure (Chung et al., 1997; Hartung et al., 1993; Leite et al., 1995). RAPD became one of the most

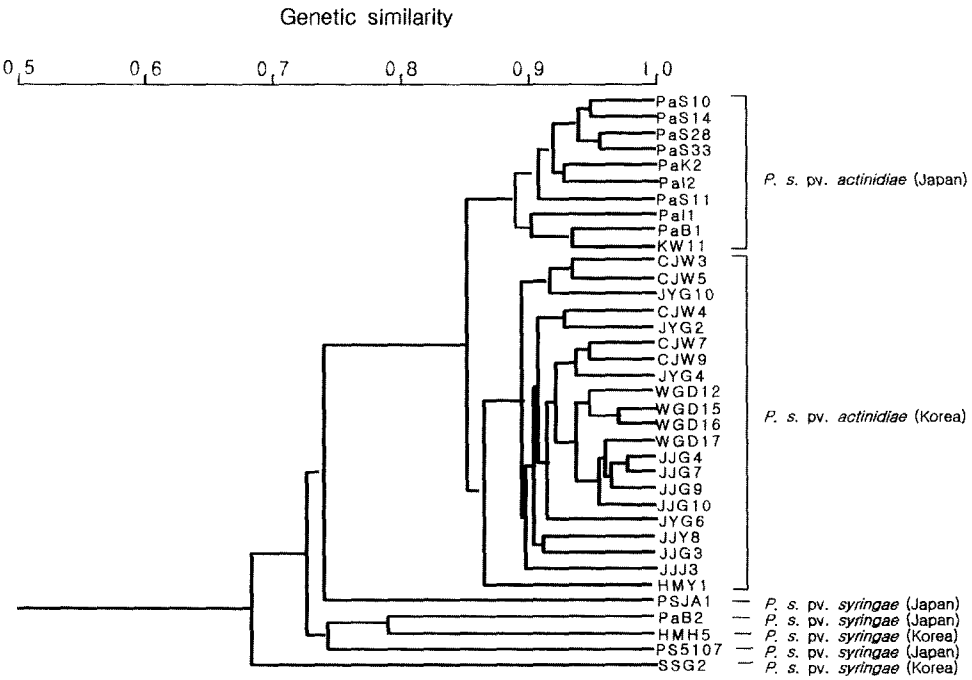

Fig. 4. Computer generated dendrogram of the 31 strains of *Pseudomonas syringae* pv. *actinidiae* and 5 strains of *P. syringae* pv. *syringae* produced from the similarity matrix using UPGMA cluster analysis of pooled random arbitrary polymorphic DNA of genomic DNAs.

powerful techniques to elucidate the structure of the populations of plant pathogens, because it makes it possible to investigate quickly and precisely genetic variations occurred among a number of strains within a short time. In this study, RAPD profiles were useful to analyze the strains of *P. syringae* pv. *actinidiae* and pv. *syringae*. All the strains of *P. syringae* pv. *actinidiae* fell into a wide cluster separated from the strains of *P. syringae* pv. *syringae*, but Korean strains of *P. syringae* pv. *actinidiae* were separated from Japanese strains. The results support that Korean and Japanese strains of *P. syringae* pv. *actinidiae* may have different phylogenic origins.

Many plant pathogenic Pseudomonads produce phytotoxins such as lipodepsipeptides, coronatine, phaeolotoxin, and tabtoxin as secondary metabolites (Bender et al., 1999). *P. syringae* pv. *actinidiae*, along with *P. syringae* pv. *phaseolicola* which causes halo blight in various legumes, is known as phaseolotoxin producer (Sawada et al., 1997; Tamura et al., 1989). Recently, however, Han et al. (2003a) found that Korean strains of *P. syringae* pv. *actinidiae* contained coronafactate ligase genes (*cfl*) but not phaseolotoxin. The production of coronatine, instead of phaseolotoxin, by the Korean strains of *P. syringae* pv. *actinidiae* confirms that Korean and Japanese strains of *P. syringae* pv. *actinidiae* have different phylogenic origins, even though they have identical nucleotide sequences of the 16S rDNA and 16S-23S internally transcribed spacer (Han et al., 2003a).

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References

Bender, C., Alarcon-Chaidez, F. and Gross, D. C. 1999. *Pseudomonas syringae* phytotoxins: Mode of action, regulation, and biosynthesis by peptide and polyketic synthetases. *Microbiol. Mol. Biol. Rev.* 63:266-292.

Chung, H. J., Kim, G. Y., Koh, Y. J., Nou, I. S. and Hwang, B. K. 1997. Genetic differentiation of strains of *Xanthomonas campestris* pv. vesicatoria by random amplified polymorphic DNA (RAPD). *Korean J. Plant Pathol.* 13:5-12.

Han, H. S., Koh, Y. J., Hur, J.-S. and Jung J. S. 2003a. Identification and characterization of coronatine-producing *Pseudomonas syringae* pv. *actinidiae*. *J. Microbiol. Biotechnol.* 13:110-118.

Han, H. S., Nam, H. Y., Koh, Y. J., Hur, J.-S. and Jung J. S. 2003b. Molecular bases of high-level streptomycin resistance in *Pseudomonas marginalis* and *Pseudomonas syringae* pv. *actinidiae*. *J. Microbiol. Biotechnol.* 41:16-21.

Hartung, J. S., Daniel, J. F. and Pruvost, O. P. 1993. Detection of *Xanthomonas campestris* pv. *citri* by the polymerase chain

reaction method. *App. Environ. Microbiol.* 59:1143-1148.

Koh, Y. J., Cha, B. J., Chung, H. J. and Lee, D. H. 1994. Outbreak and spread of bacterial canker in kiwifruit. *Korean J. Plant Pathol.* 10:68-72.

Koh, Y. J., Chung, H. J. and Kim, J. H. 1993. Blossom rot of kiwifruit caused by *Pseudomonas syringae*. *Korean J. Plant Pathol.* 9:300-303.

Koh, Y. J., Park, S. Y. and Lee, D. H. 1996. Characteristics of bacterial canker of kiwifruit occurring in Korea and its control by trunk injection. *Korean J. Plant Pathol.* 12:324-330.

Koh, Y. J., Seo, J. K., Lee, D. H., Shin, J. S. and Kim, S. H. 1999. Chemical control of bacterial canker of kiwifruit. *Plant Dis. Agric.* 5:95-99.

Leite, R. P. JR., Jones, J. B., Somodi, G. C., Minsavage, G. V. and Stall, R. E. 1995. Detection of *Xanthomonas campastris* pv. *vesicatoria* associated with pepper and tomato seed by DNA amplification. *Plant Dis.* 79:917-922.

Mazarei, M. and Mostofipour, P. 1994. First report of bacterial canker of kiwifruit in Iran. *Plant Pathol.* 43:1055-1056.

Nakajima, M. S., Yamashita, Y., Takikawa, S., Tsuyumu, S., Hibi, T. and Goto, M. 1995. Similarity of streptomycin resistance gene(s) in *Pseudomonas syringae* pv. *actinidiae* with *strA* and *strB* of plasmid RSF1010. *Ann. Phytopathol. Soc. Japan* 61:489-492.

Rohlf, F. J. 1993. *NTSYS-pc, Numerical Taxonomy and Multivariate Analysis System*. State Univ. of New York, Stone Brook.

Sambrook, J., Fritsch, E. F. and Maniatis, T. 1989. *Molecular Cloning: A Laboratory Manual*, 2nd ed. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York, USA.

Scortichini, M. 1994. Occurrence of *Pseudomonas syringae* pv. *actinidiae* on kiwifruit in Italy. *Plant Pathol.* 43:1035-1038.

Serizawa, S., Ichikawa, T., Takikawa, Y., Tsuyumu, S. and Goto, M. 1989. Occurrence of bacterial canker of kiwifruit in Japan : Description of symptoms, isolation of the pathogen and screening of bactericides. *Ann. Phytopathol. Soc. Japan* 55:427-436.

Sawada, H., Takeuchi, T. and Matsuda, I. 1997. Comparative analysis of *Pseudomonas syringae* pv. *actinidiae* and pv. *phaseolicola* based on phaseolotoxin-resistant ornithine carbamoyltransferase gene (*argK*) and 16S-23S rRNA intergenic spacer sequences. *Appl. Environ. Microbiol.* 63:282-288.

Takikawa, Y., Serizawa, S., Ichikawa, T., Tsuyumu, S. and Goto, M. 1989. *Pseudomonas syringae* pv. *actinidiae* pv. nov.: The causal bacterium of canker of kiwifruit in Japan. *Ann. Phytopathol. Soc. Japan* 55:37-444.

Tamura, K., Takikawa, Y., Tsuyumu, S. and Goto, M. 1989. Characterization of the toxin produced by *Pseudomonas syringae* pv. *actinidiae*, the causal bacterium of kiwifruit canker. *Ann. Phytopathol. Soc. Japan* 55:512.

Ushiyama, K. 1993. Studies on the epidemics and control of bacterial canker of kiwifruit caused by *Pseudomonas syringae* pv. *actinidiae*. *Bulletin of the Kanagawa Horticultural Experiment Station* 43:1-76.

Ushiyama, K., Kita, N., Aono, N., Ogawa, J. and Fujii, H. 1992a. Bacteria canker disease of wild actinidia plants as the infection source outbreak of bacterial canker of kiwifruit caused by *Pseudomonas syringae* pv. *actinidiae*. *Ann. Phytopathol. Soc. Japan* 58:426-430.

Ushiyama, K., Suyama, K., Kita, N. Aono, N., Ogawa, J. and Fujii, H. 1992b. Isolation of kiwifruit canker pathogen, *Pseudomonas syringae* pv. *actinidiae* from leaf spot of Tara vine (*Actinidia asguta* Planch). *Ann. Phytopathol. Soc. Japan* 58:476-478.