

## Difference of Physiochemical Characteristics Between Citrus Bacterial Canker Pathotypes and Identification of Korean Isolates with Repetitive Sequence PCRs

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The difference of carbon source utilization and fatty acid composition between the pathotypes of *Xanthomonas* strains, which causing citrus bacterial canker was compared, and the physiochemical characteristics were used to analyze relationship of the strains for the first time. The pattern of several carbon sources utilization and fatty acids composition reliably discriminated the pathotypes of *Xanthomonas* strains. The dendrogram which was constructed by 95 carbon source utilization profiles differentiated *X. axonopodis* pv. *citri* A, A<sup>\*</sup> and A<sup>v</sup> from the other pathotypes. When the dendrogram was drawn by combined analysis of carbon source utilization pattern and fatty acid composition, *X. axonopodis* pv. *aurantifolii* B, C and *X. axonopodis* pv. *citrumelo* formed a distinct cluster. The difference of carbon source utilization and fatty acid composition could be used effectively for the identification of pathotypes of citrus bacterial canker. The physiochemical characteristics strongly indicated that the strains isolated in Korea belong to *X. axonopodis* pv. *citri* A type. The cluster analysis by the band patterns of ERIC-, BOX- and REP-PCR allowed the discrimination of the pathotypes isolated from Korea. However, the rep-PCRs could not differentiate *X. axonopodis* pv. *citri* A types from A<sup>\*</sup> and A<sup>v</sup> types. The overall results of metabolic profiles and rep-PCRs strongly indicated that the Korean isolates are *X. axonopodis* pv. *citri* A type.

**Keywords** : citrus, diversity, pathotype, *Xanthomonas axonopodis*

Citrus bacterial canker (CBC) is an economically important disease in many tropical and subtropical countries. Several pathotypes have been described within the genus *Xanthomonas* primarily distinguished by their geographical origin and host range in addition to certain genotypic characteristics (Brunings and Gabriel, 2003). The most commonly

widespread group of *X. axonopodis* pv. *citri* strains is the main Asiatic group A, with a host range on all citrus varieties. Two groups of strains with restricted host range have been identified within pathotype A (Verniere et al., 1998). They are found in southwest Asia and Florida State in United States, and designated as *X. a.* pv. *citri* A<sup>\*</sup> and A<sup>v</sup>, respectively. A<sup>\*</sup> and A<sup>v</sup> are closely related to type A strains (Cubero and Graham, 2002, 2004) but affect only Mexican lime and Alemow. Two other canker types are described in South America, which are known as *X. axonopodis* pv. *aurantifolii* B and C. Pathotype B has a restricted host range including lemon and Mexican lime, whereas pathotype C is restricted to Mexican lime. *X. axonopodis* pv. *citrumelo* E type was later referred to as citrus bacterial spot (CBS) (Stall and Civerolo, 1993).

The variability among isolates of *Xanthomonas* causing CBC has been characterized by using different kinds of methods, such as physiological tests, phage typing, restriction analysis, and serological approaches (Egel et al., 1991; Graham et al., 1990; Hartung, 1992; Pruvost et al., 1992; Verniere et al., 1998). Both Biolog<sup>TM</sup> and MIDI<sup>TM</sup>, primarily analyzing utilization of carbon sources and composition of fatty acids, respectively, can be useful for the identification of bacterial unknowns. Strains can be grouped according to fatty acid content with the statistical programs associated with software developed for the Microbial Identification System. Several papers indicated that the *Xanthomonas* pathotypes could be differentiated by carbon source utilization pattern (Schaad et al., 2005; Verniere et al., 1998).

PCR based on repetitive sequences (rep-PCR) have been used effectively for analysis of several species of bacteria (Louws et al., 1994), and for strain identification (Cubero and Graham, 2002). Rep-PCR genomic fingerprinting makes use of DNA primers complementary to highly conserved, repetitive DNA sequences such as the 35-40 bp repetitive extragenic palindromic (REP) sequence, the 124-127 bp enterobacterial repetitive intergenic consensus (ERIC) sequence, and the 154 bp BOX element present in multiple copies in the genomes of most Gram-negative and several

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Gram-positive bacteria (Lupski and Weinstock, 1992; Versalovic et al., 1991). The primers for those repetitive sequences leads to the selective amplification of distinct genomic regions located between REP, ERIC or BOX elements. The rep-PCRs have also been used to assess variation among pathovars of *Xanthomonas* species (Cubero and Graham, 2002; Louws et al., 1994, 1995; Opgenorth et al., 1996).

In this paper, we describe the difference of carbon source utilization and fatty acid composition between the pathotypes of *Xanthomonas* strains. The difference of carbon source utilization and fatty acid composition was clustered for the first time to separate the CBC pathotypes. Rep-PCRs with ERIC, BOX and REP primers in order to compare genetic relationship and distribution of *Xanthomonas* strains isolated in Korea were also performed. These methods allowed us to evaluate the diversity of *Xanthomonas* strains collected from symptomatic citrus plants in Korea and to relate them to a worldwide collection.

## Materials and Methods

### Isolation and identification of bacteria. Diseased samples

**Table 1.** Strains of *Xanthomonas axonopodis* used in this study

Type	Name	Origin	Source <sup>a</sup>
<i>X. a. pv. citri</i> A	CFBP2859	Brazil	CIRAD
	JH410-1	China	CIRAD
	C43, CFBP1814	France	CIRAD
	CFBP2900	Japan	CIRAD
	JJ238-3, JK4-3	Korea	CIRAD
	CFBP2525	New Zealand	CIRAD
	JK148-2	Philippines	CIRAD
	M9, A-5246, M5, A-5208	USA	DPI
	<i>X. a. pv. citri</i> A <sup>*</sup>	IR01, IR02, IR03, IR04	Iran
JF90-2		Oman	CIRAD
JK2-10		Saudi Arabia	CIRAD
A-1609		USA	DPI
<i>X. a. pv. citri</i> A <sup>w</sup>	A-2032	USA	DPI
<i>X. a. pv. aurantifolii</i> B	CFBP2868, CFBP2903	Argentina	CIRAD
<i>X. a. pv. aurantifolii</i> C	CFBP2866	Brazil	CIRAD
<i>X. a. pv. citrumelo</i> (CBS)	XC05-252, A-1902, A-1887	USA	DPI
Korean isolates	SL-0870, 0874, 4021, 4024, 4026, 4028, 4029, 4034, 4036, 4040, 4041, 4042, 4043, 4044, 4045, 4046, 4047, 4049, 4050, 4052, 4054, 4056, 4057, 4059, 4060, 4062, 4063, 4064, 4066, 4067, 4068, 4070, 4071, 4072, 4073, 4090, 4091, 4093, 4095, 4096, 4098, 4099, 4100, 4468, 4469, 4474, 4477, 4478, 4483, 4484, 4492, 4493, 4500, 4501, 4510, 4511, 4516, 4517, 4518, 4519, 4520, 4525, 4526, 4528, 4529, 4530, 4537, 4538, 4539, 4547, 4548, 4553, 4554, 4556, 4558, 4560, 4562, 4564, 4566, 4567, 4568, 4915, 4916, 4917, 4918, 4920, 4922, 4925, 4926, 4928, 4931, 4932, 4933, 4934, 4935, 4937, 4943, 4945, 4946, 4947, 4949, 4950, 4952, 4953, 4954, 4955, 4956, 4958, 4990, 4993, 4995	Korea	PPD
	KACC10443		

<sup>a</sup>CIRAD; The Agricultural Research Centre for International Development, DPI; Division of Plant Industry, Florida, USA, PPD; Plant Pathology Division, NIAST, Korea, PPDSI; Plant Pests and Diseases Research Institute, Iran, KACC; Korean Agricultural Culture Collection

of citrus plants were collected from 1997 to 2005 in the southern parts of Korea, including the Jeju island. Small pieces of the tissue at the lesion margin were excised with a sterilized razor blade, and the pieces were chopped in a drop of sterile distilled water. The resulting suspension was streaked on nutrient agar or yeast peptone glucose agar (0.5% yeast extract, 0.5% peptone, 1% glucose and 1.5% agar), the single colonies were picked 3 days after incubation at 30°C. The isolated *Xanthomonas* strains were tested for their pathogenicity on *Citrus paradisi* (grapefruit) and *C. unshiu* (mandarin) at concentrations of 10<sup>5</sup> cfu/ml and for their hypersensitive reaction on pepper and tobacco at concentrations of 10<sup>8</sup> cfu/ml. All inoculated plants were kept in greenhouse with an average temperature of 26°C. The CBC strains including CBS of which the pathotype was already known were obtained as reference strain from several institutes as described in Table 1.

**Carbon source utilization.** Pure cultures of the strains were tested for utilization of the 95 carbon sources available on the GN Microplate (Biolog Inc., USA) as recommended by the manufacturer's manual. The carbon source utilization patterns were read with a microplate

reader and analyzed for the differentiation of *X. axonopodis* strains by a cluster analysis program, the fingerprinting II informatix™ software (Bio-Rad, Hercules, CA, USA).

**Fatty acid analysis.** Fatty acid profiles were generated for the selected strains. All bacterial cultures were grown on trypticase soy broth agar (BBL laboratories, Cockeysville, MD) at 28°C for 24 hr. Whole-cell fatty acid methyl esters (FAMES) were extracted and characterized as described previously (Graham et al., 1990). All numerical analysis for the FAME were pooled and subjected to the cluster analysis using the fingerprinting II informatix™ software.

**Extraction of genomic DNA.** Total genomic DNA was extracted as described by Shiotani et al. (2000) with small modification. *Xanthomonas* strains were cultured on peptone sucrose agar (1% polypeptone, 1% sucrose and 0.2% L-glutamate) or LB media at 28°C for 2 days, and the cells were harvested for total DNA extraction. Bacterial cells were washed twice with 5 M NaCl and suspended in 600 µl of Tris-EDTA (TE) buffer (pH 8.0) (Sambrook et al., 1989) containing SDS and proteinase K at a final concentration of 2% and 250 µg/ml, respectively. After incubation at 37°C for 1 h, 100 µl of 5 M NaCl was added to the solution and mixed thoroughly following incubation. Eighty microliters of cetyltrimethylammoniumbromide (CTAB)-NaCl solution (10% hexadecyltrimethyl ammonium bromide in 0.7 M NaCl) was added to the solution, which was mixed thoroughly and incubated at 65°C for 10 min. DNA was extracted using phenol chloroform isoamyl alcohol (25:24:1) and precipitated by the addition of 2 vol. of ethanol, and washed with 70% ethanol. Pelleted DNA was dried in a vacuum and dissolved in distilled water.

**DNA amplification conditions for PCR.** CBC strains as described in Table 1 were analyzed and compared with reference strains. And the isolated DNAs were used as the templates. BOX-PCR was carried out in 25 µl mixtures containing 1 X Taq buffer, 2 mM MgCl<sub>2</sub>, 4 µM primer BOX1R (Louws et al., 1994), each deoxynucleoside triphosphate (dNTP) at a concentration of 0.25 mM, and 2 U of EX-Taq polymerase (Takara); the amplification conditions consisted of 94°C for 1 min, 52°C for 1 min, and 65°C for 8 min for 30 cycles plus an initial step of 94°C for 7 min and a final step of 72°C for 10 min. ERIC-PCR was carried out in 25 µl mixtures containing 1 X Taq buffer, 2 mM MgCl<sub>2</sub>, 2 µM primer ERIC1R, 2 µM primer ERIC2 (Louws et al., 1994), each dNTP at a concentration of 0.25 mM, and 2 U of EX-Taq polymerase; the amplification conditions were the same as those used for the BOX-PCR excepting the extension at 50°C. REP-PCR was also carried out in 25 µl mixtures containing 1 X Taq buffer, 2 mM MgCl<sub>2</sub>, 50 µM

primer of REP1R-I and REP2-I, each dNTP at a concentration of 1.25 mM, and 2 U of EX-Taq polymerase; the amplification conditions were the same as those used for the BOX-PCR excepting the extension at 45°C. The PCR products were analyzed by 1.5% agarose gel electrophoresis in 1 X TBE buffer at 50 V and stained with ethidium bromide.

**Data analysis.** BOX-, ERIC-, and REP-PCR fingerprinting results were compared based on the presence or absence of fragments at a specific position (0 absence or negative; 1 presence or positive), and similarity coefficients for pairs of strains were calculated with the program NTSYS, version 2.1 (Rohlf, 2000) and clustered with UPGMA method (unweighted pair group method with arithmetic mean) to determine the genetic relationship among bacterial strains. The FA composition and carbon source utilization pattern was analyzed together using the fingerprinting II informatix™ software. A dendrogram was constructed by using UPGMA method.

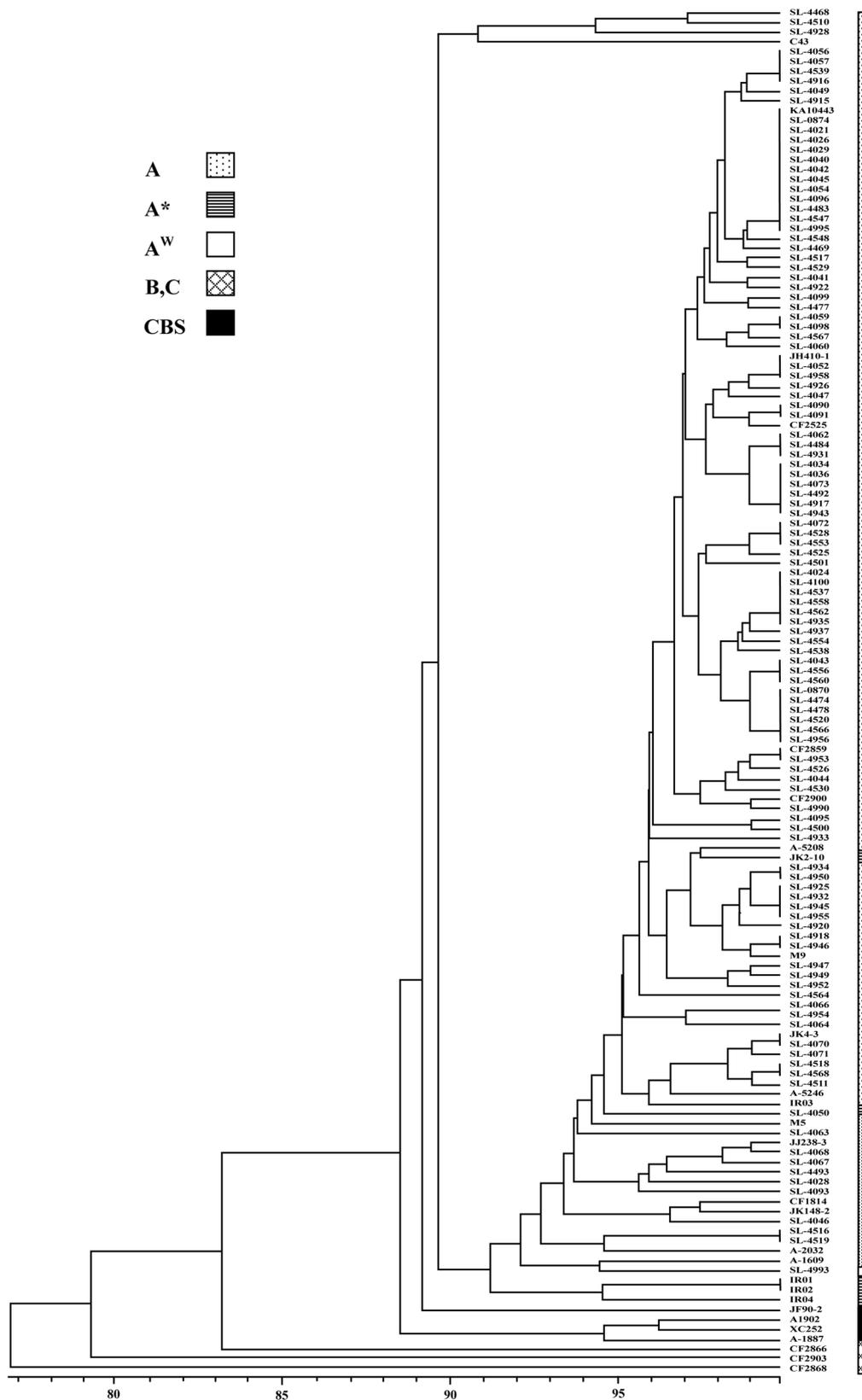
## Results

**Identification of bacterial strains.** The *Xanthomonas* strains of which the pathotype was already known were obtained from several institutes as described in Table 1. One hundred and twelve isolates of the pathogen were recovered from leaf and fruit samples collected from symptomatic citrus plants in Korea. No isolates were recovered in other regions of Korea except Jeju island, which is located southern part of Korea. The colonies were mucoid,

**Table 2.** Oxidation of carbon sources by different pathotypes of CBC and CBS

Carbohydrate	CBC pathotype					CBS
	A	A <sup>*</sup>	A <sup>w</sup>	B	C	
Glycogen	100 <sup>a</sup>	100	100	0	0	100
Dextrin	100	100	100	v	v	100
Maltose	100	100	100	0	0	100
L-Fucose	100	85.7	100	0	100	100
D-Galactose	100	100	100	0	100	100
D-Saccharic acid	v	0	0	100	v	0
Tween 40	1.8	0	0	0	0	100
Leucine	v	v	0	0	v	100

<sup>a</sup>Percentage of strains with positive results from all of the strains in Table 1. v; variable but negligible, some strains used slowly but the change of color was negligible. Representative carbon sources which showing distinctive difference are listed. The carbon source utilization patterns on the GN microplate were read with a microplate reader. A, *X. axonopodis* pv. *citri* A strain; A<sup>\*</sup>, *X. a. pv. citri* A<sup>\*</sup> strain; A<sup>w</sup>, *X. a. pv. citri* A<sup>w</sup> strain; B, C, *X. a. pv. aurantifolii* strains; and CBS, *X. a. pv. citrumelo* E strain.



**Fig. 1.** Dendrogram showing relationship between *Xanthomonas* strains by carbon source utilization pattern. The data was analyzed by the fingerprinting II informatix™ software. The lines indicate the CBC group as follows, A; *X. axonopodis* pv. *citri* A type, A\*; *X. a. pv. citri* A\* type, A<sup>w</sup>; *X. a. pv. citri* A<sup>w</sup> type, B and C; *X. a. pv. aurantifolii* B and C type, and CBS; *X. a. pv. citrumelo* E type.

**Table 3.** Difference of fatty acid profiles depending on the strains of CBC and CBS

Fatty acid <sup>a</sup>	CBC pathotype					CBS
	A	A*	A <sup>w</sup>	B	C	
11:0 ISO	4.9±1.04	2.8±0.29	2.8±0.39	4.7±0.38	3.4±0.22	3.7±0.19
13:0 ISO 3OH	5.9±1.40	2.6±0.13	2.5±0.41	6.3±0.22	5.7±0.34	3.5±0.07
15:0 ISO	9.8±0.64	12.7±1.19	13.9±0.88	8.9±0.34	6.6±0.17	8.9±1.01
15:0 ANTEISO	18.8±1.44	13.9±1.21	12.3±8.10	28.8±5.52	28.0±1.39	17.4±0.97
16:1 w7c	5.5±1.17	10.9±0.92	10.9±2.34	3.3±2.40	2.2±0.57	9.3±0.30
16:0	13.3±1.19	17.1±1.07	20.1±0.11	13.0±3.16	8.7±0.59	19.0±0.95
ISO 17:1 w9c	11.6±1.65	8.4±1.35	5.6±0.11	6.7±1.82	13.9±1.19	8.9±0.69
17:0 ISO	6.9±0.60	4.1±0.77	2.4±0.67	8.6±3.17	15.2±0.71	4.2±0.39

<sup>a</sup>11:0 ISO, 9-methyldecanoic acid; 13:0 ISO 3OH, 3-hydroxy-11-methyldodecanoic acid; 15:0 ISO, 13-methyltetradecanoic acid; 15:0 ANTEISO, 12-methyltetradecanoic acid; 16:1 w7c, cis-9-hexadecanoic acid; 16:0, hexadecanoic acid; ISO 17:1 w9c, cis-7-15-methylhexadecanoic acid; 17:0 ISO, 15-methylhexadecanoic acid. Representative fatty acids showing distinctive difference are listed.

convex, and yellow, which indicating that they produced yellow pigment and extracellular polysaccharide on nutrient agar plates as other xanthomonads. Pathogenicity and hypersensitive reaction of the isolated strains were confirmed as described in materials and methods. And they were identified into species by fatty acid and metabolic profile using MIDI and Biolog, respectively.

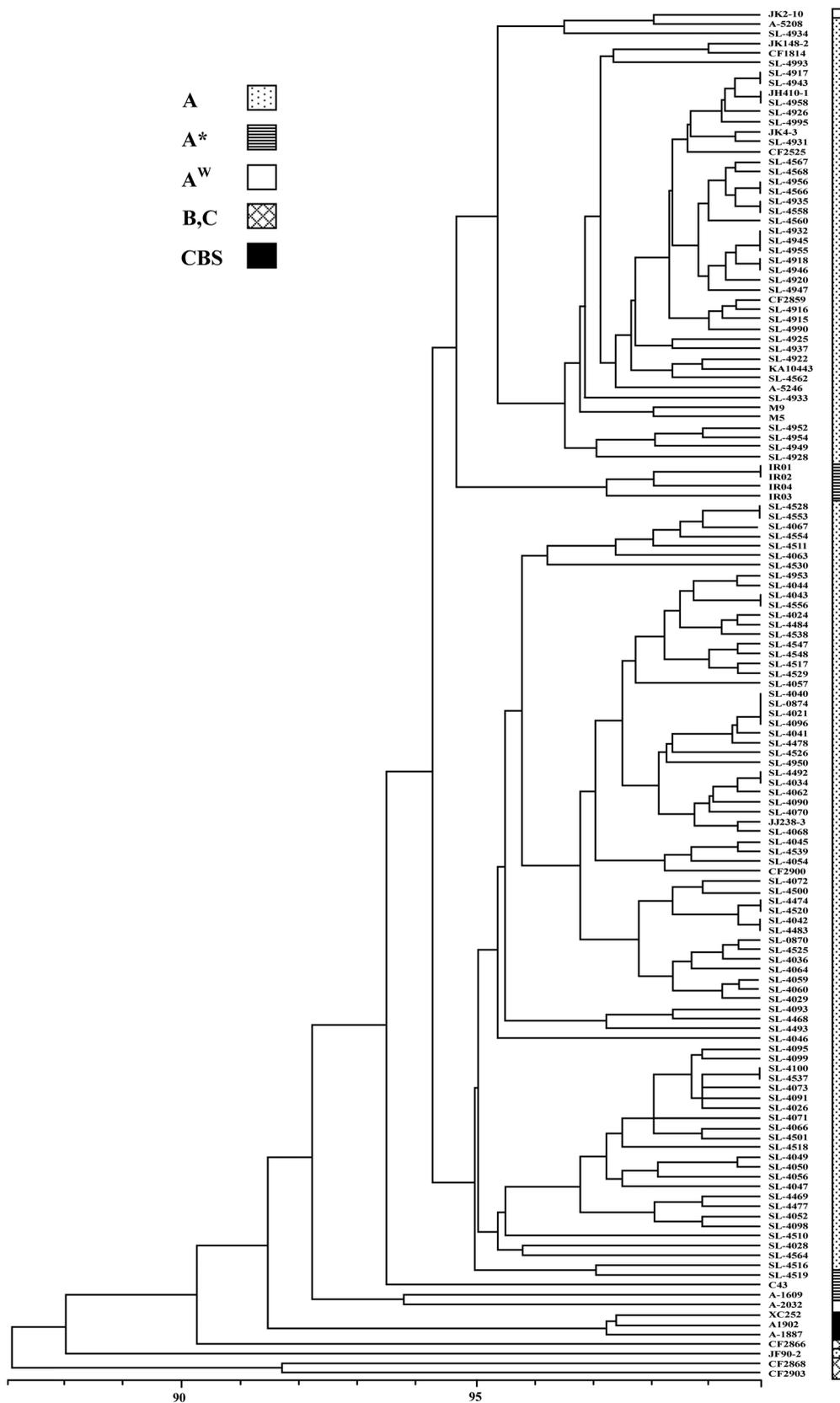
**Difference of carbon source utilization.** The pattern of 95 carbon source utilization depending on the CBC pathotypes was compared using Biolog GN microplate. Glycogen, dextrin, and maltose were used by A, A<sup>w</sup>, A\* type and CBS, but not by *X. a. pv. aurantifolii* B and C type (Table 2). Saccharic acid was oxidized only by *X. a. pv. aurantifolii* B type, and galactose was not used only by *X. a. pv. aurantifolii* B type. Tween 40 and leucine was utilized only by *X. a. pv. citrumelo* E type with negligible variation in other pathotypes, which means that the pathotype can be readily differentiated by the tween 40 and leucine. Overall, it was not possible to differentiate A, A\* and A<sup>w</sup> types by the pattern of carbon source utilization. However, *X. a. pv. aurantifolii* B, C type, and CBS can be readily differentiated from the A group by the pattern of several carbon sources. When *Xanthomonas* pathotypes were differentiated by analysis of carbon source utilization pattern (Fig. 1), bacterial strains of *X. a. pv. aurantifolii* B, C types and *X. a. pv. citrumelo* were discriminated from *X. a. pv. citri* A, A\* and A<sup>w</sup> strains with around 89% similarity. The *X. a. pv. citri* A\* and A<sup>w</sup> were clustered in the same group with *X. a. pv. citri* A by the analysis.

**Difference of fatty acid composition.** The fatty acids which show more than two times difference depending on the pathotypes of *Xanthomonas* are listed in Table 3. Several fatty acids such as 9-methyldecanoic acid, 3-hydroxy-11-methyldodecanoic acid, 9-hexadecanoic acid and 15-methylhexadecanoic acid can be used to differ-

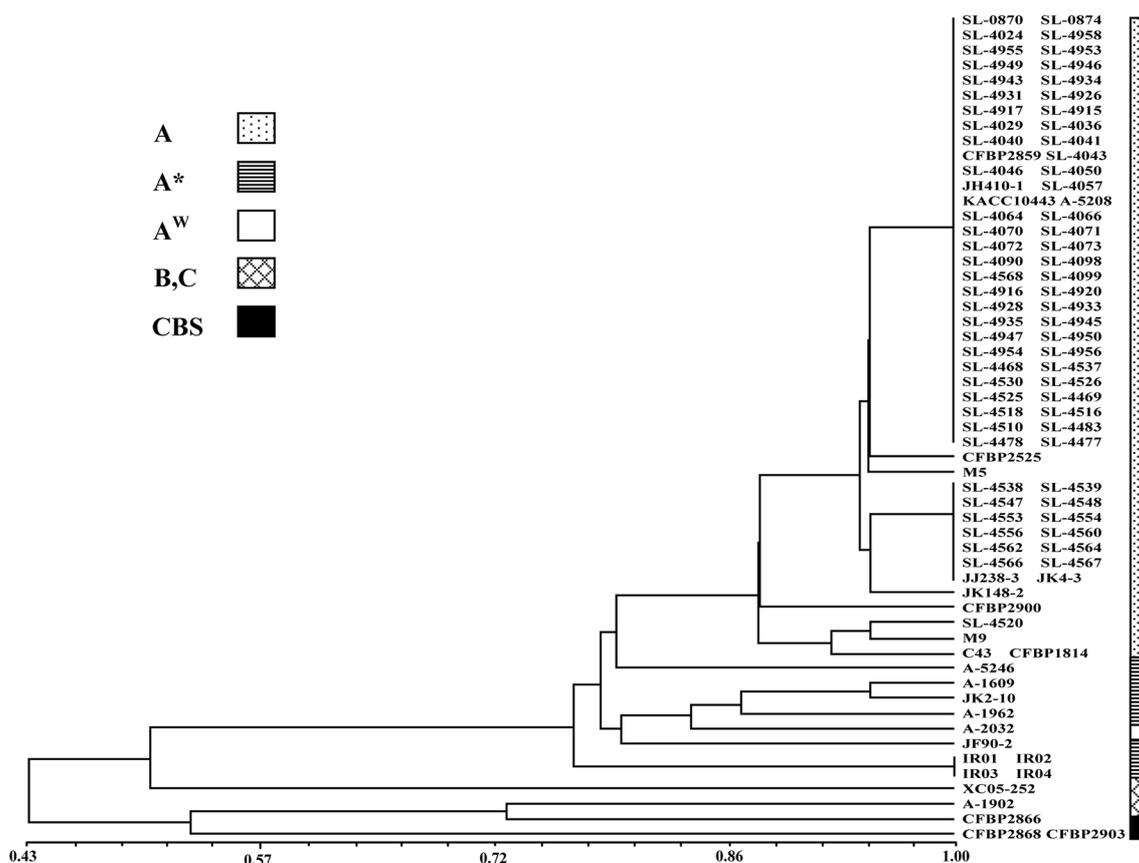
entiate *X. a. pv. citri* A\* and A<sup>w</sup> types from A types. And 12-methyltetradecanoic acid and cis-9-hexadecanoic acid can be used to detect *X. a. pv. aurantifolii* B and C type. There was a clear difference in 15-methylhexadecanoic acid and cis-7-15-methylhexadecanoic acid composition between *X. a. pv. aurantifolii* B and C types. The *Xanthomonas* pathotypes were not clearly differentiated by 50 fatty acid composition with the cluster analysis program (data not shown). When the fatty acid composition was analyzed together with carbon source utilization pattern, bacterial strains of *X. a. pv. aurantifolii* B, C type and *X. a. pv. citrumelo* E type formed a distinct cluster (Fig. 2).

***Xanthomonas* strains causing CBC classified by rep-PCRs.** Different fingerprints were generated by the products of BOX-, ERIC-, and REP-PCR. The primers corresponding to the each conserved repetitive sequences yielded PCR products ranging from approximately 100 to 40,000 bp under our PCR conditions. Cluster analysis of the data obtained from ERIC-PCR revealed two major clusters (Fig. 3). One cluster included all *X. a. pv. citri* isolates with 75% similarity and the other cluster contained all of the *X. a. pv. aurantifolii* isolates and the *X. a. pv. citrumelo* E isolates were located between the two major groups. The mean level of similarity of the tested strains was 43%. The Korean isolates grouped differently from the *X. a. pv. citri* A\* and A<sup>w</sup>, and the similarity between them was above 90%.

Analysis of the fingerprints of BOX elements yielded two main clusters, one that included all *X. a. pv. citri* and *X. a. pv. aurantifolii* strains, and one that included *X. a. pv. citrumelo* strains; the mean level of similarity between the two main clusters was 43% (Fig. 4). The *X. a. pv. citri*, *X. a. pv. aurantifolii* B and C which were included in the same main group were clearly discerned with about 50% similarity. The *X. a. pv. citri* A type could not be differentiated from A\* and A<sup>w</sup> strains with some variation, and



**Fig. 2.** Dendrogram showing relationship between *Xanthomonas* strains by combined analysis metabolic and fatty acid profiles. The data of carbon source utilization and composition of fatty acid were combined and analyzed using the fingerprinting II informatix™ software.



**Fig. 3.** Dendrogram showing relationships between *Xanthomonas* causing CBC based on ERIC-PCR analysis. Similarities were calculated by using the Dice coefficient, and clustering was achieved by UPGMA using the NTSYS version 2.1.

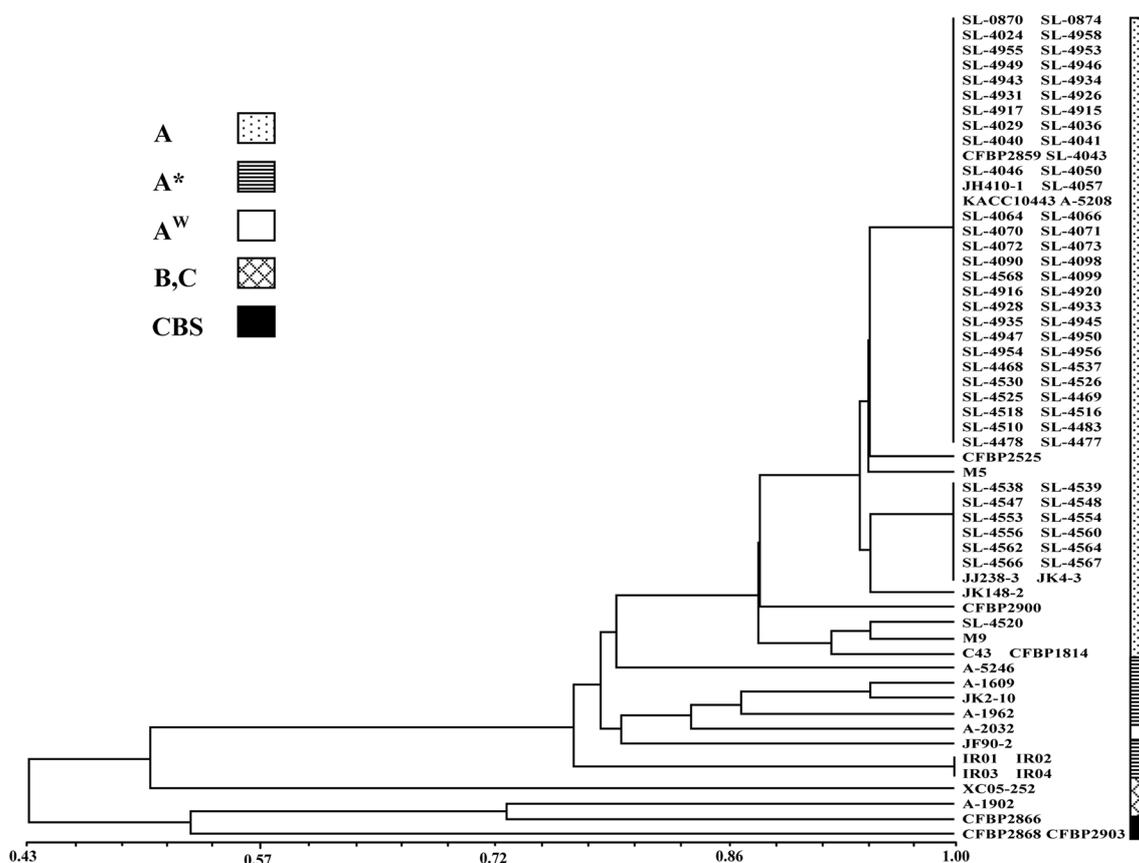
most of Korean isolates had above 80% similarity. The fingerprinting clusters by REP sequences separated the tested pathotypes into two main groups with 59% similarity (Fig. 5). One that included *X. a. pv. aurantifolii* B and one that included all of the other tested strains. In one group, the *X. a. pv. citrumelo* discerned from the other groups with an approximate 70% similarity, and *X. a. pv. aurantifolii* pathotype C was also differentiated from the *X. a. pv. citri* with a similarity about 75%. However, the *X. a. pv. citri* A, A\* and A<sup>w</sup> was not clearly discerned by the REP sequence analysis.

## Discussion

Taxonomic information can be acquired by examining the carbon source utilization profile of a bacterial strain. In this experiment the *X. a. pv. aurantifolii* B and C type were distinguishable from *X. a. pv. citri* strains by utilization of several carbon sources, such as glycogen, dextrin, and maltose (Table 2). The utilization of maltose depending on the pathotype was exactly same as reported by Schaad et al. (2005). Galactose and alaninamide were used similarly by

each pathotypes as the tests of Verniere et al. (1998). They also reported that 76.6% of the tested A types used fucose, however fucose was used by all of the tested A strains in our study. Schaad et al. (2005) reported that raffinose could not be utilized by A, A\* and A<sup>w</sup> types, but in our experiment there was some variation depending on the strains (data not shown), which needs more investigation. When the relatedness was constructed by 95 carbon source utilization pattern, the Asiatic strains of citrus canker form a discrete cluster, and they distinguished from strains of the CBS pathogen, which formed a separate cluster. In view of the general use of Biolog systems for bacterial identification, determination of carbon source utilization pattern may be a rapid and sensitive method for separation of *Xanthomonas* strains on citrus if used complementarily with other methods.

Fatty acids extracted from bacterial cells facilitate the identification and quantification of the fatty acid by gas chromatography. The fatty acid by profiles from members of the bacterial genus *Xanthomonas* are relatively complex compared to those of other plant pathogenic bacteria. The differences in relative quantity of them among strains presented the potential for distinguishing groups of strains



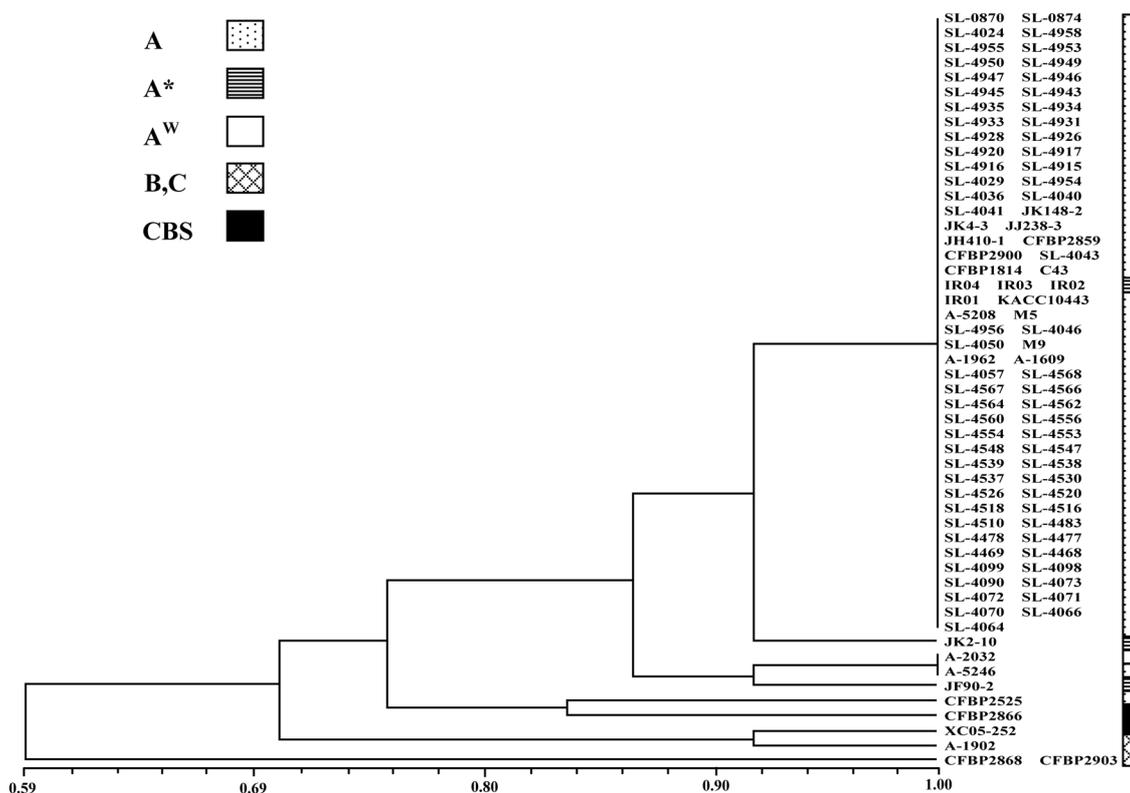
**Fig. 4.** Dendrogram of relationships based on BOX-PCR analysis. Similarities were calculated by using the Dice coefficient, and clustering was achieved by UPGMA using the NTSYS version 2.1.

within the genus as shown in Table 2. Though *X. a. pv. aurantifolii* B, C and *X. a. pv. citrumelo* E was not clearly differentiated in the dendrogram by fatty acid composition (data not shown), when the fatty acid composition was analyzed together with carbon source utilization pattern, bacterial strains of *X. a. pv. aurantifolii* B, C types and *X. a. pv. citrumelo* E type formed a distinct cluster (Fig. 2). To our knowledge this is the first report on differentiation of CBC pathotypes using combined analysis of carbon source utilization and fatty acid composition. The Korean isolates clustered in the same group as the reference of *X. a. pv. citri* A type, which indicating the pathotypes of Korean strains belong to A type.

Several techniques such as RFLP analysis, genomic fingerprinting, and rDNA analysis have been reported for the identification of the bacterial strains and pathotype assignation. Especially, REP-, ERIC-, and BOX-PCR were effectively used to generate genomic fingerprints of a variety of *Xanthomonas* isolates and to identify pathovars and strains that were previously not distinguishable by other classification methods (Cubero and Graham, 2002; Louws et al., 1994, 1995; Opgenorth et al., 1996). In this

experiment most of the bands were present in all tested strains, but there were differences in the intensity of some amplified fragments as well as in the occurrence of several polymorphic bands. The polymorphic bands in the A\*, A<sup>w</sup>, B and C types were fewer than those in the A type (data not shown). The *X. a. pv. citri* A, A\* and A<sup>w</sup> strains were clearly separated from the *X. a. pv. aurantifolii* B, C and *X. a. pv. citrumelo* E by all of the primers used. Both ERIC- and BOX-PCR allowed discrimination between the A, A\* and A<sup>w</sup> groups with slight variation, but our results of rep-PCR also indicated that A\* and A<sup>w</sup> types are close to A types.

Rep-PCR has revealed both inter and intrapathovar variability (Louws et al., 1994), but a very high degree of homogeneity was found in *Xanthomonas*, with REP and ERIC primers (Opgenorth et al., 1996). Trindade et al. (2005) reported that the ERIC-PCR revealed more polymorphic bands in *X. campestris* pv. *viticola* than the other two sets of primers. The REP element was the most conserved and the less efficient in revealing polymorphism in *X. c. pv. vesicatoria* group B strains (Louws et al., 1995). In this experiment the REP-PCR revealed less polymorphic bands between the tested strains than those of ERIC and



**Fig. 5.** Dendrogram of relationships based on REP-PCR analysis. Similarities were calculated by using the Dice coefficient, and clustering was achieved by UPGMA using the NTSYS version 2.1.

BOX primers. And REP-PCR, which identifying a smaller number of subgroups among the strains, was also the most conserved of the three sets of primers tested. These results indicate that the polymorphic bands are maybe variable according to PCR conditions but still good tool to identify diverse of pathogens.

Overall our results indicated that differentiation of the pathotypes of CBC by some of carbon source utilization and fatty acid composition could be used effectively from the isolation and identification step of the pathogen. The rep-PCR fingerprinting can be an important tool for monitoring the diversity of the CBC, which confirming the previous reports. Both the physiochemical characteristics and rep-PCR results significantly indicated that Korean isolates are all *X. a. pv. citri* A types.

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