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## Genetic Diversity of *Pectobacterium carotovorum* subsp. *brasiliensis* Isolated in Korea

Dong Hwan Lee<sup>1</sup>, Jin-Beom Kim<sup>1</sup>, Jeong-A Lim<sup>1</sup>, Sang-Wook Han<sup>2</sup> and Sunggi Heu<sup>1\*</sup>

<sup>1</sup>Division of Microbial Safety, National Academy of Agricultural Science, Rural Development Administration, Suwon 441-707, Korea

<sup>2</sup>Department of Integrative Plant Science, Chung-Ang University, Anseong 456-756, Korea

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The plant pathogenic bacterial genus *Pectobacterium* consists of heterogeneous strains. The *P. carotovorum* species is a complex strain showing divergent characteristics, and a new subspecies named *P. carotovorum* subsp. *brasiliensis* has been identified recently. In this paper, we re-identified the *P. carotovorum* subsp. *brasiliensis* isolates from those classified under the subspecies *carotovorum* and newly isolated *P. carotovorum* subsp. *brasiliensis* strains. All isolates were able to produce plant cell-wall degrading enzymes such as pectate lyase, polygalacturonase, cellulase and protease. We used genetic and biochemical methods to examine the diversity of *P. carotovorum* subsp. *brasiliensis* isolates, and found genetic diversity within the *brasiliensis* subsp. isolates in Korea. The restriction fragment length polymorphism analysis based on the *recA* gene revealed a unique pattern for the *brasiliensis* subspecies. The Korean *brasiliensis* subsp. isolates were divided into four clades based on pulsed-field gel electrophoresis. However, correlations between clades and isolated hosts or year could not be found, suggesting that diverse *brasiliensis* subsp. isolates existed.

**Keywords :** 16S rDNA, *Pectobacterium carotovorum* subsp. *brasiliensis*, PFGE, *recA*, RFLP

Bacteria belonging to *Pectobacterium carotovorum* cause soft rot disease in diverse vegetables and crops worldwide. These bacteria produce several different plant cell-wall degrading enzymes (PCWDEs) such as pectinase, polygalacturonase (Peh) and cellulase (Cel). The maceration of cell walls caused by PCWDEs results in soft rot symptoms

in host plants. Since *Pectobacterium* species can produce PCWDEs, soft rot disease can occur in the field as well as during transportation and storage. *P. carotovorum* subsp. *carotovorum* has a wide host range including potato, carrot, lettuce, onion and Chinese cabbage, but *P. atrosepticum*, *P. betavasculorum*, *P. wasabiae* and *P. carotovorum* subsp. *odoriferum* have a relatively narrow host range that includes potato, sugarbeet, horseradish and chicory, respectively (Charkowski et al., 2006; Seo et al., 2005; Toth et al., 2003).

*Pectobacterium* is a complex taxon of strains with diverse characteristics. Recently, based on new molecular biology techniques, several species in the former *Erwinia* genus have been re-classified as *Erwinia*, *Brenneria*, *Pectobacterium*, *Dickeya* and *Pantoea* (Samson et al., 2005; Waleron et al., 2002). As a result, *Erwinia carotovora* was renamed *Pectobacterium carotovorum*. In recent years, *P. carotovorum* species was classified into five subspecies, *atrosepticum*, *betavasculorum*, *carotovorum*, *odoriferum* and *wasabiae* (Hauben et al., 1998). However, the genetic differences among the subspecies *atrosepticum*, *betavasculorum* and *wasabiae* were too significant to be placed within the same species *P. carotovorum*. Gardan et al. (2003) suggested the three subspecies *atrosepticum*, *betavasculorum* and *wasabiae* should be elevated to the species level and not subspecies.

Recently, bacteria isolated from the blackleg-diseased potato in Brazil were similar to *P. carotovorum* subsp. *carotovorum* that usually cause soft rot disease, but not similar to *P. atrosepticum*, the major pathogen of the blackleg disease of potato (Duarte et al., 2004). The bacteria were termed *P. carotovorum* subsp. *brasiliensis*, a new subspecies of *P. carotovorum* (Duarte et al., 2004; Nahan et al., 2012). After this first report of the subspecies *brasiliensis*, scientists in several countries, such as the USA, South Africa, Canada, New Zealand and Korea, have reported its existence (Choi and Kim, 2013; De Boer et al., 2012; Kim

\*Corresponding author.

Phone) +82-31-290-0455, FAX) +82-31-290-0407

E-mail) sunggiheu@korea.kr

et al., 2009; Panda et al., 2012; van der Merwe et al., 2010).

The first isolation of *P. carotovorum* subsp. *brasiliensis* in Korea was reported in 2013 (Choi and Kim, 2013). In 1999, 11 bacterial strains isolated from blackleg diseased potato reportedly showed different characteristics from *P. carotovorum* subsp. *carotovorum* or *P. atrosepticum* strains (Seo et al., 2004, 2005). However, the study did not investigate whether that strain had been existed in Korea or not. Therefore, this research was conducted to demonstrate that the subspecies *brasiliensis* existed previously in Korea, and was not a new subspecies imported recently.

## Materials and Methods

**Bacterial strains, media and cultivation conditions.** *P. carotovorum* subsp. *brasiliensis* strains used in this study are listed in Table 1. The strains were isolated from various hosts showing soft rot disease symptoms between 1997 and 2013 in Korea. The strains were stored at  $-80^{\circ}\text{C}$  until use. For experiments, the strains were cultured on tryptic soy agar (TSA, BD, Franklin Lakes, NJ, USA) or in tryptic soy broth (TSB, BD) medium at  $28^{\circ}\text{C}$ , and then applied to tests.

**Table 1.** List of *Pectobacterium carotovorum* strains and 16S rDNA sequence analysis results

Previous strain name	Strain no.	Year isolated	Isolated from	16S rDNA analysis (assigned name)
<i>P. carotovorum</i> subsp.				<i>P. carotovorum</i> subsp.
<i>carotovotum</i>	30	1997	Chinese cabbage	<i>brasiliensis</i>
<i>carotovotum</i>	34	1997	Chinese cabbage	<i>brasiliensis</i>
<i>carotovotum</i>	35	1997	Chinese cabbage	<i>brasiliensis</i>
<i>carotovotum</i>	36	1997	Chinese cabbage	<i>brasiliensis</i>
<i>carotovotum</i>	49	1997	Chinese cabbage	<i>brasiliensis</i>
<i>carotovotum</i>	E3	1997	Tomato	<i>brasiliensis</i>
<i>carotovotum</i>	E10	1997	Potato	<i>brasiliensis</i>
<i>carotovotum</i>	E35	1997	Chinese cabbage	<i>brasiliensis</i>
<i>carotovotum</i>	52	1997	Chinese cabbage	<i>brasiliensis</i>
<i>carotovotum</i>	E12	1997	Chinese cabbage	<i>brasiliensis</i>
<i>carotovotum</i>	76	1998	Chinese cabbage	<i>brasiliensis</i>
<i>carotovotum</i>	113	2000	Chinese cabbage	<i>brasiliensis</i>
<i>carotovotum</i>	123	2000	Chinese cabbage	<i>brasiliensis</i>
<i>carotovotum</i>	112	2000	Chinese cabbage	<i>brasiliensis</i>
<i>carotovotum</i>	E42	2010	Chrysanthemum	<i>brasiliensis</i>
<i>carotovotum</i>	E52	2012	Eggplant	<i>brasiliensis</i>
<i>carotovotum</i>	E53	2012	Eggplant	<i>brasiliensis</i>
<i>brasiliensis</i>	N1	2013	Nepenthes	<i>brasiliensis</i>
<i>brasiliensis</i>	N2	2013	Nepenthes	<i>brasiliensis</i>
<i>brasiliensis</i>	N3	2013	Nepenthes	<i>brasiliensis</i>
<i>brasiliensis</i>	N4	2013	Nepenthes	<i>brasiliensis</i>
<i>brasiliensis</i>	N5	2013	Nepenthes	<i>brasiliensis</i>
<i>brasiliensis</i>	N6	2013	Nepenthes	<i>brasiliensis</i>
<i>brasiliensis</i>	N7	2013	Nepenthes	<i>brasiliensis</i>
<i>brasiliensis</i>	N8	2013	Nepenthes	<i>brasiliensis</i>
<i>brasiliensis</i>	N9	2013	Nepenthes	<i>brasiliensis</i>
<i>brasiliensis</i>	N10	2013	Nepenthes	<i>brasiliensis</i>
<i>brasiliensis</i>	N11	2013	Nepenthes	<i>brasiliensis</i>
<i>brasiliensis</i>	N12	2013	Nepenthes	<i>brasiliensis</i>
<i>brasiliensis</i>	N13	2013	Nepenthes	<i>brasiliensis</i>

**Pathogenicity test.** The pathogenicity test against Chinese cabbage was performed according to a previously published procedure with some modification (Lee et al., 2013a). The fresh strains grown on TSA medium for 24–48 h were harvested by a sterilized cotton swab and resuspended in dilution solution (10 mM MgCl<sub>2</sub>) to an approximate cell density of 10<sup>8</sup> cfu/mL. Ten microliters of each suspension was inoculated into Chinese cabbage, and then soft rot symptoms were observed while the Chinese cabbage was incubated at 28°C in sterilized plastic boxes with sufficient moisture. The Chinese cabbage inoculated with dilution solution was used as a negative control.

**Identification of bacteria using 16S rDNA sequence analysis.** After growth of the strains on TSA medium, 16S rRNA gene was sequenced by Macrogen, Inc. (Korea). The strains were identified based on a comparison of their nucleotide sequences with those in the GenBank database using the BLAST software on the National Center for Biotechnology Information (NCBI) server (<http://www.ncbi.nlm.nih.gov/>).

**PCWDE assays.** Extracellular enzyme assays were performed according to procedures described previously (Lee et al., 2013b). To prepare samples for the enzyme assays, the strains were cultured overnight in TSB medium at 28°C and cells were removed by centrifugation at 15,000 × g for 10 min. Supernatants were poured into wells on each assay plate. The following substances were used for semiquantitative enzyme assay media: 1% polygalacturonic acid (PGA) for pectate lyase (Pel) and Peh; 1% carboxymethyl cellulose for Cel; 1% skim milk for protease (Prt) assay. Pel and Peh assay media were developed with 4 N HCl and Prt media with 0.1% Congo Red.

**recA PCR-restriction fragment length polymorphism (RFLP) analysis.** Genomic DNA of the strains was extracted using the G-spin genomic DNA isolation kit (iNtRON Biotechnology, Seongnam, Korea), and then stored at –20°C until needed. The *recA* gene was amplified with the *recA*-specific primers Ec\_recA\_F (GGTAAAGGGTC-TATCATGCG) and Ec\_recA\_R (CCTTCACCATA-CATAATTTGGA) (Waleron et al., 2002). The ~730 bp amplified products were digested with the restriction enzymes *TasI*, *TruI*, *AluI* and *HinI* (Takara or Fermentas). The digested fragments were separated in a 2.0% agarose gel in Tris-acetate-EDTA buffer and visualized for further band pattern analysis.

**Pulsed-field gel electrophoresis (PFGE) analysis.** In ac-

cordance with the standardized PulseNet protocol maintained at the Center for Disease Control and Prevention, we performed pulsed-field gel electrophoresis (Hunter et al., 2005). Strains grown on TSA medium were collected by a cotton swab and then resuspended in 1 ml of Tris-EDTA buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0) to an OD<sub>600nm</sub> of 0.5–0.7. After lysozyme and proteinase K treatments, plugs were incubated in EC lysis buffer (6 mM Tris-HCl, 1 M NaCl, 100 mM EDTA, 0.5% Brij-58, 0.2% sodium deoxycholate, 0.5% sodium lauroly sarcosine). The plugs were digested with *SmaI* (Takara), and the genomic DNA fragments were separated in a 1% Seakem gold agarose gel using a CHEF Mapper (Bio-Rad, Hercules, CA, USA) system. Gels were stained with SYBR<sup>®</sup> Gold Stain (Invitrogen, USA). The cellular DNA from *Salmonella* serotype Braenerup H9812 was treated with *XbaI* (Takara) and that DNA fragments were used as a standard for normalization (Hunter et al., 2005). Using the Fingerprinting II software (Bio-Rad), PFGE band patterns were analyzed, and a dendrogram was clustered and analyzed using the unweighted pair group method of average linkage (UPGMA).

**Identification of strains using the Biolog system.** The carbon source usage of the isolated strains was determined using a microstation system with a GN2 MicroPlate (Biolog, Hayward, CA, USA) according to a procedure published previously (Holmes et al., 1994). Briefly, overnight cultures of bacteria on TSA plates were resuspended in 0.85% saline solution and 150 µl of suspension were dispensed per microplate well. The results obtained using the Biolog microstation set were compared to a given value provided by the Microlog software (Biolog).

**Indole production test.** The indole spot test was used to determine indole production. Strains streaked on TSA medium were incubated for 18–24 h at 28°C. A Whatman filter paper was wetted with Kovács reagent (10 g *p*-dimethylaminobenzaldehyde, 50 ml HCl and 150 ml isoamyl alcohol). After smearing the strains on the filter paper, the development of a red/pink color was observed.

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## Results and Discussion

**Identification of strains belonging to *P. carotovorum* subsp. *brasiliensis*.** Because a new subspecies *brasiliensis* of the *P. carotovorum* species was reported in Korea in 2013 (Choi and Kim, 2013), determining whether this new subspecies was imported or existed previously in Korea is important. A total of 128 isolates, identified as *P. carotovorum* subsp. *carotovorum* collected in 1997, 1998, 1999 and

2000 were maintained at  $-80^{\circ}\text{C}$ , reisolated and reidentified. Additionally, eight strains were isolated from various hosts including chrysanthemum and eggplant in 2010 and 2012, and 13 strains from nepenthes in 2013 were used for identification of the subspecies *brasiliensis*. All strains used in this present study were tested for their pathogenicity against Chinese cabbage to determine whether the preserved strains maintained their characteristics. As expected, all tested strains caused severe soft rot disease against the host (data not shown).

Because sequence analysis of 16S rRNA genes of bacterial strains was successfully used to classify bacterial isolates in *P. carotovorum* species into the subspecies level (Hauben et al., 1998), the 16S rDNA sequences of all 149 strains were identified and analyzed with the NCBI Blast program. A total of 30 isolates analyzed were identified as subspecies *brasiliensis* with 99% identity at the nucleotide level (Table 1). Among 30 isolates, 10 were isolated in 1997, 1 in 1998, 3 in 2000, and 1 in 2010. Because the first report of the subspecies *brasiliensis* was in 1999 (Duarte et al., 2004) and Korean isolates reported by Choi and Kim (2013) were collected in 2011, these data suggest that *P. carotovorum* subsp. *brasiliensis* is not a newly imported subspecies, but existed previously in Korea as *P. carotovorum* subsp. *carotovorum* and could not be identified due to a lack of phylogenetic information.

**The PCWDE production of the subspecies *brasiliensis* isolates.** Since bacteria of the genus *Pectobacterium* produce and secrete PCWDEs, macerate plant cell walls and then cause soft rot disease, the PCWDEs is an important virulence factor of the plant pathogenic bacteria. The *P. carotovorum* subsp. *carotovorum* Pcc21 strain has numerous genes involved in the production of several PCWDEs; mutants lacking enzyme production or secretion ability are also non-pathogenic (Lee et al., 2013b; Park et al., 2012). PCWDE production is also related to the plant defense response (Lee et al., 2008; Mäe et al., 2001).

Since the production and secretion of PCWDEs is an important characteristic of the genus *Pectobacterium*, the production and secretion of four PCWDEs in the strains were surveyed. All tested strains of the subspecies *brasiliensis* could produce and secrete the four PCWDE: Pel, Peh, Cel, and Prt (Table 2 and Fig. 1). The production and secretion of the major PCWDEs and the pathogenicity to Chinese cabbage suggested that all of the newly identified strains belonged to the subspecies *brasiliensis* and had strong pathogenicity in vegetables, in which they caused soft rot disease.

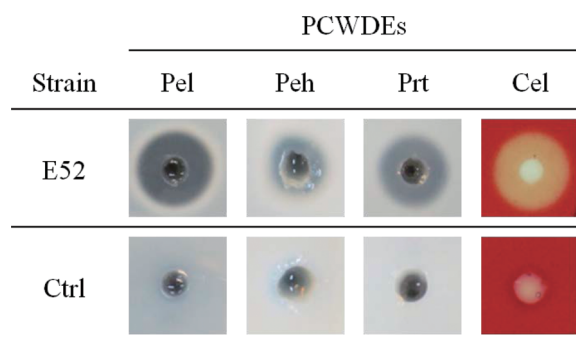
**Table 2.** Plant cell-wall degrading enzyme (PCWDE) activities of *P. carotovorum* subsp. *brasiliensis*

Strain name <sup>a</sup>	Strain no.	PCWDEs <sup>b</sup>				Indole production
		Pel	Peh	Cel	Prt	
<i>P. carotovorum</i> subsp.						
<i>brasiliensis</i>	30	+	+	+	+	+
<i>brasiliensis</i>	34	+	+	+	+	+
<i>brasiliensis</i>	35	+	+	+	+	+
<i>brasiliensis</i>	36	+	+	+	+	+
<i>brasiliensis</i>	49	+	+	+	+	+
<i>brasiliensis</i>	E3	+	+	+	+	+
<i>brasiliensis</i>	E10	+	+	+	+	+
<i>brasiliensis</i>	E35	+	+	+	+	+
<i>brasiliensis</i>	52	+	+	+	+	+
<i>brasiliensis</i>	E12	+	+	+	+	+
<i>brasiliensis</i>	76	+	+	+	+	+
<i>brasiliensis</i>	113	+	+	+	+	+
<i>brasiliensis</i>	123	+	+	+	+	+
<i>brasiliensis</i>	112	+	+	+	+	+
<i>brasiliensis</i>	E42	+	+	+	+	+
<i>brasiliensis</i>	E52	+	+	+	+	+
<i>brasiliensis</i>	E53	+	+	+	+	+
<i>brasiliensis</i>	N1	+	+	+	+	+
<i>brasiliensis</i>	N2	+	+	+	+	+
<i>brasiliensis</i>	N3	+	+	+	+	+
<i>brasiliensis</i>	N4	+	+	+	+	+
<i>brasiliensis</i>	N5	+	+	+	+	+
<i>brasiliensis</i>	N6	+	+	+	+	+
<i>brasiliensis</i>	N7	+	+	+	+	+
<i>brasiliensis</i>	N8	+	+	+	+	+
<i>brasiliensis</i>	N9	+	+	+	+	+
<i>brasiliensis</i>	N10	+	+	+	+	+
<i>brasiliensis</i>	N11	+	+	+	+	+
<i>brasiliensis</i>	N12	+	+	+	+	+
<i>brasiliensis</i>	N13	+	+	+	+	+

<sup>a</sup>Newly assigned strain name in Table 1 was used.

<sup>b</sup>Pel, pectate lyase; Peh, polygalaturonase; Cel, cellulase; Prt, protease.

**Classification of the subspecies *brasiliensis* isolates by PCR-RFLP.** A total of 30 isolates were identified as subspecies *brasiliensis* and 11 were isolated before the first subspecies *brasiliensis* isolates were reported. To investigate the genetic diversity of the subspecies *brasiliensis* isolates in Korea, the *recA* gene restriction enzyme digestion patterns of all 30 bacteria were analyzed. The



**Fig. 1.** Plant cell-wall degrading enzyme (PCWDE) activities of *P. carotovorum* subsp. *brasiliensis* strains isolated in Korea. The supernatant of *P. carotovorum* subsp. *brasiliensis* strain culture was transferred to pectate lyase (Pel), polygalacturonase (Peh), protease (Prt), and cellulase (Cel) assay media. The photographs are representative of three independent experiments using *P. carotovorum* subsp. *brasiliensis* E52. Other strains used in this study showed similar positive activities. Tryptic soy broth was used as the negative control (Ctrl); no enzyme activity was observed.

sequence analysis of 16S and 23S rRNA, *rpoS*, *gyrA*, *recA* and *groEL* genes has been used to classify plant pathogenic bacteria (Waleron et al., 2002). Additionally, in bacteria of the genus *Pectobacterium*, the *pel* gene has been used as a marker (Darrasse et al., 1994). The *recA* gene encodes the RecA protein, which is involved in the DNA repair system and SOS response; the *recA* gene DNA sequence is well conserved (Eisen et al., 1995). The RFLP patterns of the *recA* gene of the genus *Pectobacterium* using several restriction enzymes are available (Waleron et al., 2002; 2008).

Based on previous reports, the PCR-amplified *recA* DNA fragment was digested using four restriction enzymes (*TasI*, *TruII*, *AluI* and *HinFI*) separately and the fragment patterns were analyzed. The Korean *brasiliensis* isolates were not highly genetically divergent (Table 3). The RFLP patterns of the 30 different subspecies *brasiliensis* isolates were divided into group 8 and group D. Based on the Waleron's pattern designation (Waleron et al., 2002), RFLP group 8 showed the typical pattern for subspecies *carotovorum* and group D exhibited a new pattern for the Korean subspecies *brasiliensis* isolates. Although the Korean isolates were divided into two groups, group D showed a different pattern with only one of the restriction enzymes used. The genetic variation among Korean subspecies *brasiliensis* isolates was minimal, suggesting that differentiation may have begun only recently. However, further in-depth investigations should be conducted to determine whether group D is a pattern unique to the Korean strains and if group 8 should be regarded as a pattern of the subspecies *brasiliensis*

and not the subspecies *carotovorum*.

**Classification of the subspecies *brasiliensis* isolates using PFGE.** Because the analysis of RFLP based on the *recA* gene of the Korean subspecies *brasiliensis* isolates showed two different patterns, further investigations of the genetic diversity of the isolates were needed. Since the *recA*-based RFLP used a small DNA fragment (~730 bp), PFGE was used to investigate the diversity of Korean subspecies *brasiliensis* isolates and analyze the entire genome. PFGE was used for the identification of bacterial subspecies, because this method was highly sensitive and showed stable genetic differentiation of isolated bacteria (Hunter et al., 2005). The PFGE results after *SmaI* restriction enzyme digestion of the total DNA from each subspecies *brasiliensis* isolate showed more diverse genetic variation (Fig. 2). Analysis of the PFGE results using the Fingerprinting II software (Bio-Rad) indicated that the 30 Korean subspecies *brasiliensis* isolates grouped into four clades. However, no correlations between the clade and host or year of isolation were found. Therefore, diverse subspecies *brasiliensis* isolates existed previously in Korea, and new developments in technology facilitated their differentiation from the subspecies *carotovorum*.

**Biochemical characteristics of the Korean subspecies *brasiliensis* isolates.** Assays of carbon source utilization using the Biolog system and indole production of Korean subspecies *brasiliensis* isolates were conducted. Based on the results, the Biolog software identified the subspecies *brasiliensis* isolates as subspecies *carotovorum* (data not shown). In a previous report, subspecies *brasiliensis* strains were included in the heterogeneous and broader subspecies *carotovorum* group (Duarte et al., 2004). These results confirmed that carbon source utilization analysis is not sufficient to distinguish subspecies *brasiliensis* isolates from those of subspecies *carotovorum*, although several differences in biological characteristics exist. Therefore, distinguishing subspecies *brasiliensis* from the subspecies *carotovorum* is difficult.

The ability of certain bacteria to form indole from the amino acid tryptophan is an important phenotypic characteristic. Demonstration of indole production is a useful characterization test. All tested Korean subspecies *brasiliensis* isolates could produce indole (Table 2). This result is inconsistent with a previous report that isolates of the subspecies *brasiliensis* are negative for indole production (Choi and Kim, 2013; Pasanen et al., 2013). In recent studies, subspecies *brasiliensis* strains were divided into two subgroups using phylogenetic analysis based on multilocus

**Table 3.** Restriction fragment length polymorphism (RFLP) analysis of *P. carotovorum* subsp. *brasiliensis* based on the *recA* gene

Strain name <sup>a</sup>	Strain no.	Year isolated	Isolated from	<i>recA</i> RFLP pattern <sup>b</sup>				RFLP group <sup>c</sup>
				<i>TasI</i>	<i>TruII</i>	<i>AluI</i>	<i>HinI</i>	
<i>P. carotovorum</i> subsp.								
<i>brasiliensis</i>	30	1997	Chinese cabbage	4	3	2	2	8
<i>brasiliensis</i>	34	1997	Chinese cabbage	4	3	2	2	8
<i>brasiliensis</i>	35	1997	Chinese cabbage	4	3	2	2	8
<i>brasiliensis</i>	36	1997	Chinese cabbage	4	3	2	2	8
<i>brasiliensis</i>	49	1997	Chinese cabbage	4	3	2	2	8
<i>brasiliensis</i>	E3	1997	Tomato	4	3	2	2	8
<i>brasiliensis</i>	E10	1997	Potato	4	3	2	2	8
<i>brasiliensis</i>	E35	1997	Chinese cabbage	4	3	2	2	8
<i>brasiliensis</i>	52	1997	Chinese cabbage	6	3	2	2	D
<i>brasiliensis</i>	E12	1997	Chinese cabbage	6	3	2	2	D
<i>brasiliensis</i>	76	1998	Chinese cabbage	4	3	2	2	8
<i>brasiliensis</i>	113	2000	Chinese cabbage	4	3	2	2	8
<i>brasiliensis</i>	123	2000	Chinese cabbage	4	3	2	2	8
<i>brasiliensis</i>	112	2000	Chinese cabbage	6	3	2	2	D
<i>brasiliensis</i>	E42	2010	Chrysanthemum	4	3	2	2	8
<i>brasiliensis</i>	E52	2012	Eggplant	4	3	2	2	8
<i>brasiliensis</i>	E53	2012	Eggplant	4	3	2	2	8
<i>brasiliensis</i>	N1	2013	Nepenthes	4	3	2	2	8
<i>brasiliensis</i>	N2	2013	Nepenthes	4	3	2	2	8
<i>brasiliensis</i>	N3	2013	Nepenthes	4	3	2	2	8
<i>brasiliensis</i>	N4	2013	Nepenthes	4	3	2	2	8
<i>brasiliensis</i>	N5	2013	Nepenthes	4	3	2	2	8
<i>brasiliensis</i>	N6	2013	Nepenthes	4	3	2	2	8
<i>brasiliensis</i>	N7	2013	Nepenthes	4	3	2	2	8
<i>brasiliensis</i>	N8	2013	Nepenthes	4	3	2	2	8
<i>brasiliensis</i>	N9	2013	Nepenthes	4	3	2	2	8
<i>brasiliensis</i>	N10	2013	Nepenthes	4	3	2	2	8
<i>brasiliensis</i>	N11	2013	Nepenthes	4	3	2	2	8
<i>brasiliensis</i>	N12	2013	Nepenthes	4	3	2	2	8
<i>brasiliensis</i>	N13	2013	Nepenthes	4	3	2	2	8

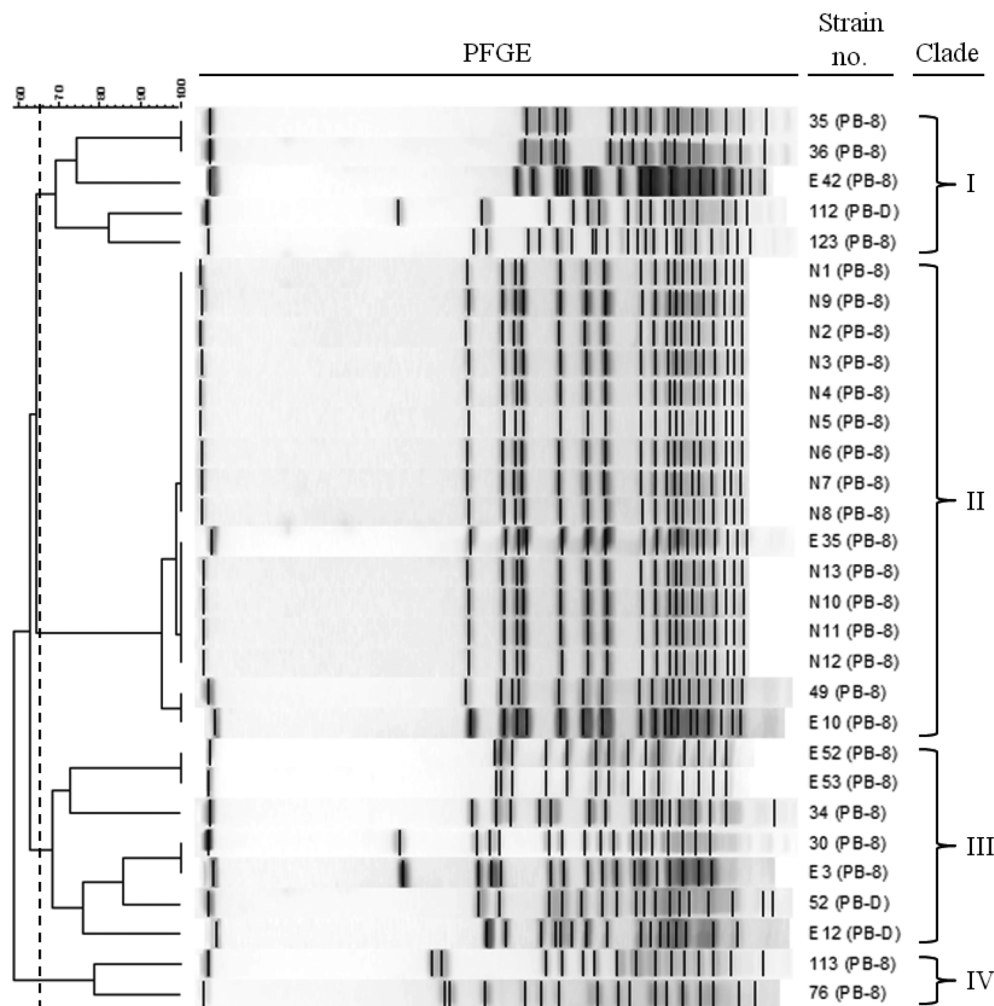
<sup>a</sup>Newly assigned strain name in Table 1 was used.

<sup>b,c</sup>PCR-RFLP pattern and group number were acquired from the previous report (Waleron et al., 2002). RFLP group D was newly designated in this research.

sequence analysis of eight housekeeping gene fragments, and showed diverse phenotypic characteristics (Nabhan et al., 2012); one third of the subspecies *carotovorum* isolates could form indole (Seo et al., 2002; Yap et al., 2004). Thus, these results suggest that not all Korean subspecies *brasiliensis* isolates can form indole; therefore, variants exist among isolates of the Korean subspecies *brasiliensis*.

In this present study, we reclassified several isolates as *P. carotovorum* subsp. *brasiliensis* previously identified as *P. carotovorum* subsp. *carotovorum*, and isolated subspecies

*brasiliensis* strains from various plants. Genetic analyses and biochemical tests indicated the genetic diversity of *P. carotovorum* subsp. *brasiliensis* strains isolated in Korea. Based on our results, we suggest that the newly identified *P. carotovorum* subsp. *brasiliensis* was not imported from a foreign country and previously existed in Korea as much as 20 years ago; further intensive comparison studies should be conducted. Knowledge of the genetic differentiation of the subspecies *carotovorum* and *brasiliensis* is important. The reported results will facilitate determination of the host



**Fig. 2.** Dendrogram of the pulsed-field gel electrophoresis (PFGE) results of *P. carotovorum* subsp. *brasiliensis* strains isolated in Korea. Four PFGE clades (I-IV) were detected based on a similarity cut-off of 65% (0.5% optimization and 1.0% tolerance). Note that PB is *P. carotovorum* subsp. *brasiliensis*, and 8 and D are *recA*-based RFLP groups in Table 3.

specificity of plant pathogens.

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