

First Report of *Pectobacterium versatile* as the Causal Pathogen of Soft Rot in Kimchi Cabbage in Korea

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In September 2021, gray-to-brown discoloration and expanding water-soaked lesions were observed on the outer and inner layers and the core of kimchi cabbage (*Brassica rapa* subsp. *pekinensis*) in fields located in Samcheok, Gangwondo, Korea. A bacterial strain designated as KNUB-02-21 was isolated from infected cabbage samples. Phylogenetic analysis based on the sequences of the 16S rRNA region and the *dnaX*, *leuS*, and *recA* genes confirmed that the strain was affiliated with *Pectobacterium versatile*. Additionally, the biochemical and morphological profiles of the isolate were similar to those of *P. versatile*. Based on these results, the isolate was identified as a novel strain of *P. versatile*. Healthy kimchi cabbage slices developed soft rot upon inoculation with *P. versatile* KNUB-02-21 and exhibited symptoms similar to those observed in the diseased plants in fields. The re-isolated strains were similar to those of *P. versatile*. Prior to our study, *P. versatile* as the causative pathogen of kimchi cabbage soft rot had not been reported in Korea.

Keywords: *Brassica rapa* subsp. *pekinensis*, Kimchi cabbage, Korea, *Pectobacterium versatile*, Soft rot

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In Korea, kimchi cabbage (*Brassica rapa* subsp. *pekinensis*) is grown in the mountains in summer and in the lowlands during fall to avoid high temperatures and disease outbreaks. Kimchi cabbage had an average cultivation area of 12,722 ha from 2019 to 2021. In Gangwondo, kimchi cabbage was cultivated over an area of approximately 1,482 ha between 2020 and 2021 (Korean Statistical Information Service, 2022). Various diseases of cabbage have been reported worldwide. *Verticillium dahliae*, *Fusarium equiseti*, and *Erysiphe cruciferarum* are the main fungal pathogens causing stem decay diseases in Korea (Afroz et al., 2021; Dumin et al.,

2021; Jee et al., 2008). In addition, soft rot disease caused by *Pectobacterium* species results in significant economic losses to dicotyledonous and monocotyledonous plants, as well as agricultural production businesses. *Pectobacterium* species produce various plant-cell-wall degrading enzymes, including cellulase, polygalacturonase, and pectinase, which are involved in causing soft rot in several plant hosts (Lee et al., 2014). Among several phytopathogenic *Pectobacterium* species, *P. brasiliense* and *P. odoriferum* have been reported to be causative pathogens of cabbage soft rot in Korea (Lee et al., 2014, 2021).

In September 2021, severe soft rot symptoms, such as gray-to-brown discoloration and expanding water-soaked lesions, were observed on kimchi cabbages in Samcheok, Gangwondo, Korea (Fig. 1). These symptoms are typical char-

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acteristics of bacterial diseases (bacterial soft rot) (Charkowski, 2018). Infected cabbage samples were used to isolate the causative pathogens. Small pieces of infected tissue were immersed in 5 ml of saline solution (0.8% NaCl) for 20 min. The resulting suspension was divided into 50 μ l and spread on King's B (Difco, Sparks, MD, USA) and nutrient agar (Difco) media and incubated at 30°C for 48 hr. The cultures were plated twice using these media for purification. A bacterial strain, designated KNUB-02-21, was isolated from infected kimchi cabbages.

For molecular analysis, total genomic DNA was extracted from the bacterial culture after 24 hr of growth on nutrient agar medium using a commercial extraction kit (HiGene Genomic DNA Prep Kit, Biofact, Daejeon, Korea), following the manufacturer's instructions. The polymerase chain reaction (PCR) protocol and primers for the 16S rRNA region were adapted from Weisburg et al. (1991) (Supplementary Table 1). ExoSAP-IT PCR Product Cleaning Reagent (Thermo Fisher Scientific, Waltham, MA, USA) was used to purify the PCR products. The 16S rRNA region sequence of KNUB-02-21 (GenBank no. LC733515) was 1,387 bp in length. A BLAST search of the National Center for Biotechnology Information (NCBI) database revealed that the sequence of the 16S rRNA region of KNUB-02-21 is highly similar to that of *P. carotovorum* subsp. *carotovorum* JB56 (GenBank no. MT579566) (99.86%), *P. brasiliense* HNP201711 (GenBank no. MN393933) (99.78%), *P. versatile* 14A (GenBank no. CP034276) (99.78%), and *P. odoriferum* WQC37 (GenBank no. MH778625) (99.64%). These results unequivocally demonstrated that the comparative analysis based solely on the 16S rRNA region sequence could not accurately identify strain KNUB-02-21 at the species level.

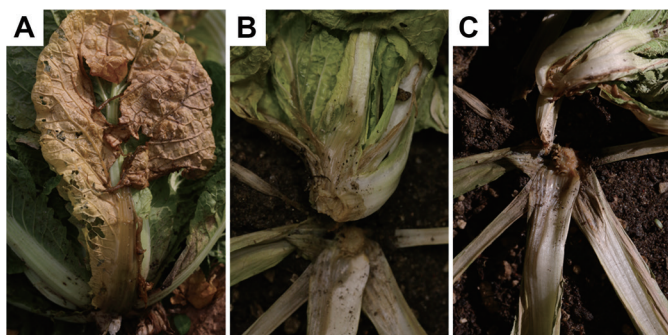


Fig. 1. Soft rot disease caused by *Pectobacterium versatile* KNUB-02-21 in kimchi cabbage in the field at Samcheok, Gangwon-do, Korea. (A) Symptoms of soft rot on the outer layer of kimchi cabbage. (B) Symptoms of soft rot on the inner layer of kimchi cabbage. (C) Symptoms of soft rot in the core of kimchi cabbage.

Based on the 16S rRNA region, only the taxonomic relationship at the genus level could be with other species; however, strain affiliation to *Pectobacterium* species and subspecies could not be precisely determined. Recent phylogenetic research using three concatenated housekeeping genes (*dnaX*, *leuS*, and *recA*) has allowed the assignment of 114 strains of the *P. carotovorum* complex to a few novel species (Portier et al., 2019). Therefore, *dnaX*, *leuS*, and *recA* of strain KNUB-02-21 were amplified, sequenced (Supplementary Table 1), and used for phylogenetic analysis.

A sequence of 511 bp for *dnaX* (GenBank no. LC733512), 522 bp for *leuS* (GenBank no. LC733513), and 714 bp for *recA* (GenBank no. LC733514) were obtained from KNUB-02-21. Based on the similarity of the *dnaX* gene sequence, *P. versatile* JB56A (GenBank no. MW930747) (100%), *P. carotovorum* subsp. *carotovorum* JB46 (GenBank no. MT632643) (100%), *P. atrosepticum* TEIC3211 (GenBank no. JN663800) (100%), *P. polaris* CFBP8603^T (GenBank no. MT684046) (97.15%), *P. parvum* YT22221 (GenBank no. CP046377) (97.06%), and *P. actinidiae* PRI-B17 (GenBank no. ON960282) (96.84%) were the closest phylogenetic relatives of the novel isolate. The *leuS* gene sequence of KNUB-02-21 exhibited 99.81% similarity to *P. parvum* YT22221 (GenBank no. CP102749) and *P. versatile* ECC15 (GenBank no. CP102749), 99.23% similarity to *P. carotovorum* RC5297 (GenBank no. CP045097), 98.85% similarity to *P. odoriferum* JK2.1 (GenBank no. CP034938), 97.71% similarity to *P. polaris* NIBIO1006 (GenBank no. CP017481), 97.13% similarity to *P. quasiquaticum* A398-S21-F17 (GenBank no. CP065178), 97.13% similarity to *P. aquaticum* A212-S19-A16 (GenBank no. CP086253), and 96.74% similarity to *P. brasiliense* BC1 (GenBank no. CP092039). The closely related *P. carotovorum* subsp. *carotovorum* BTC2 (GenBank no. MF314822), *P. versatile* 14A (GenBank no. CP034276), *P. odoriferum* JK2.1 (GenBank no. CP034938), and *P. aquaticum* A212-S19-A16 (GenBank no. CP086253) shared 99.72%, 99.58%, 97.75%, and 96.35% identity, respectively, with the *recA* gene sequence of strain KNUB-02-21. Similar to the 16S rRNA region, these results indicated that the comparative analysis based on the sequence of only one of these molecular markers did not allow precise identification of the closest relatives.

Multilocus sequence analysis is currently a popular technique for confirming with a higher degree of certainty the phylogenetic relationships of species within a genus. The combined sequences of *dnaX*, *leuS*, and *recA* genes are effec-

Table 1. Strains of *Pectobacterium* species used in this study and their GenBank accession numbers

Species	Strain no.	GenBank accession no.		
		<i>dnaX</i>	<i>leuS</i>	<i>recA</i>
<i>Pectobacterium aroidearum</i>	CFBP1457	MT683925	MT684072	MT684219
<i>Pectobacterium aroidearum</i>	CFBP2573	MT683941	MT684088	MT684235
<i>Pectobacterium aroidearum</i>	CFBP6725	MT684029	MT684176	MT684323
<i>Pectobacterium aroidearum</i>	CFBP8737	MT684054	MT684201	MT684348
<i>Pectobacterium atrosepticum</i>	CFBP1526 ^T	MK516904	MK517048	MK517192
<i>Pectobacterium betavasculorum</i>	CFBP1539 ^T	MK516905	MK517049	MK517193
<i>Pectobacterium brasiliense</i>	CFBP5392	MK516927	MK517071	MK517215
<i>Pectobacterium brasiliense</i>	CFBP6607	MK516954	MK517098	MK517242
<i>Pectobacterium brasiliense</i>	CFBP6615	MK516955	MK517099	MK517243
<i>Pectobacterium brasiliense</i>	CFBP6617 ^T	MK516956	MK517100	MK517244
<i>Pectobacterium cacticida</i>	CFBP3628 ^T	MK516923	MK517067	MK517211
<i>Pectobacterium carotovorum</i> subsp. <i>carotovorum</i>	CFBP1364	MK516896	MK517040	MK517184
<i>Pectobacterium carotovorum</i> subsp. <i>carotovorum</i>	CFBP2046 ^T	MK516909	MK517053	MK517197
<i>Pectobacterium carotovorum</i> subsp. <i>carotovorum</i>	CFBP6071	MK516950	MK517094	MK517238
<i>Pectobacterium carotovorum</i> subsp. <i>carotovorum</i>	CFBP7351	MK516962	MK517106	MK517250
<i>Pectobacterium odoriferum</i>	CFBP1878 ^T	MK516907	MK517051	MK517195
<i>Pectobacterium odoriferum</i>	CFBP3259	MK516920	MK517064	MK517208
<i>Pectobacterium odoriferum</i>	CFBP3297	MK516921	MK517065	MK517209
<i>Pectobacterium odoriferum</i>	CFBP5539	MK516929	MK517073	MK517217
<i>Pectobacterium fontis</i>	CFBP8629 ^T	MK516878	MK517022	MK517166
<i>Pectobacterium parmentieri</i>	CFBP8475 ^T	MK516972	MK517116	MK517260
<i>Pectobacterium peruvienne</i>	CFBP5834	MK516935	MK517079	MK517223
<i>Pectobacterium polaris</i>	CFBP1403	MK516898	MK517042	MK517186
<i>Pectobacterium polaris</i>	CFBP6058	MK516945	MK517089	MK517233
<i>Pectobacterium polaris</i>	CFBP7360	MT684038	MT684185	MT684332
<i>Pectobacterium polaris</i>	CFBP8603 ^T	MT684046	MT684193	MT684340
<i>Pectobacterium punjabense</i>	CFBP8604 ^T	MK516877	MK517021	MK517165
<i>Pectobacterium versatile</i>	KNUB-02-21	LC733512	LC733513	LC733514
<i>Pectobacterium versatile</i>	CFBP1118	MK516888	MK517032	MK517176
<i>Pectobacterium versatile</i>	CFBP2138	MK516912	MK517056	MK517200
<i>Pectobacterium versatile</i>	CFBP6051 ^T	MK516938	MK517082	MK517226
<i>Pectobacterium versatile</i>	CFBP8656	MK516973	MK517117	MK517261
<i>Pectobacterium wasabiae</i>	CFBP3304 ^T	MK516922	MK517066	MK517210
<i>Dickeya solani</i>	CFBP7704	MK516970	MK517114	MK517258

The strain isolated in this study is highlighted in bold.
T, the type strain.

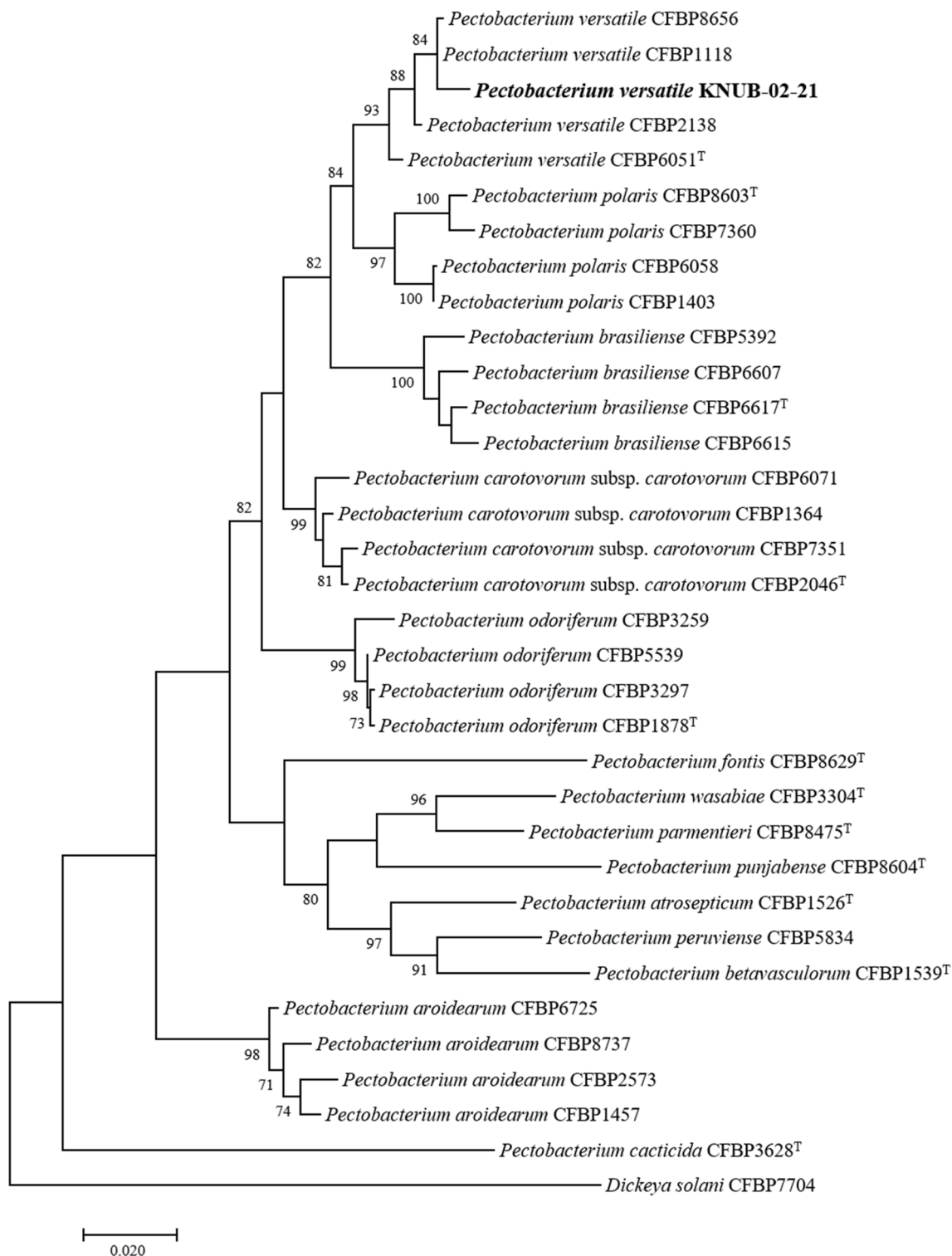


Fig. 2. Maximum-likelihood phylogenetic tree, based on concatenated partial sequences of *dnaX*, *leuS*, and *recA* genes, depicting the phylogenetic position of strain KNUB-02-21 among related species of the genus *Pectobacterium*. Bootstrap values (based on 1,000 replications) greater than 70% are displayed on the branch points. *Dickeya solani* CFBP7704 was used as the outgroup. Scale bar=0.020 substitutions per nucleotide position.

tive for the identification and classification of strains affiliated with the genus *Pectobacterium* (Portier et al., 2019). Using

the NCBI BLAST search, the sequences of the three molecular markers of strain KNUB-02-21 were compared with those of

Table 2. Biochemical test results of *Pectobacterium versatile* KNUB-02-21 and the most common soft rot pathogen *Pectobacterium* spp.

	KNUB-02-21	Pv	Pac	Paq	Pb	Pcc	Po	Pp
D-Arabitol	–	–	–/+	–	+	–	+	–
Stachyose	+	+	+	–	+	+	+	–
L-Galactonic acid- γ -lactone	+	+	+	+	+	–	+	+
Guanidine hydrochloride	+	+	+	+	+	–	+	+

+, positive reaction; –, negative reaction; –/+, ambiguous reaction; Pv, *Pectobacterium versatile*; Pac, *Pectobacterium actinidiae*; Paq, *Pectobacterium aquaticum*; Pb, *Pectobacterium brasiliense*; Pcc, *Pectobacterium carotovorum* subsp. *carotovorum*; Po, *Pectobacterium odoriferum*; Pp, *Pectobacterium polaris*.

the reference *Pectobacterium* species, and close phylogenetic neighbors were selected (Table 1). The MEGA 7 software was used to conduct phylogenetic and molecular evolutionary analyses (Kumar et al., 2016). Maximum likelihood analysis was performed using Kimura's two-parameter model and the nearest neighbor interchange heuristic search method. Bootstrap analysis was performed with 1,000 replicates to assess the stability and validity of various clusters. In the phylogenetic tree, the novel isolate KNUB-02-21 and several strains of *P. versatile* clustered together in a monophyletic clade with a high bootstrap value, indicating their affiliation with the same species (Fig. 2).

According to Portier et al. (2019), a combination of three reactions, namely assimilation of D-arabitol, stachyose, and guanidine hydrochloride or L-galactonic acid- γ -lactone can help differentiate *P. versatile*, *P. carotovorum* subsp. *carotovorum*, *P. odoriferum*, *P. brasiliense*, *P. actinidiae*, *P. aquaticum*, and *P. polaris*. Isolate KNUB-02-21 responded positively and assimilated stachyose, L-galactonic acid- γ -lactone, and guanidine hydrochloride when tested using Biolog GEN III plates (Biolog, Hayward, CA, USA). However, the strain KNUB-02-21 tested negative for D-arabitol assimilation in the Biolog GEN III test. *P. versatile* exhibits a similar response towards the four growth substrates (Table 2), further confirming the affiliation of KNUB-02-21 with this *Pectobacterium* species. The results of the phylogenetic analysis and biochemical tests supported the identification of KNUB-02-21 as a novel strain belonging to *P. versatile*.

Pathogenicity tests were conducted on kimchi cabbage to confirm Koch's postulates. The surfaces of the kimchi cabbage slices were disinfected using 70% ethanol and washed with distilled water prior to inoculation. Three kimchi cabbage slices were pierced with a sterilized blue tip and

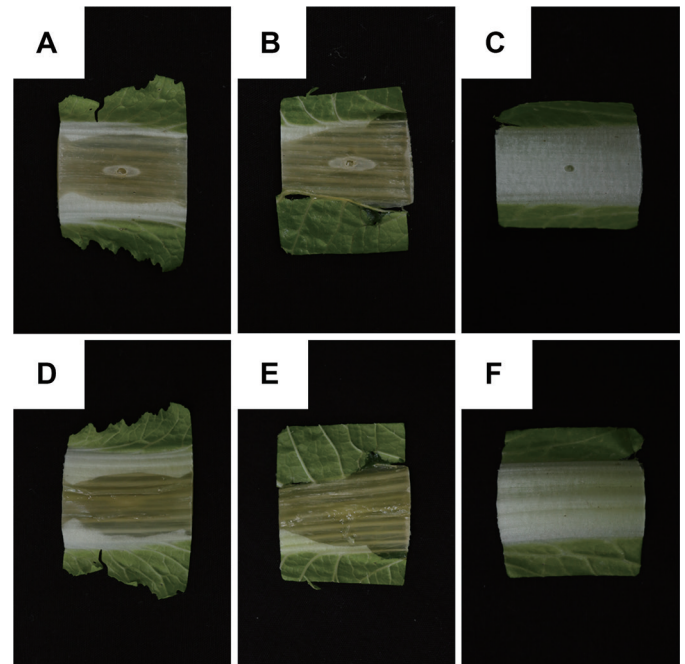


Fig. 3. Effects of *Pectobacterium versatile* KNUB-02-21 inoculation on kimchi cabbage slices. (A, B, D, E) Development of gray-to-brown discoloration and expanding water-soaked lesions in infected specimens after two days, similar to the soft rot disease symptoms observed first in kimchi cabbage. (C, F) After two days, no symptoms were observed in distilled water-inoculated, uninfected kimchi cabbage slices.

inoculated with 20 μ l of *P. versatile* KNUB-02-21 suspension at a concentration of 1×10^8 cells/ml. The plant used as an uninfected control was treated similarly but injected with 20 μ l of distilled water. Slices of inoculated kimchi cabbage were maintained under greenhouse conditions (25–30°C, 80% relative humidity). After inoculation, the kimchi cabbage slices exhibited symptoms of soft rot after one day. After two days, the symptoms of gray-to-brown discoloration were similar to those observed previously, and increased water-soaked

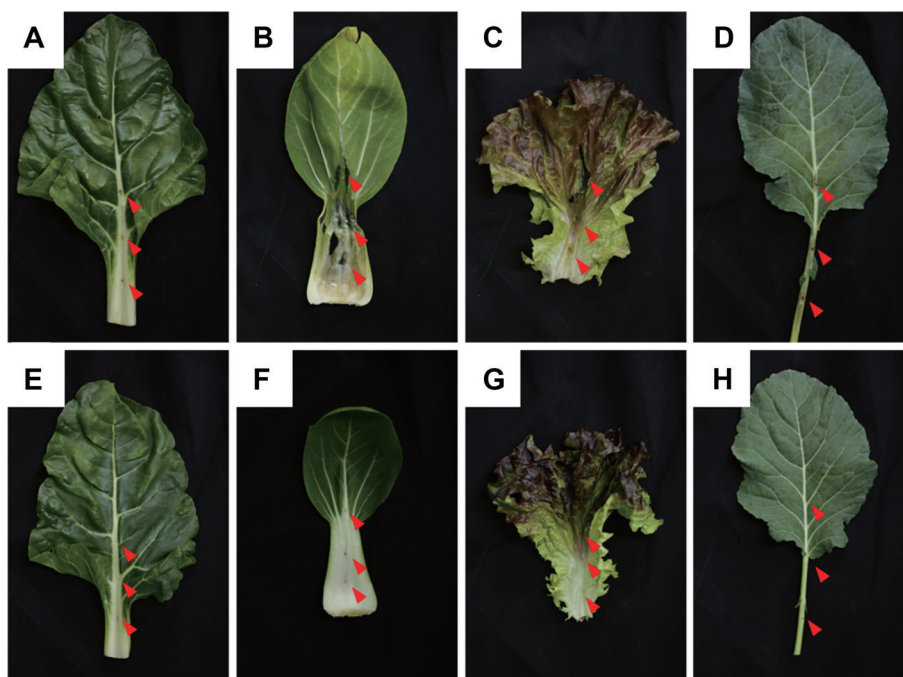


Fig. 4. Host range test for *Pectobacterium versatile* KNUB-02-21. Plants were inoculated with bacterial cells at 1×10^8 cells/ml, and incubated for two days in the greenhouse: beta (A), bok choy (B), lettuce (C), kale (D), and uninoculated Brassicaceae plants (E–H), respectively. The arrowheads indicate the area where the inoculation was performed.

appeared on the kimchi cabbage (Fig. 3A, B, D, E). However, uninoculated kimchi cabbage slices did not develop any symptoms (Fig. 3C, F). Each slice of the infected kimchi cabbage was used to re-isolate the pathogen. In each case, the isolated bacterial strain was re-identified as *P. versatile* (data not shown).

The host range of *P. versatile* KNUB-02-21 was tested using four Brassicaceae plant species: beta (*Beta vulgaris* subsp. *vulgaris*), bok choy (*Brassica rapa* subsp. *chinensis*), lettuce (*Lactuca sativa*), and kale (*Brassica oleracea*). *P. versatile* KNUB-02-21 was suspended in sterile water, and the bacterial concentration was adjusted to 1×10^8 cells/ml based on spectrophotometric readings (absorbance at 600 nm). Leaves from harvested plants were pierced with a sterilized blue tip and inoculated by injecting 20 μ l of bacterial suspension into the petiole and midrib of each leaf. The plant used as the uninoculated control sample was injected with distilled water. All the plants were maintained under greenhouse conditions (25–30°C, 80% relative humidity). After bacterial inoculation, the four Brassicaceae plant species presented symptoms of soft rot after two days (Table 3, Fig. 4).

Several species of the genus *Pectobacterium* cause disease in various host plants. *P. versatile* has been identified as the causative agent of blackleg and soft rot in various plants

Table 3. Pathogenicity assay for different Brassicaceae plants

Treatment	Brassicaceae plants			
	Beta	Bok choy	Lettuce	Kale
<i>Pectobacterium versatile</i>	+	+	+	+
Distilled water (control)	–	–	–	–

+, appearance of disease symptoms; –, no disease symptoms.

(Chen et al., 2022; Ma et al., 2021). *P. versatile* has been isolated from artichokes, bittersweet, cabbage, carrot, chicory, chrysanthemum, cyclamen, hyacinth, iris, leek, potato, and primrose (Portier et al., 2020). The phytopathogenic species *P. versatile* has been reported in Algeria, Canada, Finland, France, Morocco, the Netherlands, Spain, Syria, the United Kingdom, and the United States (Portier et al., 2020).

In this study, *P. versatile* was identified for the first time in Korea as a pathogen of soft rot in kimchi cabbage. In addition, this *Pectobacterium* species was found to cause a similar soft rot disease in four other plants, namely, beta, bok choy, lettuce, kale and cabbage of the family Brassicaceae. Our results expand our understanding of *P. versatile* distribution in Korea, enhance our knowledge of the causative pathogens

of soft rot in kimchi cabbage, and can be applied to improve control strategies to prevent economic losses.

Conflicts of Interest

No potential conflict of interest relevant to this article was reported.

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Electronic Supplementary Material

Supplementary materials are available at Research in Plant Disease website (<http://www.online-rpd.org/>).

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