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First Report of *Pectobacterium brasiliense* Causing Soft Rot on Graft Cactus in Korea

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The graft cactus (*Gymnocalycium mihanovichii*) continues to be exported to more than 20 countries worldwide. In April 2021, typical bacterial symptoms of soft rot were observed in the graft cactus (cv. Yeonbit) in Goyang, Gyeonggi-do, Korea, resulting in economic losses in cactus production. The stems turned dark brown and the flowers were covered with black rot. The bacterial strain designated as KNUB-01-21 was isolated from infected stems and flowers. The results of the morphological and biochemical tests of the isolate were similar to those of *Pectobacterium brasiliense*. For molecular analysis, the 16S rRNA region and three housekeeping genes (*dnaX*, *leuS*, and *recA*) of the strain KNUB-01-21 were amplified. Based on the results of the molecular analysis and morphological and biochemical tests, KNUB-01-21 was identified as *P. brasiliense*. The pathogenicity of KNUB-01-21 on graft cactus was confirmed by an inoculation test. Artificial inoculation using *P. brasiliense* KNUB-01-21 produced soft rot symptoms on the grafted cactus, and the same bacterium was re-isolated and re-identified. This is the first report of *P. brasiliense* causing soft rot in graft cactus in Korea.

Keywords: Graft cactus, *Gymnocalycium mihanovichii,* Pathogenicity, *Pectobacterium brasiliense,* Soft rot

In 2019, 43.2 ha of cactus was cultivated and the graft cactus (*Gymnocalycium mihanovichii*) continues to be exported to more than 20 countries worldwide (Ministry of Agriculture, Food and Rural Affairs, 2020). In addition, cactus accounted for 27.2% of Korea's total flower exports by 2020 (Korea Agro-Fisheries and Food Trade Corporation, 2020). Various diseases of the cactus have been reported worldwide. *Bipolaris cactivora*, *Glomerella cingulata*, and *Fusarium oxysporum* are the three major fungal stem decay diseases known in Korea (Chang et al., 1998; Hyun et al., 1998; Kim et al., 2000, 2004). *Pectobacterium* species are also known as causing agents of cactus diseases. *P. brasiliense* was reported in Mexico as the causal pathogen of soft rot in cactus (Mejía-Sánchez et al., 2019). *P. cacticida* was identified as the causal pathogen of soft rot of cactus in the United States (Alcorn et al., 1991), and *P. carotovorum* subsp. *carotovorum* was identified as the causal agent of soft rot in cactus in Korea (Kim et al., 2007). It is known that bacteria belonging to *Pectobacterium* species that cause soft rot in various plant hosts produce various plant cell wall-degrading enzymes, such as cellulase, polygalacturonase, and pectinase (Lee et al., 2013).

In April 2021, typical bacterial symptoms of soft rot were observed in the graft cactus (cv. Yeonbit) in Goyang, Gyeonggi-do, Republic of Korea. Infected graft cactus devel-

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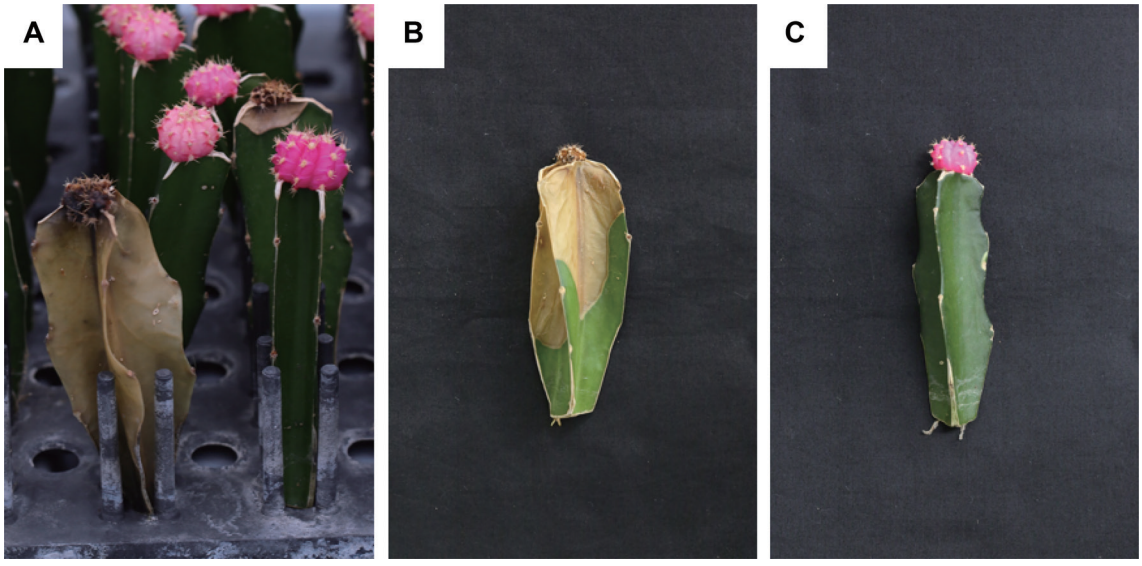

Fig. 1. Bacterial soft rot symptoms of graft cactus caused by *Pectobacterium brasiliense* KNUB-01-21. (A) Soft and black rot symptoms observed in the greenhouse. (B) Symptom induced by artificial inoculation using *Pectobacterium brasiliense* KNUB-01-21. (C) Sterilized water was used as a control.

oped a gray-green color and wilting, and these symptoms were similar to those of bacterial soft rot (Fig. 1A). An unusual outbreak of graft cactus with the same symptoms was observed in the surveyed field. These symptomatic features are similar to those of bacterial diseases, such as bacterial soft rot (Charkowski, 2018). To isolate pathogens, infected stem tissues were curled surface-sterilized with 70% ethanol for 30 sec and 1% sodium hypochlorite for 60 sec and then rinsed with sterile water. The stem tissue was placed on a glass slide, 1–2 drops of sterile water were added and left for 10 min, streaked on nutrient agar (NA; Difco, Detroit, MI, USA), and incubated at 28°C. After 3 days, the bacterial strain, designated KNUB-01-21, was isolated from NA and purified by streaking on King's medium B (KB; Difco) and NA.

For molecular analysis, total genomic DNA was extracted from strain KNUB-01-21 using the HiGene Genomic DNA Prep Kit (Biofact, Daejeon, Korea). The 16S rRNA regions were amplified using the primers 9F (5′-GAG TTT GAT CCT GGC TCA G-3′) and 1512R (5′-ACG GCT ACC TTG TTA CGA CTT-3′) (Weisburg et al., 1991). The polymerase chain reaction (PCR) cycling conditions were 94°C for 3 min, followed by 35 cycles of denaturation at 94°C for 1 min, annealing at 55°C for 1 min, and synthesis at 72°C for 1 min 30 sec; post-synthesis was carried out at 72°C for 7 min. The amplified PCR products were purified using the ExoSAP-IT PCR Product Cleaning Reagent (Thermo Fisher Scientific, Waltham, MA, USA). Sequencing was performed using SolGent (Daejeon, Korea). A sequence of 1,352 bp was obtained from the 16S rRNA region (GenBank no. LC717492). A blast search of the National Center for Biotechnology Information (NCBI) database revealed similarities of 100% between the sequence of the 16S rRNA gene of KNUB-01-21 and several strains belonging to *P. brasiliense* (GenBank nos. MN393942, MN393919), and 99.93% with *P. carotovorum* subsp. *carotovorum* PDP201711 (GenBank no. MN394009), and *P. versatile* PJ17 (GenBank no. MN393903). These results indicated that comparative analysis based on the sequence of only the 16S rRNA gene did not allow precise identification of strain KNUB-01-21 at the species level.

The identification of *Pectobacterium* species and subspecies based on 16S rRNA was not accurate, and only the taxonomic relationship between other species at the genus level could be confirmed. Recently, phylogenetic analysis using three concatenated housekeeping genes, *dnaX*, *leuS*, and *recA* allowed for the assignment of 114 strains to a novel species of the *P. carotovorum* complex and, in particular, to describe *P. brasiliense*, *P. versatile*, *P. actinidiae*, and *P. odoriferum* (Portier et al., 2019). Following this approach, *dnaX*, *leuS*, and *recA* genes of strain KNUB-01-21 were amplified and sequenced. The *dnaX* gene was amplified using *dnaXF* (5′-TAT CAG GTY CTT GCC CGT AAG TGG-3′) and *dnaXR* (5′-TCG ACA TCC ARC GCY TGA GAT G-3′) (Sławiak et al., 2009). PCR proto-

cols and primers used were previously described by Portier et al. (2019). The PCR cycling conditions for the *dnaX* gene were 94°C for 3 min, followed by 35 cycles of denaturation at 94°C for 1 min, annealing at 55°C for 1 min, and synthesis at 72°C for 2 min. The post-synthesis was performed at 72°C for 5 min. The *leuS* gene was amplified using *leuSF* (5′-TYT CCA TGC TGC CYT AYC CT-3′) and *leuSR* (5′-TCC AGT TRC GCT GCA TGG TT-3′) (Portier et al., 2019). The PCR cycling conditions for the *leuS* gene were 94°C for 10 min, followed by 31 cycles of denaturation at 94°C for 1 min, annealing at 55°C for 1 min, and synthesis at 72°C for 1 min. The post-synthesis was performed at 72°C for 5 min. The *recA* gene was amplified using *recAF* (5′-GGT AAA GGG TCT ATC ATG CG-3′) and *recAR* (5′-CCT TCA CCA TAC ATA ATT TGG-3′) (Waleron et al., 2002). The PCR cycling conditions for the *recA* gene were 94°C for 3 min, 35 cycles of denaturation at 94°C for 1 min, annealing at 47°C for 1 min, and synthesis at 72°C for 2 min. The post-synthesis was performed at 72°C for 5 min. The amplified PCR products were purified using ExoSAP-IT PCR Product Cleaning Reagent.

Sequencing was performed using SolGent. A sequence of 528, 511, and 713 bp for *dnaX* (GenBank no. LC717494), *leuS* (GenBank no. LC717495), and *recA* (GenBank no. LC717493) were respectively obtained.

The *dnaX* sequence of strain KNUB-01-21 shared 99.41%, 98.24%, and 98.04% identity with closely related *P. brasiliense* HNP201719 (GenBank no. CP046380), *P. carotovorum* subsp. *carotovorum* PCC21 (GenBank no. CP003776) and *P. atrosepticum* IPO 998 (GenBank no. GQ904832), respectively. Based on *leuS* gene sequence similarity, the close relatives of KNUB-01-21 were identified as *P. brasiliense* BC1 (GenBank no. CP009769) (99.62%), *P. carotovorum* subsp. *carotovorum* PCC21 (GenBank no. CP003776) (99.62%), and *P. quasiaquaticum* A398-S21-F17 (GenBank no. CP065178) (98.86%). The *recA* sequence of the isolate showed 100% similarity with *P. brasiliense* CFBP7079 (GenBank no. MK517247), 98.60% with *P. carotovorum* subsp. *carotovorum* 333 (GenBank no. AY264787), 98.18% with *P. aquaticum* IFB5637 (GenBank no. MW660584), and 98.17% with *P. quasiaquaticum* A477-S1-J17 (GenBank no. CP065177).

All the results indicated that comparative analysis based on the sequence of any one gene did not allow precise identification of the bacterial strain at the species level; therefore, multilocus sequence analysis was performed using concatenated sequences of the three above-mentioned genes of strain KNUB-01-21. These combined three molecular markers are highly effective in resolving species in the genus *Pectobacterium* (Portier et al., 2019). Sequences of the allied species were retrieved from the NCBI GenBank database (Table 1). Multiple sequence alignments were performed using MEGA7 (Kumar et al., 2016). A phylogenetic tree was constructed using the maximum-likelihood method (Felsenstein, 1981). Maximum-likelihood analysis was performed using the nearest-neighbor interchange heuristic search method and Kimura's two-parameter model. Strain KNUB-01-21, *P. brasiliense* CFBP5392, *P. brasiliense* CFBP6607, *P. brasiliense* CFBP6615, and *P. brasiliense* CFBP6617^{T} clustered together in a monophyletic clade with high bootstrap value, strongly supporting their affiliation with the same species (Fig. 2).

The isolated *P. brasiliense* was characterized according to the methods described by Czajkowski et al. (2015) and Schaad et al. (2001). Strain KNUB-01-21 was gram-negative, motile, bacillar, and facultatively anaerobic. However, the isolate did not fluoresce in KB medium, did not grow at 37°C in NA, and did not utilize sucrose-reducing substances or indole. Growth was observed in the 5% NaCl treatment. KNUB-01-21 showed cavity formation on crystal violet pectate and produced acid from lactose, maltose, α-methyl glucoside, and trehalose. The strain was sensitive to antibiotics such as amikacin, ampicillin, cephaletin, cefinaxone, chloramphenicol, enonaxin, gentamicin, notilmicin, and trimethoprim-sulfatomethoxasol, but it was resistant to penicillin, erythromycin, and dicloxacillin. The results of these morphological and biochemical tests for strain KNUB-01-21 were the same as those for *P. brasiliense* (Supplementary Table 1).

To determine the pathogenicity of *P. brasiliense* KNUB-01-21, pathogenicity tests were conducted on the grafted cactus. The surface of the grafted cactus was disinfected with 70% ethanol and washed with distilled water below inoculation. Graft cactus was inoculated with a 20 ml suspension (1×10^{8} cells/ml) of strain KNUB-01-21. Plants inoculated with 10 ml distilled water were used as mock-infected plants. The inoculated graft cactus was maintained under greenhouse conditions (25–30°C, relative humidity 80%). After inoculation, symptoms of soft rot were observed in the grafted cactus flowers after 2 days. The same symptoms, including gray-green color and wilting, occurred as the first discovered symptoms that appeared on the graft cactus 7 days after flowering (Fig. 1B). However, no symptoms were observed in the mock-infected graft cactus (Fig. 1C). The pathogen was

Table 1. *Pectobacterium* species used in this study for phylogenetic analysis and GenBank accession numbers

Species	Strain no.	GenBank accession no.		
		dnaX	***leuS***	***recA***
Pectobacterium aroidearum	CFBP1457	MT683925	MT684072	MT684219
Pectobacterium aroidearum	CFBP2573	MT683941	MT684088	MT684235
Pectobacterium aroidearum	CFBP6725	MT684029	MT684176	MT684323
Pectobacterium aroidearum	CFBP8737	MT684054	MT684201	MT684348
Pectobacterium atrosepticum	CFBP1526^T	MK516904	MK517048	MK517192
Pectobacterium betavasculorum	CFBP1539^T	MK516905	MK517049	MK517193
Pectobacterium brasiliense	**KNUB-01-21**	**LC717494**	**LC717495**	**LC717493**
Pectobacterium brasiliense	CFBP5392	MK516927	MK517071	MK517215
Pectobacterium brasiliense	CFBP6607	MK516954	MK517098	MK517242
Pectobacterium brasiliense	CFBP6615	MK516955	MK517099	MK517243
Pectobacterium brasiliense	CFBP6617^T	MK516956	MK517100	MK517244
Pectobacterium cacticida	CFBP3628^T	MK516923	MK517067	MK517211
Pectobacterium carotovorum subsp. *carotovorum*	CFBP1364	MK516896	MK517040	MK517184
Pectobacterium carotovorum subsp. *carotovorum*	CFBP2046^T	MK516909	MK517053	MK517197
Pectobacterium carotovorum subsp. *carotovorum*	CFBP6071	MK516950	MK517094	MK517238
Pectobacterium carotovorum subsp. *carotovorum*	CFBP7351	MK516962	MK517106	MK517250
Pectobacterium odoriferum	CFBP1878^T	MK516907	MK517051	MK517195
Pectobacterium odoriferum	CFBP3259	MK516920	MK517064	MK517208
Pectobacterium odoriferum	CFBP3297	MK516921	MK517065	MK517209
Pectobacterium odoriferum	CFBP5539	MK516929	MK517073	MK517217
Pectobacterium fontis	CFBP8629^T	MK516878	MK517022	MK517166
Pectobacterium parmentieri	CFBP8475^T	MK516972	MK517116	MK517260
Pectobacterium peruviense	CFBP5834	MK516935	MK517079	MK517223
Pectobacterium polaris	CFBP1403	MK516898	MK517042	MK517186
Pectobacterium polaris	CFBP6058	MK516945	MK517089	MK517233
Pectobacterium polaris	CFBP7360	MT684038	MT684185	MT684332
Pectobacterium polaris	CFBP8603^T	MT684046	MT684193	MT684340
Pectobacterium punjabense	CFBP8604^T	MK516877	MK517021	MK517165
Pectobacterium versatile	CFBP1118	MK516888	MK517032	MK517176
Pectobacterium versatile	CFBP2138	MK516912	MK517056	MK517200
Pectobacterium versatile	CFBP6051^T	MK516938	MK517082	MK517226
Pectobacterium versatile	CFBP8656	MK516973	MK517117	MK517261
Pectobacterium wasabiae	CFBP3304^T	MK516922	MK517066	MK517210

The isolated strain is shown in bold.

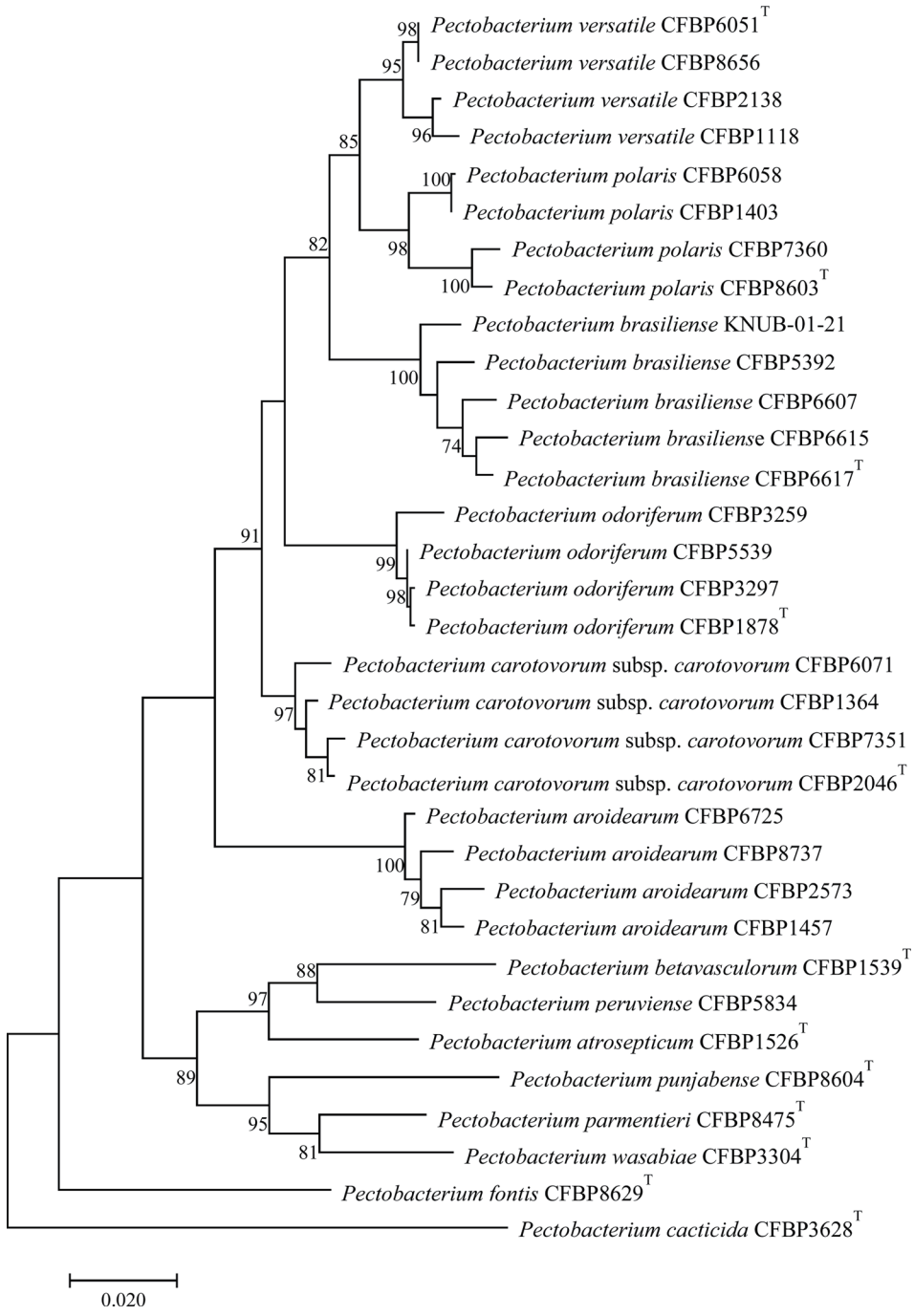

Fig. 2. Maximum-likelihood phylogenetic tree, based on concatenated partial sequences of *dnaX*, *leuS*, and *recA* genes, showing the phylogenetic position of strain KNUB-01-21 among related species of the genus *Pectobacterium*. Bootstrap values (based on 1,000 replications) greater than 70% are shown at branch points. *Pectobacterium cacticida* CFBP3628^T was used as the outgroup. Scale bar, 0.020 substitutions per nucleotide position.

re-isolated from each diseased graft cactus, and the isolated bacterial strain was re-identified as *P. brasiliense* (data not shown).

Various *Pectobacterium* species have been reported as crop pathogens. Among them, *P. brasiliense* is one of the most dangerous phytopathogens, and 19 different plant species belonging to 10 families have been reported as hosts of this agent (Oulghazi et al., 2021). The first isolation of *P. brasiliense* from Korea was reported in 2012 (Choi and Kim, 2012). Moreover, *P. brasiliense* has been identified in Korea as

the causal pathogen of soft rot in amaranth, paprika, potato, and tomato (Choi and Kim, 2012; Jee et al., 2018; Lee et al., 2013).

In Korea, the bacterial pathogen causing soft rot on the cactus was reported to be *P. carotovorum* subsp. *carotovorum* (Kim et al., 2007). Our study is the first report of *P. brasiliense* as the causal agent of soft rot in graft cactus in Korea. Our results increase the awareness of *P. brasiliense* distribution in Korea, thereby improving our understanding of cactus soft rot associated with *Pectobacterium* species and can be used to develop control methods 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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