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First Report of *Pectobacterium brasiliense* Causing Bitter Melon Soft Rot Disease in Korea

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In the Goesan region, located in Chungcheongbuk-do, Korea, a significant outbreak of soft rot infections was documented in August 2021, affecting fruits of *Momordica charantia*, commonly known as bitter melon or bitter gourd. The symptoms included a noticeable transition to yellowing in the affected fruits, eventually leading to their collapse. The bacterial strain KNUB-09-21 was isolated from the diseased fruits. Molecular analysis, using the sequences of the 16S rRNA region and three housekeeping genes (*dnaX*, *recA*, and *leuS*), along with the results of compound utilization in the API ID 32 GN system, provide strong evidence for the identification of the isolate KNUB-09-21 as *Pectobacterium brasiliense*. The pathogenicity of strain KNUB-09-21 on *M. charantia* was confirmed through a controlled inoculation test. Within two days, inoculated fruits displayed soft rot symptoms closely resembling those observed in naturally affected fruits. This is the first report of soft rot on *M. charantia* in Korea.

Keywords: Korea, *Momordica charantia*, Pathogenicity, *Pectobacterium brasiliense*, Soft rot

Momordica charantia, commonly known as bitter melon or bitter gourd, was the most extensively cultivated among subtropical crops such as asparagus, banana, coffee, dragon fruit, feijoa, guava, hooker chives, mango, okra, papaya, passion fruit, and turmeric until 2018 (Jeong et al., 2020). In Korea's agricultural landscape, the area dedicated to *M. charantia* cultivation averaged approximately 84.2 ha between 2017 and 2019. Within the specific region of Chungcheongbuk-do, the cultivation area for *M. charantia* averaged around 5 ha from 2017 to 2019 (Jeong et al., 2020). *M. charantia* is susceptible to various fungal diseases such as anthracnose and soft rot (Kim et al., 2015; Kwon and Jee, 2005). Bacterial soft rot diseases have been documented in Japan (Kubo et al., 2009). However, to date, there have been no reported instances of bacterial diseases affecting *M. charantia* in Korea.

Soft rot is a highly destructive ailment that has a global impact on vegetables, affecting regions where fleshy storage tissues of vegetables and ornamentals are present (Appy et al., 2023; Wu et al., 2023). Among bacterial plant pathogens, there exists a group of pectinolytic organisms known as soft rot Pectobacteriaceae, which encompasses two genera: *Pectobacterium* and *Dickeya* (Adeolu et al., 2016). *Pectobacterium*, recognized as one of the top 10 economically significant plant pathogenic bacteria, demonstrates a wide range of host compatibility, leading to the development of soft rot symptoms in both live plants and harvested crops (Ma et

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al., 2007; Mansfield et al., 2012). The invasion of the vascular systems of vegetables, ornamentals, and fruit trees by *Pectobacterium* strains can result in crop losses at various stages, including field production, harvest, storage, and transportation, with significant economic consequences (Czajkowski et al., 2011; Koh et al., 2012; Pérombelon, 2002).

The genus *Pectobacterium* is known for its production of enzymes, including cellulase, pectinase, and polygalacturonase, which actively degrade plant cell walls and membranes (Hugouvieux-Cotte-Pattat et al., 2014; Maung et al., 2022). Under favorable climatic conditions, infected tissue exhibits a characteristic water-soaked, slimy, and rotten appearance (Barras et al., 1994; Hugouvieux-Cotte-Pattat et al., 1996).

Within the same species, the genus *Pectobacterium* encompasses diverse strains with varying biochemical, physiological, and genetic characteristics (Duarte et al., 2004; Portier et al., 2019). The use of molecular techniques for differentiation and advanced diagnostic methods has led to the reclassification of strains with distinct biochemical and physiological traits (Nabhan et al., 2012; Portier et al., 2019). Currently, this genus comprises 20 species with validly published names and several other proposed taxa are awaiting their validation (Hong et al., 2023b).

P. brasiliense, a member of the genus *Pectobacterium*, is recognized for its exceptional virulence. It was first identified as an atypical strain of *Erwinia carotovora* causing severe blackleg disease in Brazilian potato plants by Duarte et al. (2004). Initially suggested as a new subspecies of *P. carotovorum*, it was later classified as a separate species (Nabhan et al., 2012; Portier et al., 2019). *P. brasiliense* has recently gained worldwide attention as a significant threat to global potato production (Öztürk and Umar, 2022).

The presence of soft rot induced by *P. brasiliense* in Korea was initially documented in 2012 in paprika (Choi and Kim, 2013). Since then, *P. brasiliense* has been identified in various plant species in Korea, including cucumber, graft cactus, melon, and paprika (Choi and Kim, 2013; Hong et al., 2023a; Park et al., 2022, 2023).

In August 2021, cases of soft rot diseases were observed on *M. charantia* fruits in Goesan, Chungcheongbuk-do, Korea. The affected fruits displayed a distinct transition to yellowing, followed by a subsequent collapse, revealing sunken lesions on the fruit surface and exhibiting typical soft rot symptoms (Fig. 1A, B). Tissue samples from the affected *M. charantia* were extracted for analysis. To isolate the pathogens, the affected *M. charantia* fruits underwent a surface sterilization process lasting 90 sec, using a 1% hypochlorite solution, followed by thorough rinsing with sterile distilled water. After sterilization, a 100 µl aliquot of liquid suspension was applied to the surface of a CVP medium (Hélias et al., 2012). Distinctive colonies developed within the CVP medium after a three-day incubation period at 28°C. These colonies were characterized by their white-gray color, circular morphology, smooth edges, and noticeable cavities, primarily due to their pectin metabolism capability. To ensure the purity of the cultures, successive streaking techniques were employed on nutrient agar (NA; Difco, Franklin Lakes, NJ, USA) medium. One representative bacterial isolate, designated as KNUB-09-21, was selected for further comprehensive analysis. This strain was stored in 30% glycerol stocks at −20°C.

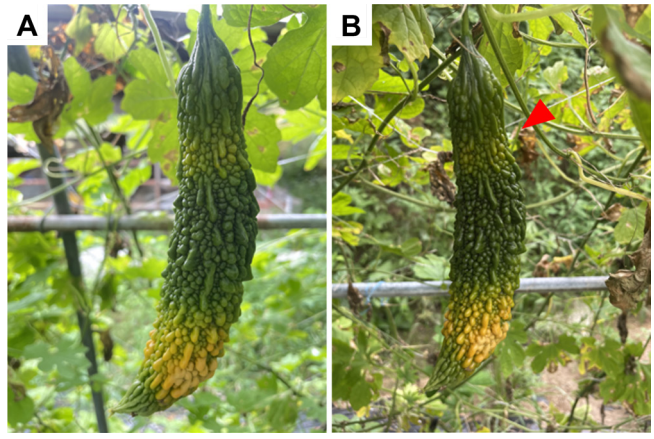

Fig. 1. Display of soft rot symptoms in *Momordica charantia* plants from Goesan, Chungcheongbuk-do, Korea. (A) The infected fruits show a clear shift to yellow. (B) The area with the collapsed structure was softened and gave off an unpleasant odor. Arrowhead points to the part of the structure that has collapsed.

For molecular analysis, the total genomic DNA was extracted from 24 hr cultures grown on NA using a commercial extraction kit, following the guidelines presented in the HiGene Genomic DNA Prep Kit (Biofact, Daejeon, Korea). The 16S rRNA region was amplified through a polymerase chain reaction (PCR) protocol adapted from Weisburg et al. (1991). To effectively purify the PCR products, the ExoSAP-IT PCR Product Cleaning Reagent (Thermo Fisher Scientific, Waltham, MA, USA) was employed. The sequencing of the 16S rRNA region of the strain KNUB-09-21 was successful, resulting in a fragment of 1,387 base pairs (GenBank no. LC782270). A BLAST search against the NCBI database revealed a striking similarity between the 16S rRNA sequence of KNUB-09-21

Table 1. Compilation of PCR primers utilized in this study

Gene	Primer sequence 5′→3′	Reference
16S rRNA	Forward: GAG TTT GAT CCT GGC TCA G	Weisburg et al. (1991)
	Reverse: ACG GCT ACC TTG TTA CGA CTT	
dnaX	Forward: TAT CAG GTY CTT GCC CGT AAG TGC	Sławiak et al. (2009)
	Reverse: TCG ACA TCC ARC GCY TGA GAT G	
leuS	Forward: TYT CCA TGC TGC CYT AYC CT	Portier et al. (2019)
	Reverse: TCC AGT TRC GCT GCA TGG TT	
recA	Forward: GGT AAA GGG TCT ATC ATG CG	Waleron et al. (2002)
	Reverse: CCT TCA CCA TAC ATA ATT TGG	

and various strains within the genus *Pectobacterium*, including *P. brasiliense*, *P. carotovorum* subsp. *carotovorum*, and *P. versatile*, ranging from 99.78% to 99.93%. This comparison definitively established the affiliation of the strain KNUB-09-21 with the genus *Pectobacterium*. This genetic closeness provides valuable insights into the taxonomic classification of the strain within this specific microbial group.

Recently, a comprehensive analysis was conducted to clarify the classification of *Pectobacterium* species. This analysis utilized three housekeeping genes (*dnaX*, *leuS*, and *recA*) to refine the taxonomic categorization of various members within the genus *Pectobacterium* (Portier et al., 2019). In our study, the PCR protocols for amplifying the *dnaX*, *leuS*, and *recA* genes were adapted from Sławiak et al. (2009), Portier et al. (2019), and Waleron et al. (2002), respectively. Using the primer sets outlined in Table 1, the genetic sequences of the strain underwent amplification, subsequently being submitted to GenBank under the corresponding accession numbers LC782271, LC782272, and LC782273. For the alignment of multiple sequences, the software MEGA7 was employed, following the approach outlined by Kumar et al. (2016). The resulting sequences (*dnaX*, 511 bp; *leuS*, 486 bp; *recA*, 734 bp) were utilized for the subsequent phylogenetic analysis with other *Pectobacterium* species (Table 2).

In the construction of the phylogenetic tree, the maximum-likelihood analysis was conducted, employing Kimura's two-parameter model. This was complemented by the application of the nearest-neighbor interchange heuristic search approach, in accordance with the methodology proposed by Felsenstein (1981). The phylogenetic analysis revealed a monophyletic clade uniting the KNUB-09-21 isolate with a set of *P. brasiliense* strains (CFBP 5392, CFBP 6607, CFBP 6615, and CFBP 6617^{T}), characterized by a high bootstrap value. This compelling evidence strongly supported their membership within the same species (Fig. 2), thus solidifying the taxonomic relationship and shedding light on the phylogenetic context of the analyzed strains.

The strain KNUB-09-21 underwent an evaluation of compound utilization using the API ID 32 GN system (Biomérieux, Marcy l'Etoile, France), following the manufacturer's provided guidelines. The results of this assessment revealed that KNUB-09-21 exhibited positive reactions to several compounds, including N-acetyl-glucosamine, D-glucose, inositol, D-mannitol, D-melibiose, L-rhamnose, salicin, L-serine, and sucrose. Conversely, the isolate demonstrated negative responses to L-alanine, L-fucose, L-histidine, lactic acid, D-maltose, propionic acid, and D-sorbitol. The comprehensive findings from these conventional biochemical assays were in alignment with the results of the molecular analysis, thus affirming the accurate identification of the strain KNUB-09-21 as *P. brasiliense* (Portier et al., 2019). This harmonization between traditional biochemical assessments and advanced molecular characterization provides robust validation for the strain's taxonomic classification within the context of *P. brasiliense*.

To fulfill Koch's postulates, pathogenicity assessments were carried out using *P. brasiliense* KNUB-09-21 on *M. charantia* plants. The fruits of *M. charantia* were sterilized with 70% ethanol and subsequently rinsed with distilled water before the inoculation process. Inoculation involved applying a 20 µl suspension (1×10^8 cells/ml) of *P. brasiliense* KNUB-09-21 to *M. charantia* via syringe injection. Simultaneously, a control group was established by inoculating the plants with a 20 µl volume of distilled water. The inoculated

Table 2. GenBank and culture accession numbers of the isolates incorporated in this study

Species	Strain no.	GenBank accession no.		
		dnaX	***leuS***	***recA***
Pectobacterium aroidearum	CFBP 1457	MT683925	MT684072	MT684219
Pectobacterium aroidearum	CFBP 2573	MT683941	MT684088	MT684235
Pectobacterium aroidearum	CFBP 6725	MT684029	MT684176	MT684323
Pectobacterium aroidearum	CFBP 8737	MT684054	MT684201	MT684348
Pectobacterium atrosepticum	CFBP 1526^T	MK516904	MK517048	MK517192
Pectobacterium betavasculorum	CFBP 1539^T	MK516905	MK517049	MK517193
Pectobacterium brasiliense	**KNUB-09-21**	**LC782271**	**LC782272**	**LC782273**
Pectobacterium brasiliense	CFBP 5392	MK516927	MK517071	MK517215
Pectobacterium brasiliense	CFBP 6607	MK516954	MK517098	MK517242
Pectobacterium brasiliense	CFBP 6615	MK516955	MK517099	MK517243
Pectobacterium brasiliense	CFBP 6617^T	MK516956	MK517100	MK517244
Pectobacterium cacticida	CFBP 3628^T	MK516923	MK517067	MK517211
Pectobacterium carotovorum subsp. *carotovorum*	CFBP 1364	MK516896	MK517040	MK517184
Pectobacterium carotovorum subsp. *carotovorum*	CFBP 2046^T	MK516909	MK517053	MK517197
Pectobacterium carotovorum subsp. *carotovorum*	CFBP 6071	MK516950	MK517094	MK517238
Pectobacterium carotovorum subsp. *carotovorum*	CFBP 7351	MK516962	MK517106	MK517250
Pectobacterium odoriferum	CFBP 1878^T	MK516907	MK517051	MK517195
Pectobacterium odoriferum	CFBP 3259	MK516920	MK517064	MK517208
Pectobacterium odoriferum	CFBP 3297	MK516921	MK517065	MK517209
Pectobacterium odoriferum	CFBP 5539	MK516929	MK517073	MK517217
Pectobacterium fontis	CFBP 8629^T	MK516878	MK517022	MK517166
Pectobacterium parmentieri	CFBP 8475^T	MK516972	MK517116	MK517260
Pectobacterium peruviense	CFBP 5834	MK516935	MK517079	MK517223
Pectobacterium polaris	CFBP 1403	MK516898	MK517042	MK517186
Pectobacterium polaris	CFBP 6058	MK516945	MK517089	MK517233
Pectobacterium polaris	CFBP 7360	MT684038	MT684185	MT684332
Pectobacterium polaris	CFBP 8603^T	MT684046	MT684193	MT684340
Pectobacterium punjabense	CFBP 8604^T	MK516877	MK517021	MK517165
Pectobacterium versatile	CFBP 1118	MK516888	MK517032	MK517176
Pectobacterium versatile	CFBP 2138	MK516912	MK517056	MK517200
Pectobacterium versatile	CFBP 6051^T	MK516938	MK517082	MK517226
Pectobacterium versatile	CFBP 8656	MK516973	MK517117	MK517261
Pectobacterium wasabiae	CFBP 3304^T	MK516922	MK517066	MK517210

The isolated strain is shown in bold.

plants were maintained in a controlled greenhouse environment (28°C, relative humidity 80%). After two days, the infected fruits emitted a foul odor, displayed a transition to a yellow color on their surface, and ultimately underwent structural collapse (Fig. 3A). Furthermore, the internal region of the fruit turned dark brown (Fig. 3B). These symptoms

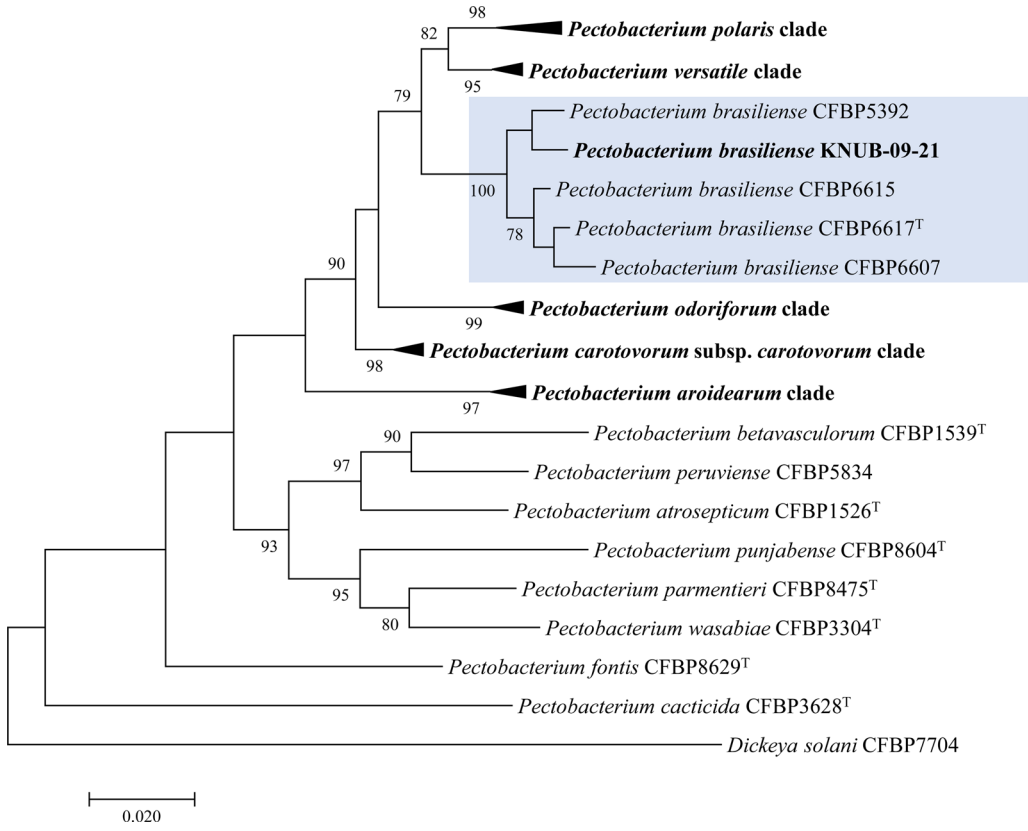

Fig. 2. Maximum-likelihood phylogenetic tree, constructed from concatenated sequences (*dnaX*+*leuS*+*recA*), illustrating the phylogenetic position of *Pectobacterium brasiliense* KNUB-09-21 among closely related *Pectobacterium* species. The isolated strain is highlighted in bold. Bootstrap values (based on 1,000 replications) of >70% are displayed at the branch points. *Dickeya solani* CFBP 7704 was used as the outgroup. The scale bar represents 0.020 substitutions per nucleotide position.

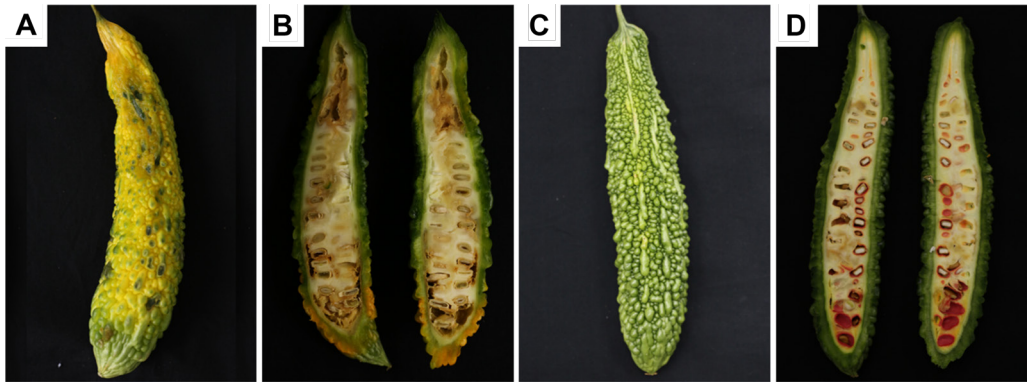

Fig. 3. Results of the pathogenicity test for *Pectobacterium brasiliense* KNUB-09-21. (A) The surface exhibited yellowing and collapse. (B) The fruit's interior has darkened to a brown color. (C, D) *Momordica charantia* inoculated with sterile water (control).

observed closely resembled those found in naturally affected *M. charantia* plants from Goesan. In contrast, the control group did not exhibit any noticeable symptoms (Fig. 3C). Re-identification of the bacterial strain isolated from each symptomatic fruit confirmed its classification as *P. brasiliense* (data not shown).

M. charantia has held the distinction of being the largest cultivated crop among subtropical crops in Korea, surpassing various other fruits and vegetables (Jeong et al., 2020). This crop is susceptible to a range of bacterial, fungal, and viral pathogens, including *Colletotrichum gloeosporioides* and *Rhizopus stolenifer* (Kim et al., 2015; Kwon and Jee, 2005).

Notably, while bacterial soft rot diseases have been reported globally, there have been no documented cases of bacterial diseases affecting *M. charantia* in Korea. Bacterial soft rot, induced by pectinolytic soft rot bacteria belonging to the genera *Pectobacterium* and *Dickeya*, is considered one of the most destructive diseases of vegetables worldwide (Ma et al., 2007). The genus *Pectobacterium*, known for its wide host compatibility, can trigger soft rot symptoms in various plant species due to its ability to produce enzymes that degrade plant cell walls (Hugouvieux-Cotte-Pattat et al., 2014; Maung et al., 2022). The use of phylogenetic analysis involving housekeeping genes (*dnaX*, *leuS*, and *recA*) and investigations into compound utilization has proven effective in classifying *Pectobacterium* species (Portier et al., 2019).

Our study, utilizing a comprehensive investigative approach that includes analyses of the 16S rRNA region sequence, multilocus sequence, and thorough evaluation of biochemical traits, definitively identifies *P. brasiliense* as the causative agent of the observed soft rot in *M. charantia* in Korea. Despite recent advancements in pathogen diagnostic methods for soft diseases that allow for sensitive and specific detection of target pathogens (Jin et al., 2022), there is a lack of comprehensive investigation regarding the survey of bacterial pathogens in *M. charantia*. This underscores the need for further investigation of potential bacterial diseases that can affect *M. charantia*. Our findings provide valuable insights that can contribute to the development of effective plant pathology strategies. Importantly, they lay a solid foundation for designing targeted control measures to combat soft rot and mitigate associated economic losses caused by this identified phytopathogen. In conclusion, our results provide foundational data that can inform future plant pathology efforts, offering a promising direction for effective disease management.

Conflicts of Interest

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

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