

First Report of Melon Soft Rot Disease Caused by *Pectobacterium brasiliense* in Korea

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In May 2021, characteristic soft rot symptoms, including soft, watery, slimy, black rot, wilting, and leaf collapse, were observed on melon plants (*Cucumis melo*) in Gokseong, Jeollanam-do, Korea. A bacterial strain, designated KNUB-06-21, was isolated from infected plant samples, taxonomically classified, and phylogenetically analyzed using 16S rRNA and housekeeping gene sequencing. Strain KNUB-06-21 was also examined for compound utilization using the API ID 32 GN system and strain KNUB-06-21 was identified as *Pectobacterium brasiliense*. Subsequent melon stem inoculation studies using strain KNUB-06-21 showed soft rot symptoms similar to field plants. Re-isolated strains shared phenotypic and molecular characteristics with the original *P. brasiliense* KNUB-06-21 strain. To our knowledge, ours is the first report of *P. brasiliense* causing melon soft rot disease in Korea.

Keywords: *Cucumis melo*, Korea, Melon, *Pectobacterium brasiliense*, Soft rot

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Melon (*Cucumis melo*) belongs to the family Cucurbitaceae, subfamily Cucurbitoideae, tribe Melothriaceae, subtribe Cucumerinae, and the genus *Cucumis*. The fruit exhibits many morphological colors, shapes, flavors, and textures, along with diverse biochemical properties that vary depending on region and climate (Kim et al., 2021). In Korea, melons are grown on 3,595 hectares of land, with an annual production of 144,834 tons in 2021 (Food and Agriculture Organization Corporate Statistical Database, 2021). However, globally, bacterial diseases can affect melon production and are highly prevalent. In Serbia, *Pectobacterium brasiliense* has

been identified as the causal agent of melon soft rot, while *P. carotovorum* subsp. *carotovorum* was identified as the causal agent of melon soft rot in Korea (Yi and Kim, 1996; Zlatković et al., 2019). As a highly aggressive phytopathogen, *P. brasiliense* has been reported in several global regions, including Asia, Africa, Europe, Oceania, and North and South America (Charkowski, 2018; Gottsberger and Huss, 2016; Hua et al., 2020; Jaramillo et al., 2017; Naas et al., 2018; Panda et al., 2012; Park et al., 2022, 2023).

In May 2021, typical melon soft rot symptoms, including soft, watery, slimy, black rot, wilting, and collapse were observed in Gokseong, Jeollanam-do, Korea. Affected plants showed soft rot symptoms on the infected tissue surfaces and water-soaked lesions on the infected stems (Fig. 1A, B). Disease incidence was approximately 15% of the melons in

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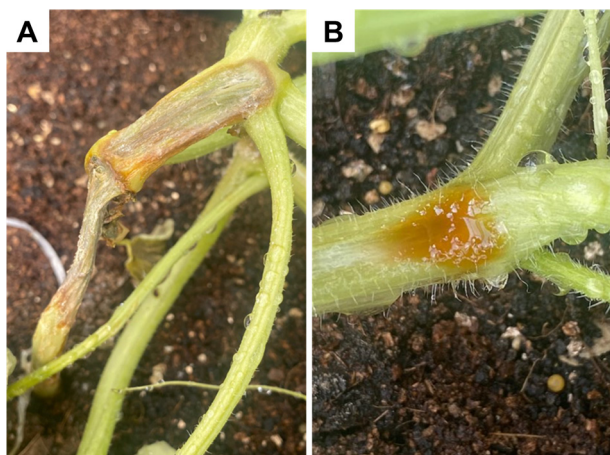


Fig. 1. Soft rot symptoms caused by *Pectobacterium brasiliense* KNUB-06-21 on melon stems. (A) Soft rot symptoms on infected tissue surfaces and water-soaked infected stems. (B) Stem surface turned dark brown.

the investigated region, as observed in the comprehensive survey.

To isolate pathogens from infected plants, the affected stem surfaces sterilized in 1% hypochlorite solution for 90 sec and rinsed in sterile distilled water. Bacterial isolation was carried out on selective crystal violet pectate medium (Hyman et al., 2001) at 26°C for 48 hr. Strains positive for pectolytic cavity formation were selected and purified on nutrient agar (NA; Difco, Franklin Lakes, NJ, USA). A total of 2 isolates with identical colony morphology were obtained from the affected stem. Among these isolates, a single colony, designated as KNUB-06-21, was selected in a random manner for further investigation.

Genomic DNA of strain KNUB-06-21 was extracted using a commercial extraction kit (HiGene Genomic DNA Prep Kit, Biofact, Daejeon, Korea) following manufacturer's instructions. The 16S rRNA gene underwent polymerase chain reaction (PCR) using 9F/1512R primers (Weisburg et al., 1991) and yielded a 1,352 base pair (bp) fragment (GenBank no. LC773684), which after sequencing, was BLAST searched in the National Center for Biotechnology Information (NCBI) database. BLAST analyzes revealed 100% 16S rRNA gene similarity with several *P. brasiliense* strains (GenBank nos. CP009769, CP020350, MK910250, MN393919, and MN393942).

For accurate identification of the strain KNUB-06-21, three housekeeping genes (*dnaX*, *leuS*, and *recA*) were amplified

using PCR protocols and primers as described by Portier et al. (2020). A 521 bp fragment was generated for *dnaX* gene, 511 bp for *leuS* gene, and 714 bp for *recA* gene (GenBank nos. LC773681, LC773682, and LC773683). The highest *dnaX* gene homology was 100% with *P. brasiliense* (GenBank no. LC717494). Similarly, the strain KNUB-06-21 exhibited a high level of sequence homology 100% with *P. brasiliense* (GenBank nos. CP059959, LC717493, and MK517247) for the *recA* gene and with *P. brasiliense* (GenBank no. LC717495) for the *leuS* gene, confirming their close genetic relationship. Concatenated *dnaX*, *leuS*, and *recA* gene sequences were used in phylogenetic analyses and sequences from closely related neighbors were retrieved from NCBI (Table 1). Using MEGA7, a phylogenetic tree was constructed using the maximum-likelihood method with a bootstrap of 1,000 replicates (Kumar et al., 2016). As depicted in Fig. 2, strain KNUB-06-21 grouped with *P. brasiliense* clade in the phylogenetic tree, exhibiting a bootstrap value of 100%. This result indicates that it is a strain belonging to *P. brasiliense*.

The API ID 32 GN system (Biomérieux, Marcy l'Etoile, France) was used to investigate compound utilization by strain KNUB-06-21 according to manufacturer's instructions. KNUB-06-21 responded positively to N-acetyl-glucosamine, L-alanine, D-glucose, inositol, D-maltose, D-mannitol, D-melibiose, L-rhamnose, salicin, L-serine, and sucrose utilization. However, it responded negatively to L-fucose, L-histidine, 3-hydroxybutyric acid, propionic acid, and D-sorbitol utilization. These results were consistent with the characteristics of the type strain of *P. brasiliense* (Supplementary Table 1) reported by Portier et al. (2019).

Strain KNUB-06-21 pathogenicity was tested by inoculating the strain onto melon plant stems. Bacterial colonies, grown for 48 hr on NA at 26°C, were picked using sterile toothpicks, inserted into stems at 5 cm above the stem base, and inoculation sites covered with vaseline. Control plants were treated with toothpicks dipped in sterile water. Inoculated plants were maintained in greenhouse conditions (25–30°C and relative humidity=80%). Typical soft rot symptoms appeared at 3–4 days after inoculation and were represented by wilting leaves on stems (Fig. 3A), water-soaked lesions on leaves (Fig. 3B), and detailed examination of a cross-section of an infected melon stem revealed the presence of a hollow stalk (Fig. 3C). In contrast, control plants showed no infectious symptoms (Fig. 3D–F). From infected plants, pathogen was successfully re-isolated and, based on

Table 1. *Pectobacterium* species used in this study for phylogenetic analyses and 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>	CFBP 2573	MT683941	MT684088	MT684235
<i>Pectobacterium aroidearum</i>	CFBP 6725	MT684029	MT684176	MT684323
<i>Pectobacterium aroidearum</i>	CFBP 8737	MT684054	MT684201	MT684348
<i>Pectobacterium atrosepticum</i>	CFBP 1526 ^T	MK516904	MK517048	MK517192
<i>Pectobacterium betavasculorum</i>	CFBP 1539 ^T	MK516905	MK517049	MK517193
<i>Pectobacterium brasiliense</i>	KNUB-06-21	LC773681	LC773682	LC773683
<i>Pectobacterium brasiliense</i>	CFBP 5392	MK516927	MK517071	MK517215
<i>Pectobacterium brasiliense</i>	CFBP 6607	MK516954	MK517098	MK517242
<i>Pectobacterium brasiliense</i>	CFBP 6615	MK516955	MK517099	MK517243
<i>Pectobacterium brasiliense</i>	CFBP 6617 ^T	MK516956	MK517100	MK517244
<i>Pectobacterium cacticida</i>	CFBP 3628 ^T	MK516923	MK517067	MK517211
<i>Pectobacterium carotovorum</i> subsp. <i>carotovorum</i>	CFBP 1364	MK516896	MK517040	MK517184
<i>Pectobacterium carotovorum</i> subsp. <i>carotovorum</i>	CFBP 2046 ^T	MK516909	MK517053	MK517197
<i>Pectobacterium carotovorum</i> subsp. <i>carotovorum</i>	CFBP 6071	MK516950	MK517094	MK517238
<i>Pectobacterium carotovorum</i> subsp. <i>carotovorum</i>	CFBP 7351	MK516962	MK517106	MK517250
<i>Pectobacterium odoriferum</i>	CFBP 1878 ^T	MK516907	MK517051	MK517195
<i>Pectobacterium odoriferum</i>	CFBP 3259	MK516920	MK517064	MK517208
<i>Pectobacterium odoriferum</i>	CFBP 3297	MK516921	MK517065	MK517209
<i>Pectobacterium odoriferum</i>	CFBP 5539	MK516929	MK517073	MK517217
<i>Pectobacterium fontis</i>	CFBP 8629 ^T	MK516878	MK517022	MK517166
<i>Pectobacterium parmentieri</i>	CFBP 8475 ^T	MK516972	MK517116	MK517260
<i>Pectobacterium peruviense</i>	CFBP 5834	MK516935	MK517079	MK517223
<i>Pectobacterium polaris</i>	CFBP 1403	MK516898	MK517042	MK517186
<i>Pectobacterium polaris</i>	CFBP 6058	MK516945	MK517089	MK517233
<i>Pectobacterium polaris</i>	CFBP 7360	MT684038	MT684185	MT684332
<i>Pectobacterium polaris</i>	CFBP 8603 ^T	MT684046	MT684193	MT684340
<i>Pectobacterium punjabense</i>	CFBP 8604 ^T	MK516877	MK517021	MK517165
<i>Pectobacterium versatile</i>	CFBP 1118	MK516888	MK517032	MK517176
<i>Pectobacterium versatile</i>	CFBP 2138	MK516912	MK517056	MK517200
<i>Pectobacterium versatile</i>	CFBP 6051 ^T	MK516938	MK517082	MK517226
<i>Pectobacterium versatile</i>	CFBP 8656	MK516973	MK517117	MK517261
<i>Pectobacterium wasabiae</i>	CFBP 3304 ^T	MK516922	MK517066	MK517210
<i>Dickeya solani</i>	CFBP 7704	MK516970	MK517114	MK517258

^TType strain. The isolated strain is in bold.

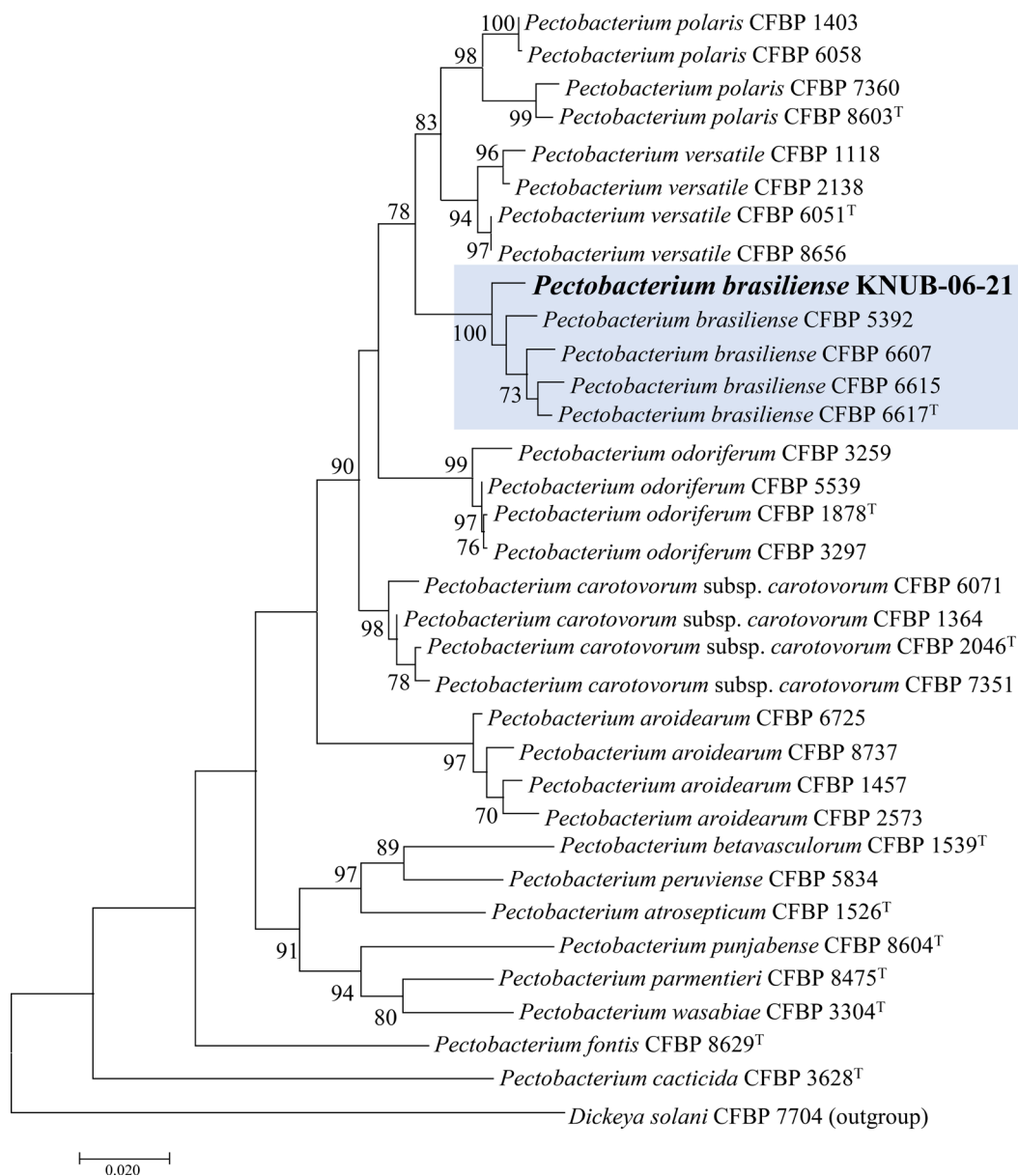


Fig. 2. Maximum-likelihood phylogenetic tree, based on concatenated sequences (*dnaX*+*leuS*+*recA*), shows the phylogenetic position of strain KNUB-06-21 among the closest members of the *Pectobacterium*. The isolated strain is shown in bold. KNUB-06-21 is in bold. Bootstrap values (based on 1,000 replications) >70% are shown at branch points. *Dickeya solani* CFBP 7704 was used as an outgroup. Scale bar=0.020 substitutions/nucleotide position.

its morphological characteristics and the 16S rRNA, *dnaX*, *leuS*, and *recA* gene sequences, was identified as *P. brasiliense* (data are not shown).

P. brasiliense has been reported as a soft rot pathogen on different crops (Meng et al., 2017; Onkendi and Moleleki, 2014). The isolated strain KNUB-06-21 was identified using 16S rRNA and housekeeping gene sequencing, biochemical analyses, and pathogenicity studies on melon stems. To the best of our knowledge, this is the first report of *P. brasil-*

ense causing melon soft rot in Korea. Our findings contribute to an increased understanding of *P. brasiliense* diversity associated with melon, and highlight the importance of early detection and management strategies preventing pathogen spread. Further research is required to investigate *P. brasiliense* epidemiology and ecology in melon production areas, and develop effective control measures to minimize economic losses caused by this pathogen.

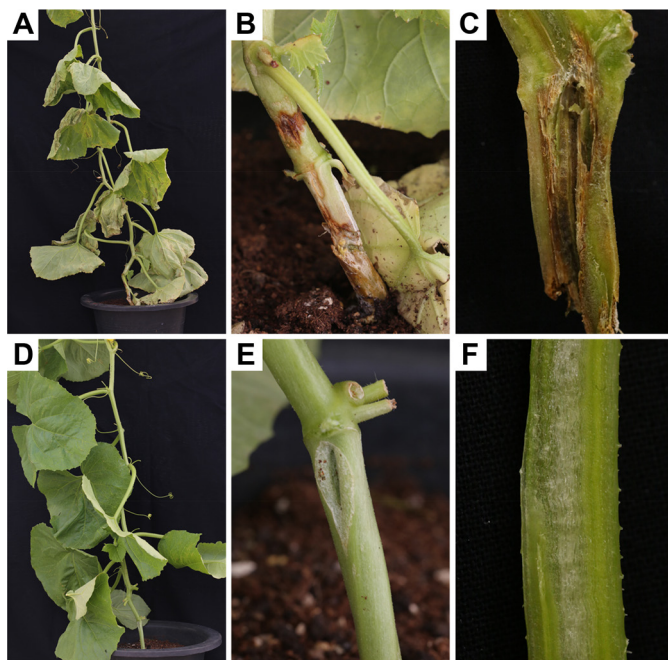


Fig. 3. *Pectobacterium brasiliense* KNUB-06-21 pathogenicity results. (A) Inoculated melon plants show leaf wilting symptoms. (B) Water-soaked lesions on melon plants. (C) Cross-section of an infected melon stem showing a hollow stalk. (D–F) Control melon stems showing no infectious symptoms.

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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