

# First Report of Soft Rot Caused by *Pectobacterium brasiliense* on Cucumber in Korea

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Wilted and water-soaked lesion symptoms were observed on cucumbers in greenhouses located in Daejeon, Chungcheongnam-do, Korea, in June 2021. A bacterial strain, designated KNUB-04-21, was isolated from the cucumbers, which was subsequently identified as *Pectobacterium brasiliense* through a phylogenetic analysis based on sequences of the 16S rRNA region, *dnaX*, *leuS*, and *recA* genes. The biochemical characteristics of KNUB-04-21 were also similar to those of *P. brasiliense* through investigation using the API ID 32 GN system. The pathogenicity of KNUB-04-21 was confirmed by inoculating it into healthy cucumber plants. The reisolated strains were also found to be same to the original strain. To our knowledge, this is the first report of *P. brasiliense* being identified as the causative agent of cucumber soft rot in Korea.

**Keywords:** Cucumber, *Cucumis sativus*, Pathogenicity, *Pectobacterium brasiliense*, Soft rot

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In 2020, the production area of cucumber (*Cucumis sativus*) was 4,721 ha with an annual yield of 335,596 tons in Korea (Korean Statistical Information Service, 2022). However, cucumbers are susceptible to various diseases during their growth period, including soft rot caused by *Pectobacterium* species, which can result in significant economic losses for agricultural production. Several species of *Pectobacterium*, including *P. brasiliense* and *P. carotovorum* subsp. *carotovorum*, have been reported as causative agents of cucumber soft rot in China and Malaysia (Meng et al., 2017; Nazerian et al., 2011). Soft rot caused by various strains of *P. brasiliense* has been reported in Asian countries, including China, Japan, and Korea, affecting a variety of plants, such as cucumber, graft-cactus, napa cabbage, potato, radish, and tobacco (Fuji-

moto et al., 2017; Jee et al., 2020; Meng et al., 2017; Park et al., 2022, 2023; Wang et al., 2017).

In June 2021, numerous cucumber samples exhibiting wilted and water-soaked lesions symptoms were collected from Daejeon, Chungcheongnam-do, Korea. The affected cucumber plants showed symptoms of soft rot on the surface of infected tissues, water soaking in infected stems, and wet rot on the edges or centers of leaves (Fig. 1A, B). Furthermore, conspicuous symptoms of liquid production became apparent on the infected stem, indicating the presence of exudates or secretions resulting from the infection (Fig. 1C). These symptoms, reminiscent of bacterial soft rot as described by Charkowski (2018), provide visible evidence of the pathogenic impact on the stem tissue. The presence of liquid production on the infected stems serves as a characteristic hallmark, reinforcing the resemblance to bacterial soft rot and confirming the manifestation of the disease symptoms.

To isolate the pathogenic bacterial agent, cucumber tis-

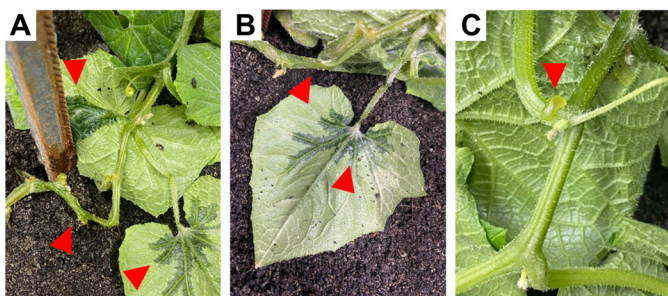
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**Fig. 1.** Symptoms of soft rot disease caused by *Pectobacterium brasiliense* on cucumber plants in Daejeon, Chungcheongnam-do, Korea. (A, B) Stems and leaves of cucumber were wilted and have water-soaked lesions caused by *P. brasiliense* KNUB-04-21. (C) Liquid production was observed from the infected stem. Arrowheads indicate the invasion of soft rot.

sues exhibiting symptoms of infection were immersed in a 5 ml saline solution (0.85% NaCl) for 20 min. Subsequently, a 50  $\mu$ l aliquot of the resulting suspension was uniformly spread onto nutrient agar (NA; Difco, Franklin Lakes, NJ, USA) medium and incubated for 24 hr at 27°C. After the purification process using NA and streaking on King's medium B (Difco), the bacterial strain was successfully isolated from samples collected in the region of Daejeon and was designated KNUB-04-21, serving for further analyses.

For molecular analysis, the total genomic DNA of KNUB-04-21 was extracted from 24 hr cultures grown on NA using HiGene Genomic DNA Prep Kit (Biofact, Daejeon, Korea). The polymerase chain reaction (PCR) protocol used in this study for amplifying the 16S rRNA region was based on the methodology described by Weisburg et al. (1991). The 16S rRNA region of KNUB-04-21 (GenBank no. LC744204) was 1,352 bp in its sequence length. After a BLAST search in the National Center for Biotechnology Information (NCBI) database, it was found that the 16S rRNA gene sequence of KNUB-04-21 displayed similarities of 99.78% and 99.70% with that of *P. brasiliense* BP201611 (GenBank no. MN393922) and *P. carotovorum* subsp. *carotovorum* PDP201711 (GenBank no. MN394009), respectively. These findings clearly demonstrated that relying solely on the comparative analysis of the 16S rRNA gene sequence was insufficient to accurately identify the strain KNUB-04-21 at the species level. These data also underscore the limitations of using the 16S rRNA gene as a standalone marker for species identification and emphasize the need for using additional molecular markers or comprehensive genomic approaches to achieve a more precise and

reliable taxonomic classification. Therefore, following Portier et al. (2019), the genes *dnaX*, *leuS*, and *recA* of KNUB-04-21 were subjected to PCR amplification and sequencing.

The PCR protocols used in this study to amplify *dnaX*, *leuS*, and *recA* were based on the methodology outlined by Sławiak et al. (2009), Portier et al. (2019), and Waleron et al. (2002), respectively (Supplementary Table 1). Fragments containing 509, 518, and 715 bp were obtained for *dnaX*, *leuS*, and *recA*, respectively. All obtained gene sequences were registered in the NCBI with the following GenBank accession numbers: *dnaX*, LC744201; *leuS*, LC744202; *recA*, LC744203. Based on the similarity of the *dnaX* sequence, KNUB-04-21 exhibited a similarity of >98% with *P. brasiliense* NY1563A (GenBank no. MW979072) and *P. carotovorum* subsp. *carotovorum* PCC21 (GenBank no. CP003776). The *leuS* sequence also exhibited a similarity of >98% with *P. brasiliense* 130 (GenBank no. CP092039), *P. carotovorum* subsp. *carotovorum* PCC21 (GenBank no. CP003776), and *P. quasiquaticum* A398-S21-F17 (GenBank no. CP065178). Furthermore, the *recA* sequence of KNUB-04-21 demonstrated a similarity of >98% with *P. brasiliense* CFBP6615 (GenBank no. MK517243) and *P. carotovorum* subsp. *carotovorum* 333 (GenBank no. AY264787).

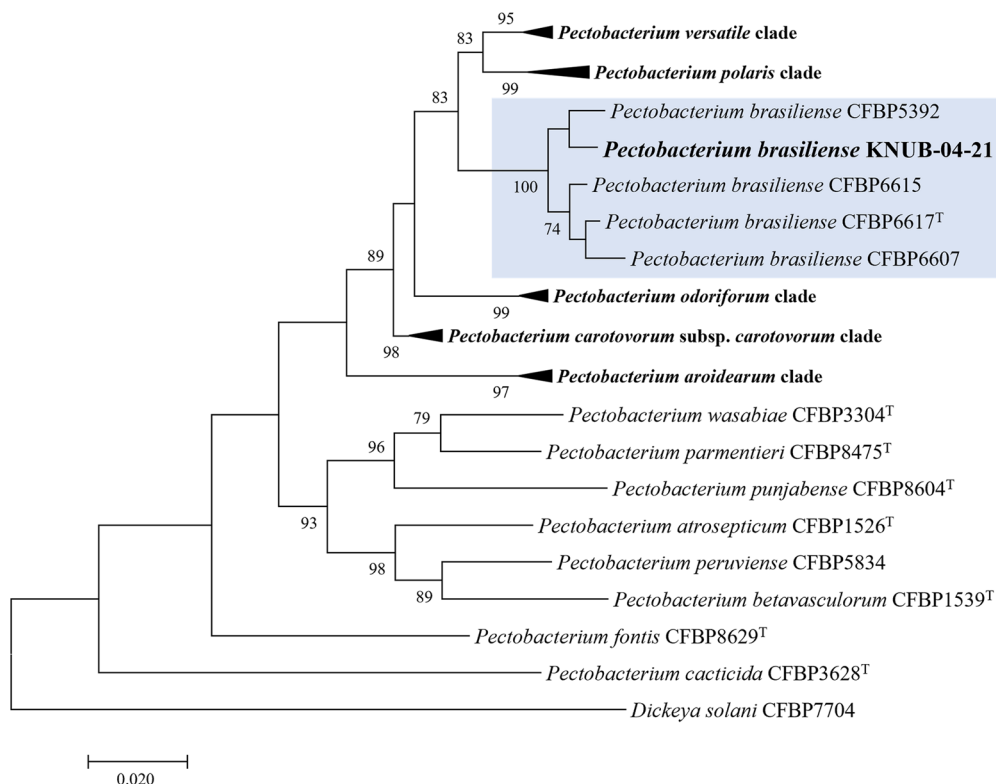
The NCBI BLAST was used to compare the sequences of the three housekeeping genes with those of the reference *Pectobacterium* species in the genomic databases (Table 1). Phylogenetic and molecular evolutionary analyses were conducted using the MEGA 7 software (Kumar et al., 2016). Maximum likelihood analysis was performed using Kimura's two-parameter model and the nearest neighbor interchange heuristic search method. The reliability and robustness of the obtained clusters were evaluated using bootstrap analysis with 1,000 replicates. The constructed phylogenetic tree revealed that KNUB-04-21 and multiple *P. brasiliense* strains formed a monophyletic clade with a high bootstrap value, strongly suggesting that they belong to the same species (Fig. 2).

To characterize the strain KNUB-04-21, it was subjected to a comprehensive analysis using the API ID 32 GN system (Biomérieux, Marcy l'Etoile, France) according to standardized procedures recommended by the manufacturer. The inoculum of KNUB-04-21 was prepared by selecting a colony from the surface of NA medium. KNUB-04-21 demonstrated positive assimilation responses to various compounds, including D-glucose, D-maltose, D-mannitol, D-melibiose, ino-

**Table 1.** List of *Pectobacterium* species and *Dickeya solani* used in phylogenetic analysis with their corresponding 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 <sup>T</sup>	MK516904	MK517048	MK517192
<i>Pectobacterium betavasculorum</i>	CFBP1539 <sup>T</sup>	MK516905	MK517049	MK517193
<i>Pectobacterium cacticida</i>	CFBP3628 <sup>T</sup>	MK516923	MK517067	MK517211
<b><i>Pectobacterium brasiliense</i></b>	<b>KNUB-04-21</b>	<b>LC744201</b>	<b>LC744202</b>	<b>LC744203</b>
<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 <sup>T</sup>	MK516956	MK517100	MK517244
<i>Pectobacterium carotovorum</i> subsp. <i>carotovorum</i>	CFBP1364	MK516896	MK517040	MK517184
<i>Pectobacterium carotovorum</i> subsp. <i>carotovorum</i>	CFBP2046 <sup>T</sup>	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 <sup>T</sup>	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 <sup>T</sup>	MK516878	MK517022	MK517166
<i>Pectobacterium parmentieri</i>	CFBP8475 <sup>T</sup>	MK516972	MK517116	MK517260
<i>Pectobacterium peruviense</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 <sup>T</sup>	MT684046	MT684193	MT684340
<i>Pectobacterium punjabense</i>	CFBP8604 <sup>T</sup>	MK516877	MK517021	MK517165
<i>Pectobacterium versatile</i>	CFBP1118	MK516888	MK517032	MK517176
<i>Pectobacterium versatile</i>	CFBP2138	MK516912	MK517056	MK517200
<i>Pectobacterium versatile</i>	CFBP6051 <sup>T</sup>	MK516938	MK517082	MK517226
<i>Pectobacterium versatile</i>	CFBP8656	MK516973	MK517117	MK517261
<i>Pectobacterium wasabiaae</i>	CFBP3304 <sup>T</sup>	MK516922	MK517066	MK517210
<i>Dickeya solani</i>	CFBP7704	MK516970	MK517114	MK517258

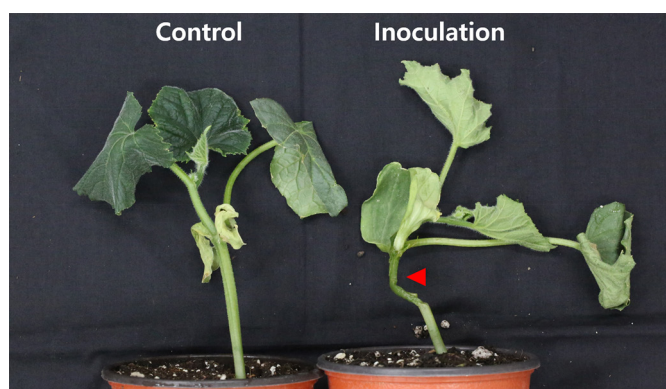
<sup>T</sup>The type strain. The strain isolated in this study is highlighted in bold.



**Fig. 2.** Maximum-likelihood phylogenetic tree, based on concatenated partial sequences of *dnaX*, *leuS*, and *recA* genes, showing the phylogenetic position of *Pectobacterium brasiliense* KNUB-04-21 among related species of the genus *Pectobacterium*. Bootstrap values (based on 1,000 replications) greater than 70% are shown at branch points. *Dickeya solani* CFBP7704 was used as the outgroup. The scale bar represents 0.020 substitutions per nucleotide position.

sitol, L-alanine, L-rhamnose, L-serine, N-acetylglucosamine, salicin, and sucrose. Conversely, KNUB-04-21 exhibited negative utilization responses to 3-hydroxybutyric acid, D-sorbitol, L-fucose, L-histidine, and propionic acid. These findings are consistent with the characteristics outlined by Portier et al. (2019) for the reference strain *P. brasiliense*.

To fulfill Koch's postulates and establish the pathogenicity of KNUB-04-21, pathogenicity tests were conducted on cucumber plants. Before inoculation, the cucumber surface was sterilized using 70% ethanol, followed by rinsing with distilled water. A 1 ml suspension of KNUB-04-21 with a concentration of  $1 \times 10^8$  cells/ml was used for inoculation. As a control, a mock infection was performed by inoculating the cucumber with 1 ml of distilled water. The experiment was conducted under controlled greenhouse conditions, maintaining a temperature range of 25–30°C and a relative humidity of 80% throughout the study period. At 2 days after inoculation, the cucumber stems exhibited characteristic symptoms of wilting and water-soaked lesions, as shown in Fig. 3. In contrast, the cucumber stems subjected to mock



**Fig. 3.** Effect of inoculating *Pectobacterium brasiliense* KNUB-04-21 on the cucumber stem. Two days after inoculation, symptoms such as wilting and water-soaked lesions were observed in the stem of the cucumber. However, no symptoms were observed in the control plant. Arrowheads indicate the invasion of bacterial cells and soft rot areas.

infection exhibited no symptoms (Fig. 3). Reidentification of the bacterial strain isolated from each symptomatic cucumber confirmed its classification as *P. brasiliense*, although the details of this reidentification are not described in this report.

These data provide strong evidence supporting the phytopathogenic nature of KNUB-04-21 and its association with the observed disease symptoms in the cucumber plants.

Several species of *Pectobacterium* have been identified as disease-causing agents for various crops. Among them, *P. brasiliense* has been reported to infect 19 different plant species belonging to 10 families (Oulghazi et al., 2021). In Korea, the first detection of *P. brasiliense* was performed in 2012 (Choi and Kim, 2013). Furthermore, *P. brasiliense* is a pathogen of amaranth, Chinese cabbage, chrysanthemums, eggplant, graft-cactus, napa cabbage, nepenthes, paprika, pepper, potato, radish, and tomato (Choi and Kim, 2013; Jee et al., 2018, 2020; Lee et al., 2014; Park et al., 2022, 2023). Low-cost, sensitive, and specific methods were recently developed for efficient detection of target pathogens belonging to the genus *Pectobacterium* (Jin et al., 2022; Oulghazi et al., 2021). This study describes the identification of *P. brasiliense* as the causal agent of cucumber soft rot in Korea. This finding not only expands our understanding of the distribution of *P. brasiliense* in Korea but also enhances our knowledge of the range of causative agents responsible for cucumber soft rot. Moreover, this study can contribute to the development of more efficient management strategies for minimizing the economic damage caused by this disease.

### Conflicts of Interest

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

### Acknowledgments

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### Electronic Supplementary Material

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

### References

Charkowski, A. O. 2018. The changing face of bacterial soft-rot diseases. *Annu. Rev. Phytopathol.* 56: 269-288.

- Choi, O. and Kim, J. 2013. *Pectobacterium carotovorum* subsp. *brasiliense* causing soft rot on paprika in Korea. *J. Phytopathol.* 161: 125-127.
- Fujimoto, T., Yasuoka, S., Aono, Y., Nakayama, T., Ohki, T., Sayama, M. et al. 2017. First report of potato blackleg caused by *Pectobacterium carotovorum* subsp. *brasiliense* in Japan. *Plant Dis.* 101: 241.
- Jee, S., Choi, J.-G., Hong, S., Lee, Y.-G. and Kwon, M. 2018. First report of soft rot by *Pectobacterium carotovorum* subsp. *brasiliense* on amaranth in Korea. *Res. Plant Dis.* 24: 339-341.
- Jee, S., Choi, J.-G., Lee, Y.-G., Kwon, M., Hwang, I. and Heu, S. 2020. Distribution of *Pectobacterium* species isolated in South Korea and comparison of temperature effects on pathogenicity. *Plant Pathol. J.* 36: 346-354.
- Jin, Y. J., Jo, D., Kwon, S.-W., Jee, S., Kim, J.-S., Raman, J. and Kim, S.-J. 2022. A new approach using the SYBR green-based real-time PCR method for detection of soft rot *Pectobacterium odoriferum* associated with kimchi cabbage. *Plant Pathol. J.* 38:656-664.
- Korean Statistical Information Service. 2022. Data by policy. URL [https://kosis.kr/statHtml/statHtml.do?orgId=101&tblId=DT\\_1ET0027](https://kosis.kr/statHtml/statHtml.do?orgId=101&tblId=DT_1ET0027) [15 September 2022].
- Kumar, S., Stecher, G. and Tamura, K. 2016. MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. *Mol. Biol. Evol.* 33: 1870-1874.
- Lee, D. H., Kim, J.-B., Lim, J.-A., Han, S.-W. and Heu, S. 2014. Genetic diversity of *Pectobacterium carotovorum* subsp. *brasiliensis* isolated in Korea. *Plant Pathol. J.* 30: 117-124.
- Meng, X., Chai, A., Shi, Y., Xie, X. Ma, Z. and Li, B. 2017. Emergence of bacterial soft rot in cucumber caused by *Pectobacterium carotovorum* subsp. *brasiliense* in China. *Plant Dis.* 101: 279-287.
- Nazerian, E., Sijam, K., Zainal Abidin, M. A. and Vadmalai, G. 2011. First report of soft rot caused by *Pectobacterium carotovorum* subsp. *carotovorum* on cucumber in Malaysia. *Plant Dis.* 95: 1474.
- Oulghazi, S., Sarfraz, S., Zaczek-Moczydłowska, M. A., Khayi, S., Ed-Dra, A., Lekbach, Y. et al. 2021. *Pectobacterium brasiliense*: genomics, host range and disease management. *Microorganisms* 9: 106.
- Park, K.-T., Hong, S.-M., Back, C.-G., Kang, I.-K., Lee, S.-Y., Ten, L. N. et al. 2023. *Pectobacterium brasiliense* as a causative agent for soft rot of radish in Korea. *Res. Plant Dis.* 29: 64-71.
- Park, K.-T., Hong, S.-M., Back, C.-G., Kim, S. Y., Lee, S.-Y., Kang, I.-K. et al. 2022. First report of *Pectobacterium brasiliense* causing soft rot on graft cactus in Korea. *Res. Plant Dis.* 28: 172-178.
- Portier, P., Pédrón, J., Taghouti, G., Fischer-Le Saux, M., Caullireau, E., Bertrand, C. et al. 2019. Elevation of *Pectobacterium carotovorum* subsp. *odoriferum* to species level as *Pectobacterium odoriferum* sp. nov., proposal of *Pectobacterium brasiliense* sp. nov. and *Pectobacterium actinidiae* sp. nov., emended description of *Pectobacterium carotovorum* and description of *Pectobacterium versatile* sp. nov., isolated from streams and symptoms on diverse plants. *Int. J. Syst. Evol. Microbiol.* 69: 3207-3216.
- Ślawiak, M., van Beckhoven, J. R. C. M., Speksnijder, A. G. C. L., Cza-

- jkowski, R., Grabe, G. and van der Wolf, J. M. 2009. Biochemical and genetical analysis reveal a new clade of biovar 3 *Dickeya* spp. strains isolated from potato in Europe. *Eur. J. Plant Pathol.* 125: 245-261.
- Waleron, M., Waleron, K., Podhajska, A. J. and Łojkowska, E. 2002. Genotyping of bacteria belonging to the former *Erwinia* genus by PCR-RFLP analysis of a *recA* gene fragment. *Microbiology* 148: 583-595.
- Wang, J., Wang, Y. H., Dai, P. G., Chen, D. X., Zhao, T. C., Li, X. L. et al. 2017. First report of tobacco bacterial leaf blight caused by *Pectobacterium carotovorum* subsp. *brasiliense* in China. *Plant Dis.* 101: 830.
- Weisburg, W. G., Barns, S. M., Pelletier, D. A. and Lane, D. J. 1991. 16S ribosomal DNA amplification for phylogenetic study. *J. Bacteriol.* 173: 697-703.