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First Report of *Pectobacterium aroidearum* Causing Soft Rot on *Ficus carica* in Korea

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In July 2021, symptoms of soft rot were observed on the stems of *Ficus carica* in Yeongam, Jeollanamdo, Korea. To accurately diagnose the cause, infected stem was collected and bacterial strain was isolated. Among these, the pathogenic strain KNUB-08-21 was identified as *Pectobacterium aroidearum* through 16S rRNA gene sequencing and phylogenetic analysis based on the concatenated sequences of the *dnaX*, *leuS*, and *recA* genes. The affiliation of the isolate with this bacterial species was also confirmed by its biochemical characteristics obtained using API ID 32 GN system. Artificial inoculation confirmed the strain's pathogenicity in figs, causing significant damage to both stems and fruits. To our knowledge, this is the first report of *P. aroidearum* causing soft rot disease in *F. carica* in Korea.

Keywords: *Ficus carica*, Identification, Pathogenicity, *Pectobacterium aroidearum*, Soft rot

Ficus carica, also known as the fig, is a member of the Moraceae family, which includes around 40 genera. The *Ficus* genus is one of the largest angiosperm genera, comprising over 800 species of trees, epiphytes, and shrubs found in tropical and subtropical regions globally (Singh et al., 2011). The Asian-Australasian region is home to the highest diversity, with approximately 500 *Ficus* species (Badgujar et al., 2014). In 2020, Korea produced about 3,460 tons of figs (Statistics Korea, 2023). Only a few bacterial diseases have been reported in *Ficus* species, particularly edible figs. These diseases include crown gall caused by *Agrobacterium tumefaciens*; Pseudomonas leaf spot caused by *Pseudomonas cichorii*; and Xanthomonas leaf spot caused by *Xanthomonas campestris* pv. *fici* (Bouzar and Jones, 2001; Campoverde and Palmateer, 2011; Elboutahiri et al., 2009). Additionally, *Agrobacterium larrymoorei* was isolated from gall formations on weeping figs (Mousavi et al., 2020). However, while soft rot is a disease reported worldwide, there has been limited research on *Ficus* species. Bacterial soft rot is a common disease in agricultural ecosystems (Charkowski, 2018). It primarily affects plant storage organs like tubers, rhizomes, and bulbs (Ma et al., 2007), but can also appear in fleshy plant organs such as succulent stems and leaves or densely packed leaf vegetables like lettuce (Ma et al., 2007). The pectinolytic soft rot Pectobacteriaceae, a group of bacterial plant pathogens, consists of two genera: *Pectobacterium* and *Dickeya* (Adeolu et al., 2016). Recently, *Pectobacterium aroidearum* has been reported to affect various plants, including alocasia, konjac, Chinese cabbage, and pumpkin (Chen et al., 2020; Mikicińsk et al., 2023; Moraes et al., 2017; Wei et al., 2020; Xie et al., 2018; Xu et al., 2020). However, *P. aroidearum* has never been reported to cause soft rot disease in *F. carica*.

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In July 2021, soft rot symptoms were observed on stems of *F. carica* in Yeongam, Jeollanamdo, Korea (Fig. 1A, B). Diseased stems were collected for isolation of pathogens to an accurate diagnosis. Infected tissue fragments were immersed in a 5 ml solution of saline (0.8% NaCl) for 20 min. The resulting suspension was then divided into 50 μl and spread onto nutrient agar (NA; Difco, Detroit, MI, USA) media, followed by an incubation period of 48–72 hr at 30°C. After 3 days, white-gray circular colonies exhibiting the typical cultural characteristics of bacterial strains were obtained on NA. Single colonies were picked, purified by repeated streaking on fresh NA plates, and a randomly chosen strain, designated as KNUB-08-21, was used for further comprehensive analysis.

To test the pathogenicity of the bacterial strain, surface-sterilized stems with holes in the center were filled with 100 μl of bacterial suspension (1×10^9 cells/ml) of strain KNUB-08-21 to test the ability to cause soft rot. As a control, a mock infection was conducted by inoculating the stem with 100 μl of distilled water. Inoculated plants were kept at 25°C and 80% relative humidity. Three days later, bacterial strain caused symptoms similar to those in the field (Fig. 1C). In contrast, the control did not exhibit any noticeable symptoms (Fig. 1D). Based on the result of the pathogenicity test, the bacterial strain, designed KNUB-08-21, was selected for further detailed investigation.

To identify strain KNUB-08-21, genomic DNA was extracted from it utilizing the HiGene Genomic DNA Prep Kit (Biofact, Daejeon, Korea) in accordance with the manufacturer's instructions. Subsequently, the 16S rRNA gene was subjected to polymerase chain reaction (PCR) employing the 9F/1512R primers as outlined by Weisburg et al. (1991). The 16S rRNA gene of strain KNUB-08-21 was sequenced and found to be comprised of 1,351 base pairs in total length (GenBank accession no. LC779908). A BLAST search in the NCBI database showed a high similarity between the 16S rRNA gene sequence of KNUB-08-21 and those of *P. aroidearum* CEP2

Fig. 1. Soft rot symptoms on *Ficus carica* stems. (A, B) Soft rot caused by *Pectobacterium aroidearum* KNUB-08-21 on *F. carica* in the field in Yeongam, Jeollanamdo, Korea. (C) Soft rot induced by *P. aroidearum* KNUB-08-21 through artificial inoculation on the stems. (D) Sterilized water was used as a control.

Table 1. PCR primers for amplifying three housekeeping genes (*dnaX*, *leuS*, and *recA*)

Gene	Primer	Sequence (5′-3′)	Annealing temperature (°C)	Reference
dnaX	*dnaXF*	GAG TTT GAT CCT GGC TCA G	55	Sławiak et al., 2009
	dnaXR	ACG GCT ACC TTG TTA CGA CTT		
leuS	*leuSF*	TYT CCA TGC TGC CYT AYC CT	55	Portier et al., 2019
	leuSR	TCC AGT TRC GCT GCA TGG TT		
recA	*recAF*	GGT AAA GGG TCT ATC ATG CG	47	Waleron et al., 2002
	recAR	CCT TCA CCA TAC ATA ATT TGG		

PCR, polymerase chain reaction.

Table 2. Strains of *Pectobacterium* species used in the study and their GenBank accession numbers

Species	Strain no.	GenBank accession no.		
		dnaX	***leuS***	***recA***
Pectobacterium aroidearum[a]	KNUB-08-21[a]	LC779905[a]	LC779906[a]	LC779907[a]
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$^{\mathrm{T}}$	MK516904	MK517048	MK517192
Pectobacterium betavasculorum	CFBP 1539$^{\mathrm{T}}$	MK516905	MK517049	MK517193
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$^{\mathrm{T}}$	MK516956	MK517100	MK517244
Pectobacterium brasiliense	KNUB-01-21	LC717494	LC717495	LC717493
Pectobacterium brasiliense	KNUB-03-21	LC738892	LC738894	LC738893
Pectobacterium cacticida	CFBP 3628$^{\mathrm{T}}$	MK516923	MK517067	MK517211
Pectobacterium carotovorum	CFBP 1364	MK516896	MK517040	MK517184
Pectobacterium carotovorum	CFBP 2046$^{\mathrm{T}}$	MK516909	MK517053	MK517197
Pectobacterium carotovorum	CFBP 6071	MK516950	MK517094	MK517238
Pectobacterium carotovorum	CFBP 7351	MK516962	MK517106	MK517250
Pectobacterium fontis	CFBP 8629$^{\mathrm{T}}$	MK516878	MK517022	MK517166
Pectobacterium odoriferum	CFBP 1878$^{\mathrm{T}}$	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 parmentieri	CFBP 8475$^{\mathrm{T}}$	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$^{\mathrm{T}}$	MT684046	MT684193	MT684340
Pectobacterium punjabense	CFBP 8604$^{\mathrm{T}}$	MK516877	MK517021	MK517165
Pectobacterium versatile	CFBP 1118	MK516888	MK517032	MK517176
Pectobacterium versatile	CFBP 2138	MK516912	MK517056	MK517200
Pectobacterium versatile	CFBP 6051$^{\mathrm{T}}$	MK516938	MK517082	MK517226
Pectobacterium versatile	CFBP 8656	MK516973	MK517117	MK517261
Pectobacterium wasabiae	CFBP 3304$^{\mathrm{T}}$	MK516922	MK517066	MK517210
Dickeya solani	CFBP 7704	MK516970	MK517114	MK517258

[a] The strain isolated in this study.

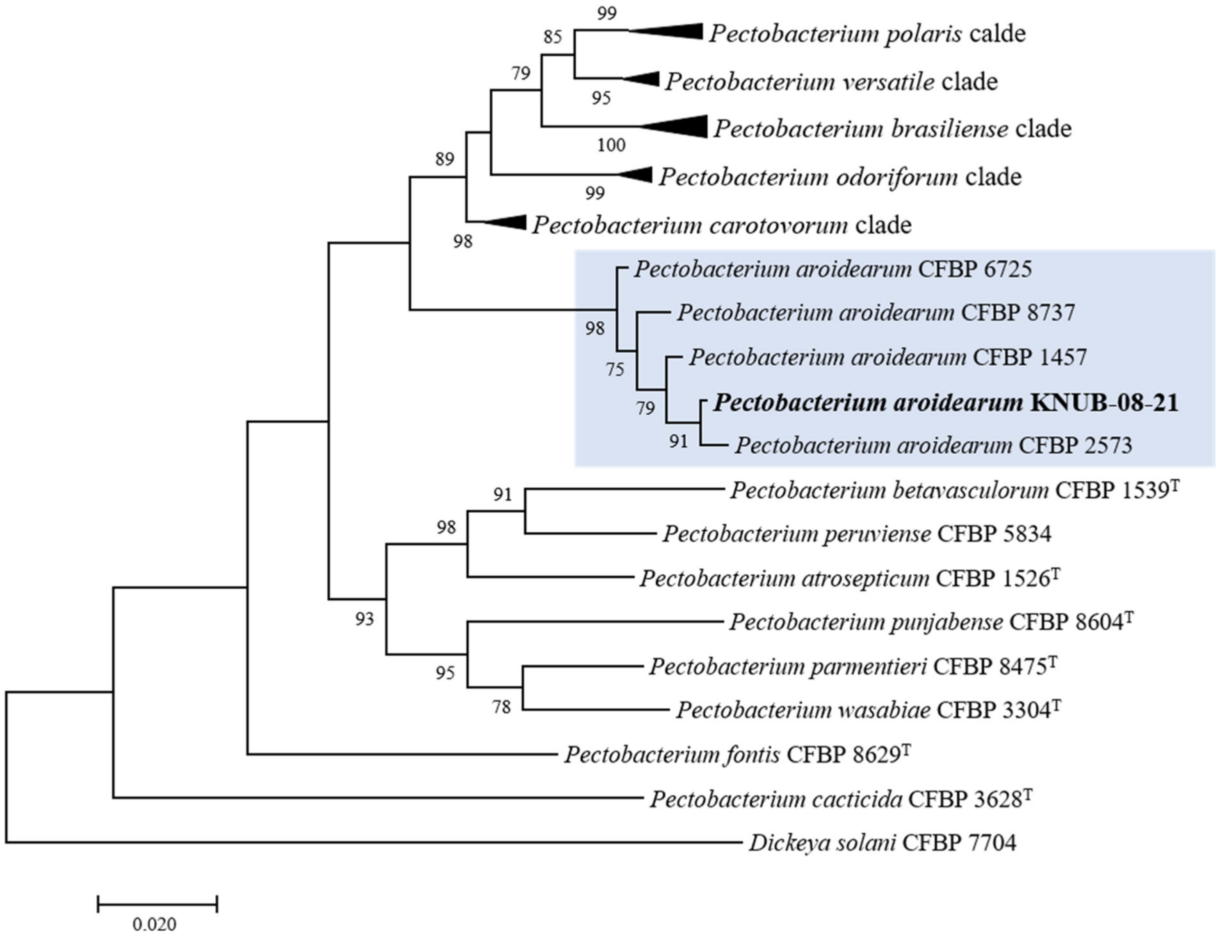

Fig. 2. Maximum-likelihood phylogenetic tree showing the relationship between *Pectobacterium aroidearum* KNUB-08-21 and other *Pectobacterium* species based on concatenated sequences of *dnaX*, *leuS*, and *recA* genes. Bootstrap values (based on 1,000 replications) more than 70% are displayed on the branch points. *Dickeya solani* CFBP 7704 was used as the outgroup. Scale bar: 0.020 substitutions per nucleotide position.

(GenBank no. MN904952) (99.56%), *P. carotovorum* HG-49 (GenBank no. CP032619) (99.56%), and *P. colocasium* PL155 (GenBank no. CP118921) (99.19%). This result suggests that strain KNUB-08-21 belongs to the genus *Pectobacterium*. However, an accurate identification of the isolate solely based on 16S rRNA gene was not achievable.

For precise identification of the isolated *Pectobacterium* strain, three housekeeping genes (*dnaX*, *leuS*, and *recA*) were amplified using the protocols and primers previously reported by Portier et al. (2020). The strain KNUB-08-21 was amplified using *dnaXF*/*dnaXR*, *leuSF*/*leuSR*, and *recAF*/*recAR* primers to analyze *dnaX*, *leuS*, and *recA* genes (Table 1). Multiple sequence alignment (*dnaX*, 480 bp; *leuS*, 530 bp; *recA*, 609 bp) was executed utilizing the MEGA7 software program (Kumar et al., 2016). The GenBank accession numbers of reference sequences of *Pectobacterium* species used in this study are shown in Table 2. A well-supported monophyletic clade, consisting of strain KNUB-08-21 and several members of *P. aroidearum* (CFBP 1457, CFBP 2573, CFBP 6725, and CFBP 8737), strongly indicates their belonging to the same species (Fig. 2).

The isolate KNUB-08-21 underwent compound utilization analysis utilizing the API ID 32 GN system (Biomérieux, Marcy l'Etoile, France) in accordance with the manufacturer's instructions. The results showed that strain KNUB-08-21 was able to utilize N-acetyl-glucosamine, L-arabinose, D-glucose, inositol, D-mannitol, D-melibiose, L-rhamnose, L-serine, and sucrose. However, it was unable to utilize L-alanine, glycogen, L-histidine, itaconic acid, lactic acid, D-maltose, L-proline, propionic acid, and valeric acid. The strain KNUB-08-21 displayed almost all the characteristic features consistent with the type strain of *P. aroidearum* described by Nabhan

Table 3. Utilization of various compounds as the sole carbon source by *Pectobacterium aroidearum* KNUB-08-21 and *Pectobacterium aroidearum* SCRI 109^{T}

Compound	***P. aroidearum*** **KNUB-08-21**	***P. aroidearum*** **SCRI 109^{T}**
N-Acetyl-glucosamine	+	+
L-Alanine	–	–
L-Arabinose	+	+
D-Glucose	+	–
Glycogen	–	+/–
L-Histidine	–	–
Inositol	+	+
Itaconic acid	–	–
Lactic acid	–	+
D-Maltose	–	+/–
D-Mannitol	+	+
D-Melibiose	+	+
L-Proline	–	–
Propionic acid	–	–
L-Rhamnose	+	+
L-Serine	+	+
Sucrose	+	+
Valeric acid	–	–

Data for *P. aroidearum* SCRI 109^{T} are from Nabhan et al. (2013). '+', indicates positive reaction; '+/–', indicates weakly positive reaction; '–', indicates negative reaction.

et al. (2013), except for its inability to metabolize D-glucose and lactic acid (Table 3). This difference can be considered as intraspecific variability among various strains belonging to *P. aroidearum*. Overall, the outcomes of conventional biochemical tests corroborate the molecular analysis results, thus confirming the accurate identification of strain KNUB-08-21 as *P. aroidearum*.

To investigate whether the pathogenicity of the *P. aroidearum* KNUB-08-21, as confirmed in *F. carica,* can induce disease symptoms in other parts of the plant besides the stems. Artificial inoculation was conducted on the fruit to determine the potential development of symptoms. Before inoculation, the fruit surfaces were sterilized using 70% ethanol and subsequently rinsed with distilled water. Inoculation was carried out using a 100 µl suspension of *P. aroidearum* KNUB-08-21 at a concentration of 1×10^{8} cells/ml. As a control, a mock infection was conducted by inoculating the fruit with 100 µl of distilled water. The inoculated fruits were stored at 25°C with humidity levels exceeding 80%. After 2 days, symptoms of soft rot began to appear in the fruits inoculated with the suspension of *P. aroidearum* KNUB-08-21, accompanied by the onset of a foul odor. After 5 days, the fruits began to split, revealing soft and decayed internal tissues (Fig. 3A). After 7 days, the fruits completely detached from the stems and fell to the ground. Furthermore, the interior of the infected fruits displayed typical symptoms of soft rot (Fig 3B). In contrast, control plants showed no infectious symptoms (Fig. 3C, D).

Several strains belonging to *P. aroidearum* have primarily been isolated from monocotyledonous plants, including *Zantedeschia aethiopica* in South Africa, *Saccharum* spp. in Ja-

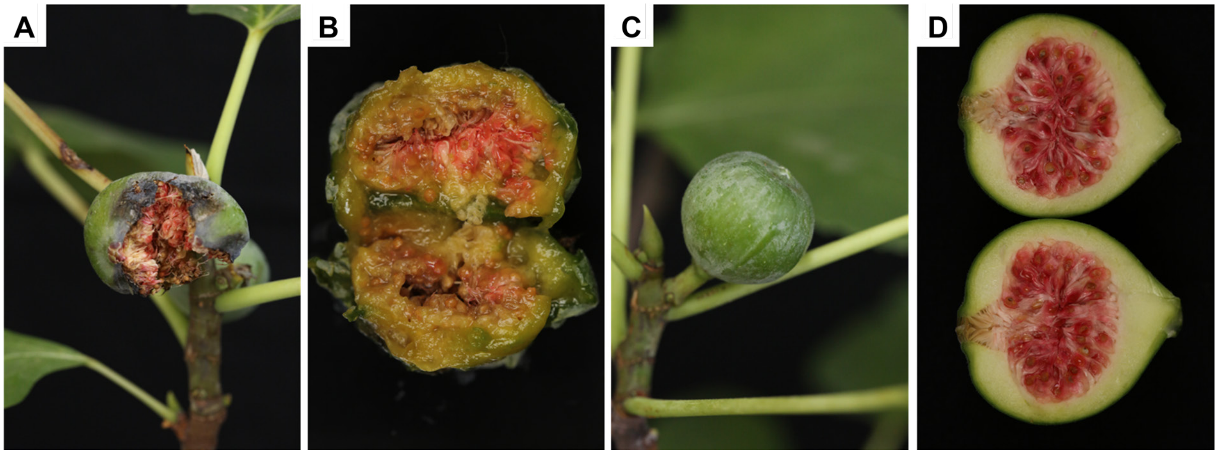

Fig. 3. Pathogenicity of *Pectobacterium aroidearum* KNUB-08-21 on fruits of *Ficus carica*. (A) Fruits inoculated with *P. aroidearum* KNUB-08-21 suspension exhibit onset of soft rot symptoms, accompanied by a putrid smell. Upon splitting open, internally soft and decayed tissues become visible. (B) Characteristic symptoms of soft rot are evident within infected fruits. (C, D) Control plants display no infection symptoms.

maica, *Persea americana*, *Ornithogalum dubium* in Israel, and *Amorphophallus konjac* in East Asia (Li et al., 2022; Nabhan et al., 2013; Sun et al., 2019). Recent studies have confirmed the presence of *P. aroidearum* in several plant species, including alocasia, konjac, Chinese cabbage, and pumpkin (Chen et al., 2020; Mikicińsk et al.,2023; Moraes et al., 2017; Wei et al., 2020; Xie et al., 2018; Xu et al., 2020). However, there have been no recorded cases of *P. aroidearum* causing soft rot disease in figs until now.

Through comprehensive analysis encompassing 16S rRNA gene sequence analysis, multilocus sequence analysis, and meticulous assessment of physiological characteristics, *P. aroidearum* was confirmed as the causative agent isolated from *F. carica*, which represents soft rot disease in Korea. Moreover, it was noted that symptomatology presents as stem browning; however, when inoculated within the fruit, the disease swiftly advances over time, resulting in substantial damage. Additionally, substantiation has been made that inoculating at distinct anatomical sites within the host can yield divergent levels of disease severity. These findings corroborate the results of prior research demonstrating that the genus Pectobacterium can induce soft rot in various anatomical regions (Ma et al., 2007).

Our findings enhance the understanding of the diversity of *P. aroidearum* linked with *F. carica* and underscore the importance of timely detection and strategic management protocols to impede the spread of the pathogen. Further investigations are imperative to scrutinize the epidemiology and ecology of *P. aroidearum* in areas dedicated to *F. carica* production. Moreover, there is a pressing need to devise efficacious control measures aimed at mitigating the economic repercussions induced by this pathogenic agent. This is pivotal for diminishing potential losses in the agricultural sector.

Conflicts of Interest

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

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