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Bacterial blight on *Sansevieria cylindrica* caused by *Pseudomonas* sp.

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Abstract

The African spear or cylindrical snake plant (*Sansevieria cylindrica*) is commonly used as an ornamental indoor succulent plant. In May 2019, we observed bacterial blight in *S. cylindrica* grown for interior decoration and air purification in Jinju, South Korea. These symptoms eventually led to leaf desiccation. Similar leaf blight symptoms have been frequently documented on social media; however, as an imported species, the pathogens of *S. cylindrica* have not been reported in South Korea. Based on pathogenicity tests; levan production, oxidase production, pectinolytic activity, arginine dihydrolase production, and tobacco hypersensibility tests; analytical profile index API tests; and phylogenetic analysis based on multilocus sequence analysis using the 16S rRNA, *gyrB*, *rpoD*, and *rpoB* gene sequences, we identified the causative pathogen as *Pseudomonas* sp. This study is the first to report leaf blight caused by *Pseudomonas* sp. on *S. cylindrica*.

Keywords Bacterial blight · Multi-locus sequence analysis · *Pseudomonas* sp. · *Sansevieria cylindrica*

The African spear or cylindrical snake plant (*Sansevieria cylindrica*; family Asparagaceae) is a succulent native to Africa. *S. cylindrica* is a popular indoor ornamental plant due to its long, striped, greenish-gray subcylindrical leaves; it also exhibits strong air purification effects and is easy to cultivate (Wolverton et al. 1989; Takawira and Nordal 2003). In Korea, potted *Sansevieria* plants are often presented as gifts at opening ceremonies and other auspicious events, and it has been used as a traditional medicine for various diseases in Africa (Tanveer et al. 2017). *Sansevieria* species have been investigated for their pharmacological activity, producing antimicrobial, antioxidant, antitumor, and antidiabetic effects (Da Silva et al. 2003). The chemical constituents of *S. cylindrica* include steroids, flavonoids, saponins, tannins, and phenolic acids (Said et al. 2015; Tanveer et al. 2017).

In May 2019, leaf blight was observed in *S. cylindrica* plants used for interior decoration and air purification in Jinju, South Korea. Similarly infected plants were easily found in commercial shops growing *S. cylindrica* for decoration; these were eventually discarded. Many individuals who grow *S. cylindrica* indoors have tried to determine the cause of its leaf blight by uploading photographs on social media. Because little is known about the pathogens associated with *S. cylindrica* in Korea, the aim of this study was to isolate and identify the causative agent of leaf blight on *S. cylindrica.*

Symptoms on *S. cylindrica* begin from the bottom of the leaf and progress to the tip, eventually leading to severe desiccation of the entire leaf (Fig. 1a). To isolate the pathogen from infected plants, we cut tissue Sects. (0.5 cm × 0.5 cm) including lesions and healthy areas of leaves using a scalpel, and then the sections were surface-sterilized with 1% hypochlorite solution and rinsed with sterilized distilled water. The tissue sections were placed in a 1.5-mL microtube containing sterilized distilled water, crushed with a pipette tip, soaked for 10 min, and then mixed evenly. We serially diluted 100 µL of bacterial suspension and then spread it onto a 0.5 × trypticase soy agar (TSA) plate. The plates were incubated at 28°C for 2 days, and the resulting colonies were re-streaked onto a new TSA plate. We tested 20 isolated colonies for hypersensitivity reaction (HR) in tobacco leaves. Three HR-inducing bacteria (MHGNU B109a–c)

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Fig. 1 Symptoms of bacterial blight caused by *Pseudomonas* sp. on *Sansevieria cylindrica*

were selected and subjected to levan production, oxidase production, pectinolytic activity, arginine dihydrolase production, and tobacco hypersensibility (LOPAT) tests (Ewing et al. 1960; Klement et al. 1964; Schaad et al. 2001). The three isolates were found to be positive for oxidase production and negative for levan and arginine dihydrolase production and pectinolytic activity. The physiological and biochemical characteristics of the bacterial isolates were tested using the analytical profile index (API) 20NE system (bioMérieux, Marcy l'Etoile, France). All three isolates utilized D-glucose, L-arabinose, D-mannose, D-mannitol, potassium gluconate, capric acid, malic acid, and trisodium citrate, and did not utilize N-acetyl-glucosamine, D-maltose, adipic acid, or phenylacetic acid. All three isolates were negative for the production of indole, urease, β-glucosidase, protease, and β-galactosidase. Nitrate was not reduced to nitrite. All three isolates showed non-fluorescence on King's medium B. All three isolates exhibited the same traits in LOPAT and API tests, suggesting that they are the same bacterial species. To confirm the identity of the bacterial pathogens, we analyzed the 16S rRNA gene sequences of the three isolates. Total DNA extraction and polymerase chain reaction amplification were performed as previously described (Choi et al. 2017) using the primers 27mF (5′-AGAGTTTGATCMTGGCTCAG-3′) and 1492mR (5′-GGYTACCTTGTTACGACTT-3′) (Lane 1991). The 16S rRNA gene sequences (1388 bp) of

the three isolates were identical, and that of MHGNU B109a was deposited into GenBank (accession no. MW617234). The DNA sequences were analyzed using the BLASTN program and compared with sequences in the National Center for Biotechnology Information GenBank database. The 16S rRNA gene sequence of the isolate was 99.93% homologous with *Pseudomonas* sp. strain p34 from *Quercus pyrenaica* rhizosphere in Spain (Lasa et al. 2019). A representative isolate, MHGNU B109a, was deposited into the Korea Agricultural Culture Collection (KACC 21450).

For phylogenetic analysis, we amplified three housekeeping genes, *gyrB*, *rpoD*, and *rpoB*, of KACC 21450 with the following primer sets: UP-1E (5′-CAGGAAACA GCTATGACCAYGSNGGNGGNAARTTYRA-3′) and APrU (5′-TGTAAAACGACGGCCAGTGCNGGRTCY TTYTCYTGRCA-3′) (Hall et al. 2016); PsEG30F (5′-ATYGAAATCGCCAARCG-3′) and PsEG790R (5′-CGG TTGATKTCCTTGA-3′) (Mulet et al. 2009); and LAPSs (5′-TGGCCGAGAACCAGTTCCGCGT-3′) and LAPs27 (5′-CGGCTTCGTCCAGCTTGTTCAG-3′) (Tayeb et al. 2005), respectively. The resulting sequences of the *gyrB* (920 bp), *rpoD* (687 bp), and *rpoB* (1074 bp) genes were deposited into GenBank (accession nos. MW721596, MW727275, and MW727274, respectively). Phylogenetic analysis based on multi-locus sequence analysis (MLSA) and comparison with sequences in the *Pseudomonas aeruginosa* Metabolome Database (http://genome.ppws.vt.edu/cgi-bin/MLST/home.pl) were performed as described previously (Mulet et al. 2009). A phylogenetic tree based on the concatenated nucleotide sequences of the 16S rRNA gene and three housekeeping genes was generated using the MEGA 7.0 software and the maximum likelihood method (Kumar et al. 2016). The phylogenetic trees based on MLSA showed that KACC 21450 was closely clustered with *Pseudomonas abietaniphila* (Fig. 2). However, we did not identify KACC 21450 as *P. abietaniphila* because this isolate exhibits different phenotypes than the *P. abietaniphila* type strain DSM 17554T, being positive for the utilization of D-maltose and the production of urease and arginine dihydrolase (Saati-Santamaría et al. 2018). Further study is required to determine the species of this pathogen.

The pathogenicity test was carried out as described previously (Choi et al. 2013). Leaves of commercially purchased *S. cylindrica* were disinfected with 70% ethanol, and 100 µL of bacterial suspension (~ 10^8 CFU/ml) of KACC 21450 in sterilized distilled water was injected into three leaves of *S. cylindrica* using a syringe. Sterile distilled water was used for negative control inoculation. The inoculated plants were placed in a greenhouse at 25°C for 10 days and then evaluated for disease symptoms. In the pathogenicity tests, water soaking symptoms appeared on inoculated leaves 3–4 days after inoculation. The diseased lesions became large and rapidly dried up and either broke or took on a mummified

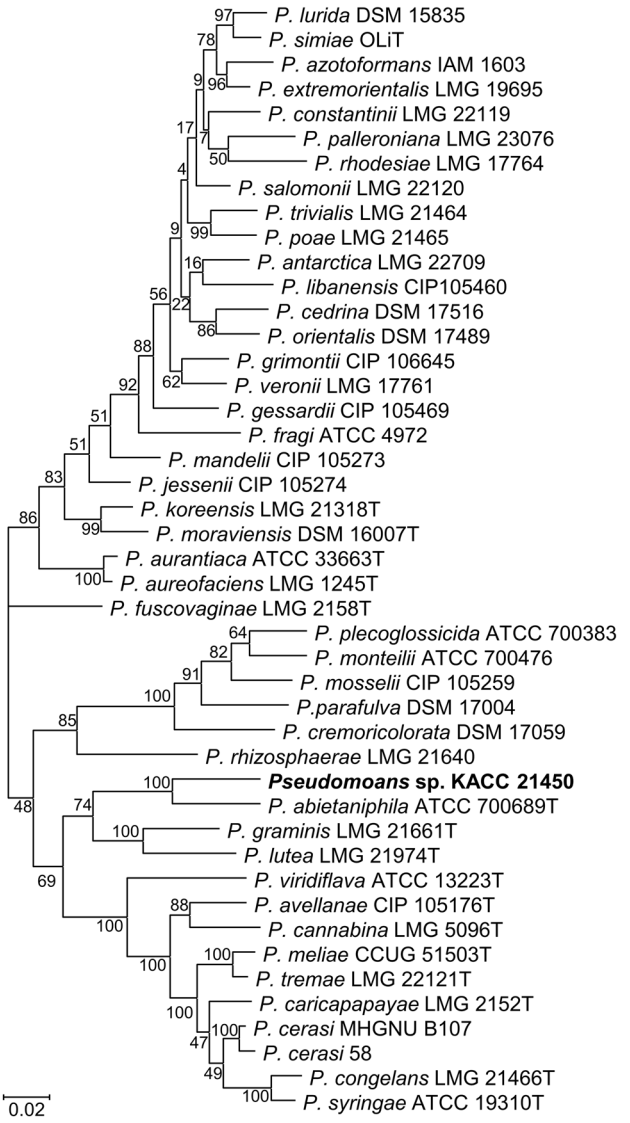

Fig. 2 Phylogenetic tree of *Pseudomonas* species obtained using the maximum likelihood method based on four concatenated genes (16S rRNA, *gyrB*, *rpoD*, and *rpoB*). The percentages of trees in which associated taxa were clustered together are shown next to the branches. The tree is drawn to scale, with branch lengths measured in numbers of substitutions per site. Bars indicate numbers of nucleotide substitutions per site. The isolate used in the present study is indicated in bold

appearance (Fig. 3a and b). These symptoms were similar to those observed in natural infection. No symptoms were observed on healthy control plants inoculated with sterile distilled water (Fig. 3c and d). To fulfill Koch's postulates, the bacteria were re-isolated from the artificially induced lesions to confirm the 16S rRNA gene sequence.

Thus, the causative bacterial pathogen from *S. cylindrica* was identified as *Pseudomonas* sp. based on physiological and molecular analyses and pathogenicity tests. To our knowledge, this study is the first to report leaf blight caused

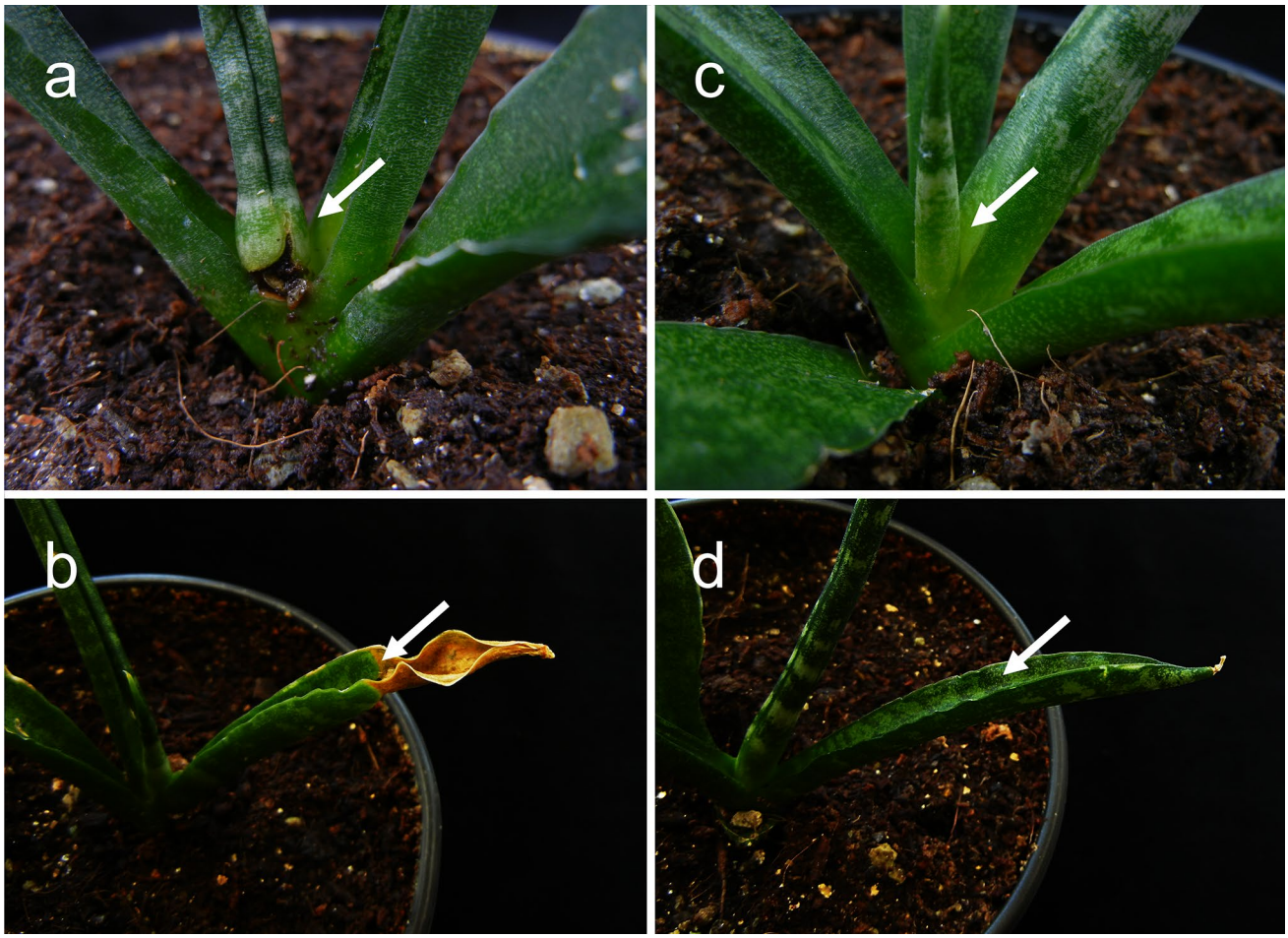

Fig. 3 Pathogenicity tests. (**a**, **b**) Symptoms induced by artificial inoculation of KACC 21450 on *Sansevieria cylindrica*. (**c**, **d**) Negative control. Photos were taken 7 and 12 days after inoculation in subcylindrical and noncylindrical leaves, respectively. Arrows indicate inoculation sites

by *Pseudomonas* sp. on *S. cylindrica*. These results provide important information for commercial growers of *S. cylindrica*, to improve production and survival of this popular indoor air-purifying and decorative plant.

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