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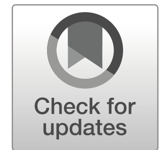

Bacterial blight on *Dracaena sanderiana* caused by *Burkholderia cepacia*

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Abstract

In 2018, severe bacterial blight was observed on *Dracaena sanderiana* plants imported and cultivated by a commercial nursery in Jinju, South Korea. This indoor plant is popularly grown for indoor air purification to prevent sick building syndrome (SBS). The diseased plants had severe blight symptoms, with leaf chlorosis and crumpling of stem and leaves. To identify the causal agent, we performed bacterial isolation, pathogenicity tests, physiological analysis, sequencing of the 16S rRNA region and *recA* gene, and phylogenetic analysis; the bacterial pathogen was identified as *Burkholderia cepacia*. This is the first report of bacterial blight on *D. sanderiana* caused by *B. cepacia*. Although there is no clear epidemiological information on the inoculum source, the recent occurrence of the disease indicates that *B. cepacia* is a potential threat to the production of indoor dracaena plants, which are increasingly used for air purification in SBS. Because *D. sanderiana* is grown in indoor living spaces, *B. cepacia* infection could threaten human health as well.

Keywords *Dracaena sanderiana* · Blight · *Burkholderia cepacia* · 16S rRNA · *recA*

Dracaena sanderiana, also called 'Lucky Bamboo' or 'Ribbon Dracaena', is a flowering plant in the family *Asparagaceae*. It is a small, shrubby plant with slim stems and flexible strap-shaped leaves. *D. sanderiana* is native to Cameroon in tropical West Africa, where it grows on the ground in tropical rainforests (Damen et al. 2018). It is a popular indoor plant that can survive in various indoor environments and has many cultivars (USDA, ARS 2008; Damen et al. 2018). *D. sanderiana* is often propagated from short cuttings in water. In Korea, it is called lucky bamboo because it is believed to bring happiness and prosperity when it is grown in houses and workplaces; this belief has made it more popular. Although it is attracting attention as an air purification plant to eliminate sick building syndrome (SBS), which is becoming a serious problem (Orwell et al. 2006; Dela Cruz et al. 2014), there are no accurate data on imports into South Korea.

Okhee Choi and Yeyeong Lee contributed equally to this work.

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In 2018, bacterial blight was observed on *D. sanderiana* that was grown commercially in a nursery in Jinju, South Korea. The diseased *D. sanderiana* had severe symptoms of blight and leaf chlorosis (Fig. 1). Since *D. sanderiana* is a recently imported plant, no diseases have been reported in South Korea; however, bacterial leaf spot caused by *Xanthomonas campestris*, and Stewart's wilt caused by *Pantoea stewartii*, have been recorded in Korea (Korean Society of Plant Pathology 2009; Choi and Kim 2013).

In this study, we isolated and identified the causal plant pathogen from wilted *D. sanderiana* plants. We performed bacterial isolation, pathogenicity tests, molecular identification, and physiological tests. Here, we report the occurrence of bacterial blight caused by *Burkholderia cepacia* on *D. sanderiana* for the first time. The occurrence of this disease indicates that *B. cepacia* poses a potential risk to the production of ornamental *D. sanderiana* plants.

Bacteria were isolated as described previously (Choi and Kim 2013) with some modifications. Tissues (× 5 mm) from wilted stems of plants with leaf chlorosis were added to a 1.5-ml microtube containing 0.5 ml of sterile distilled water, macerated with a pipette tip, left to soak for 10 min, and vortexed vigorously to make a suspension. A 100 μl aliquot of a 1/10 serial dilution was spread on 1/10 tryptic soy broth agar

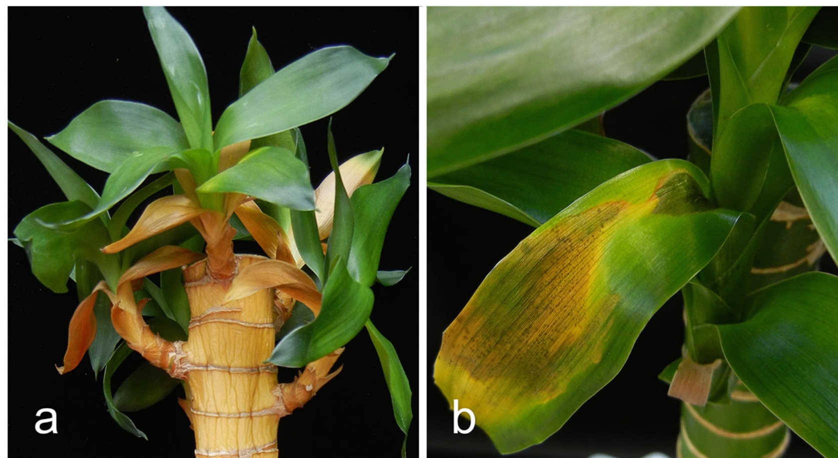

Fig. 1 Natural occurrence of bacterial blight and leaf chlorosis caused by *Burkholderia cepacia* on *Dracaena sanderiana*. **a** Blight; **b** leaf chlorosis

(TSBA) and incubated at 28 °C for 3 days. The resulting dominant colonies were streaked onto new TSBA. Gram-negative, rod-shaped, and non-spore forming yellow colonies of bacteria were consistently isolated from infected tissues on TSBA. The yellow colonies were circular and opaque with smooth margins, and produced non-fluorescent yellow pigment. A representative isolate was deposited in the Korean Agricultural Culture Collection (KACC 21282).

Pathogenicity tests were performed as described previously (Choi and Kim 2013) with some modifications. Three bacterial isolates including KACC 21282 were cultured on TSBA for 2 days. Bacterial suspensions (~10^8 CFU/ml) in sterile distilled water were inoculated on the stems and leaves of *D. sanderiana* purchased from nurseries using the syringe infiltration method. The inoculated plants were kept in a greenhouse at 25 °C for 10 days. Sterile distilled water was used as a negative control. The stems and leaves of *D. sanderiana* inoculated with the bacterial isolates showed the typical symptoms of blight (Fig. 2a) and leaf chlorosis (Fig. 2b), whereas those infiltrated with sterile distilled water showed no symptoms after 10 days. Koch's postulates were fulfilled as the 16S rRNA gene sequence was identical for bacterial isolates with confirmed pathogenicity.

Three isolates including KACC 21282 were tested for the tobacco leaf hypersensitive reaction and potato rot induction. All bacterial isolates were negative in the potato rot assays; however, they induced a hypersensitive reaction in tobacco leaves.

The physiological and biochemical characteristics of the bacteria were determined using the API 20NE system (BioMérieux, Marcy l'Etoile, France). The optical density of the bacterial suspension was adjusted as recommended by the manufacturer. In biochemical tests, KACC 21282 utilized D-glucose, L-arabinose, D-mannose, D-mannitol, N-acetyl-glucosamine, potassium gluconate, caprate, adipate, malate, trisodium citrate, and phenyl acetate, while it did not utilize D-maltose. The isolate was positive for catalase, oxidase, β-glucosidase, protease, and β-galactosidase, but negative for the production of arginine dihydrolase, urease, and indole. The isolate did not reduce nitrates to nitrites.

To confirm the identity of the bacteria, the 16S rRNA and *recA* genes were amplified with primer pairs 27mF (5′-AGAGTTTGATCMTGGCTCAG-3′) and 1492mR (5′-GGYTACCTTGTTACGACTT-3′) and BCR1 (5′-TGACCGCCGAGAAGAGCAA-3′) and BCR2 (5′-CTCTTCTTCGTCCATCGCCTC-3′), respectively (Lane 1991; Mahenthiralingam et al. 2000). DNA extraction and PCR amplification were as described previously (Choi et al. 2019). PCR was performed on a thermal cycler (T100; Bio–Rad, Hercules, CA, USA) using PCR premix (Bioneer, Daejeon, Korea), 1 µl genomic DNA, and 1 mM each primer, under the following conditions: 98 °C for 2 min; 30 cycles of 98 °C for 30 s, 55 °C for 30 s, and 70 °C for 1 min; and a final 4-min extension at 72 °C. The PCR products were examined by electrophoresis in 0.8% agarose gels, purified using Expin Gel SV (GeneAll Biotechnology, Seoul, Korea) and cloned using pGEM-T Easy Vector (Promega, Madison, WI, USA), according to the manufacturers' instructions. Sequencing was performed with primers M13F and M13R using a BigDye Terminator Cycle Sequencing kit (Applied Biosystems, Foster City, CA, USA), following the manufacturer's instructions. Using BLAST, searches revealed that the 16S rRNA (1,495 bp; GenBank accession no. MK779204) and *recA* gene (1,040 bp; MK779203) sequences of KACC 21282 shared 99.87 and 99.9% similarity with *B. cepacia* SE-1 (KF681774) (Zhu et al. 2016) and ATCC17759 (AF143788) (Mahenthiralingam et al. 2000; Mahenthiralingam and Vandamme 2005), respectively.

The DNA sequences (MK779203 and MK779204) were analysed using the program BLAST and compared using the NCBI/GenBank database (http://www.ncbi.nlm.nih.gov/blast/). A phylogenetic tree was generated using MEGA 4.0 with the neighbor joining method and Tajima–Nei distance model (Tamura et al. 2007). The sequences of related bacterial strains were downloaded from the GenBank database. A phylogenetic tree was constructed using partial *recA* sequences

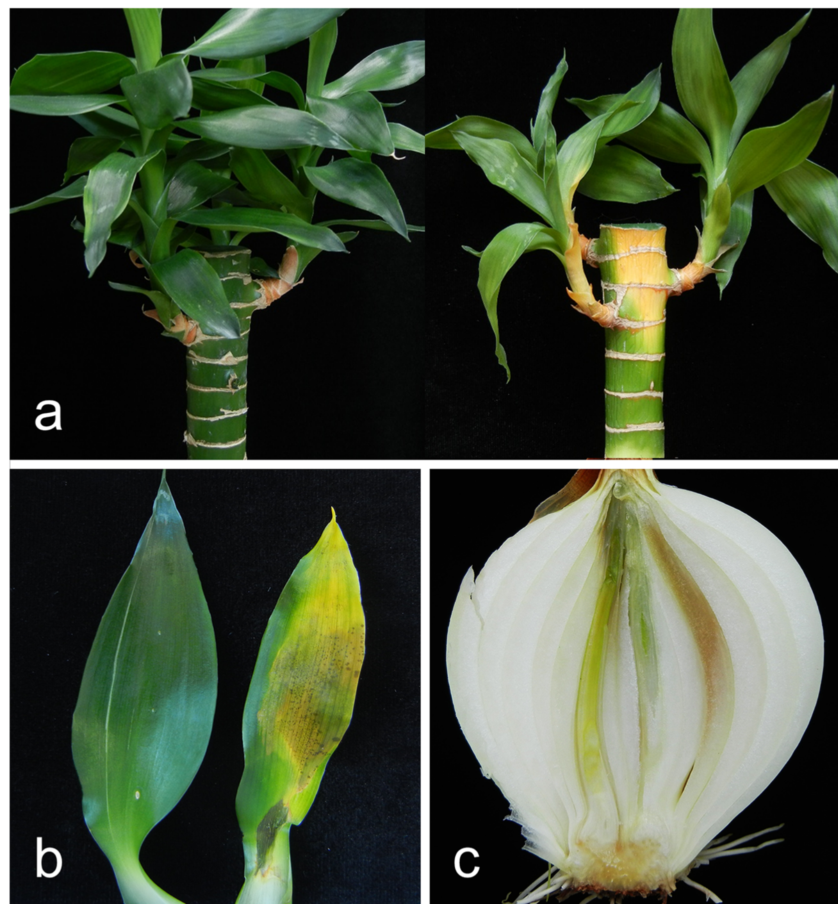

Fig. 2 Pathogenicity of *Burkholderia cepacia* causing bacterial blight on *Dracaena sanderiana*. **a** Symptoms developed after artificial inoculation on the stem of *D. sanderiana* (right) and a negative control inoculated with water (left); **b** leaf chlorosis after artificial inoculation on a *D. sanderiana* leaf (right) and a negative control inoculated with water (left); **c** pathogenicity of *B. cepacia* on onion bulb

against the database of type strains of *B. cepacia* complex (Jacobs et al. 2008). These results confirmed that KACC 21282 from *D. sanderiana* was in the same cluster as *B. cepacia* (Fig. 3).

B. cepacia is reported to cause onion rot, and we also performed a pathogenicity test on onion bulbs. For onion bulb pathogenicity tests, three onion bulbs purchased from a market were each surface-disinfested with 70% ethanol, dried, and

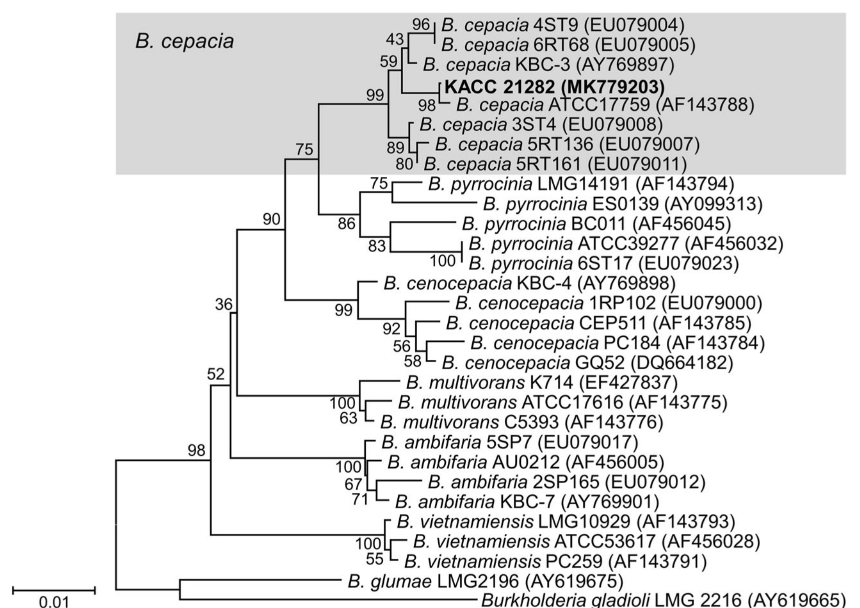

Fig. 3 Phylogenetic tree produced from *recA* gene sequences showing the phylogenetic relationships among *Burkholderia* spp. using the neighbor-joining method. DNA sequences from the NCBI nucleotide database were aligned using ClustalW. The numbers above the branches are the bootstrap values. The isolate infecting *Dracaena sanderiana* is in bold

injected with 200 μl of bacterial suspension (~10^8 CFU/ml) of KACC 21282 using syringe infiltration. The bulbs were placed in plastic boxes at 100% relative humidity and incubated at room temperature for 7 days. Sterile distilled water was used as a negative control inoculation. KACC 21282 caused soft rot on the onion bulbs after 7 days after inoculation (Fig. 2c).

Combining the symptoms and results of the pathogenicity assay, API tests, and 16S rRNA and *recA* gene sequencing, the pathogen causing blight on *D. sanderiana* was identified as *B. cepacia*. This is the first report that *B. cepacia* causes blight on indoor *D. sanderiana*.

Isolates of the *B. cepacia* complex often cause pneumonia in immunocompromised patients with underlying lung diseases such as cystic fibrosis and chronic granulomatous diseases (Isles et al. 1984; Mahenthiralingam and Vandamme 2005). Some isolates cause bacterial soft rot on onions, while others are biological control agents with potential agricultural benefits (Parke and Gurian-Sherman 2001; Sotokawa and Takikawa 2004). The bacterium occurs naturally in various environments, including soil, the rhizosphere, urban habitats, and river water (Miller et al. 2002; Dalmastri et al. 2003; Ramette et al. 2005). Because *D. sanderiana* plant is grown in indoor living spaces, *B. cepacia* infection could pose a risk to human health. Although epidemiological information on the inoculum source is unclear, the recent occurrence of the disease indicates that *B. cepacia* is a potential threat to the production of indoor plants, which are increasingly used for air purification, such as in SBS.

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