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Research Note

Characterization of *Myrothecium roridum* Isolated from Imported Anthurium Plant Culture Medium

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Abstract During an investigation of microorganisms and pests in plant culture media from imported anthurium pots, a fungal isolate (DUCC4002) was detected. Based on its morphological characters including colony shape on potato dextrose agar, the microstructures of spores observed by light and scanning electron microscopy and the results of phylogenetic analysis using an internal transcribed spacer rDNA sequence, the fungal isolate was identified as *Myrothecium roridum*. Pathogenicity testing on anthurium leaves revealed that the fungus could colonize and produce sporodochia on the inoculated leaves. This is the first report of *M. roridum* detected in imported plant culture medium in Korea.

Keywords Anthurium, Import, *Myrothecium roridum*, Plant culture medium

Anthurium is a genus of flowering plants belonging to the Araceae family that includes approximately 1,000 known species. Many species of *Anthurium* can be grown as houseplants or in shady spots outdoors in mild climates. *Anthurium* is an important cutting flower race; it is produced in subtropical regions such as Colombia, Brazil, and Ecuador, and it is the second largest traded item in the world flower market [1, 2]. This plant is highly susceptible to infection by various bacteria and fungi. For example, blight caused by *Xanthomonas axonopodis* pv. *dieffenbachiae* was first reported on *Anthurium* in 1971, and bottom rot caused by the fungal pathogen *Fusarium oxysporum* and the fungus-like organism *Phytophthora parasitica* has also been reported [3-5]. The fungal pathogen *Myrothecium roridum* has also been known to cause leaf spot on anthurium leaves [6]. In Korea, anthurium is imported as both seeds and young plants in pots. In the case of young plants, anthuriums are potted in plant culture media such as plant by-products or natural plant residues including coco-peat, coconut bark, moss, and peat moss. These plant culture medium components could potentially become contaminated with pathogenic fungi, bacteria, and other pests during cultivation and transportation. Therefore, the plant culture medium of imported potted plants should be cause for suspicion in the importing countries. Consequently, the detection of potent plant pathogens in the plant culture medium of imported plant pots should be an important issue in the plant quarantine process. In a preliminary investigation of fungi in imported, potted anthurium plant culture media, several fungi were isolated. One of these isolates, called DUCC4002, was identified as *Myrothecium roridum*. In this study, we report on its identification and pathological properties on *Anthurium andraeanum*.

DUCC4002 was isolated from a plant culture medium sample obtained from one of the potted anthurium plants that were imported from the People's Republic of China in April 2012. The plant culture medium was soaked in sterile water for 5 min with shaking. After centrifugation at 3,500 ×g for 10 min, the supernatant was removed, diluted 1,000-fold with sterile water and plated on potato dextrose agar (PDA; Difco, Detroit, MI, USA) containing streptomycin (200 μg/mL) and incubated for 3 days at 25°C. After fungal colonies grew up, the hyphal tips were detached and transferred to new PDA plates containing antibiotics and incubated for 7 days at 25°C. Single spores were isolated from a new colony generated on a new plate according to basic plant pathologyl methods [7]. Single spore isolates were maintained on PDA plates until completion of the experiments.

For morphological studies, the fungal isolate DUCC4002 was grown on PDA plates for 7 days at 25°C. Colony patterns were examined and photographed. Morphological characteristics were observed using a phase-contrast microscope

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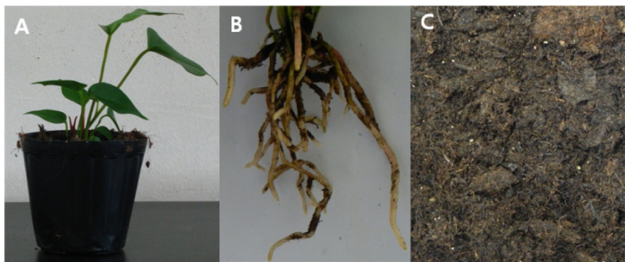

Fig. 1. Photographs of an imported anthurium plant pot and culture medium used for *Myrothecium roridum* isolation. A, Anthurium plant in a plastic pot; B, Anthurium roots pulled out from the pot; C, Anthurium culture medium from the pot.

(Axioskop 40; Carl Zeiss, Jena, Germany) and a scanning electron microscope (SEM; Hitachi, Tokyo, Japan). Fungal samples for SEM were prepared as described by Yun *et al.* [8] using 1% osmic acid. Superficially, the imported anthurium plant did not show any abnormal features, disease symptoms, or wounds caused by insects or pathogens (Fig. 1A and 1B). The peat moss that was used as a plant culture medium was tinged with brown coloration (Fig. 1C). From the culture medium, we obtained several fungal colonies. According to colony morphology and microscopic observations, these fungal colonies were identified as *Trichoderma* spp., *Penicillium* spp., and an unknown fungal species. For *Trichoderma* identification, *Trichoderma atroviride* (KOSPFG0000125700), *T. reesei* (KOSPFG0000125731), and *T. harzianum* (KOSPFG0000125714) were used as reference species.

The unknown fungal species was coded as DUCC4002 and its morphological characteristics were observed in detail. When we observed a colony of PDA-cultured DUCC4002, the peripheral area was white and the central area was yellowish (Fig. 2A). When the fungus was incubated on the PDA for more than 30 days, black conidiomata developed. Sporodochia were sessile, and setae were absent. Images of the conidia were observed by phase-contrast microscopy and SEM (Fig. 2B~2F). Conidia were 5~6 × 1~1.2 μm in size and cylindrical in shape with rounded ends similar to those of *M. roridum* [6]. The conidial mass was green in color despite staining with lactophenol blue solution (Fig. 2B and 2C).

To identify the fungus DUCC4002 isolate at the molecular level, it was grown on a cellophane-layered PDA plate for 7 days at 25°C. Fungal mycelia were prepared by scrapping the mycelium mat with a sterile scalpel. For polymerase chain reaction (PCR) amplification, genomic DNA was extracted from the prepared mycelium mat as described previously [9]. Primers ITS1 (5'-TCCGTAGGTGAACCTGCGG-3') and ITS4 (5'-TCCTCCGCTTATTGATATGC-3') were used to amplify an internal transcribed spacer (ITS) rDNA region [10]. The PCR amplification was performed using previously described conditions [9]. The sequence of a 473 bp fragment of the ITS rDNA PCR product was determined by Macrogen (Seoul, Korea) using primers ITS1 and ITS4. When we used the DUCC4002 ITS rDNA sequence as a query to search the GenBank DNA database of the National Center for Biotechnology Information (NCBI; http://www.ncbi.nlm.nih.gov), we found that it showed 100% sequence similarity with that of *M. roridum* BBA71015 (AJ302001). The full-length ITS nucleotide sequence of DUCC4002 was edited with Chromas v.2.31 (Technelysium Pty. Ltd., South Brisbance, QLD, Australia) and was used to search the GenBank using the BLAST tool. Reference ITS rDNA sequences of related taxa were down-loaded from GenBank. The DUCC4002 and down-loaded ITS rDNA sequences were aligned using ClustalW [11]. Phylogenetic analysis was performed using MEGA

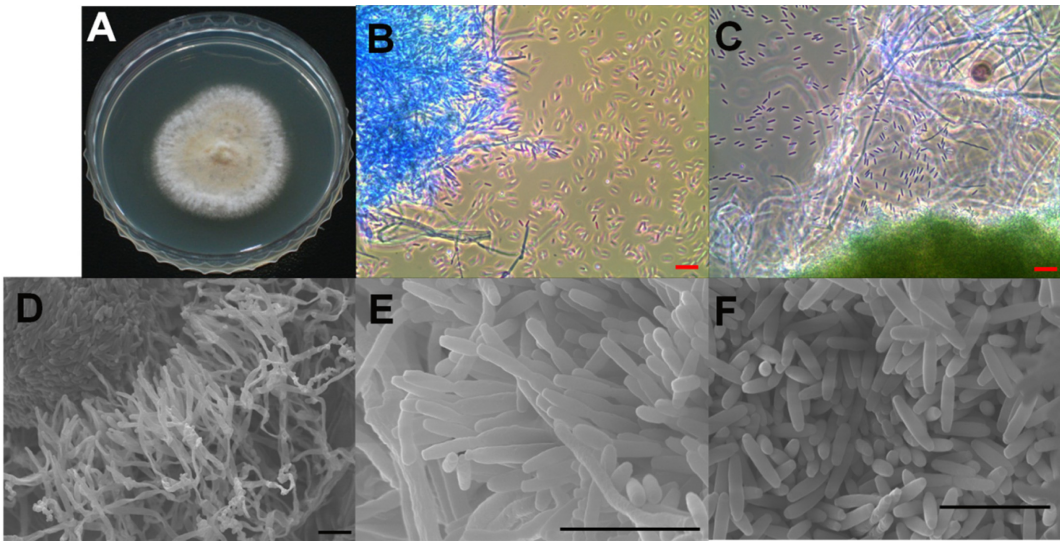

Fig. 2. Morphological features of *Myrothecium roridum*. A, Colony morphology on potato dextrose agar; B, C, Light microscopy images of mycelia and spores; D~F, Scanning electron microscopy images of mycelia and spores (scale bars: B~F = 10 μm).

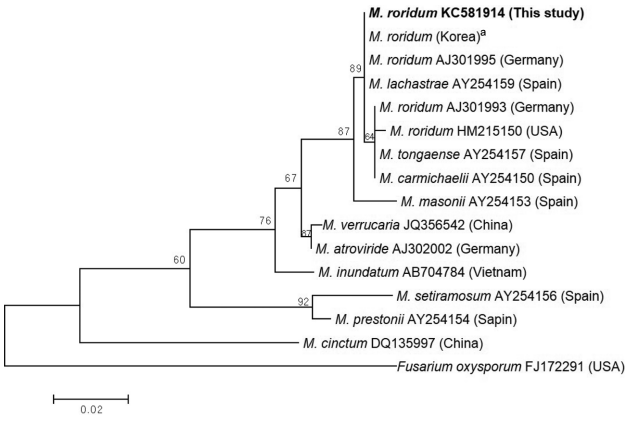

Fig. 3. Phylogenetic relationships between *Myrothecium roridum* isolates and related species inferred using a neighbor-joining method with internal transcribed spacer (ITS) rDNA sequences. Bootstrap values based on 1,000 replications are shown above the branches. Scale bar, 0.02 nucleotide substitutions per site. The DUCC4002 isolate from this study is *M. roridum* KC581914. GenBank accession numbers are shown after the fungus name. [a]ITS rDNA sequence of a Korean isolate (KACC4892) that was downloaded from the Rural Development Administration Genebank website (http://www.genebank.go.kr).

ver. 5.05 [12]. A phylogentic tree was constructed using the neighbor-joining method [13] with 1,000 bootstrap replications. The sequence of the ITS1~ITS4 region sequence of *Fusarium oxysporum* was used as outgroup for the phylogenetic analysis. In the phylogenetic tree, the ITS rDNA sequence of DUCC4002 was grouped with known *M. roridum* sequences retrieved from GenBank (Fig. 3). Together with the morphological study finding, this result supported that DUCC4002 is *M. roridum*. We deposited the DUCC4002 culture as *M. roridum* in the Dankook University Culture Collection and deposited its full-length ITS rDNA sequence into GenBank under accession number KC581914.

To investigate its pathogenicity, *M. roridum* DUCC4002 was inoculated on PDA and incubated for 20 days at 25°C. Conidia were collected from the PDA-grown *M. roridum* DUCC4002 using sterile water. The ontained conidial suspension was adjusted to 10^9 conidia/mL and was used as the inoculum. The wound/drop inoculation method was used to inoculate anthurium leaves as previously described [14]. Twenty-five days after inoculation, the applied *M. roridum* produced necrosis symptoms on the anthurium leaves (Fig. 4A and 4B). Sporodochia were produced on the edges of the necrotic portions of the anthurium leaves (Fig. 4C). When we observed sporodochia under a phase-contrast microscope, we observed that they contained hyphae and green conidial mass. We re-isolated conidia from the inoculated leaves with disease symptoms, and confirmed that they were indeed *M. roridum*. Therefore, we demonstrated that isolate DUCC4002 is pathogenic *M. roridum*.

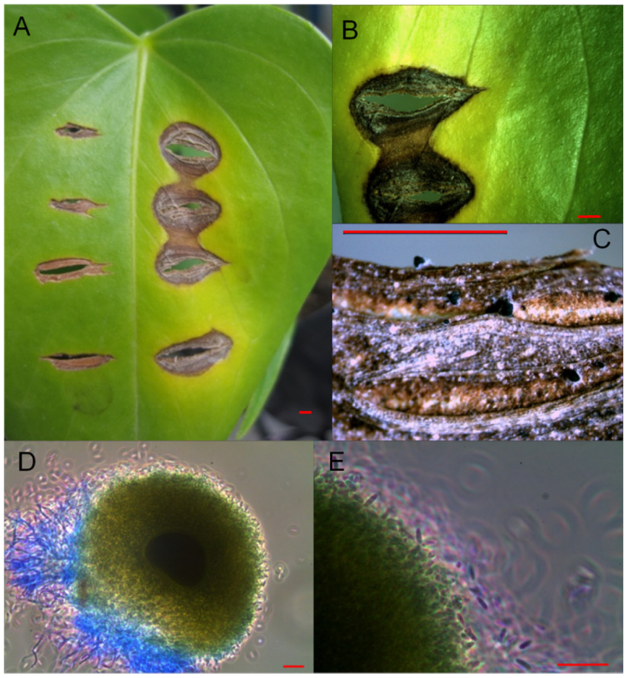

Fig. 4. Symptom development after inoculation of *Myrothecium roridum* DUCC4200 on anthurium leaves. A, The scars on the left half of the leaf were inoculated with sterile water (control). Those on the right half were inoculated with *M. roridum* DUCC4200, and show disease symptoms of browned and yellowed leaf tissues; B, Black spots of *M. roridum* sporodochia formed on the inoculation sites of an anthurium leaf; C, A light stereomicroscopy image of sectioned necrotic leaf tissue with sporodochia; D, E, Light microscopy images of a sporodochium with spores (scale bars: A~C = 1 mm, D, E = 10 μm).

M. roridum has been reported to cause necrosis on anthurium in Brazil [6]. This pathogen can produce toxins in host cells and colonize on the host cells after the host cell dies [15, 16]. *M. roridum* has also been reported to cause disease on diverse plants such as snap dragons, tomato, pansy, violet, cowpea, and soybean, and has been collected from decayed plant tissues and soils [17]. In Korea, *M. roridum* has been reported to cause leaf spot on soybean and watermelon [18, 19], and has been reported as a potential biocontrol agent against weedy plants [20]. However, there have been no previous reports of its pothogenicity on anthurium in Korea. This is first report of *M. roridum* detection in imported anthurium plant pots and risk assessment in Korea. How the pathogen was introduced into the plant culture medium remains unknown. Considering that the number of anthurium imports is increasing and that these imports are distributed to domestic growers in diverse regions, if *M. roridum* is present in the plant culture medium of imported plant pots, it could be a potential source of pathogen spread. In addition, because most plant culture media from plant pots are reused for compost, *M. roridum* contamination in plant culture media

could result in secondary infection of other plants. Therefore, we suggest proper plant quarantine operations are needed to hinder the introduction of *M. roridum* from foreign sources.

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