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Identification of *Microdochium bolleyi* Associated with Basal Rot of Creeping Bent Grass in Korea

Sung Kee Hong*, Wan Gyu Kim, Hyo Weon Choi and Sang Yeob Lee

Plant Pathology Division, National Institute of Agricultural Science and Technology, Rural Development Administration, Suwon 441-707, Korea

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Symptoms of basal rot occurred sporadically on creeping bent grasses growing at a golf course in Hampyeong, Korea in April 2007. Ten isolates of *Microdochium* sp. were obtained from leaves and crowns of the diseased bent grasses. All isolates were identified as *Microdochium bolleyi* based on morphological, cultural, and molecular characteristics. This is the first report on *M. bolleyi* associated with basal rot on creeping bent grass in Korea.

KEYWORDS : Basal rot, Creeping bent grasses, *Microdochium bolleyi*

Creeping bent grass (*Agrostis stolonifera* L.) is a cool-season specialty grass primarily used for golf course putting greens, lawn bowling greens, and lawn tennis facilities. Rhizoctonia rot, brown patch, and yellow patch are major diseases of creeping bent grass, and anthracnose basal rot is a minor concern (Farr *et al*., 2008). *Colletotrichum graminicola* (Ces.) Wilson and *Microdochium bolleyi* (Sprauge) De Hoog and Herm.-Nijh. [= *Idriella bolleyi* (Sprague) von Arx, *Aureobasidium bolleyi* (Sprague) von Arx, *Gloeosporium bolleyi* Sprague] are associated with anthracnose basal rot (Farr *et al*., 2008; Hillman *et al*., 2004; Hodges and Campbell, 1996). In Korea, *M. oryzae* (Hashioka and Yokogi) Samuel and Hallett has been reported as a causal agent of brown leaf blight of rice (Cho and Shin, 2004). Also, *M. nivale* (Fr.) Samuels and Hallett [= *Micronectriella nivalis* (Schaffnit) Booth, *Fusarium nivale* f. sp. *graminicola* (Berk and Broome) Snyder and Hansen] can exhibit snow mold on wheat (Cho and Shin, 2004).

Symptomatic leaves of creeping bent grass were found sporadically on a golf course in Hampyeong, Korea in 2007. The appearance of the damage was similar to basal rot caused by *Microdochium* spp. Several diseases such as anthracnose, Pythium blight, yellow patch, and brown patch have been reported on bent grass in Korea (Cho and Shin, 2004). However, there is no record on *Microdochium* species causing basal rot on creeping bent grass. The present study was conducted to confirm the presence of *Microdochium* species and to briefly describe the symptoms of basal rot of creeping bent grasses grown in Korea.

Materials and Methods

Isolation of fungi from symptomatic plants. Diseased leaves and crowns of creeping bent grasses were carefully observed with the unaided eye and 10 samples were collected from a golf course located in Hampyeong, Korea in 2007. The diseased plant parts were sterilized in 1% sodium hypochlorite for 1 min followed by several rinses with sterile distilled water. Pieces of the lesions (each 5 mm × 5 mm) were placed on the surface of water agar in Petri plates. After incubation at 24°C for 3 days, 10 single spore isolates were obtained from the lesion pieces. The isolates were cultured on potato dextrose agar (PDA) and used for identification.

Morphological and cultural characteristics. The isolates were cultured on PDA in darkness at 24°C to observe morphological characteristics. The size and shape of 100 spores were examined by light microscopy after 14 days of incubation. To ascertain the growth rate and mycelium color of each isolate, each was cultured on PDA, oatmeal agar (OA), and malt-extract agar (MA) in darkness at temperatures ranging from 15-31°C for 7 days.

Sequencing and phylogenetic analyses. Isolate M0701 was selected from the 10 isolates for analysis of internal transcribed spacer (ITS) sequences of ribosomal DNA (rDNA). To extract genomic DNA, the isolate was cultured on potato dextrose broth at 23°C for 4 days. Genomic DNA was extracted using a DNeasy™ kit (QIAGEN, Valencia, CA, USA) following the manufacturer's instructions. The rDNA ITS region was amplified by polymerase chain reaction (PCR) with primers ITS1 and ITS4 (White *et al*., 1990). PCR amplification was performed in 50 μl of a mixture containing 3 μl of 2.5 mM dNTP, 5 μl of 10× buffer, 0.4 μl of 100 μM primers, 0.3 μl of 1.5 unit *Taq* polymerase, and 1 μl of genomic DNA. The thermal cycler parameters were programmed for 1 cycle of dena-

*Corresponding author <E-mail : sukihong@rda.go.kr>

turation at 95°C for 4 min, 35 cycles of denaturation at 95°C for 1 min, annealing at 58°C for 1 min, extension at 72°C for 1 min, and a final extension at 72°C for 7 min. The PCR product was purified by a Wizard PCR clean-up system (Promega, Madison, WI, USA) and cloned into the plasmid pGEM T easy™ vector (Promega). Recombinant plasmid DNAs were selected and sequenced on both strands. The ITS sequences of *Microdochium* sp. isolate M0701 were generated and used for phylogenetic analysis. The data set for the analysis was obtained from sequences of *Microdochium* spp. and related fungal species from the GenBank database. Sequences were edited using a computer package (DNASTAR, Madison, WI, USA) and aligned by the CLUSTAL W method (Thompson et al., 1994). A phylogenetic tree for ITS analysis was obtained from the data by neighbor-joining methods using MEGA ver. 4.0 software as previously described (Tamura *et al*., 2007), and sequence distance was calculated with a Tamura-Nei parameter model. Bootstrap analysis was performed with 1,000 replications to determine the support for each clade.

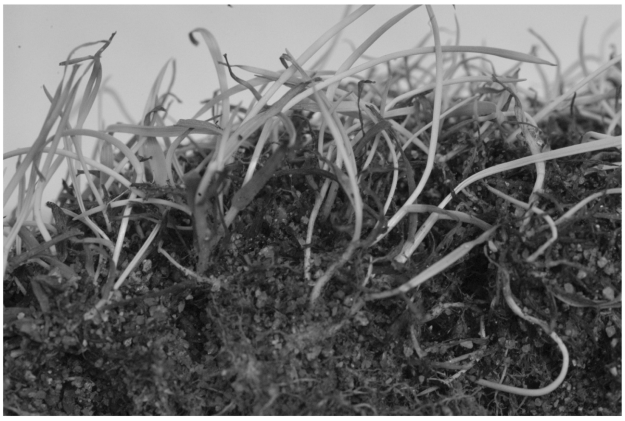

Fig. 1. Basal rot on creeping bent grass from a selected golf course.

Results and Discussion

Symptoms. The disease symptoms that developed sporadically on creeping bent grasses in the investigated golf course were limited to leaves and crowns (Fig. 1). The diseased leaves were mainly on the lower portion of the affected vegetation. Typically, the leaves faded to yellow and then turned to brown or reddish brown without distinct spots. There was no distinct margin between healthy and diseased tissues on an affected plant. Severely diseased plants resulted in a patch but were replaced with healthy plants in the field a few weeks later. Therefore, it is considered that this disease is not epidemic in the growing season.

Morphological and cultural characteristics. Recently developed colonies on PDA were white, and dark-colored chylamydospores formed leading to discoloration of

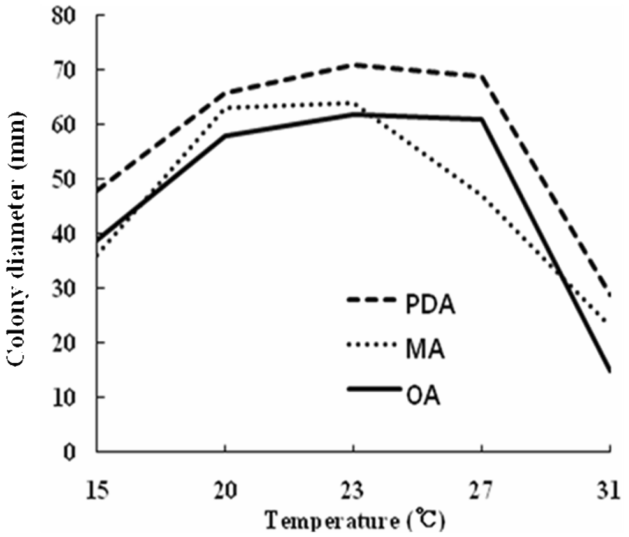

Fig. 3. Mycelial growth of *Microdochium bolleyi* isolates on PDA, MA, and OA after a week of incubation at different temperatures in darkness.

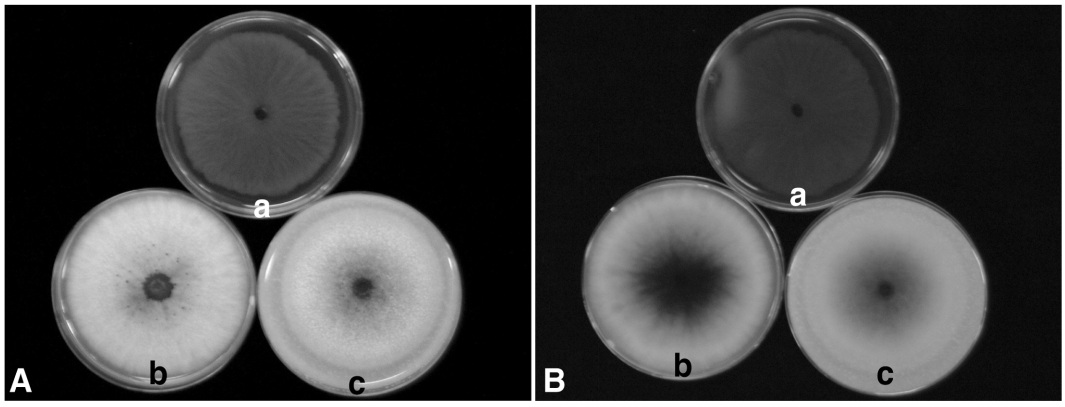

Fig. 2. Colonial appearance of *Microdochium bolleyi* grown on MA (a), PDA (b) and OA (c) incubated at 23°C for 7 days. A, front side; B, reverse side.

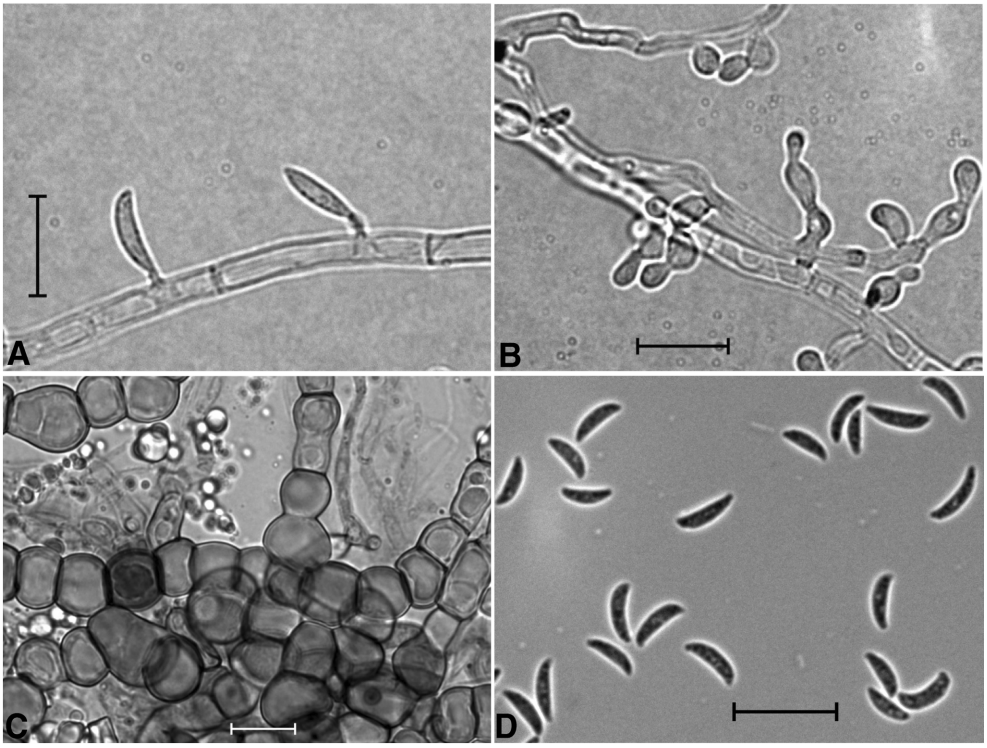

Fig. 4. Morphological features of *Microdochium bolleyi* isolated from basal rot of creeping bent grass. A, conidia on cylindrical conidiogenous cells; B, conidia on ampule-shaped conidiogenous cells; C, chlamydospores produced in chain or cluster in PDA; D, conidia. All scale bars represent 10 μm.

with mycelial growth being optimal at 23°C (Fig. 3). Two distinct types of conidiogenous cells were evident: ampullate and cylindrical (Figs. 4A and 4B). They formed laterally along with hyaline hyphal cells. Both types were hyaline and thin-walled. The size of the types were 3.1~6.4 × 2.5~3.8 μm (average 4.9 × 3.2 μm) and 1.5~2.7 (~5.9) × 0.8~1.4 μm (average 2.1 × 1.0 μm), respectively. Chlamydospores that formed in chain or clusters in older cultures were brown, swollen, thick walled, and were 5.2~14.9 × 4.4~11.3 μm (average 8.9 × 8.3 μm) in size (Fig. 4C). Conidia formed either singly or in a cluster on each locus of the conidiogenous cells (Fig. 4D). The conidia were hyaline, smooth, one-celled, thin-walled, lunate, and 5.0~8.7 × 1.6~2.3 μm (average 6.4 × 1.9 μm) in size. All 10 isolates were identified as *Microdochium bolleyi* (Sprauge) de Hoog and Herm.-Nijh. based on their

Table 1. Comparison of morphological characteristics of *Microdochium bolleyi* isolates

Structure	Characteristics	
	Present isolates	De Hoog & Hermanides-Nijhof (1977)
Conidium		
Shape	Lunate	Lunate
Cell	One	One
Color	Hyaline	Hyaline
Size (μm)	5.0~8.7 × 1.6~2.3 (average 6.4 × 1.9)	5.5~8.5 × 1.6~2.2
Conidiogenous cell		
Shape	Ampullate or cylindrical	Cylindrical and globose or subglobose
Color	Hyaline	Hyaline
Size (μm), ampullate	3.1~6.4 × 2.5~3.8 (average 4.9 × 3.2)	2~4.5 × 2~3.5
Size (μm), cylindrical	1.5~2.7 (~5.9) × 0.8~1.4 (average 2.1 × 1.0)	–[a]
Chlamydospore		
Production	Chain or cluster	Chain or cluster
Color	Brown	Brown
Size (μm)	5.2~14.9 × 4.4~11.3 (average 8.9 × 8.3)	6~9

[a]Not described.

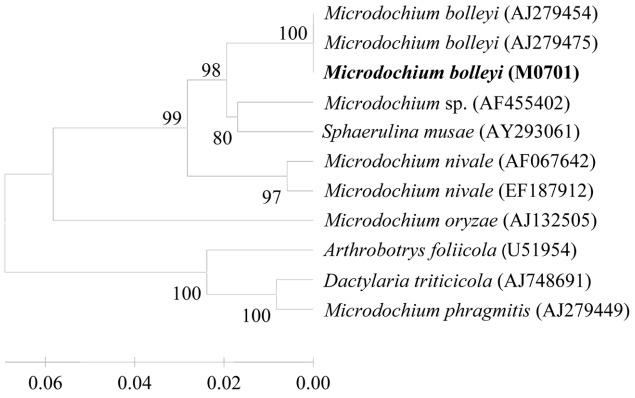

Fig. 5. Phylogenetic tree of *Microdochium* spp. and other fungi based on entire ITS sequences of nuclear rDNA. The tree was constructed by the neighbor-joining method. The values of each clade are confidence levels from a 1000 replicate bootstrap sampling. Numbers in parentheses are accession numbers found in the GenBank database.

morphological and cultural characteristics (Table 1). These characteristics were consistent with previous observations (De Hoog and Hermanides-Nijhof, 1977; Von Arx, 1982).

Phylogenetic analysis. A phylogenetic tree based on rDNA-ITS sequences of *Microdochium* spp. and related fungal species demonstrated that isolate M0701 had the same sequence as *M. bolleyi* but different from *M. nivale* and *M. oryzae*, and is distantly related to *Microdochium phragmitis* Syd (Fig. 5).

M. bolleyi is a causal fungus of root rot and seedling blight of cereals and grasses (Sprague, 1948). However, the fungus has been regarded as a saprophyte or inhabitant in soil (Gams and Domsch, 1969), weak or potential pathogen of flax and creeping bent grass (Black and Brown, 1986; Hodges and Campbell, 1996). It was reported that the fungus is a potential biocontrol agent against cereal pathogens (Kirk and Deacon, 1987; Lascaris and Deacon, 1994). The present study reports for the first time that *M. bolleyi* is associated with basal rot of creeping bent grass in Korea. Whether the fungus is the cause of the disease remains to be ascertained.

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