Muribasidiospora gordoniae sp. nov. occurring on Gordonia axillaris in Taiwan

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ABSTRACT. The genus *Muribasidiospora* producing muriform basidiospores belongs to Exobasidiaceae. Three species, *Muribasidiospora hesperidium*, *M. indica* and *M. celtidis*, have been recorded. *Muribasidiospora gordoniae*, which causes a prominant red leaf spot disease on *Gordonia axillaris*, is a new species in Taiwan based on the host and its morphology. Additionally, analysis of the internal transcribed region (ITS1-5.8S rDNA–ITS2) reveals *M. gordoniae* is closely related to the genus *Exobasidium*, especially those species of it occurring on *Camellia* species.

Keywords: Exobasidiaceae; *Gordonia axillaris*; Internal transcribed spacer; *Muribasidiospora* disease; Taiwan; Taxonomy.

INTRODUCTION

The genus Muribasidiospora Kamate & Rajendren was first established in 1968 (Rajendren, 1968) and belongs to Basidiomycota, Ustilaginomycetes, Exobasidiales, Exobasidiaceae (Kirk et al., 2001). All members in the genus are phytoparasites, and only three species—M. hesperidium (Maire) Kamat & Rajendren, M. indica Kamat & Rajendren, and M. celtidis (Ramakrishnan, T. S. & K.) Kamat & Rajendren—have been recorded (Rajendren, 1968). Their respective hosts are Celtis tetrandra (Ulmaceae) infected by M. celtidia, Rhus mysorensis (Anacardiaceae) by M. indica, and R. oxyacantha by M. hesperidum (Rajendren, 1968; Donk, 1974; Crous et al., 2003). Muribasidiospora hesperidium and M. celtidis originally belonged to Exobasidium viz. E. hesperidium Maire and E. celtidis Ramakrishnan T. S. & K. but Raiendren revised the two species as Muribasidiospora owing to their production of muriform basidiospores (Rajendren, 1968). The taxonomy of Muribasidiospora and Exobasidium are similar, but the differences are that Muribasidiospora bears muriform basidiospores, 2-4 sterigmata, sterile hyphidia-like hyphal elements between the basidia and basidia with thickened cell walls at the base (Begerow et al., 2001).

The other characteristics are sporulation by rupturing epidermal cells, basidiospore germination by budding to produce cylindrical conidia, and the formation of yeast-like colonies (Begerow et al., 2001). Phylogenetically, *Muribasidiospora* is closely related to *Exobasidium*, based on the 5' region of nuclear large subunit of rDNA (LSU rDNA) (Begerow et al., 2001 and 2002).

A red leaf spot disease of *Gordonia axillaris* (Roxb.) Dietr. was discovered in May, 2006 in Taiwan. *Gordonia axillaris*, which belongs to Theaceae is distributed from 100 m to 2,200 m above sea level in Taiwan. *Gordonia axillaris* is native to Taiwan, and some people plant them as ornamentals. The new disease was collected in Anmashan, located 2,000 m above sea level, and the weather is usually humid and cool. Owing to its different hosts and morphology, the pathogen was identified as a new species, which was named *Muribasidiospora gordoniae* Shih, Hsieh et Fu. Up to now, no *Muribasidiospora* diseases have been recorded in Taiwan, and the new species on *G. axillaris* will be described. On the other hand, the ITS sequence of *M. gordoniae* is also analyzed in this article.

MATERIALS AND METHODS

The specimen was collected in Anmashan, Taichung, Taiwan on 5 May 2006. Hymenium were scraped and stained with lactophenol containing 0.05% cotton blue. Cross sections of the infected tissue were made by hand to observe the hymenium on infected cells. The germination

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of basidiospores was performed on water agar. The fresh tissue was attached to the lid of the petri dish to allow basidiospores to fall on to the agar, and the germinated basidiospores were then transfered to a potato sucrose agar (PSA) medium. All isolates were grown at 22°C. Half of the dried herbarium material was deposited at the Department of Plant Pathology and Microbiology, National Taiwan University (NTUPPM), and half went to the herbarium at the National Museum of Natural Science, Taiwan (TNM). The living culture was deposited in the Bioresource Collection and Research Center (BCRC).

Genomic DNA was extracted with a VIOGENE Plant Genomic DNA Mini System. The internal transcribed spacer (ITS) region, composed of ITS1, 5.8S rDNA and ITS2, was amplified with primer pairs, ITS1 (5'-TCCGTAGGTGAACCTGCGG-3') and ITS4 (5'-TCCTCCGCTTATTGATATGC-3'). The PCR product was purified using a VIOGENE PCR-M Clean Up System. The purified rDNA was then sequenced with the ITS1 and ITS4 primers. The obtained ITS sequence was blasted in the NCBI database (National Center for Biotechnology Information).

RESULT

Muribasidiospora gordoniae Shih, Hsieh et Fu, sp. nov. (Figure 1A-I)

Etymology. Named after the host plant, Gordonia axillaris (Roxb.) Dietr.

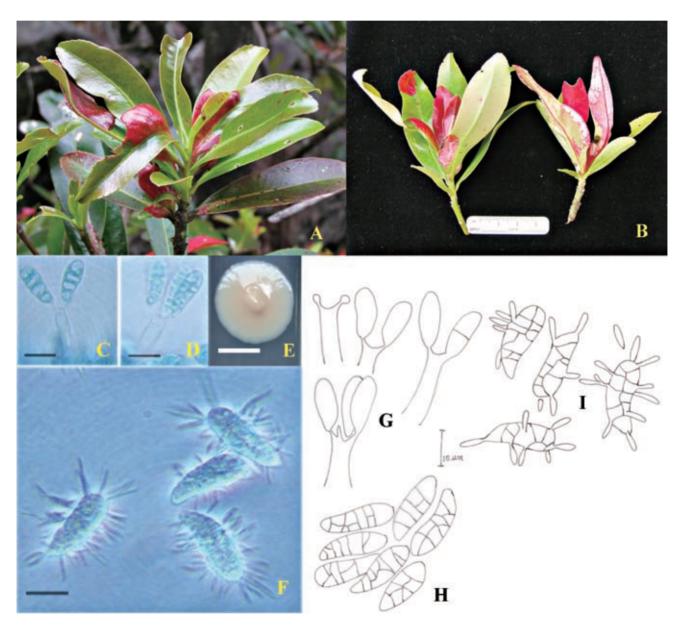


Figure 1. *Muribasidiospora gordoniae* sp. nov. occurring on *Gordonia axillaris*. A-B, symptoms; C-D: basidia bearing 2-3 sterigmata; E: colonies on PSA at 22°C after 47 days growth; F: germination of basidiospores; G-I: draw-line of hymenium; G: basidia; H: basidiospores; I: germination of basidiospores. Bar: C-D, F & G-I = $10 \mu m$; E = 5 mm.

Hymenium hypophyllum. Basidia hyalina, cylindrica, 120-170 × 5-7 μm, terminaliter cum 2-3 sterigmatibus subulatis, 3-4 × (1-)2-2.5 μm. Basidiosporae primo continuis dein muriform septatis, 15-21 × 5.5-7 μm. Conidia hyalina, 4-11 × 0.6-1.5 μm.

Holotype. **TAIWAN.** Taichung: Anmashan, in folia on *Gordonia axillaris* (Roxb.) Dietr. 5 May 2006, H.-H. Shih, TNM F0020737 (NTUPPM-623), BCRC34277 (living culture).

Fungi infecting lower young leaves and forming red leaf spots. Leaf spots conspicuous, red, irregular, slightly hypertrophic; abaxial lesion surface covered with thick, protruding, white and powdery hymenium, making the leaf slightly curled.

Hymenium composed of basidia, basidiospores and conidia. Basidia rupturing from epidermis, cylindrical, fewer thickened at the basal basidia, $120\text{-}170 \times 5\text{-}7 \mu m$, with 2-3 sterigmata. Sterigmata subulate, $3\text{-}4 \times (1\text{-})2\text{-}2.5 \mu m$. Basidiospores broad obovoid, muriform septa, $15\text{-}21 \times 5.5\text{-}7 \mu m$. Conidia short fusiform to filiform, bacilliform, $4\text{-}11 \times 0.6\text{-}1.5 \mu m$. Basidiospores on water agar germinating to produce short bacilliform conidia in the periphery by budding without germ tubes. Colonies on PSA yeast-like, sticky, smooth, pale yellow at the surface of the culture, about 1 cm at 22°C after 47 days growth, consisting of abundant budding secondary spores. The optimal growth temperature is $20\text{-}25^{\circ}\text{C}$.

The ITS sequence (accession number in NCBI database: EU262600) of *Muribasidiospora gordoniae* is composed of 598 bases. A BLAST search in the NCBI database revealed that the ITS sequence of *M. gordoniae* to be closely related to the *Exobasidium* genus, especially to those species occurring on *Camellia* species such as *E. reticulatum* (AB180377, 92% identity) and *E. gracile* (AB180371, 88% identity). Up to now, ITS sequences of *Muribasidiospora* species are absent from the NCBI database, and that of *M. gordoniae* is the first one.

DISCUSSION

The morphology of *Muribasidiospora* is similar to *Exobasidium*, but production of muriform basidiospores by *Muribasidiospora* is the most important character distinguishing it from *Exobasidium*. Few *Muribasidiospora* species have ever been recorded, but all of them cause leaf-spot symptoms (Rajendren, 1968; Crous et al., 2003). In this study, *M. gordoniae* induces red leaf-spots on *Gordonia axillaris*, and its morphology is typical of *Muribasidiospora*, except for the thickened cell wall at the basal basidia. In this specimen, many long cylindrical basidia were found, but the lack of sterigmata on the top of basidia indicated immaturity. The basidiospore size of *M. gordoniae* is different from that

of M. hesperidium (12-25 \times 7-9 μ m) and of M. indica (12-20 × 8-16 µm) (Rajendren, 1968; Crous et al., 2003). The reference on M. celtidis is, regrettably, unavailable for comparison. M. gordoniae is thought to be a new species, owing to the different hosts and morphology. In the previous study, the phylogenetic relationship between Exobasidium and Muribasidiospora was closer (Begerow et al., 2001). The recorded hosts of Muribasidiospora are Rhus (Anacardiaceae) and Celtis (Ulmaceae), but those hosts aren't infected by Exobasidium. However, Gordonia axillaris can be infected by E. monosporum (Sawada, 1922) and M. gordoniae in Taiwan. The leaves infected by E. monosporum produce near circular, yellow green spots on the surface. Analyzing the ITS sequence, M. gordoniae was more similar to Exobasidium species occurring on Camellia than to those on Ericaceae. The suspected reason is that both Camellia and Gordonia belong to Theaceae. However, the ITS sequence of *E. monosporum* is absent, and it may be more interesting to pursue the relationship between E. monosporum and M. gordoniae.

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台灣地區大頭茶新種病害 (Muribasidiospora gordoniae)

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Muribasidiospora 屬於擔子菌之外擔子菌科,具有磚格狀之擔孢子,目前世界上已記錄有 3 種,分別為 Muribasidiospora hesperidium、M. indica 和 M. celtidis。於臺灣中部地區首次發現感染大頭茶葉片,形成紅色不規則的塊斑之真菌性病害。由於其寄主植物和病原形態之差異性,此病原菌被鑑定為新種,並命名為 Muribasidiospora gordoniae。此外,分析 ITS 序列,M. gordoniae 和感染茶屬植物之外擔子菌(Exobasidium)之序列相似度較高。

關鍵詞:外擔子菌科;ITS;大頭茶;Muribasidiospora病害;臺灣;分類。