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First Report of *Ampelomyces quisqualis* from Sycamore and Crape Myrtle and Its Potential as a Mycoparasite of Powdery Mildew

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ABSTRACT : During screening fungi as potential biological control agents for plant parasitic fungi, a fungal strain, EML-FAM3, was isolated from powdery mildew leaf lesions caused by *Erysiphe platani* on sycamore (*Platanus occidentalis* L.), and another strain, EML-FAMC1, from *Erysiphe australiana* on crape myrtle (*Lagerstroemia indica* L.). Based on the morphological characteristics and phylogenetic analysis of the internal transcribed spacers (ITS1 and ITS2) and 5.8S rDNA, the strains were identified as *Ampelomyces quisqualis*. To our knowledge, this is the first report of new mycohosts, *E. platani* and *E. australiana,* of the mycoparasite *A. quisqualis* on sycamore and crape myrtle plants. The hyperparasite may represent the potential for controlling *E. platani* and *E. australiana* epidemics.

KEYWORDS : ***Ampelomyces quisqualis*, Hyperparasite, *Lagerstroemia indica*, *Platanus occidentalis*, Powdery mildew**

Ampelomyces spp. are common intracellular mycoparasites of powdery mildews. Pycnidial fungi belonging to the haploid ascomycete genus *Ampelomyces* are common mycoparasites occurring intracellularly in mycelia of powdery mildews (Erysiphaceae) worldwide [1, 2]. The natural co-occurrence of *A. quisqualis* on various Erysiphaceae species has been reported in different geographic regions [3-6]. *Ampelomyces* species have been associated with *ca.* 65 fungal species of eight genera of the order Erysiphales in different regions globally [2]. In Korea, *Ampelomyces* has been revealed on 19 species of the genus *Erysiphe* and occurs on a wide range of plants [7]. To our knowledge, there were no previous published literature records of this species as a mycoparasite of powdery mildew disease on sycamore (*Platanus occidentalis* L.) and crape myrtle (*Lagerstroemia indica* L.) hosts.

Many researches on *A. quisqualis* has focused on its potential use as a biocontrol agent against powdery mildews on various crops and woody plants [8, 9]. At present, a few *Ampelomyces* isolates have already been registered as biocontrol agents of powdery mildews, e.g. AQ10 biofungicide is commercially used for the biocontrol of grape powdery mildew [10].

Recent research on the phylogenetic analysis based on the internal transcribed spacer (ITS) rDNA sequences revealed that the species could be classified into five different genetic clades, showing that different physiological forms exist within genetically diverse strains of the species [11]. *P. occidentalis* is widely planted as a street tree distributed in South Korea and is useful in the rehabilitation of various sites with saturated soils. Moreover, it has been used for furniture, veneer, and flooring. Crape myrtle (*L. indica*) is used as a decorative tree. It flowers early in the summer and produces seedpods that contains the alkaloids of interesting biological activity [12].

Powdery mildew has frequently been observed on sycamore plants in Jeonnam province, South Korea since it was reported by Lee et al. [13]. The use of biocontrol agents is encouraged for the control of this disease to enhance environmental sustainability.

The objectives of the present study were to investigate the co-occurrence of a mycoparasite, *A. quisqualis* with

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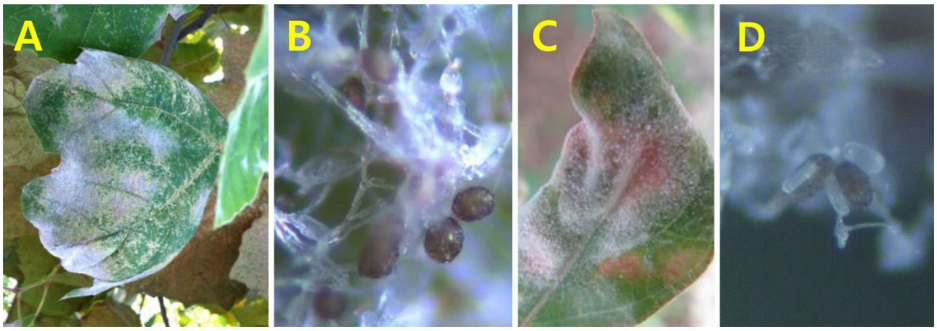

Fig. 1. Co-occurrence of *Ampelomyces quisqualis* on leaf lesions (A, C) of powdery mildews caused by *Erysiphe platani* on sycamore and *Erysiphe australiana* on crape myrtle in Korea and pycnidia of *A. quisqualis* (B, D) on their hosts.

E. platani and *E. australiana* as new mycohosts, which cause powdery mildew on sycamore plants and crape myrtle, to describe the morphological characteristics of *Ampelomyces* species, and to determine the molecular phylogenetic relationship inferred from the ITS rDNA sequence data of the fungus and related species.

To obtain *Ampelomyces* isolates, leaves (Fig. 1A, 1C) with powdery mildew symptom caused by *Erysiphe platani* on sycamore and *Erysiphe australiana* on crape myrtle with a higher production of pycnidia were collected. The samples of infected leaves were examined under a stereo-microscope, and *Ampelomyces* pycnidia were isolated using the single-spore isolation method (Fig. 1B, 1D). They were then transferred with a glass needle to potato dextrose agar (PDA). Pure isolates were transferred to slant tubes, and deposited at the Environmental Microbiology Laboratory Herbarium (EMLH; Chonnam National University, Gwangju, Korea) as EML-FAM3 and EML-FAMC1. Genomic DNA was directly extracted from pycnidia using the HiGene Genomic DNA prep kit for

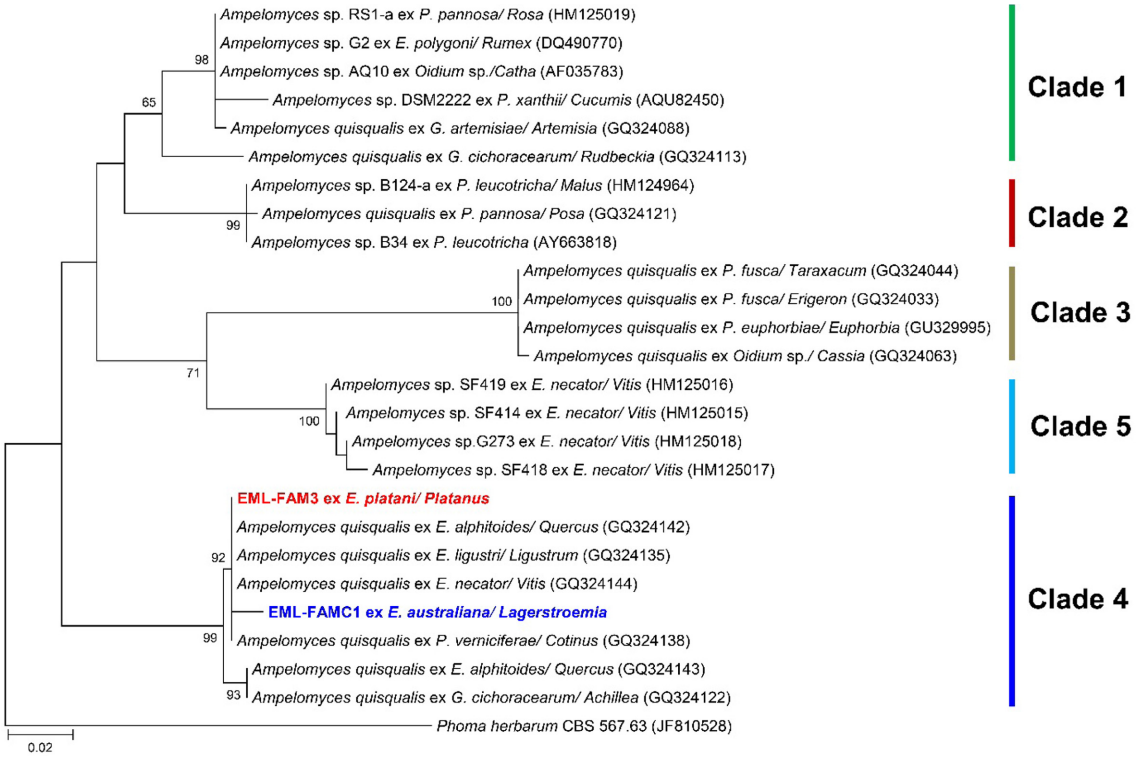

Fig. 2. Phylogenetic tree based on maximum likelihood analysis of internal transcribed spacer rDNA sequences for EML-FAM3 and EML-FAMC1. *Phoma herbarium* was used as an outgroup. Bootstrap values are shown above/below branches supported by more than 50% from 1,000 replications. Each clade was made based on the previous phylogenetic system constructed by Pintye et al. [11].

fungi (Biopact Corp., Daejeon, Korea). The internal transcribed spacers (ITS1 and ITS2) and 5.8S gene were amplified using primers ITS1 (5- CTTGGTCATTTAGAGG AAGTAA-3) and ITS4 (5-TCCTCCGCTTATTGATATGC-3) following the method by White et al. [14]. The sequences were initially aligned using CLUSTAL X [15], and edited manually. Phylogenetic analyses were performed using MEGA 6 [16] with the default settings. Phylogenetic trees were constructed from the data using maximum likelihood (ML). The sequences of EML-FAM3 and EML-FAMC1 strains were deposited in the GenBank database with accession numbers, KC878305 and KU948156, respectively. A BLASTn search revealed that the rDNA ITS sequences of EML-FAM3 and EML-FAMC1 represented high similarities of 99.8% (491/492 bp) and 99.1% (313/316 bp) with *A. quisqualis* (GenBank accession no. GQ 324135). The phylogenetic tree of the ITS region (Fig. 2) revealed that the isolates EML-FAM3 and EML-FAMC1 were significantly grouped with *A. quisqualis*. These species belonged to clade 4, which includes *A. quisqualis* strains from *E. ligustri*, *P. verniciferae*, *E. alphitoides*, *E. necator* and *G. cichoracearum*.

To confirm the molecular species identification, the morphology of the isolates EML-FAM3 and EML-FAMC1

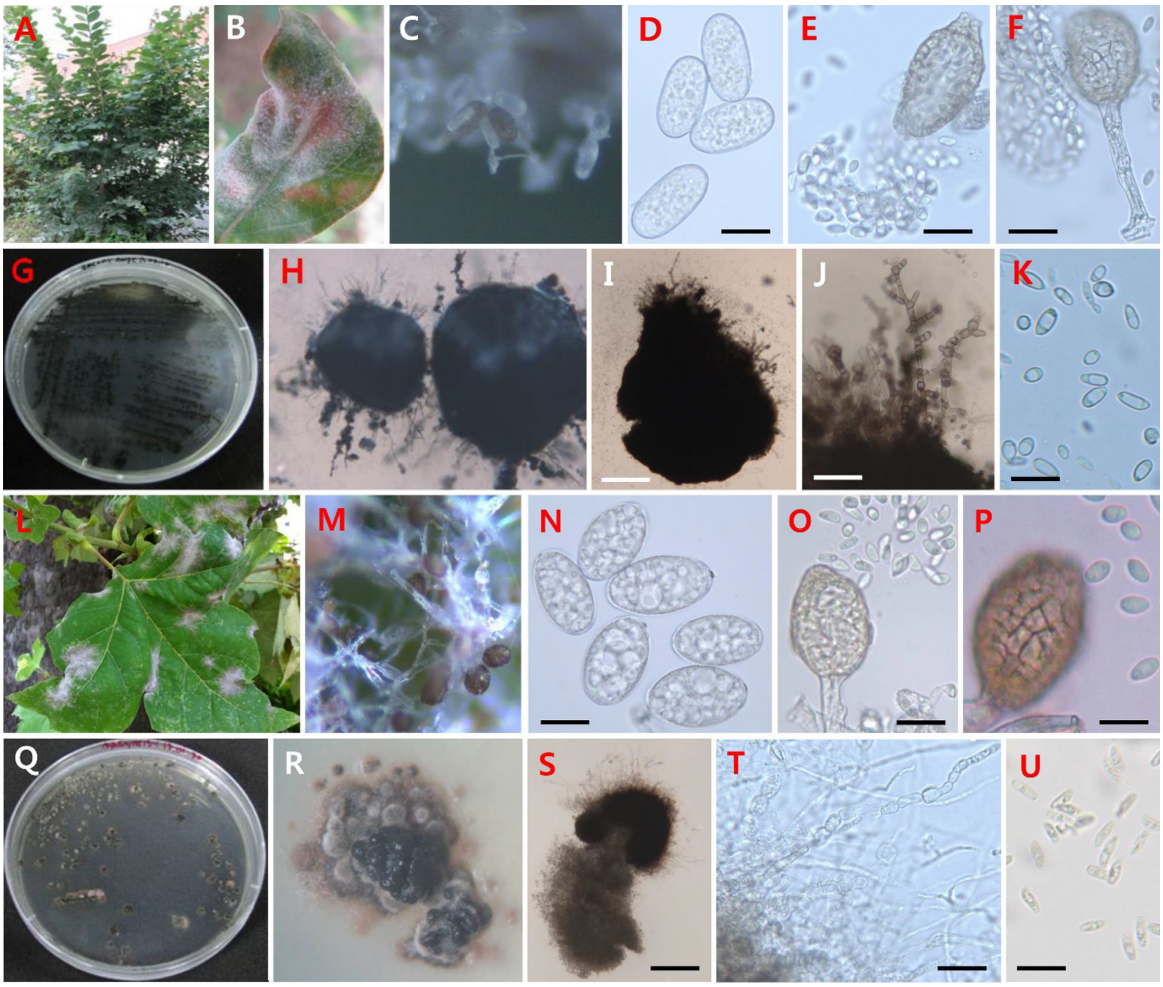

Fig. 3. Sycamore and crape myrtle plants infected powdery mildew with its mycoparasite, *Ampelomyces quisqualis*. A, B, Leaf lesion of powdery mildew caused by *Erysiphe australiana* on crape myrtle plants; C, Mycoparasite *A. quisqualis* on *E. australiana* under stereo-microscope; D, Conidia of *E. australiana*; E, F, Pycnidia and pycnidiospores of *A. quisqualis*; G, Streak plate with colonies of EML-FAMC1 on potato dextrose agar (PDA) medium; H~K, Fungal masses consisting of pycnidia and pycnidiospores on colony; L, Leaf lesion of powdery mildew caused by *Erysiphe platani* on sycamore plant; M, Mycoparasite *A. quisqualis* on *E. platani* under stereo-microscope; N, Conidia of *E. platani*; O, P, Pycnidia and pycnidiospores of *A. quisqualis*; Q, Streak plate with colonies of EML-FAM3 on PDA medium; R, Fungal masses consisting of pycnidia and pycnidiospores on colony; S, Pycnidiospores exuded from pycnidia; T, Appendages of pycnidia; U, Pycnidiospores (scale bars: D~F, J, K, N, P, T, U = 20 μm; I, S = 200 μm).

were examined under light microscope (DFC 290; Leica Microsystems, Wetzlar, Germany).

Ampelomyces strains, EML-FAMC1 and EML-FAM3, were observed on crape myrtle (Fig. 3A~3D) and sycamore (Fig. 3L~3N) with powdery mildew infection, respectively. Pycnidia of EML-FAMC1 were vary in shape, globose to obovoid, light brown to dark brown, measured 35~38 × 23.5~26.5 μm. Pycnidiospores were globose to oblong, measured 5.5~6.5 × 2.5~3.5 μm (Fig. 3E, 3F). While the pycnidia of EML-FAM3 were globose, dark brown, and measured 41.5~51.0 × 33.5~43.0 μm. Pycnidiospores were globose to oblong, measured 7.5~8.5 × 3.0~3.5 μm (Fig. 3O, 3P). The pycnidia and conidia of EML-FAMC1 (Fig. 3G) and EML-FAM3 (Fig. 3Q) isolates were incubated on potato dextrose agar (PDA) to determine their morphological characteristics. The color of the mycelia and pycnidia of EML-FAMC1 (Fig. 3H, 3J) and EML-FAM3 (Fig. 3R, 3T) were observed after 14 and 30 days at 25°C in the dark. EML-FAMC1 isolate: Pycnidia were ovate to lemon-shaped, dark olivaceous. Pycnidiospores were globose to oblong, measured 6.0~7.0 × 2.5~3.5 μm (Fig. 3I, 3K). EML-FAM3 isolate: Pycnidia were globose, dark brown. Pycnidiospores exuded from pycnidia were globose to oblong, measured 5.5~7.5 × 3.0~3.5 μm (Fig. 3S, 3U). Two isolates exhibited slow growth on PDA and an irregular margin, and mycelia were immersed or superficially formed.

When fungal masses of *A. quisqualis* EML-FAM3 were treated on the leaves infected by powdery mildew on ubame oak (*Quercus phillyraeoides* A. Gray) leaf at 27°C for 24 hr, the conidial germination of the powdery mildew spores were significantly reduced (data not shown). Powdery mildew is a notorious disease and generally difficult to control. Our data suggests that the EML-FAM3 strain isolated from sycamore plant may reduce the incidence of powdery mildew by suppressing conidial germination. More studies on the application of the *Ampelomyces* strains as biocontrol strategies are needed.

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