Characterization of a *Septobasidium* sp. Associated with Felt Disease of *Schisandra chinensis*

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Abstract Extensive disease surveys performed during the summers of 2013 and 2014 in *Schisandra chinensis* orchards resulted in the finding of a *Septobasidium* sp. associated with felt disease. The fungus was characterized to be symbiotic with a scale insect (*Pseudaulacaspis cockerelli*). Morphological and molecular characteristics of the *Septobasidium* isolates were investigated. The isolates were morphologically and phylogenetically close to *S. bogoriense*. We tentatively describe this isolate as a *Septobasidium* sp., mainly because of the limited amount of information available on the internal transcribed spacer region of the ribosomal DNA of *Septobasidium* spp.

Keywords Felt disease, Phylogenetic analysis, *Schisandra chinensis*, *Septobasidium*

Schisandra chinensis (Turcz.) Baill. [Schisandraceae], native to northern China and the Russian Far East, is a deciduous woody vine. The dried fruit of this plant is used medicinally. The berries of *S. chinensis* are called “Omija” in Korean, which literally translates to “five-flavor berry” because of the salty, sweet, sour, pungent (spicy), and bitter flavors [1]. *S. chinensis* is one of ten major medicinal crops in Korea. As of 2013, 9,575 metric tons were produced from a 2,367 ha cultivation area [2]. More than ten fungal agents are thought to be associated with diseases of *S. chinensis* worldwide [3]. *S. chinensis* in Korea is susceptible to several

fungi, such as *Botrytis cinerea* (gray mold), *Erysiphe schisandrae* (powdery mildew), *Penicillium* sp. (blue mold), *Pestalotiopsis guepinii* (leaf blight), *Phoma* sp. (leaf spot), *Phytophthora citrophthora*, *P. drechsleri*, and *P. nicotianae* (*Phytophthora* root rot) [4].

We found felt-like symptoms commonly occurring in most *S. chinensis* orchards in Korea during our extensive survey of phytopathogens on *S. chinensis*. Since felt disease on *S. chinensis* has not been previously recorded, we identified and characterized the causal agent of the disease with morphological features and molecular analyses.

Symptomatology and scale insect association. We conducted extensive surveys of twelve *S. chinensis* farms in Jangsu County, one of the major producing areas in Korea [5], during the summers of 2013 and 2014. Hundreds of *S. chinensis* plants (cv. Cheongsun) exhibited felt disease symptoms as a result of infections by a previously unknown fungus. The disease incidence was approximately 10%.

The initial symptoms were white to grey mycelial mats on some areas of the branches. Each mat progressively expanded until the mats coalesced to occupy larger areas and finally girdled the branches. The damage was apparently greater on older branches (over four years) that exceeded 50% disease incidence in some orchards (Fig. 1A). The symptoms were characterized by whitish gray to dark gray felts on the branches (Fig. 1B~1D).

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Fig. 1. Felt disease caused by *Septobasidium* sp. on *Schisandra chinensis* (cv. Cheongsun). A, An orchard was damaged by felt disease and was ready to be burnt for the next cropping; B~D, Basidiomata formed on branches; E, Scale insect on branches; F, G, Nymph and female of *Pseudaulacaspis cockerelli* (scale insect) on branch.

Careful observation of felts that formed on the branches showed that the hyphal mats covered and embedded scale insects (Fig. 1E). The scale insects on *S. chinensis* were in the nymphal developmental stage. They were brown, cylindrical to obclavate, and 2~3 mm in size. Females were small, bright yellow, oval-shaped, 2~3 mm in size, and hidden underneath the pear-shaped, white armor. Males were protected by their armor until they grow into tiny, winged adults. The male armor was elongate, snow-white, feebly tricarinate, and about 1 mm in size (Fig. 1F and 1G). These morphological characteristics were consistent with those of *Pseudaulacaspis cockerelli* (Cooley) (Insecta: Hemiptera: Coccoidea: Diaspididae), which is known to associate with *S. chinensis* in Korea [6].

Morphological characteristics of *Septobasidium* sp.

Stems and branches of *S. chinensis* with typical felt symptoms were collected from several orchards. Small pieces of felt tissue containing fungal structures were removed from the plant tissue, mounted in a drop of water, and examined with differential-interference contrast light microscopy (Zeiss AX10 microscope; Zeiss, Jena, Germany) to observe morphological characteristics. Measurements of the fungal

structures were performed at 400× and 1,000× magnifications.

Samples for scanning electron microscopy were directly obtained by peeling the fungal structures from felts on *S. chinensis* stems. These samples were dehydrated in a graded series of ethyl alcohol at room temperature and coated with a thin layer of gold in an argon atmosphere. Prepared samples were observed with a scanning electron microscope (JSM-5410LV; JEOL, Tokyo, Japan). Samples were also rapidly frozen using liquid nitrogen and stored at -80°C until further processing. The frozen samples were cut into 200 µm sections on glass slides with a freezing microtome (LEICA CM 3000; Leica Microsystems, Wetzlar, Germany) at -20°C for microscopy.

Basidiomata on the branches of living trees were 2~15 cm long, 1~5 cm wide, resupinate, patchy, velvety or spongy, perennial, initially grayish-white, and later becoming grayish-brown to dark brown. The surface of the basidioma was usually smooth initially, but was broken by a few cracks at maturity (Fig. 1C and 1D). Hyphal mats were 500~800 µm thick, and were composed of three layers. Thin subicula were brown and 30~100 µm thick. Pillars were brown, 100~200 µm high, 50~90 µm wide, and loosely filled with hyphae. Hyphae of pillars branching out to form a layer

Fig. 2. Morphological characteristics of *Septobasidium* sp. on *Schisandra chinensis* (cv. Cheongsun). A, B, Sections of basidiomata; C, Hypha; D~F, Hymenium and probasidia; G, Basidia.

were 2~6 μm thick (Fig. 2A and 2B). Hyphae of basidiomata were rather loosely arranged to form irregularly anastomosing networks. The old hyphae of the inner region were brown, copper-colored, and 2~7 μm in size. However, the young hyphae of the outer region were hyaline. Hymenial layers were composed of densely packed, 30~90 μm wide, much entangled brownish hyphae that gave rise to hyaline, branched, and irregularly twisted hyphae (Fig. 2C~2E). Probasidia that developed on the surfaces of basidiomata were ovoid or subglobose and 6.4~12.5 μm long (Fig. 2F). Basidia were smooth, cylindrical to slightly curved, 4-celled, and 23~30 $\times$ 2~5 μm in size (Fig. 2G). Basidiospores were not seen. Based on the morphological characteristics, the

fungus on *S. chinensis* was placed in the genus *Septobasidium* [7-9]. A representative voucher specimen was deposited in the Korea University Herbarium (accession No. KUS-F28660).

Phylogenetic analysis of *Septobasidium* sp. To confirm our tentative identification based on morphological characteristics, small pieces of hyphal mats were removed from the stems of four samples from the farms surveyed, rinsed 2~3 times with sterilized distilled water, and dried for further processing. DNA was extracted using a DNeasy Plant Mini Kit (Qiagen Inc., Valencia, CA, USA) following the manufacturer's instructions. The complete internal

transcribed spacer (ITS) region of ribosomal DNA (rDNA) was amplified using ITS1/ITS4 primers as described by White *et al.* [10]. The amplified PCR products were separated on a 1.5% agarose gel, followed by purification with a PCR purification kit (Core-one; Core-Bio, Seoul, Korea). Both amplicon strands were sequenced using the same primers used for the initial amplification. The reactions were monitored using BigDye Terminator Cycle Sequencing Kits (Applied Biosystems, Foster City, CA, USA) as indicated by the manufacturer and analyzed on an ABI 3130 automated DNA sequencer (Applied Biosystems). The possible identity of the isolates was established by comparing their ITS sequences with those in the GenBank database (National Center for Biotechnology Information [NCBI] US National Institute of Health, Bethesda, MD, USA; <http://www.ncbi.nlm.nih.gov/BLAST>).

Phylogenetic analysis of the ITS sequence data was conducted by means of a neighbor-joining (NJ) method using MEGA, ver. 6.0 [11] and the sequence distance was calculated under the Tamura-Nei parameter model. *Sporobolomyces gracilis* (AF444578) was used as an outgroup for the phylogenetic analysis. Bootstrap analysis was performed with 1,000 replications to determine the support for each clade.

The ITS sequences from four representative isolates were deposited in GenBank with accession numbers of HQ267951 (578 bp), HQ267957 (577 bp), HQ267958 (578 bp), and HQ267959 (578 bp). BLAST analysis of the ITS sequences in GenBank demonstrated a 93% nucleotide identity with a sequence of *Septobasidium broussonetiae* (HM209416) and 92% nucleotide identity with *S. bogoriense* (HM209414). The generated NJ tree showed that four isolates from *S. chinensis* formed a well-supported clade that was sister to a clade consisting of *S. bogoriense* and *S. broussonetiae* (Fig. 3).

Identification and discussion. The genus *Septobasidium* was established by Patouillard [7], and more than 200

species of the genus have been described [8, 9]. *Septobasidium* is known to have a wide host range [8, 9]. The *Septobasidium* isolates from *S. chinensis* in Korea were morphologically similar to *S. bogoriense* in size and shape of basidiomata, as well as in the shape of the probasidia and basidia [12].

Nevertheless, the species could not be identified. Recently, morphological characteristics and host information of more than 20 species of *Septobasidium* have been introduced from China [13–27], Costa Rica [28, 29], and the southeastern United States [29]. Unfortunately, most *Septobasidium* spp. have been described without appropriate ITS sequence data. Therefore, we tentatively describe this isolate as a *Septobasidium* sp. until morphologically similar species of *Septobasidium* are described with ITS sequence data.

Felt disease on *S. chinensis* has never been reported to occur in Korea, but two species of *Septobasidium*, *S. bogoriense* Pat. and *S. tanakae* (Miyabe) Boedijn & B.A. Steinm, have been reported in the forms of ‘felt’ and ‘brown felt’ on Japanese plum, kiwifruit, mulberry, spindle tree, chestnut, Japanese flowering cherry, red-leaved hornbeam, Korean paulownia, konara oak, and *Juglans sinensis* [4]. The genus *Septobasidium* colonizes on branches, twigs, trunks, or leaves of numerous woody plants including *Acer*, *Camellia*, *Carya*, *Citrus*, *Cornus*, *Liquidambar*, *Magnolia*, and *Quercus* [8]. Importantly, they live in symbiotic relationship with scale insects; the fungus obtains its nutrition from the scale insect, while during winter season the fungus serves as blanket for scale insect to protect it from low temperature [8]. Damage to the plant was often considered attributed to certain species of *Septobasidium*, but it is most likely due to the activity of feeding insects, rather than direct parasitism of the fungus [30]. For example, Reinert and Lauderdale [31] reported that *Pseudaaulacaspis cockerelli*, which is also associated with the present Korean isolates, feeds on both leaves and stems of diverse plants.

In Korea, *Schisandra chinensis* plantations have considerably increased in recent years associated with healthy fruits. This could be a reason of the relatively rapid expansion of

Fig. 3. A neighbor-joining tree based on the internal transcribed spacer rDNA sequences of *Septobasidium* sp. from *Schisandra chinensis*, including *Septobasidium* spp. retrieved from GenBank. Numbers above the branches represent bootstrap values. Isolates obtained in this study are shown in boldface.

this pathogen. Currently, the best control measures of felt disease on *S. chinensis* are to repress scale insects in setting up orchards or plantations and to remove the infected stem, but pruning wounds are vulnerable for penetration, as in the cases of other fruit crops. As a result, fungicide treatment is mainly required to reduce the crop losses by this pathogen. Hence, there is an urgent need to develop eco-friendly control methods and resistant cultivars against this pathogen.

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