Identification of *Calonectria colhounii* Associated with Basal Stem Rot on Blueberry Seedlings Imported from the United States of America

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Basal stem rot symptoms were found on blueberry seedlings imported from the United States of America in 2008. The fungus obtained from the diseased seedlings was identified as *Calonectria colhounii* based on morphological and molecular characteristics. The consignments of the blueberry seedlings infected with *C. colhounii* were destroyed to prevent introduction of the fungus to Korea.

KEYWORDS : Basal stem rot, Blueberry, *Calonectria colhounii*

Blueberries belong to the genus *Vaccinium* in the family *Ericaceae*. High bush blueberry (*V. corymbosum* L.), one of the most commercially cultivated blueberry species, has long been grown in North America. Cultivation has recently expanded to temperate and subtropical regions including Europe, Australia, Chile and New Zealand, as well as China, Japan and Korea. In Korea, the cultivated area has increased in response to consumer demand for healthy foods enriched in antioxidants and other beneficial compounds.

With the increasing importation of blueberry seedlings, the likelihood of the introduction of a non-native pathogen into Korea has also increased. During the import inspection of high bush blueberry seedlings imported from the United States at Incheon International Airport in 2008, basal stem rot was found on some blueberry seedlings. The symptoms first appeared as white mycelia with conidiophores and conidia on the affected parts, followed by the production of yellow, circular and rough perithecia on the white mycelia in about 10 days (Fig. 1A). Fungal isolates were obtained from the diseased seedlings and their mycological features were observed.

Colonies of the isolates that developed on potato dextrose agar during incubation at 25°C in the dark consisted of white to cream-colored aerial mycelia, with irregular margins and a dark-red color on the reverse side (Fig. 1B and 1C). Perithecia on the diseased seedlings were initially yellow and turn brown over time, mostly globose or subglobose in shape, solitary, superficial, with a warty wall, 240–410 µm in height and 280–490 µm in diame-

ter. Asci in perithecia were unitunicate, clavate, without a differentiated apex, and tapered to a long and thin stalk that was 88.4–121.7 × 10.0–14.1 µm in size. Ascospores aggregated in the upper third of the ascus were fusoid or slightly straight with round ends, straight or slightly curved, 3-septate, colorless, and 29.7–57.9 × 3.1–6.3 µm in size (Fig. 1D and 1E).

Conidiophores on the diseased plants consisted of a stipe bearing a penicillate or subverticillate arrangement of fertile branches; the stipe was septate, hyaline, straight, smooth and 160–270 × 4–5 µm in size. Sterile filament terminated in a narrowly clavate vesicle 2.7–6 µm in diameter and 20.3–67.2 µm in length at the apical septum. Conidiophore branches arose laterally from a stipe and were 50–88 × 23–85 µm in size. Primary branches were mostly aseptate or rarely one septate, and were 20.3–31.3 × 4–6 µm in size. Secondary and tertiary branches were aseptate, 18.8–23.5 × 4–5 µm and 12.5–17.2 × 4–5 µm in size, respectively. Conidia were cylindrical, hyaline, straight, rounded at both ends, 3-septate and 39.1–81.4 × 3.8–5.2 µm in size. Each terminal branch produced 1–4 phialides that were mostly cylindrical or reniform, hyaline, aseptate and measured 7.8–11.0 × 4–6 µm (Fig. 1F and 1G).

All the isolates were identified as *Calonectria colhounii* Peerally (anamorph *Cylindrocladium colhounii* Peerally) based on their morphological and cultural characteristics. The morphological characteristics of *C. colhounii* were similar to those described previously (Table 1) [1].

To confirm the results of morphological identification, comprehensive internal transcribed spacers (ITS1, 5.8S and ITS2) of ribosomal DNA and the partial β-tubulin

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Fig. 1. Morphological features of *Calonectria colhounii*. A, Sporulation on surface of diseased blueberry seedlings (scale bar = 200 μ m); B, C, Surface and reverse colonies; D, Perithecium (scale bar = 300 μ m); E, Four-spored asci and three septate ascospores (scale bar = 10 μ m); F, A conidiophore and three septate conidia (scale bar = 10 μ m); G, A vesicle (scale bar = 10 μ m).

Table 1. Morphological characteristics of *Calonectria colhounii* isolated from blueberry seedlings

Structure		<i>C. colhounii</i>	
		Present isolate	Peerally [1]
Anamorph			
Conidiophore	Arrangement	Penicillate or subverticillate	Penicillate
	Primary branch (μ m)	20.3~31.3 $\times$ 4~6	13.0~26.0
	Secondary branch (μ m)	18.8~23.5 $\times$ 4~5	7.8~18.2
	Tertiary branch (μ m)	12.5~17.2 $\times$ 4~6	7.8~10.4
Conidium	Shape	Cylindrical	Cylindrical
	Color	Hyaline	Hyaline
	Size (μ m)	39.1~81.4 $\times$ 3.8~5.2	38.3~84.2 $\times$ 3.4~5.7
	Number of septum	3	3
Vesicle	Shape	Narrowly clavate	Narrowly clavate
Teleomorph			
Perithecium	Shape	Globose or subglobose	Globose or subglobose
	Size (height, μ m)	210~410	247~463
	(diameter, μ m)	280~490	309~515
Ascus	Color	Yellow (fresh), brown (old)	Yellow (fresh), brown (old)
	Shape	Clavate	Clavate
	Number of ascospore	4	4
Ascospore	Size (μ m)	88.4~121.7 $\times$ 10.0~14.1	104~156 $\times$ 13.0~18.2
	Shape	Straight, curved	Straight, curved
	Size (μ m)	29.7~57.9 $\times$ 3.1~6.3	33.8~84.2 $\times$ 4.4~7.8
	Number of septum	3	3

gene (BT) were amplified using the primers ITS1/ITS4 [2] and T1/CYLUB1R [3, 4], and the resulting products were purified and sequenced. The two gene sequences were compared with sequences available in the GenBank database by the BLAST search. Phylogenetic trees were constructed by the neighbor-joining method with Kimura's two-parameter distance model [5] using MEGA version 4.0 [6].

An ITS-based phylogeny yielded very few variations; as

a result, the present isolate not only clustered together with *C. colhounii* and *C. eucalypti*, but was also differentiated from the other *Calonectria* species with low bootstrap support (Fig. 2). Therefore, DNA sequences obtained from the BT gene were employed to resolve these taxa. The BT sequences were more parsimony-informative than the ITS sequences, and resulted in much better resolution for delimitation of *C. colhounii* from the other *Cylindrocladium* species. A phylogenetic tree generated from the

Fig. 2. Neighbor-joining tree based on internal transcribed spacer region sequences showing relationship among *Calonectria* species. The numbers above each branch indicate bootstrap values of distance. The bootstrap values were obtained after a bootstrap test with 1,000 replications. The bar represents 0.01 substitutions per site.

Fig. 3. Neighbor-joining tree based on partial β -tubulin sequences showing relationship among *Calonectria* species. The numbers above each branch indicate bootstrap values of distance. The bootstrap values were obtained after a bootstrap test with 1,000 replications. The bar represents 0.02 substitutions per site.

BT sequences placed the present isolate in the same clade with *C. colhounii* isolates from *Eucalyptus grandis* Hill ex Maiden (DQ190557) and *Rhododendron* sp. (DQ190560) obtained in the U.S., and clearly distinguished the isolate from other *Calonectria* species with high bootstrap support (Fig. 3). However, the clade including the present isolate was more distantly related with another clade of *C. colhounii* isolates from *Arachis pintoi* Krap. et Greg. in Australia (DQ190561 and DQ190562) than *Calonectria eucalypti* Lombard, Wingf. and Crous, a new species determined using morphological and DNA sequence comparisons (Fig. 3) [7]. This result indicates that the *C. colhounii* species complex might include species hitherto not described.

C. colhounii has been reported on 14 host genera in 10 families in Africa, Asia, Australia, Central America and North America [8]. The fungus causes a variety of diseases including root rot of goldenseal (*Hydrastis canadensis*), leaf spot on blueberry [9], sentry palm (*Howea forsteriana*) [10], stiff bottlebrush (*Callistemon rigidus*), wintergreen (*Gaultheria procumbens*) [11, 12] and leaf blight on *Leucospermum* spp., Ohia (*Metrosideros collinus*), New Zealand Christmas tree (*M. excelsus*) and areca palm (*Chrysalidocarpus lutescens*) [10]. Although the majority of disease reports associated with *C. colhounii* have been limited to some medicinal herbs, shrubs and ornamental plants, the fungus could also be a threat to economically important agricultural crops such as the host plants blueberry, eucalyptus and fig [8]. In addition, *Calonectria ilicicola* Boedijn and Reitsma causing stem and root rot and *Calonectria kyotensis* Terash. causing root and crown rot on blueberry plants have been reported [8, 13]. In Korea, *C. ilicicola* has been reported as a pathogen causing black root rot of soybean, and *C. kyotensis* as a pathogen causing brown leaf spot of common jujube (*Zizyphus jujuba* var. *inermis* Rehd.) and Japanese Azalea (*Rhododendron japonicum* Terash) [14]. This suggests that during the growth season of blueberry, the plant might be damaged by *C. ilicicola* and *C. kyotensis* distributed in Korea.

The consignments of the blueberry seedlings infected with *C. colhounii* were destroyed to prevent the introduction of the fungus to Korea. This is the first report that *C. colhounii* is associated with basal stem rot on blueberry seedlings.

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