Genetic Diversity and Pathogenicity of Cylindrocarpon destructans Isolates Obtained from Korean Panax ginseng

Jeong Young Song1, Mun Won Seo1, Sun Ick Kim1, Myeong Hyeon Nam1, Hyoun Sub Lim1 and Hong Gi Kim1*

1Department of Applied Biology, Chungnam National University, Daejeon 305-764, Korea
2Keumsan Ginseng & Medicinal Crop Experiment Station, Chungcheongnam-do Agricultural Research & Extension Services, Geumsan 312-823, Korea
3Nonsan Strawberry Experiment Station, Chungcheongnam-do Agricultural Research & Extension Services, Nonsan 320-862, Korea

Abstract We analyzed the genetic diversity of Cylindrocarpon destructans isolates obtained from Korean ginseng (i.e., Panax ginseng) roots by performing virulence tests and nuclear ribosomal gene internal transcribed spacer (ITS) and mitochondrial small subunit (mt SSU) rDNA sequence analysis. The phylogenetic relationship analysis performed using ITS DNA sequences and isolates from other hosts helped confirm that all the Korean C. destructans isolates belonged to Nectria/Neonectria radicicola complex. The results of in vivo and ex vivo virulence tests showed that the C. destructans isolates could be divided into two groups according to their distinctive difference in virulence and the genetic diversity. The highly virulent Korean isolates in pathogenicity group II (PG II), together with foreign isolates from P. ginseng and P. quinquefolius, formed a single group. The weakly virulent isolates in pathogenicity group I, together with the foreign isolates from other host plants, formed another group and exhibited a greater genetic diversity than the isolates of PG II, as confirmed by the mt SSU rDNA sequence analysis. In addition, as the weakly virulent Korean isolates were genetically very similar to the foreign isolates from other hosts, they were likely to originate from hosts other than the ginseng plants.

Keywords Cylindrocarpon destructans, Genetic diversity, Ginseng root rot, Panax ginseng, Pathogenicity

Cylindrocarpon destructans (teleomorph: Nectria/Neonectria radicicola), a soil-borne pathogenic fungus, can cause a primary root rot disease in ginseng (Panax ginseng and P. quinquefolius), reduce the yield of ginseng production, and result in great economic losses [1, 2]. In addition, C. destructans has been reported to lead to replant failure, due to its ability to survive in the soil for more than ten years after the harvest of ginseng [3]. C. destructans was originally described as Ramuraria destructans according to the root rot symptoms of American ginseng (P. quinquefolius) by Zinssmeister [4]. In South Korea, it had been identified only in ginseng, strawberries and peonies [5] since it was firstly discovered in ginseng roots by Chung [6], although it was shown to affect the roots of diverse woody plants and to cause one of the most severe fungal diseases worldwide [7-11].

A large number of pathogenic fungi in the genera of Cylindrocarpon, Fusarium, and Cylindrocladium, which belong to Nectriaceae (Hypocreales), show taxonomically close association with C. destructans [12]. Mantiri et al. [13] proposed the presence of approximately 125 species in the genus of Cylindrocarpon, and Booth [14] divided this genus into four groups, namely C. magnusianum, C. cylindroides, Nectria mammnoida, and C. destructans, according to the presence of microconidia and chlamydospores. Furthermore, Samuels and Brayford [15] reviewed the existing classification system and categorized Nectria radicicola, including the asexual generation of C. destructans, into three variations, known as var. radicicola (anamorph: C. destructans var. destructans), var. coprosmae (anamorph: C. destructans var. coprosmae), and var. macroconidiales (anamorph: C. macroconidiales), based on their morphological and culture characteristics.

Mantiri et al. [13] modified Booth’s [14] classification and redefined the genus of Nectria into three clades (i.e.,
clade I: Nectria cocinea/galligena group; clade II: N. mammoidae/veuillotiana group; and clade III: N. radicicola group) through the sequence analysis of mitochondrial rDNA, and included C. destructans into clade III. Seifert et al. [16] conducted DNA sequence analysis of nuclear ribosomal internal transcribed spacer (ITS) region and partial β-tubline gene using C. destructans isolates from diverse hosts and closely related species. They reported that N. radicicola complex isolates in the clade III established by Mantiri et al. [13] could be further divided into subclades a and b, and that all the isolates from Korean and Japanese ginseng (P. ginseng) and Canadian ginseng (P. quinquefolius) are genetically close to each other and belong to subclade b. Recently, several attempts have been made to establish a new type of classification system for the Nectria/Neonectria complex and to analyze the genetic diversity using various morphological, pathogenic, and genetic analytical approaches [9, 17-19]. Although the genetic variation of Korean C. destructans population that causes root rot in P. ginseng has been evaluated by random amplified polymorphic DNA analysis [20, 21], the pathogenic and taxonomical characteristics of P. ginseng in Korea have been rarely studied. As the genetically distinct populations may show difference in host range, aggressiveness, and susceptibility to disease control treatment, a better understanding of the genetic and pathogenic variations in C. destructans will be important for developing suitable root rot disease management strategies [16].

In this study, ITS region and mitochondrial small subunit (mt SSU) rDNA which were frequently used for analyzing the genetic diversity of phytopathogenic fungi [22, 23] were sequenced from Korean ginseng isolates, and compared with those from the foreign isolates to locate the taxonomic position of the C. destructans group. The genetic diversity of C. destructans isolates was analyzed by evaluating their virulence both in vivo and ex vivo. The data obtained from this study will be useful for constructing effective treatment strategies against ginseng root rot diseases.

### MATERIALS AND METHODS

**Isolation of pathogenic fungus.** In this study, C. destructans was isolated from diseased ginseng roots that demonstrated typical symptoms of root rot; the ginseng roots were obtained from the main ginseng-cultivating regions of South Korea (Table 1). The diseased tissues on the collected ginseng roots showing brown discoloration were washed in running water. The tissues of both healthy and rotten parts were sliced into 5-mm pieces, treated with 2% NaOCl for 1 min for surface disinfection, washed two or three times in sterilized water, and placed on filter paper for dehydration. The affected tissues, whose surface had been previously disinfected, were placed on water agar or on pentachloronitrobenzene agar medium [24] and cultured at 15°C for seven days. Subsequently, the spores were isolated from the hypha grown on the tissues cultured in potato dextrose agar (PDA) medium at 15°C for ten days. Individual spores were then isolated, inoculated, and cultured in PDA medium at 20°C for 2 wk and re-inoculated into PDA medium for virulence tests. Furthermore, their morphological characteristics on PDA media were observed for identification of the isolates.

**Isolation of genomic DNA from C. destructans.** After the pure culture, the isolates were inoculated into a potato dextrose broth medium and subject to stationary culture at 20°C for ten days for isolating the genomic DNA. The mycelia were then harvested, split into 1.5-mL Effendorf

### Table 1. A list of Korean ginseng (Panax ginseng) isolates of Cylindrocarpon destructans used in this study

| Isolate | Geographic origin | Age of ginseng (year) | Accession No. | Pathogenicity group |
|---------|-------------------|-----------------------|---------------|--------------------|
| CY001   | Wanjoo, Jeonbuk   | 4                     | KF894988      | KF895011           | PG I               |
| CY003   | Yejiu, Gyeonggi   | 6                     | KF894989      | KF895012           | PG I               |
| CY008   | Icheon, Gyeonggi  | 4                     | KF894991      | KF895014           | PG I               |
| CY010   | Goesan, Chungbuk  | 4                     | KF894992      | KF895015           | PG II              |
| CY030   | Yeongju, Gyeongg  | 4                     | KF894993      | KF895016           | PG I               |
| CY033   | Icheon, Gyeonggi  | 6                     | KF894994      | KF895017           | PG II              |
| CY036   | Goesan, Chungbuk  | 4                     | KF894995      | KF895018           | PG II              |
| CY037   | Okcheon, Chungbuk | 4                     | KF894996      | KF895019           | PG II              |
| CY050   | Boryeong, Chungham| 3                     | KF894997      | KF895020           | PG II              |
| CY055   | Boryeong, Chungham| 3                     | KF894999      | KF895022           | PG II              |
| CY060   | Goesan, Chungbuk  | 6                     | KF895000      | KF895023           | PG II              |
| CY063   | Hongcheon, Gangwon| 4                     | KF895001      | KF895024           | PG I               |
| CY066   | Goesan, Chungbuk  | 4                     | KF895002      | KF895025           | PG II              |
| CY075   | Yangju, Gyeonggi  | 6                     | KF895003      | KF895026           | PG II              |
| CY076   | Goesan, Chungbuk  | 4                     | KF895004      | KF895027           | PG II              |

**ITS region**, internal transcribed spacer region; **mt SSU rDNA**, mitochondrial small subunit ribosomal DNA.

*Pathogenicity group: PG I (lesion size < 8.1 mm, diseased rate < 81%) and PG II (lesion size > 8.0 mm, diseased rate > 80%).
for each treatment. In the obtained to provide a mean value for the six replicate roots size of the lesions. An average value of the lesion size was stored it in an incubator for 4 wk, before measuring the (5 mm in diameter) containing the cultured mycelia, and placed a piece of paper towel wetted with sterilized water onto the ginseng root pierced using plastic pipette tips in a sealed container disinfected with 70% ethanol, fixed it

P D A  m e d i u m  f o r  1 4  d a y s  w e r e  u s e d  a s  a n  i n o c u l u m .  W e

disinfect with 2% NaOCl. And the isolates cultured in

Fig. 1. Optical images showing symptoms of root rot due to Cylindrocarpon destructans on wounded 2-year-old ginseng plants ex vivo (A), and field-grown ginseng plants affected by weakly (PG I) and highly (PG II) aggressive C. destructans isolates in vivo (B).

Virulence test. Ex vivo and in vivo tests were performed to determine the virulence of C. destructans isolates obtained from the ginseng roots (Table 1, Fig. 1). In the ex vivo test, the surface of a 2-year-old ginseng plant was disinfected with 2% NaOCl. And the isolates cultured in PDA medium for 14 days were used as an inoculum. We placed a piece of paper towel wetted with sterilized water in a sealed container disinfected with 70% ethanol, fixed it onto the ginseng root pierced using plastic pipette tips (5 mm in diameter) containing the cultured mycelia, and stored it in an incubator for 4 wk, before measuring the size of the lesions. An average value of the lesion size was obtained to provide a mean value for the six replicate roots for each treatment. In the in vivo test, ginseng seedlings were dipped in the mycelia and spores suspension, which was prepared by suspending the ground isolates cultured at 20°C for 14 days after inoculation into a PDA medium in 40 mL of sterilized water, and transplanted into the pre-planting field for ginseng cultivation. Seven months later, an average value of diseased rate (%) was assessed to provide a mean value for the ten roots for each treatment. The ginseng isolates were divided into two groups according to the results of the virulence tests. The isolates showing an average lesion size less than 8.1 mm and diseased rate lower than 81% were categorized as pathogenicity group I (PG I); and those with an average lesion size more than 8.0 mm and diseased rate higher than 80% were classified as pathogenicity group II (PG II). The ginseng isolates were divided into two groups according to the results of the pathogenicity group. The isolates with an average lesion size more than 8.0 mm and diseased rate higher than 81% were categorized as pathogenicity group I (PG I); and those with an average lesion size less than 8.1 mm and diseased rate lower than 80% were classified as pathogenicity group II (PG II).

Genetic relatedness analysis. PCR amplification of approximately 600-bp region of ITS and mt SSU rDNA region was performed using ITS1 and ITS4 primers [26] and NMS1 and NMS2 primers [27], respectively. For each amplification, a 0.5 pmol primer, 2 ng of genomic DNA, 0.2 mM deoxynucleotide triphosphate (dNTP), 10 mM Tris-HCl, 50 mM KCl, 1.5 mM MgCl2, and 2.5 units of Taq DNA polymerase were added to sterilized water to obtain a final volume of 50 μL. The PCR conditions used in analyzing the ITS region were as follows: an initial denaturation step at 95°C for 3 min; followed by 35 cycles of 95°C for 35 sec, 55°C for 1 min, and 72°C for 1 min; and a final elongation step at 72°C for 8 min. The PCR conditions for mt SSU rDNA region analysis were as follows: an initial denaturation step at 95°C for 3 min; followed by 34 cycles of 95°C for 1 min, 56°C for 1 min, and 72°C for 1 min; and a final elongation step at 72°C for 4 min. Each of the PCR products were analyzed by electrophoresis on a 1.5% agarose gel and stained with ethidium bromide for visualizing its amplification and size.

A Solgent PCR Purification Kit (Solgent Co., Ltd., Seoul, Korea) was used to purify the amplified PCR products, according to the manufacturer’s protocol and sequenced by Genotech Co., Ltd. (Daejeon, Korea). Sequences generated from the present study were deposited in Genbank (Table 1). The PHYLIP 3.57c Package [29] was used to produce a neighbor-joining tree based on Kimura’s two-parameter model [30], and an analysis of 1,000 bootstrap samples was carried out to assess its reliability.

**RESULTS AND DISCUSSION**

Virulence test. Virulence tests were performed on all the Korean ginseng isolates of C. destructans that formed white or brown colonies with microconidia, macroconidia, and chlamydospores on PDA media (data not shown). C. destructans isolates obtained from the diseased ginseng tissues can be divided into two groups based on their virulence (Table 1, Fig. 1) determined by the in vivo and ex vivo tests. In the in vivo test, an entire rotten root and weakened ginseng tissues were observed in the ginseng plants with withered leaves in the aerial part or in case of leaves failing to grow; and in such a case, normal ginseng growth was not observed, and the isolates were found to be highly virulent (PG II) after inoculation. Whereas, in the case where the root rot was found only in some parts of the plants, normal ginseng growth was observed, and weakly virulent isolates (PG I) were identified post-inoculation. Kim [31] observed that the symptoms of root rot in ginseng growth varied with the soil types, plant ages, and cultivating seasons, and he assumed that they also varied with the differences in the virulence among the C. destructans isolates rather than with the environmental factors. Matuo and Miyazawa [7] reported that the C. destructans isolates obtained from tea plants and cedar trees were weakly virulent against ginseng seedlings, although the isolates from ginseng plants were highly virulent. In this study, remarkable difference in virulence was identified
among the \textit{C. destructans} isolates grown on \textit{P. ginseng}, and the isolates could be clearly divided into two groups—PG I and PG II—on the basis of their virulence.

**Analysis of genetic diversity.** DNA sequence analysis of ITS region is often used to examine the genetic diversity of phytopathogenic fungi [22]. Seifert \textit{et al.} [16] reported the presence of two groups, subclades a and b, in clade III, including the \textit{Nectria/Neonectria radicicola} complex. In this study, the DNA sequences of ITS region from 15 \textit{C. destructans} isolates of rotten ginseng roots collected from several different regions in Korea were used to analyze the genetic relatedness of \textit{C. destructans} isolates with \textit{Nectria/Neonectria radicicola} complex, as suggested by Seifert \textit{et al.} [16] (Table 2, Fig. 2). All the Korean \textit{P. ginseng} isolates of \textit{C. destructans}, together with the isolates in clade III, formed a single group, with 98% bootstrap support. Among them, the highly virulent \textit{C. destructans} (PG II) isolates from diverse ginseng-cultivation regions in Korea showed 100% similarity, and together with Japanese (IFO31881, IFO31882) and Canadian (NSAC SH 1, NSAC SH 2.5) ginseng isolates in clade III, formed the subclade a. Moreover, they showed 100% DNA sequence homology to the Japanese \textit{P. ginseng} isolates, but not to the Canadian \textit{P. quinquefolius} isolates. Though Seifert \textit{et al.} [16] reported that all Korean, Japanese, and Canadian ginseng isolates belonged to subclade a in clade III, we observed 97.0–100% similarity among the weakly virulent \textit{P. ginseng} isolates in PG I, which formed subclade b, along with the foreign isolates from diverse hosts. This finding indicates a great genetic diversity among the weakly virulent isolates, which is contrary to the results observed for the highly virulent isolates that form a single group with a high genetic homology.

Mantiri \textit{et al.} [13] elucidated phylogenetic relationships among \textit{Neonectria} species with \textit{Cylindrocarpon} anamorphs using mt SSU rDNA region, and identified four well-supported clades or groups of the \textit{Neonectria} complex. Based on this result, we analyzed the genetic relatedness between the DNA sequences of the PCR products obtained from Korean ginseng isolates of \textit{C. destructans} and the DNA sequences of foreign \textit{Neonectria/Cylindrocarpon} isolates and closely related species, which belonged to the \textit{Neonectria} complex, as identified by GeneBank (Table 2, Fig. 3). In the analysis of the ITS region, both weakly (PG I) and highly (PG II) virulent isolates formed a monophyletic clade or group, and yet they formed different clusters in group III of \textit{Neonectria}. Apart from the isolates in PG II, which showed 100% DNA sequence homology to each

| Species                     | Strain No. | Host                  | Accession No. |
|-----------------------------|------------|-----------------------|---------------|
| \textit{C. cylindroides}     | CR6        | \textit{Pseudotsuga menziesii} | AY295301     |
|                             | c2cun2ah2  | \textit{Pseudotsuga menziesii} | AF315201     |
| \textit{C. obtusisporum}    | 94-1356    | \textit{Picea mariana} | AY295304     |
|                             | JAT 1366   | \textit{Prunus armenica} | AY295306     |
| \textit{C. radicicola}      | AR2553     | Bark                  | AF220968     |
|                             | RTDF14     | \textit{Pseudotsuga menziesii} | AF315203     |
|                             | FM242.1    | \textit{Abies rubra}  | AF315204     |
| \textit{Cylindrocarpon sp.} | JAT 1591   | \textit{Prunus armeniaca} | AY295308     |
|                             | JAT 1590   | \textit{Prunus armeniaca} | AY295308     |
| \textit{Nectria cinnabarina} | CBS 279.48 | \textit{Acer pseudoplatanus} | AY163025    |
| \textit{N. radicicola}      | IMI 376404 | \textit{Malus} sp.   | AJ007357     |
|                             | IMI 061356 | \textit{Narcissus sp} | AJ007354     |
|                             | CTR71-322  | Wood                  | AF220969     |
|                             | IMI 375719 | \textit{Malus} sp.   | AJ007356     |
|                             | IMI 375717 | -                     | AJ007355     |
|                             | IMI 376403 | \textit{Abies} sp.  | AJ007351     |
|                             | IMI 376408 | \textit{Arbutus menziesii} | AJ007352    |
| \textit{Neonectria galligena} | JR0609B-2 | \textit{Malus pumila} | AF315206     |
| \textit{Neon. liriodendri}  | USSO150    | \textit{Vitis} sp.   | AY997585     |
|                             | UST148     | \textit{Vitis} sp.   | AY997584     |
| \textit{Neon. radicicola}   | JAT1378    | \textit{Corylus} sp. | AY295328     |
|                             | IFO31881   | \textit{Panax ginseng}, Japan | AY295323 |
|                             | IFO31882   | \textit{Panax ginseng}, Japan | AY295324 |
|                             | NSAC SH 2.5 | \textit{Panax quinquefolius}, Canada | AY295314 |
|                             | CBS 129083; NSAC-SH-1 | \textit{Panax quinquefolius}, Canada | AY295311 |
|                             | 94-1628    | \textit{Picea} sp.  | AY295315     |
|                             | JAT 1551   | \textit{Prunus} sp.  | AF315202     |
|                             | CR20       | \textit{Pseudotsuga} | AY295317     |
|                             | CR26       | \textit{Pseudotsuga} | AY295318     |
| \textit{Neon. ramulariae}   | CBS 730.87 | -                     | AJ279446     |
other and formed a specific group, the isolates in PG I showed 99.5~100% homology, and together with the foreign isolates from various hosts, formed an individual cluster.

Cabral et al. [17] recently renamed the *Nectria/Neonectria radicicola* complex as *Ilyonectria radicicola* complex after analyzing its morphological characteristics and multi-gene relatedness, and reclassified the fungi in this complex into 15 species under the genus of *Ilyonectria* based on their morphological characteristics and genetic diversity. They also reported the presence of genetically diverse fungi within the group of *P. quinquefolius* isolates, which were originally divided into four species: *I. mors-panacis*, *I. robusta*, *I. panacis*, and *I. crassa*. In particular, both Japanese *P. ginseng* isolates and those highly virulent to *P. quinquefolius* were included in *I. mors-panacis*. The Japanese isolates (IFO31881) in this study showed 100% hereditary homology to the highly virulent Korean isolates in PG II group. Thus, they were expected to belong to *I. mors-panacis*, as per the classification scheme of Cabral et al. [17].

In this study, we found that the *P. ginseng* isolates collected from several regions in Korea could be divided into two distinct groups on the basis of their virulence, although they all belonged to the same complex of *Nectria/Neonectria radicicola*. All the highly virulent isolates were...
Genetic Diversity of C. destructans from Korean Ginseng

ACKNOWLEDGEMENTS

This study was supported by the research grant funded by Chungnam National University, Korea.

REFERENCES

1. Chung HS. Studies on Cylindrocarpon destructans (Zins.) Scholten causing root rot of ginseng. Rep Tottori Mycol Inst Jpn 1975;12:127-38.
2. Rahman M, Punja ZK. Factors influencing development of root rot on ginseng caused by Cylindrocarpon destructans. Phytopathology 2005;95:1381-90.
3. Kang SW, Yeon BY, Hyeon GS, Bae YS, Lee SW, Seong NS. Changes of soil chemical properties and root injury ratio by progress years of post-harvest in continuous cropping soils of ginseng. Korean J Med Crop Sci 2007;15:157-61.
4. Zinssmeister CL. Ramularia root rots of ginseng. Phytopathology 1918;8:557-71.
5. Korean Society of Plant Pathology. List of plant diseases in Korea. 5th ed. Suwon: Korean Society of Plant Pathology; 2009. p. 91, 284, 288.
6. Chung HS. Ginseng disease. Research reports of the Korean Society of Plant Protection. Seoul: Korean Society of Plant Protection; 1979. p. 107-44.
7. Matuo T, Miyazawa Y. Scientific name of Cylindrocarpon sp. causing root rot of ginseng. Ann Phytopathol Soc Jpn 1984;50:649-52.
8. Domsch KH, Gans W, Andreason TH. Compendium of soil fungi. Vol. 1. Nectria radicicola Gerlach & L. Nilsson. New York: Academic Press; 1980. p. 503-8.
9. Halleen F, Fourie PH, Crous PW. A review of black foot disease of grapevine. Phytopathol Mediterr 2006;45:555-67.
10. Tevelopedmihin YT, Mazzola M, Mostert L, McLeod A. Cylindrocarpon species associated with apple tree roots in South Africa and their quantification using real-time PCR. Eur J Plant Pathol 2011;129:637-51.
11. Abreo E, Martinez S, Bettucci L, Lupo S. Morphological and molecular characterisation of Campylocarpon and Cylindrocarpon spp. associated with black foot disease of grapevines in Uruguay. Aust Plant Pathol 2010;39:446-52.
12. Rossman AY, Samuels GJ, Rogerson CT, Lowen R. Genera of black foot disease of grapevines (Nectriales, Ascomycetes). Stud Mycol 1999;42:1-248.
13. Mantiri FR, Samuels GJ, Rahe JE, Honda BM. Phylogenetic relationships in Neonectria species having Cylindrocarpon anamorphs inferred from mitochondrial ribosomal DNA sequences. Can J Bot 2001;79:334-40.
14. Booth C. The genus Cylindrocarpon. CAB Int Mycol Inst Mycol Pap 1966;104:1-56.
15. Samuels GJ, Brayford D. Variation in Nectria radicicola and its anamorph, Cylindrocarpon destructans. Mycol Res 1990;94:433-42.
16. Seifert KA, McMullen CR, Yee D, Reelder RD, Dobinson KF. Molecular differentiation and detection of ginseng-adapted isolates of the root rot fungus Cylindrocarpon destructans. Phytopathology 2003;93:1533-42.
17. Cabrera A, Groenewald JZ, Rego C, Oliveira H, Crous PW. Cylindrocarpon root rot: multi-gene analysis reveals novel species within the Ilyonectria radicicola species complex. Mycol Prog 2012;11:655-88.
18. Chaverri P, Salgado C, Hirooka Y, Rossman AY, Samuels GJ. Delimitation of Neonectria and Cylindrocarpon (Nectriaceae, Ascomycetidae, Ascomycetes) and related genera with Cylindrocarpon-like anamorphs. Stud Mycol 2011;68:57-78.
19. Luo J, Zhuang WY. Three new species of Neonectria (Nectriaceae, Ascomycetes) with notes on their phylogenetic positions. Mycologia 2010;102:142-52.
20. Jun NJ. Study on new host range and genetic diversity for ginseng root rot pathogen, Cylindrocarpon destructans in Korea [dissertation] Daejeon: Chungnam National University; 2008.
21. Seo MW, Kim SL, Song JY, Kim HG. Genetic diversity of Korean Cylindrocarpon destructans based on virulence assay and RAPD analysis. Kor J Mycol 2011;39:16-21.
22. O’Donnell K. Ribosomal DNA internal transcribed spacers are highly divergent in the phytopathogenic ascomycete.
Fusarium sambucinum (Gibberella pulicaris). Curr Genet 1992;22:213-20.
23. O’Donnell K, Nirenberg HI, Aoki T, Cigelnik E. A multigene phylogeny of the Gibberella fujikuroi species complex: detection of additional phylogenetically distinct species. Mycoscience 2000;41:61-78.
24. Punja ZK. Fungal pathogens of American ginseng (Panax quinquefolium) in British Columbia. Can J Plant Pathol 1997;19:301-6.
25. Doyle JJ, Doyle JL. Isolation of plant DNA from fresh tissue. Focus 1990;12:13-5.
26. White TJ, Bruns T, Lee S, Taylor J. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis MA, Gelfand DH, Sninsky JJ, White TJ, editors. PCR protocols: a guide to methods and applications. New York: Academic Press; 1990. p. 315-22.
27. Li KN, Rouse DI, German TL. PCR primers that allow intergeneric differentiation of ascomycetes and their application in Verticillium spp. Appl Environ Microbiol 1994;60:4324-31.
28. Chun J. Computer-assisted classification and identification of actinomycetes [dissertation]. Newcastle upon Tyne: University of Newcastle upon Tyne; 1995.
29. Felsenstein J. Confidence limits on phylogenies: an approach using the bootstrap. Evolution 1985;39:783-91.
30. Kimura M. A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. J Mol Evol 1980;16:111-20.
31. Kim SI. Characteristics of occurrence and cultural, chemical control of ginseng root rot [dissertation]. Daejeon: Chungnam National University; 2006.