Chlamydospore Induction from Conidia of *Cylindrocarpon destructans* Isolated from Ginseng in Korea

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Abstract *Cylindrocarpon destructans* causes root rot disease in ginseng and can survive for a long time, producing chlamydospores. We optimized conditions to induce chlamydospore production from the conidia of *C. destructans*, isolated from Korean ginseng. This will provide the basis for testing the efficacy of control agents targeting these chlamydospores.

Keywords Chlamydospore, Conidium, *Cylindrocarpon destructans*, Ginseng root rot

The ascomycete fungus *Cylindrocarpon destructans* (teleomorph: *Neonectria radiculicola*) causes root rot disease in ginseng (a member of the genus *Panax* in the family Araliaceae), and is a major causal agent of replant failure during ginseng cultivation [1, 2]. This fungus produces chlamydospores, which are the thick-walled resting spores, in soil and infected plants. These spores contribute to long-term survival of this fungus under unfavorable conditions [3]. *C. destructans* strains isolated from American ginseng (*Panax quinquefolius*) cultivated in North America, are known to produce chlamydospores from both the conidia and the hyphae. Korean strains, isolated from Korean ginseng (*P. ginseng*), produce chlamydospores only at the hyphal tips or within the hyphae, but do not form chlamydospores from the conidia [3-5]. Chlamydospores germinate from the primary inoculum when favorable conditions for infection are present; however, the natural inoculum levels of this fungus are unknown [6, 7].

A chemical strategy has been adopted to manage ginseng

root rot in pre-planted fields. However, this method is expensive for soil-borne disease, and the effects of chemicals diminish over time as it takes at least 4 years before ginseng is harvested. Alternatively, a biological strategy has been widely performed; several *Bacillus* strains were shown to have a highly antagonistic effect against *C. destructans* [8]. However, in most tests aimed at confirming the efficacies of such biological agents, only the inhibition levels of mycelial growth have been measured, and not inhibition of chlamydospore germination. As chlamydospores play a role for primary inoculum, it is necessary to test control agents against the chlamydospores of this fungus. However, hyphae-associated chlamydospores produced by *C. destructans* strains isolated from Korean ginseng obstruct the isolation of chlamydospores. To overcome this obstacle, it is possible to induce chlamydospores from the conidia of this fungus. Thus, in this study, we have attempted to optimize the conditions for the conversion of conidia to chlamydospores, through modifying the medium composition and culture conditions.

To optimize these conditions, we added dried ginseng powder to potato dextrose broth (PDB) or Czapek-Dox broth (CDB) [4]. For preparation of ginseng powder, 4-yr-old ginseng was sliced and dried at 60°C for 48 hr. The dried ginseng was ground with a laboratory mill, and the powder was added to each medium (at 5% w/v) before autoclaving. A mycelial agar plug (5 mm diameter) was inoculated in each medium, and the medium was incubated at various temperatures. Chlamydospore production was observed under a light microscope and a JSM-6700F scanning electron microscope (SEM; JEOL, Tokyo, Japan) at the Joint Instrumentation Center of Dong-A University (Busan, Korea), as previously described [9, 10].

When each strain was incubated in PDB at 20°C in an

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Fig. 1. Chlamydospore formation from conidia in *Cylindrocarpon destructans*. The fungal strain was incubated at 20°C for 4 days in an orbital shaker. The culture was transferred to 4°C, 20°C, 25°C, or 30°C and incubated for 5 additional days without shaking (scale bar = 10 μ m). Black and white arrows indicate a conidium and chlamydospore, respectively, and the black arrowhead indicates a mycelium. PDB, potato dextrose broth; PDB + G, PDB supplemented with 5% ginseng powder; CDB, Czapek-Dox broth; CDB + G, CDB supplemented with 5% ginseng powder.

orbital shaker (200 rpm), chlamydospores were not produced even though lipid bodies accumulated after 9-day incubation. However, in PDB supplemented with ginseng powder (PDB + G), chlamydospores were produced at the hyphal tips and within the hyphae after the same incubation time. The production of chlamydospores in CDB medium was observed after 7-day incubation and the addition of ginseng powder (CDB + G) increased the chlamydospore production. However, the majority of chlamydospores were associated with the hyphae and only a few conidia were converted into chlamydospores.

To induce conversion of conidia to chlamydospores, the fungal strain was incubated for 4 days at 20°C in an orbital shaker (200 rpm). Cultures were then incubated at 4°C, 20°C, 25°C, or 30°C, and incubated for an additional 5 days without shaking. In PDB medium, chlamydospore-like structures were induced in hyphae at 20°C and 25°C, but were not observed at 4°C and 30°C. In PDB + G medium, some conidia were converted into chlamydospores at 4°C and 20°C. When the fungal strain was cultured at 30°C, most mycelia were broken (Fig. 1). In CDB medium, the conversion of conidia into chlamydospores was often observed at 4°C, 20°C, and 30°C, but chlamydospore formation was associated with the hyphae at 25°C. In CDB + G medium, conversion was highly increased, compared to that in CDB medium at all temperatures tested, and most chlamydospores showed clear double-layered cell walls (Fig. 1). SEM observation showed that the chlamydospore cells were oval or globose (Fig. 2), concordant with previously

Fig. 2. Scanning electron microscope imaging of chlamydospores converted from conidia. The fungal strain was incubated in CDB + G for 4 days at 20°C in an orbital shaker. The culture was moved to 4°C for an additional 5-day incubation without shaking. Black and white arrows indicate a conidium and chlamydospore, respectively. CDB + G, Czapek-Dox broth supplemented with 5% ginseng powder.

Fig. 3. Assay of chlamydospore formation from conidia of *Cylindrocarpon destructans* isolated from Korean ginseng. A~G, Chlamydospores originated from conidia; H~M, Strains that do not form chlamydospores from conidia. Each strain was incubated in CDB + G for 4 days at 20°C in an orbital shaker. The culture was moved to 4°C for an additional 5-day incubation without shaking. Black and white arrows indicate a conidium and chlamydospore, respectively, and the black arrowhead indicates a mycelium. CDB + G, Czapek-Dox broth supplemented with 5% ginseng powder.

Table 1. Conidia production in *Cylindrocarpon destructans* strains isolated from ginseng in Korea

Total No. of isolates	Level of conidial production (conidia/mL) ^a			
	10 ⁴ ~10 ⁵	10 ⁵ ~10 ⁶	10 ⁶ ~10 ⁷	10 ⁷ ~10 ⁸
97	15	26	55	1

CDB + G, Czapek-Dox broth supplemented with ginseng powder.

^aEach strain was incubated in 50-mL CDB + G medium for four days at 20°C in an orbital shaker (200 rpm) and the number of conidia was counted using a hemocytometer under a light microscope.

Table 2. Estimated conversion percentages of conidia to chlamydospores in *Cylindrocarpon destructans* isolates

Total No. of isolates	Conversion percentages ^a					
	0	< 5%	5~10%	10~20%	20~30%	> 90%
97	39	7	17	28	5	1

CDB + G, Czapek-Dox broth supplemented with ginseng powder.

^aEach strain was incubated in 50-mL CDB + G medium for four days at 20°C in an orbital shaker (200 rpm) and moved to 4°C for additionally 5-day incubation without shaking. The number of conidia and conidia-originated chlamydospores were counted using a hemocytometer under a light microscope.

reports [11].

To determine if naturally occurring strains of *C. destructans* could also produce chlamydospores from conidia, we isolated 97 *C. destructans* strains from infected ginseng roots. First, the conidia production level of all strains was tested. For this, each strain was incubated in 50 mL of CDB + G medium for 4 days at 20°C in an orbital shaker (200 rpm) and the number of conidia was counted using a hemocytometer, under a light microscope (Fig. 3). The experiment was repeated twice with 3 replicates for each trial. The production level varied depending on the strain, but all strains produced more than 10⁴ conidia per milliliter (Table 1). When the 4-day-old culture was moved to 4 and 25°C for additional 5-day incubation without shaking, 39 out of 97 strains did not produce chlamydospores from conidia; however, all strains produced hyphae-associated chlamydospores (Table 2). To check the conversion efficacy of conidia into chlamydospores, we counted the number of chlamydospores and conidia in these conditions. In all strains except 1, fewer than 30% of conidia were converted into chlamydospores; however, more than 90% of conidia were converted into chlamydospores in 1 strain (Table 2).

In this study, we optimized culture conditions for chlamydospore formation from conidia in *C. destructans*. Addition of ginseng powder to CDB medium resulted in a higher production of hyphae-associated chlamydospores. When strains were incubated in CDB + G medium for 4 days at 20°C in an orbital shaker (200 rpm), and the culture was moved to 4°C or 25°C for an additional 5-day incubation

without shaking, conidia were converted into chlamydospores even though the conversion ratio varied depending on the strain. This study showed that *C. destructans* strains can rapidly produce chlamydospores from both hyphae and conidia at higher or lower than optimal temperatures for vegetative growth, and this rapid chlamydospore production might promote survival of this fungus during summer and winter. This result provides a resource to test the efficacy of control agents against chlamydospores of *C. destructans*, and can be applied to the development of such strategies to neutralize this fungal disease.

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