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Characteristics and pathogenicity of *Cladobotryum mycophilum* isolated from cobweb disease of button mushroom (*Agaricus bisporus*) in Korea

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ABSTRACT : Cobweb disease symptoms were observed in a mushroom farm in Buye, Korea during a disease survey in 2008-2011. Five isolates of *Cladobotryum* sp. were obtained from the infected caps and stipes. These isolates of *Cladobotryum* sp. were identified as *C. mycophilum* based on their morphological, cultural characteristics and analysis of the ITS sequences. Early symptoms were noticed as round, fleshy, yellowish brown lesions on mushroom caps. Late symptoms progressed when the parasitic fungus formed white cobweb circular colonies on dead or damaged pinheads, spread on the surface of the casing, and covered entirely fruiting bodies. Optimal temperature and pH for mycelial growth on MEA is 23℃ and 6.0. Microscopically the spores of the fungus are large and most 2~3 celled produced on vertically branched conidiophores. Mushroom caps turned dark brown and shrunk due to soft rot. Testing of sensitivity to selected fungicides showed that isolate was highly resistance to Mancozeb and Thiophanate-methyl, moderately sensitivity to Iprodione, and highly sensitivity to Benomyl, Prochloraz-Mn and Carbendazim.

KEYWORDS : *Agaricus bisporus*, *Cladobotryum mycophilum*, Cobweb disease, Mushroom

Mushroom is cultivated as one of the major economical crops in many areas of the Korea. The total production has steadily increased approximately 186,400 M/T in 2007 to 216,276 M/T in 2010. The button mushroom, *Agaricus bisporus*, showed the 5th production to 22,635 M/T in 2010. Several *Cladobotryum* species have been recorded from cultivated mushrooms by various authors and include *C. dendroides*, *C. multiseptatum*, *C. mycophilum* and *C. varium*(Sinden, 1971; De Hoog, 1978; Sharma *et al.*, 1992). Cobweb disease of mushrooms is caused by a few species of *Cladobotryum*, most notably *C. dendroides* and *C. mycophilum*. Both of these occur on other mushroom species growing in the wild(Rogerson and Samueis, 1993, 1994). Until the late 1980's the disease was rarely seen on mushroom farms worldwide, and was easily controlled by a variety of fungicides used in the industry(Fletcher *et al.*, 1986). But some fungicide(Mertect and Topsin) resistance was reported by Lockley and Gay(1983). This disease was first reported to be caused by *C. mycophilum* by Back *et al.* (2010) in Korea. This study was conducted to investigate characateristics and pathogenicity of the casual fungus causing cobweb disease of button mushroom in Korea.

Disease incidence and symptoms. Diseased fruiting bodies of *A. bisporus* with symptoms resembling cobweb disease were observed in 10 mushroom farms. Early symptoms were noticed as round, fleshy, yellowish brown lesions on mushroom caps(Fig. 1. A). Late symptoms progressed when the parasitic fungus formed white cobweb circular colonies on dead or damaged pinheads, spread on the surface of the casing, and covered entirely fruiting bodies(Fig. 1. B). Mushroom caps turned

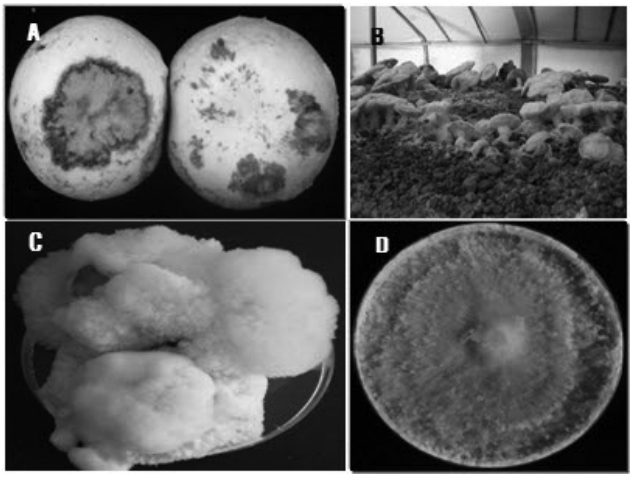

Fig. 1. Symptoms of coweb disease on button mushroom caused by *Cladobotryum mycophilum*. A, B : Typical symptoms on fruiting body in mushroom field. C : Symptoms induced by artificial inoculation in pot. D : Colony of *C. mycophilum* grown for 7days malt extract agar (MEA) .

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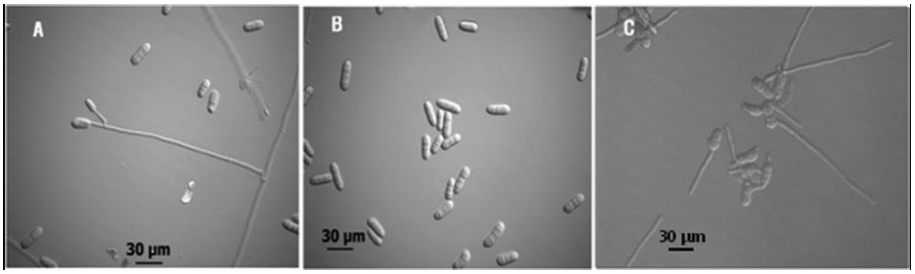

Fig. 2. Morphological characteristics of *Cladobotryum* mycophilum isolated from button mushroom. A ; Conidiophores and conidia in head, B : Conidia, C : Germination of conidia.

dark brown and shrunk due to soft rot. On occasion, the mycelium and infected mushrooms might have some reddish/orange color and on agar some isolates have a 'camphor-like' odor. Colonies growing on the casing are usually circular and rapidly overwhelm mushrooms, causing rapid decay. The pathogen grows very rapidly across the beds, especially under moist and humid conditions. These symptoms correspond with symptoms caused by *Cladobotryum* isolates.

Pathogenicity test. Pathogenicity assay on mushroom pilei showed that each of the twenty isolates had high virulence level for button mushrooms. The symptoms were not produced when sterile water was used as a negative control. All isolates induced severe disease symptoms on mushroom pilei. The growth of the pathogenic mycelia was recorded two days after inoculation. Three days after inoculation, the sporocarps were completely covered with white cottony mycelia with abundant sporulation, resembling the symptoms of natural infection(Fig. 1 C). The pilei were completely rotten, soft and decayed on the fourth day of incubation. There were no significant differences in levels of symptom development among the different isolates.

Mycological characteristics. The isolates formed white, cottony, aerial mycelium on MEA at 25℃. Optimal spore germination temperature is 25℃. Under these conditions, the pathogen can germinate and grow in 7 days. The fungus abundantly sporulates in contact with mushroom tissue and the dry spores are easily dislodged when watering or salting an infected area. The fungus sporulated and mycelium turned yellow three days after innoculation. After a few more days, conidia and colonies turned reddish. Microscopically the spores of the fungus are large and most 2~3 celled produced on vertically branched conidiophores(Fig. 2). The colonies grow as fast as 15~20 mm per day on agar plates(Fig. 1. D). Optimal temperature and pH for mycelial growth on MEA is 23℃ and 6.0(Fig. 3, 4).

Internal transcribed spacer (ITS) sequence analysis. DNA was isolated from pure cultures of fungus using the Qiagen Genomic DNA Isolation Kit (Qiagen, USA). The rDNA ITS region of the *Cladobotryum* isolate was amplified with conserved fungal primers

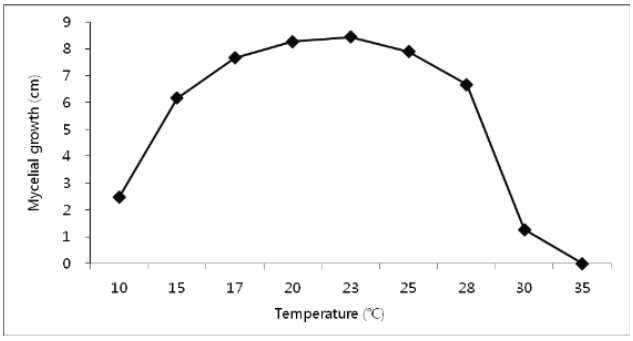

Fig. 3. Growth properties of the *Cladobotryum mycophilum* isolated from button mushroom. The isolated was grown at different temperature on malt extract agar (MEA) for 3 days.

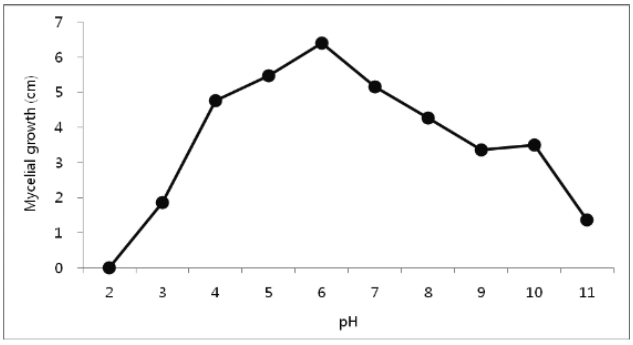

Fig. 4. pH properties of the *Cladobotryum mycophilum* isolated from button mushroom. The isolated was grown at different pH on malt extract agar (MEA) for 3 days.

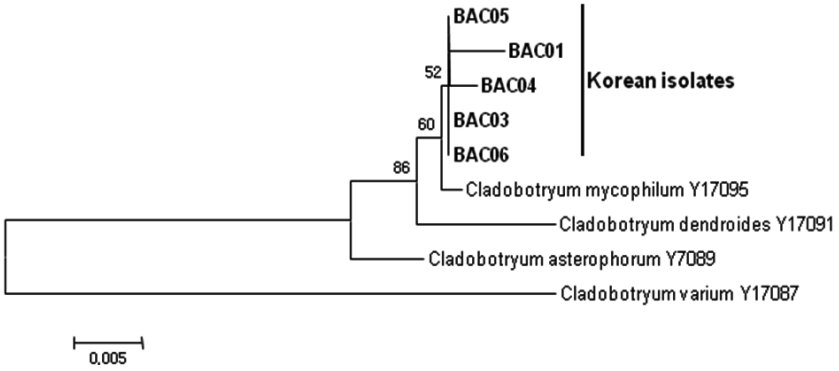

Fig. 5. Phylogenetic relationships of of *Cladobotryum mycophilum* based on internal transcribed spacer(ITS) sequences. Numerical values on branches are the boots trstrap values as percentage of bootstrap replication from 1000 replicate analysis.

Table 1. Similarities(%)(upper right) and evolutionary distances(lower left) between rDNA ITS region sequences of species of the genus *Cladobotryum*

	1	2	3	4	5	6	7	8	9
1		100	100	99.8	99.8	99.6	98.6	98.6	91.5
2	0		100	99.8	99.8	99.6	98.6	98.6	91.5
3	0	0		99.8	99.8	99.6	98.6	98.6	91.5
4	0.2	0.2	0.2		99.6	99.4	98.4	98.4	91.8
5	0.2	0.2	0.2	0.4		99.4	98.4	98.4	91.3
6	0.4	0.4	0.4	0.6	0.6		98.2	98.0	91.3
7	1.2	1.2	1.2	1.4	1.4	1.6		97.8	92.2
8	1.4	1.4	1.4	1.6	1.6	1.8	2.3		91.1
9	7.5	7.5	7.5	7.3	7.7	7.9	7.2	8	

1: BAC01, 2: BAC03, 3: BAC04, 4: *Cladobotryum_mycophilum* Y17095, 5: BAC05, 6: BAC06, 7: *Cladobotryum_asterophorum* Y7089, 8: *Cladobotryum_dendroides* Y17091, 9: *Cladobotryum_varium* Y17087.

ITS1 and ITS4. The size of the ITS fragment was approximately 580 bp, which includes ITS1, 5.8S and ITS4 regions. The sequences were compared with the published *Cladobotryum* fungi sequences available in the NCBI database. The strain is closely related to strains of the type species of *Cladobotryum mycophilum* (Fig. 5). This strain showed 99% ITS region sequence similarity to *Cladobotryum mycophilum*, according to blast search for homologous(Table 1). Phylogenetic trees were constructed using the neighbour-joining method (Saitou and Nei, 1987) with the MEGA3.0 program (Kumar *et al.*, 2004); bootstrap percentages were based on 1000 replications (Felsenstein, 1985).

Testing of sensitivity to selected fungicides. Sensitivity analysis was done with isolates grown on MEA amended with the following fungicides: Benomyl 50%, Iprodione 50%, Prochloraz-Mn complex 50%, Mancozeb 75%, Carbendazim 60% and Thiophanate-methyl 70%. The preliminary concentrations of all selected fungicides were 1, 2, 10 and 20 ppm. The plates were inoculated with inverted mycelial agar disk (10 mm) taken from the edge of 7 days old culture of *C. mycophilum* isolate, placed centrally on fungicide-amended and fungicide-free medium (control) and incubated at 25℃. Colony diameter was measured after 4 days of growth. Growth of colonies on the fungicide-amended medium was given as a percentage of the control. In these in vitro investigations of the sensitivity of *C. mycophilum* isolate to the selected fungicides, studied isolate showed highly resistance to Mancozeb and Thiophanate-methyl; and moderate sensitivity to Iprodione; and high sensitivity to Benomyl , Prochloraz-Mn and Carbendazim (Table 2).

Table 2. In vitro sensitivity of *Cladobotryum mycophilum* isolate to selected fungicides

Fungicides	Concentration(ppm)	Mycelial growth inhibition rate(%)
Prochloraz –Mn	1	82.4
	2	91.0
	10	100
	20	100
Benomyl	1	51.8
	2	93.7
	10	100
	20	100
Iprodione	1	18.2
	2	59.7
	10	98.1
	20	99.3
Carbendazim	1	92.5
	2	100
	10	100
	20	100
Thiophanate–methyl	1	0
	2	3.8
	10	54.7
	20	64.6
Mancozeb	1	0
	2	0
	10	34.9
	20	59.7

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