シイタケとトリコデルマの菌糸間相互作用におよぼす培地の炭素源と窒素源の影響

誌名	日本植物病理學會報 = Annals of the Phytopathological Society of Japan
ISSN	00319473
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発行元	日本植物病理學會
巻/号	45巻2号
掲載ページ	p. 261-264
発行年月	1979年4月

農林水産省 農林水産技術会議事務局筑波産学連携支援センター
Tsukuba Business-Academia Cooperation Support Center, Agriculture, Forestry and Fisheries Research Council Secretariat

日植病報 45 : 261-264 (1975)
Ann. Phytopath. Soc. Japan 45 : 261-264 (1979)

短　報
Phytopathological Note

Effect of Carbon and Nitrogen Sources in Media on the Hyphal Interference between *Lentinus edodes* and Some Species of *Trichoderma**

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時本景亮**・小松光雄**：シイタケとトリコデルマの菌糸間相互作用におよぼす培地の炭素源と窒素源の影響*

Lentinus edodes (Berk.) Sing., "Shiitake" mushroom, is well known as one of the most important edible fungi in Japan. Many reports on the biological natures of the fungus have been published[1], however, *Trichoderma* spp., such as *T. viride, T. harzianum, T. polysporum,* etc., remain to be of major importance as injurious inhabitants[2]. They often attack and kill mycelia of *L. edodes,* and therby reduce remarkably the mushroom yield.

The study was undertaken to ascertain the mode of the antagonistic action of *Trichoderma* hyphae to those of *L. edodes.* A part of the work was already published preliminarily[3,4].

The fungi used in the study were as follows: *Lentinus edodes* (Berk.) Sing. (TMI-655), *Trichoderma polysporum* (TMI-60146), *T. viride* (TMI-60147), and *T. harzianum* (TMI-60622). These *Trichoderma* species were isolated from the bed-logs of *L. edodes.* Isolate TMI-60147 is slightly pathogenic to *L. edodes* on the bed-log, and on the contrary, isolates TMI-60146 and 60622 cause severe damage. The basal medium used throughout this work contained (per liter of distilled water): d-glucose 20 g, casamino acids 2.0 g, $MgSO_4 \cdot 7H_2O$ 0.5 g, KH_2PO_4 1.0 g, $FeSO_4 \cdot 7H_2O$ 1.0 mg, $ZnSO_4 \cdot 7H_2O$ 0.2 mg, $Co(NO_3)_2 \cdot 6H_2O$ 0.1 mg, adenine-HCl 5.0 mg, thiamine-HCl 0.1 mg, and agar-agar powder 15 g. The medium was sterilized by autoclaving for 15 min. at 121 C. All cultures were grown in 9-cm petri dishes containing 20 ml of the medium. A disk (5 mm in diameter) of *Trichoderma* colony which had been stored at 5 C in an electric refrigerator was placed on *L. edodes* mycelium which achieved a diameter of approximately 80-90 mm in a petri dish. After several days' incubation at 25 C, the rejecting reaction of *L. edodes* against *Trichoderma* was determined by measuring the size of the damaged circle of the former. Various carbon and nitrogen sources were examined separately for their ability to support the rejecting reaction of *L. edodes* against *Trichoderma* attack. If necessary, a replacement culture using a cellophane membrane was employed.

Amounts and kinds of carbon and nitrogen sources in the medium affected the antagonistic effect between *L. edodes* and other fungi used. Media high in carbon were unfavorable to the antagonistic effect of *Trichoderma* to *L. edodes,* whereas

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Table 1. Effect of casamino acids and glucose concentrations on the antagonistic property between *L. edodes* and *Trichoderma*

Glucose conc.	*Trichoderma* employed	Casamino acids conc. 0.0	0.1	5.0g/l	Mean	
0.0	TMI-60147	9.4*	10.5	12.0	10.6	
	TMI-60146	10.3	13.9	20.1	14.8	16.5
	TMI-60622	12.0	18.6	41.3	24.0	
5.0	TMI-60147	8.9	8.1	15.0	10.7	
	TMI-60146	11.9	11.8	21.4	14.8	15.7
	TMI-60622	18.1	17.1	29.3	21.5	
20.0g/l	TMI-60147	7.9	8.0	15.0	10.3	
	TMI-60146	11.1	9.3	15.1	11.8	14.5
	TMI-60622	18.1	16.6	29.1	21.3	
Mean	TMI-60147	8.7	8.9	14.0		
	TMI-60146	11.1	11.6	18.9		
	TMI-60622	16.1	17.5	33.2		
		12.0	12.7	22.0		15.6

L. edodes mycelium which achieved a diameter of 80-90 mm on cellophane membrane in the basal medium was replaced to each medium tested, and then *Trichoderma* mycelia were inoculated. After 3 days' incubation, diameters of the damaged circle of *L. edodes* was determined. *Average (mm) of 4 samples.

Differences among means of the damaged circle in casamino acids concentrations and *Trichoderma* employed are significant ($p < 0.001$), respectively.

media rich in nitrogen source favored *Trichoderma* attack (Table 1). On media containing glucose, mannose, starch, etc., *L. edodes* developed the ability to reject the *Trichoderma* attack, whereas on media with xylose, sucrose, cellulose, etc., the former was attacked by the latter continuously (Table 2). The composition of medium after the inoculation of *Trichoderma* affected the antagonism, though no effect could be observed in the medium before the inoculation (Table 3). The kind of nitrogen source caused no clear difference in the antagonism except nitrite compounds (Table 4). By a heat treatment, the rejecting reaction of *L. edodes* was lost and the damaged circle of *L. edodes* in glucose medium changed to be similar to that in sucrose medium (Table 5). The rejection of *L. edodes* correlated clearly with the browning of the mycelium at the contact zone with *Trichoderma*.

These results suggest that the rejection of *L. edodes* against *Trichoderma* is an active reaction, and the damage of the mushroom cultivation by *Trichoderma* may be reduced by the control of nutritional condition. *Trichoderma* species produce various antifungal substances[5,6,7,8,9] and some enzymes which degrade *L. edodes* mycelium[10,11], however, the role of them in the pathogenecity to *L. edodes* remain to be unknown.

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Table 2. Effect of carbon sources on the interaction between *Lentinus edodes* and *Trichoderma*

Carbon source	*Trichoderma*		
	T. viride (TMI-60147)	*T. polysporum* (TMI-60146)	*T. harzianum* (TMI-60622)
Sodium acetate	+*	+*	+*
Glycerin	−	−	−
Arabinose	−	−	−
Ribose	−	−	−
Xylose	−	−	−
Fructose	+	+	+
Galactose	−	−	−
Glucose	+	+	+
Mannose	+	+	+
Sorbose	+	+	+
Mannitol	+	+	+
Lactose	−	−	−
Maltose	+	+	+
Sucrose	−	−	−
Trehalose	+or−	+or−	+or−
Dextrin	+	+	+
Starch**	+	+	+
Cellulose**	−	−	−
None	−	−	−

All individual carbon sources were added to the basal medium at levels equivalent to glucose-carbon of the basal medium. See Table 1 for procedures. + : Rejecting reaction (*L. edodes* mycelium rejected the invasion of *Trichoderma* with brownish pigmentation), − : Non-rejecting reaction. * No brownish pigment was observed. ** Replacement culture was not employed.

Table 3. Effect of carbon sources of medium on rejecting reaction in replacement culture

Carbon source Pre-Rep*	*Trichoderma*		
	TMI-60147	TMI-60146	TMI-60622
Glu-Glu	+	+	+
Glu-Man	+	+	+
Glu-Su	−	−	−
Glu-Xy	−	−	−
Su-Glu	+	+	+
Su-Man	+	+	+
Su-Su	−	−	−
Su-Xy	−	−	−

* "Pre" and "Rep" mean the cultures before and after the time of medium replacement, respectively. See Tables 1 and 2 for procedures and symbols. Glu: Glucose, Man: Mannose, Su: Sucrose, Xy: Xylose.

Table 4. Effect of nitrogen sources on the interaction between *L. edodes* and *Trichoderma*

Nitrogen source	*Trichoderma*		
	TMI-60147	TMI-60146	TMI-60622
KNO_2	−	−	−
$NaNO_2$	−	−	−
KNO_3	+	+	+
$NaNO_3$	+	+	+
NH_4Cl	+	+	+
NH_4-Tartrate	+	+	+
L-Glutamine	+	+	+
L-Tyrosine	+	+	+
Casamino acids	+	+	+

All individual nitrogen sources were added to the basal medium at levels equivalent to casmino acids-nitrogen. See Tables 1 and 2 for procedures and symbols.

Table 5. Disappearance of the rejecting reaction of *L. edodes* mycelium against *Trichoderma* by heat treatment

Trichoderma inoculated	*L. edodes* mycelium			
	On glucose medium		On sucrose medium	
	Heated	Un-heated	Heated	Un-heated
TMI-60147	26.3*	6.5	26.0	10.0
TMI-60146	28.3	11.3	26.5	16.2
TMI-60622	56.0	15.4	52.0	21.8

Mycelial colonies of *L. edodes* on cellophane membrane were heated by steam (100 C) for 20 min, and then the organism was placed on glucose or sucrose medium for *Trichoderma* inoculation. * Diameter (mm) of damaged circle of *L. edodes* after 2 days' incubation of *Trichoderma*.

The authors are indebted to Dr. S. Nishimura, Tottori University, for kindly reading of the manuscript, and also Dr. N. Hiratsuka, Director of Tottori Mycological Institute, for his support of this work.

(Received January 8,1979)