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Effects of culture conditions of *Lentinula edodes* (Shiitake mushroom) on the disease resistance of *Lentinula edodes* against *Trichoderma harzianum* in the sawdust cultures.

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Abstract

The effect of ingredients of the sawdust cultures of *Lentinula edodes* on the disease resistance of the mycelial culture against *Trichoderma harzianum* was studied by spraying spore suspensions of the pathogen to the small mycelial cultures of the mushroom. Water content affected strongly on the disease severity, but water activity was not so important. Nitrogen source content and the kind of nitrogen source affected the disease severity. The kind of carbon source also affected disease severity. The sawdust mycelial cultures of *L. edodes* containing cellulose acetate and similar cellulose derivatives as carbon source were disease resistance.

Introduction

Although recetly sawdust cultivation of *L. edodes* increased rapidly instead of bedlog cultivation in Japan, *Trichoderma harzianum* is an important pathogen in the cultivation of *Lentinula edodes*, "Shiitake mushroom". Concerning the physiological aspects of the interaction between *L. edodes* and *T. harzianum,* several studies have been reported (Oga and Kondo, 1978; Kawamura et al., 1980; Tokimot, 1985; Badham, 1991). But the test using sawdust cultures similar to the practical cultivation and that of the effect of pure compound have not been performed. We have already developed a method to assay varietal difference of the disease resistance of *L. edodes*, in which spore suspension of *T. harzianum* was sprayed over sawdust cultures of the mushroom (Ohmasa et al., 1995). In this report, we describe the results of experiments on the effects of ingredients of the media of sawdust cultures of *L. edodes* on the disase resistance against *T. harzianum*.

Materials & methods

Organisms.

The strains of *L. edodes*, SA134, SA168, SA170, and *T. harzianum*, SA321, used in this study were stock cultures of our laboratory.

Culture of *Lentinula edodes.*

Culture of *L. edodes* was performed as described previously (Ohmasa et al., 1995) except for the composition of media. The sawdust ricebran meium containing 5 parts of sawdust and 1 part of ricebran containing various amounts of water was used in the tests of water contens. In the test of water activity, water containing various amounts of glycerol was used in place of water and the total amount of water and glycerol was 65% of the weight of media.

In the tests of the effects of nytrogen source and carbon source, synthetic

or semisynthetic liquid media (Table 1) which were modified media of that

Table 1. Composition of the synthetic liquid media mixed with sawdust in this study.

carbon source*	35.0 - 48.0 g
nitrogen source**	9.6 - 48.0 g
KH_2PO_4	1.0 g
$MgSO_4$-$7H_2O$	0.5 g
$CaCl_2$-$2H_2O$	0.1 g
$FeSO_4$-$7H_2O$	1.0 mg
H_3BO_4	1.0 mg
$ZnSO_4$-$7H_2O$	0.9 mg
$MnSO_4$-$4H_2O$	0.8 mg
$(NH_4)_6Mo_7O_{24}$-$4H_2O$	0.2 mg
$CuSO_4$-$5H_2O$	1.15 mg
$Co(NO_3)_2$-$6H_2O$	0.1 mg
Adenine sulfate dihydrate	5.0 mg
Thiamine hydrochloride	0.1 mg
Distilled water	1000 ml
pH	5.6

* : Usually, glucose was used.
** : Usually, casamino acids was used.

reported by Tokimoto (Tokimoto, 1985) to contain more carbon and nitrogen sources, were prepared and mixed with sawdust and used for *L. edodes* cultivation. The final humidity of the obtained sawdust media (synthetic sawdust media) was 65%.

The sawdust ricebran media inoculated with *L. edodes* were cultured for 45 days and the synthetic sawdust media were cultured for 50 days at 25C.

Treatment of the sawdust culture of *Lentinula edodes* with *Trichoderma harzianum.*

Treatment of the sawdust culture of *L. edodes* with *T. harzianum* was performed as described previously (Ohmasa et al., 1995).

Assessment of disease severity.

The relative area of the invaded part of the surface of the inoculated sawdust cultures of *L. edodes* was visually determined on 7th, 9th, and 12th day after inoculation of *T. harzianum.* The relative area of the invaded part was converted to the disease severity index as shown in Table 2.

Table 2. Relationship between the relative area of the invaded part of the surface of sawdust cultures of *L. edodes* and the disease severity index.

Relative area of the invaded part (%)	Disease severity index
0 - 5	1
6 - 10	2
11 - 25	3
26 - 50	4

Results

Effects of water content and water activity of the media on the disease severity.

Dependence of disease severity on the initial water content of the sawdust ricebran media for *L. edodes* cultivation was tested using two strains, SA134 and SA168, in the range between 50% and 75%. Although difference in the disease severity of two strains under the same water content was observed, the disease severity, in general, increased as the water content of the media increased. A result is shown in Table 3.

Table 3. Effect of water content of the medium on the disease severity of *L. edodes*.

Water content of the medium (%)**	Disease siverity index***
50	1.6 (1.3)
55	1.2 (0.4)
60	2.0 (1.4)
65	2.8 (2.0)
70	3.8 (1.6)
75	5.0 (0.0)

* : Values for the strain, SA 134, are shown.

** : The initial value of the water content. The sawdust ricebran medium was used.

*** : The disease severity indexes at 12th day after inoculation of *T. harzianum* are shown. Values in the parentheses are standard deviations.

The effect of the water activity (a_w) of the sawdust ricebran media used for *L. edodes* cultivation was tested under the a_w between 1.00 and 0.83. The mycelia of *L. edodes* grew well between a_w of 1.00 and 0.98 and didn't grow below 0.95. The difference in the disease severity was not observed under these conditions (data not shown).

Effects of nitrogen source contents and the kind of nitrogen source of the media

The nitrogen content of the medium for *L. edodes* cultivation was varied by changing the amount of casamino acids in the synthetic medium. Three strains, SA134, SA168, and SA170, were used in this experiment. Although dependence of the disease severity on the nitrogen content varied among strains, the disease severity increased, in general, as the nitrogen content increased. A result is shown in Table 4.

The effect of the kind of the nitrogen source on the disease severity was tested for two strains, SA134, and SA168, using the following substances as the nitrogen source of the synthetic sawdust media; casamino acids,

Table 4. Effects of nitrogen source contents of the media on the disease severity.

Nitrogen source contents of the medium* (g/l for the synthetic liquid media)	Disease severity index**
(Strain SA134)	
48.0	4.7 (0.8)
35.4	3.8 (1.6)
22.2	2.8 (1.6)
9.6	2.8 (1.5)
(Strain SA170)	
48.0	5.0 (0.0)
35.4	4.8 (0.4)
22.2	3.5 (1.6)
9.6	1.8 (1.3)

* : Casamino acids were used as the nitrogen source of the synthetic media.
** : The disease severity indexes at 7th day after inoculation of *T. harzianum* are shown. Values in the parentheses are standard deviations.

polypeptone, yeast extract, L-glutamic acid, L-valine, L-arginine, L-aspartic acid, and L-proline. The disease severity changed with the kind of the nitrogen source. For SA134, the mycelial culture obtained with L-proline was resistant to *T. harzianum* and showed low disease severity (data not shown).

Effects of the kind of carbon source of the media

The effect of the kind of the carbon source on the disease severity was tested for two strains, SA134 and SA168, using the following substances as the carbon source of the synthetic sawdust media; D-glucose, D-xylose, D-galactose, carboxymethyl cellulose (CMC), and starch. A result is shown in Table 5. The disease severity changed with the kind of carbon source. Especially the mycelial culture of *L. edodes* with cellulose acetate was resistant to *T. harzianum* and showed very low disease severity, although growth rate of *L. edodes* was not so fast on this carbon source. The growth of *L. edodes* on the CMC medium was very poor.

Then, we tested the following substances as the carbon source which have similar chemical structure to cellulose acetate; cellulose phtalate acetate

Table 5. Effects of the kind of carbon source of the media on the disease severity.

Kind of carbon source*	Disease severity index**
D-glucose	5.0 (0.0)
D-xylose	3.5 (1.0)
D-galactose	4.6 (0.5)
carboxymethyl cellulose (CMC)	4.6 (0.9)
cellulose acetate	1.0 (0.0)
starch	5.0 (0.0)

* : The compounds listed in this table are used as the carbon source of the synthetic media.

** : The disease severity indexes at 12th day after inoculation of *T. harzianum* are shown. Values in the parentheses are standard deviations.

(phtalate about 35%), cellulose acetate propionate (acetate 2.5%, propionate 45%), and cellulose acetate butylate (acetate 29.5%, butylate 17%). The growth of *L. edodes* on the carboxy methyl cellulose and the cellulose phtalate acetate media was very poor, and the mycelial cultures were not resistant to *T. harzianum*. The mycelial cultures produced with cellulose acetate propionate and cellulose acetate butylate as the carbon source were very resistant to *T. harzianum* as cellulose acetate. Alkaline lignin was also tested as the carbon source. The growth of *L. edodes* on the media with the lignin as the sole carbon source was very poor and the mycelial culture was not resistant to *T. harzianum*.

Discussion/Conclusions

Water content showed important effects on disease severity. As shown in Table 3, the disease severity index increased as the water content of the sawdust ricebran medium increased. The mycelia grown in the media with 70% or more water content were attacked severely. Similar effects were reported by Oga and Kondo (1978) and Badham (1991). These facts seem to indicate that the pathogenicity of *T. harzianum* become stronger at higher water content.

Nitrogen content also showed important effects. As given in Table 4, the disease severity index became higher as the content of casamino acids increased. This is in consistent with the result of Tokimoto (1985). According to Table 4, the response of SA 134 and SA 170 are not parallel, and SA 170 is more sensitive to the increase of casamino acids.

The kind of nitrogen source also affected disease severity. Although only several nitrogen source were tested, L-proline remarkably increased the disease resistance of the strain, SA 134, compared with other nitrogen source at relatively high nitrogen content. The reason is not clear at present.

The kind of carbon source was also important. In our test, cellulose acetate increased, but CMC did not increased disease resistance of the sawdust cultures of *L.*

CMC could be used by *L. edodes*, when small amount of glucose was present and promoted disease resistance. Because we did not added glucose, mycelial growth was very poor in the CMC medium. The mycelial growth in the cellulose acetate media was also increased by the addition of glucose and decreased when large amount of casamino acids was present.

Conclusions

Disease severity of mycelia of *Lentinula edodes* by *T. harzianum* was strongly affected by culture conditions.

1) Higher water content of sawdust-rice bran media resulted in higher disease severity.
2) Disease severity was influenced scarcely by water activity of the sawdust-rice bran media within the range in which mycelia of *Lentinula edodes* grew well.
3) Higher contents of nitrogen source (casamino acids) resulted in higher disease severity.
   Disease severity was also influenced by the kind of amino acid.
   The sawdust mycelial cultures of SA 134 became resistant against *T. harzianum* when L-proline was used as nitrogen source.
4) Disease severity was affected by the kind of carbon source, and cellulose acetate and some similar cellulose derivatives was good carbon source, for example, for high disease resistance against *T. harzianum*.

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