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NOTE

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Influence of contamination by *Penicillium brevicompactum* and *Trichoderma harzianum* during *Lentinula edodes* spawn run on fruiting in sawdust-based substrates

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Abstract Substrates with two kinds of supplements, raw and deoiled rice bran, were artificially infected with *Penicillium brevicompactum* or *Trichoderma harzianum* on days 0, 26, 61, and 90 after inoculation with *Lentinula edodes*. With *P. brevicompactum* infection, there was no significant difference in the yield and size of the fruit-bodies among either infected and uninfected substrates, raw and deoiled rice bran substrates, or days when the substrates were infected. However, the irregularly shaped fruit-bodies, which were commercially of low value, yielded greatly on raw rice bran substrates infected on days 0 and 26, whereas the substrates infected with *T. harzianum* on any day were covered with conidia and fatally damaged.

Key words *Lentinula edodes* · *Penicillium brevicompactum* · Rice bran · Sawdust-based cultivation · *Trichoderma harzianum*

Introduction

In facility cultivation of edible mushrooms using sawdust-based substrates, pest fungi contaminate the substrates during inoculation, spawn run, and harvest. So far, pest fungi have been identified on the substrates of *Flammulina velutipes* (Curt.: Fr.) Sing. (Tanabe and Tabata 1968), *Pleurotus ostreatus* (Jacq.: Fr.) Kummer (Kono and Terashita 1982), and *Pholiota nameko* (T. Ito) S. Ito & Imai *in* Imai (Yoshida and Takao 1982).

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Lentinula edodes (Berk.) Pegler has a history of more than 100 years of cultivation on bed logs in fields, and the pest fungi invading bed logs, especially *Trichoderma* spp., have been reported (Komatsu 1976; Tokimoto 1985). In recent facility cultivation, damage of sawdust-based substrates of *L. edodes* by pest fungi during harvest have been reported (Togashi et al. 1996; Agriculture, Forestry and Fisheries Research Council Secretariat, Japan 1997); however, influence of the contamination during a spawn run has not been recorded. Contamination during a spawn run is only experientially known to cause quantity and quality reduction of the fruit-bodies. In this article, to examine how the various contamination conditions during a spawn run influence the fruiting, we artificially infected raw and deoiled rice bran substrates with the pest fungi *Penicillium brevicompactum* Dierckx and *Trichoderma harzianum* Rifai aggr. at four different intervals after the inoculation of *L. edodes* and compared the yield, size ratio, and shape of the fruit-bodies. These two fungal species were two of the main species that have dominated in more than 70% of the facilities for *L. edodes* surveyed (Togashi et al. 1996), and *P. brevicompactum* and *T. harzianum* especially were identified with high frequency (Agriculture, Forestry and Fisheries Research Council Secretariat, Japan 1997).

Materials and methods

As the strain of *L. edodes*, a commercial strain, No. 600 (Hokken, Tochigi, Japan), was used. As the pest fungi, a *P. brevicompactum* strain, KRCF-182, and a *T. harzianum* strain, Tricho sp. 1, isolated from naturally contaminated *L. edodes* sawdust-based substrates and stored at the Forestry and Forest Products Research Institute, Japan, were used. Materials were measured on a dry weight basis unless otherwise mentioned. Hardwood sawdusts (16–30 mesh and about 9 mesh) and hardwood wood chips (about 4 mesh) purchased from Hokken were used as the main ingredients. Raw and deoiled rice brans from Boso Fat & Oil (Funabashi, Japan) were used as the supplements.

For the mycelial growth tests in dual culture, *L. edodes* and either *P. brevicompactum* or *T. harzianum* were inoculated onto potato dextrose agar (PDA) media (Eiken, Tokyo, Japan) in Petri dishes and cultivated at 25°C, and the mycelia on agar plugs (4mm in diameter) taken from near the margins of the growing colonies were used as the inocula. To prepare the sawdust medium, 27% (w/w; the same hereafter) of the 16–30 mesh sawdust, 9% of each rice bran, and 65% of distilled water were mixed; 40g (wet wt) of each kind of medium was contained in ten open-end tubes (28mm in diameter, 245mm long) to a length of 100mm. The media were autoclaved at 121°C for 60min. The *L. edodes* mycelia were inoculated at one end of the open-end test tube and the mycelia of each pest fungus at the other end to make contact in the middle portion of the media. The inocula were incubated at 25°C. The leading lines of the mycelia of each fungus were marked on the test tubes every 7 days after contact of the mycelia of *L. edodes* with each pest fungus. The lengths between the marked lines at the four sides 90° apart were measured for each tube, and the means were recorded.

For the cultivation test, 18% of the 9 mesh sawdust, 9% of the wood chips, 9% of each rice bran, and 65% of tapwater were mixed; 1.2kg (wet wt) of each kind of sawdust-based substrate was contained in polypropylene bags (130 × 100 × 120mm) and autoclaved at 121°C for 70min after the inside reached 121°C. Then, 25g of the purchased *L. edodes* spawn was inoculated onto the substrate in each bag and incubated at 20°C for the total spawn run of 120 days. For the latter 30 days of the spawn run, they were held under fluorescent light (500–1000lx) for 12h/day. After the spawn run, the bags were removed, and the colonized substrates were maintained at a 12-h cycle of 16°C with light and 12°C in the dark, with relative humidity at 100% for 2h twice a day and otherwise at 90%. The first flush occurred from day 8 to 19, and the second and third flushes from day 33 to 40 and from day 47 to 60, respectively, after immersing the colonized substrates in water for 3h.

To estimate the mycelial biomass in the substrates, the ergosterol contents of the mixed three samples each from the three raw and deoiled bran substrates were measured on days 26, 61, and 90 after inoculation with *L. edodes*. The ergosterol extraction procedure was modified from Seitz et al. (1979), and the measurement was done according to the method described previously (Ohga and Wood 2000).

For the infection, the mycelia of the two pest fungi on agar plugs were inoculated on PDA media in test tubes and cultivated at 25°C. Sterilized distilled water was added to the conidia of each pest fungus to a final conidium concentration of 2×10^6/ml. One milliliter of the conidium suspensions of each of the two pest fungi was syringed onto the substrate surfaces in 12 bags on days 0, 26, 61, and 90 after inoculation with *L. edodes*. The infected and uninfected substrate surfaces were observed, and the conidia produced by the pest fungi and color changes according to the mycelial growth of *L. edodes* were noted.

All fruit-bodies were picked when the veils had broken. Marketable fruit-bodies and irregularly shaped ones, which had nonround pilei, were freshly counted and weighed according to the pileus diameters: SS, under 3cm; S, 3–4cm; M, 4–6cm; L, 6–8cm; and LL, over 8cm. Results were analyzed statistically at the 5% level by two-factor analysis of variance (ANOVA).

Results and discussion

Mycelial growth of *L. edodes* after contact with *P. brevicompactum* or *T. harzianum* in the sawdust media is shown in Fig. 1. The *L. edodes* mycelia grew further after contact with *P. brevicompactum*. In contrast, the *L. edodes* mycelia that contacted *T. harzianum* did not reach the opposite end but stopped part way. The *L. edodes* mycelia in the raw bran media showed faster growth than those in the deoiled bran media.

The ergosterol contents of the sawdust-based substrates with raw and deoiled bran on days 26, 61, and 90 after inoculation with *L. edodes* are shown in Fig. 2. Ergosterol

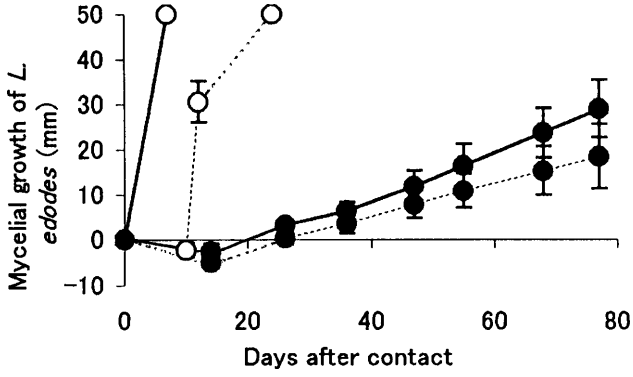

Fig. 1. Mycelial growths of *Lentinula edodes* after contact with *Penicillium brevicompactum* or *Trichoderma harzianum* in sawdust media with raw or deoiled rice bran in open-end tubes. *Zero* on the axis of the ordinate indicates the contact point; *positive numbers* indicate forward growth of the *L. edodes* mycelia. –○– and --○--, mycelial growth of *L. edodes* after contact with *P. brevicompactum* in the media with raw and deoiled rice bran; –●– and --●--, *T. harzianum* on the media with raw and deoiled rice brans, respectively. *Lines*, standard deviations. $n = 10$

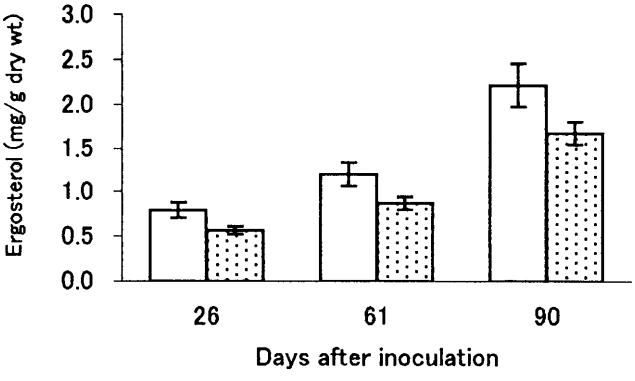

Fig. 2. Ergosterol contents of sawdust-based substrates with raw and deoiled rice bran on days 26, 61, and 90 after inoculation with *L. edodes*. *Open* and *dotted bar graphs*, substrates with raw and deoiled rice bran, respectively; *lines*, standard deviations. $n = 9$

content in the raw and deoiled substrates increased gradually according to mycelial growth. Higher content of ergosterol in the raw rice bran substrate was observed, which meant that mycelial biomass was greater in the raw rice bran substrate than in the deoiled bran substrate.

When the substrates with raw and deoiled rice bran were infected with the pest fungi after inoculation with *L. edodes* in the cultivation test, the surface appearances of both substrates showed the same tendency. The inoculated and uninfected substrates were wholly covered with *L. edodes* white mycelia on day 26 and with the brown pigmented mycelia after day 61. After infection with *P. brevicompactum* at the same time as the inoculation, the conidia were seen on day 6. However, on day 26, they were not seen, and the substrates were covered with *L. edodes* white mycelia, the same as the uninfected substrates. The substrates infected with *P. brevicompactum* after being entirely covered with *L. edodes* mycelia on days later than 26 looked like the uninfected substrates. In contrast, when the inoculated substrates were infected with *T. harzianum* on days 0 and 26, the conidia were seen on days 6 and 61, respectively, and they caused fatal damage to the substrates. However, when the substrates were infected with *T. harzianum* on day 61 after being covered with the brown-pigmented mycelia of *L. edodes*, the conidia were not seen for 30 days.

The average and standard deviations of the total yields of *L. edodes* fruit-bodies on the substrates with raw and deoiled rice bran infected with *P. brevicompactum* on days 0, 26, 61, and 90 and without infection after the inoculation ranged from 305 ± 87 g to 441 ± 77 g per 1.2 kg substrate. No statistical differences were observed either among substrates with raw and deoiled rice bran, infected and uninfected substrates, or days when the substrates were infected. The numerical ratios of *L. edodes* fruit-bodies divided into five different sizes on the substrates with raw and deoiled rice bran infected by *P. brevicompactum* were calculated. The average SS-, S-, M-, L-, and LL-sized fruit-bodies on the raw rice bran substrate were 3%, 6%, 30%, 34%, and 27%, respectively, and those on the deoiled rice bran substrate were 3%, 9%, 29%, 29%, and 30%, respectively. There was no tendency of fruit-body size difference as a result of substrates with raw and deoiled bran, infected and uninfected substrates, or days when the substrates were infected.

Figure 3 shows the numerical ratio of the irregularly shaped fruit-bodies of *L. edodes* against the total on the raw and deoiled rice bran substrates infected with *P. brevicompactum*. On the raw rice bran substrate infected on days 0 and 26, the numbers of irregularly shaped fruit-bodies were greater. On the deoiled rice bran substrate, there was no distinct difference among the days when the substrates were infected.

In the dual cultures, the *L. edodes* mycelia kept growing after contacting the *P. brevicompactum* mycelia, whereas they stopped growing after contacting the *T. harzianum* mycelia. In the cultivation test, the surface appearance of the sawdust-based substrates infected with *P. brevicompactum* was the same as that of the uninfected substrates, whereas the substrates infected with *T. harzianum* were covered with the conidia and fatally damaged. Pest fungi were divided into the two groups, competitors and pathogens; the former competed with the mushrooms for the substrate nutrition and possibly caused secondary infection with other pathogens, and the latter attacked the cultivated mushrooms (Stamets and Chilton 1983; Furukawa and Nobuchi 1996). The *P. brevicompactum* strain used here might be categorized as the competitor and the *T. harzianum* strain as the pathogen.

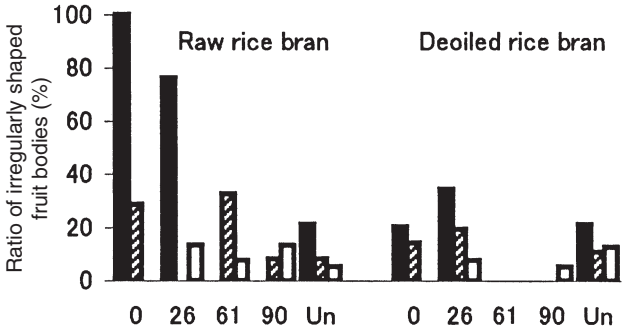

Fig. 3. Numerical ratio of irregularly shaped fruit-bodies compared to total on sawdust-based substrates with raw and deoiled rice bran infected with *P. brevicompactum* on days 0, 26, 61, and 90 after inoculation with *L. edodes*. *Un* (on abscissa), uninfected substrates; *solid bars*, first flush; *shaded bars*, second flush; *open bars*, third flush

There were no significant differences in the yield and size ratio of the fruit-bodies between substrates infected or not infected with *P. brevicompactum*, or substrates with raw and deoiled rice bran. However, the irregularly shaped fruit-bodies yielded greatly on the raw bran substrate infected on days 0 and 26 after the inoculation. Although the *L. edodes* mycelia grew more in the raw rice bran substrate than in the deoiled rice bran substrate, the mycelia of *P. brevicompactum* were suspected to also grow more in the uncolonized raw rice bran substrate than in the deoiled rice bran substrate on the earlier days of the *L. edodes* spawn run.

For the days when the substrates were infected with *P. brevicompactum*, infection at the same time as inoculation resulted in apparent conidia production, but infection on days later than day 26 showed no apparent difference from the uninfected substrates. Also, on the substrates infected with *T. harzianum* on days 0 and 26 after inoculation, the conidia were seen after 5 and 25 days, respectively, whereas on the substrates infected on days 61 and 90, the conidia were not seen until more than 30 days later. These facts might also relate to the larger biomass of the *L. edodes* mycelia in the substrates on the later days of the spawn run.

The large mycelial biomass revealed the defense phenomenon against invasion by the pest fungi, *Hypocrea* spp., and nutrient conditions of the medium were important factors (Ohga and Kondo 1978, 1981). It is also reported that the competition between *L. edodes* and *Trichoderma* spp. greatly differed according to the substrates (Badham 1991). With contamination with even the competitive pest fungus, the mycelial biomass of *L. edodes* in the substrates greatly influenced quality reduction of the fruit-bodies, and sub-

strate nutrition and the time when the substrates were infected were some of the factors affecting mycelial biomass.

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