Agronomie 18 (1998) 515–520

Original article

Reductions in the incidence of *Trichoderma* spp. using substrate supplementation with peat and an alternative spawn during cultivation of *Lentinula edodes* on pasteurised wheat straw

Gerardo Mata*, Jean-Michel Savoie**, Philippe Delpech, Jean-Marc Olivier

Station de recherches sur les champignons, Inra, BP 81, 33883 Villenave d'Ornon cedex, France

(Received 4 August 1998; accepted 24 September 1998)

Abstract – Shiitake, *Lentinula edodes*, is an Asian mushroom which is now produced in Western countries thanks to new cultivation methods. One of the major limitation of the cultivation method on pasteurised wheat straw used in France and other European countries is the growth of antagonistic fungi, *Trichoderma* spp., in the cultivation substrate. By taking into account findings on the interactions between *L. edodes* and *Trichoderma* spp., two improvements in the cultivation method were tested: substrate supplementation with peat and the use of a new support of activated inoculum. Cumulated data for seven strains of *L. edodes* under conditions of natural contamination by *Trichoderma* spp. were reported. Both changes in cultivation methods improved the competitive ability of *L. edodes*, decreased the incidence of *Trichoderma* spp., and increased the overall yield of harvested mushrooms. (© Inra/Elsevier, Paris.)

edible mushrooms / cultivation method / Shiitake / *Lentinula edodes* / *Trichoderma*

Résumé – Diminution de l'impact de *Trichoderma* spp. pendant la culture de *Lentinula edodes* sur paille de blé pasteurisée par la supplémentation du substrat avec de la tourbe et l'emploi d'une semence de champignon alternative. Le Shiitaké, *Lentinula edodes*, est un champignon comestible asiatique qui est produit actuellement en occident grâce au développement de nouvelles méthodes de culture. L'une des limites principales de la méthode de culture sur paille de blé pasteurisée qui est utilisée en France et dans les autres pays européens est la croissance de champignons antagonistes, *Trichoderma* spp., dans les substrats de culture. En prenant en compte des connaissances sur les interactions entre *L. edodes* and *Trichoderma* spp., nous avons testé deux améliorations de la méthode de culture : la supplémentation du substrat avec de la tourbe et l'utilisation d'un nouveau support d'inoculum activé. Un cumul des données

Communicated by Gérard Guyot (Avignon, France)

* Present address: Instituto de Ecologia, Departamento Hongos, Apartado Postal 63, Xalapa 91000, Veracruz, Mexico
** Correspondence and reprints
Tel: +33.05.56.84.31.61; fax: +33.05.56.84.31.78; e-mail: savoie@bordeaux.inra.fr

recueillies avec sept souches de *L. edodes* dans des conditions de contamination naturelles par *Trichoderma* spp. est reporté. Les deux changements apportés à la méthode de culture améliorent l'aptitude compétitive de *L. edodes*, diminuent l'impact de *Trichoderma* spp., et augmentent le rendement en champignons. (© Inra/Elsevier, Paris.)

champignons comestibles / méthode de culture / Shiitaké / *Lentinula edodes* / *Trichoderma*

1. INTRODUCTION

The commercial cultivation of Shiitake, *Lentinula edodes* (Berk.) Pegler, started in Europe in the 1960s using the traditional outdoor wood log method [16]. The length of the process motivated the development of alternative production methods for this mushroom. The synthetic log system using sterilised sawdust supplemented with available carbon and nitrogen sources packed in small units is now used throughout the world. The advantages of this method include less time required to complete a growing cycle and greater biological efficiency of mushroom production [12]. The major disadvantages are dictated by the need for sterilisation. The initial investment cost is relatively high, the method is still excessively labour-intensive and energy-consuming [10, 12] and fructification cannot be obtained under axenic conditions. Delpech and Olivier [4] proposed the use of low temperature treated (pasteurised) wheat straw as a substrate for Shiitake cultivation. Wheat straw is an abundant and widely available resource with a low lignin content and this alternative allows a considerable saving of energy [10]. Most French growers are using the cultivation method on pasteurised wheat straw [4], and recent surveys show that French production is currently about 500 tons of Shiitake a year.

Whatever the cultivation method used, one of the largest production problems remains the competition with other fungi [1]. Different species of *Trichoderma* including *T. viride*, *T. harzianum*, *T. polysporum*, commonly cause injury to mycelial growth and fruit body formation of *L. edodes* [14]. In the pasteurised wheat straw culture, spores of *Trichoderma* spp. originating from air and materials contaminate the substrate during the inoculation of the substrate with *L. edodes*. As a consequence the competition between *Trichoderma* spp. and *L. edodes* occurs during the first weeks and the initial rate of substrate colonisation by the antagonistic fungi is an important factor of the competitive interaction. If *L. edodes* rejects the attack by *Trichoderma* spp. at this stage, no other problem is encountered afterward. Strains of *L. edodes* are able to reject the attack by *Trichoderma* spp. under temperature and nutritive conditions favourable to them [1] and if their mycelium has colonised enough space before contact [13]. It is commonly observed that a brownish pigment is produced in the contact zone between the two mycelia when *L. edodes* rejects the attack of *Trichoderma* spp. This reaction is associated with an over-production of laccases induced by extracellular metabolites of *Trichoderma* spp. [13]. Otherwise laccases are also induced by water soluble lignin derivatives after a period of adaptation and favour the mycelial growth of *L. edodes* in the presence of these growth limiting compounds [7].

For improving the competitiveness of *L. edodes* during the first days after inoculation (spawning), the mycelium in the inoculum material (spawn) has to be vigorous, adapted to the components of the cultivation substrate [9] and able to colonise all the particles of the substrate, whereas spores of *Trichoderma* spp. should not encounter favourable conditions for rapid germination and mycelial growth. For these reasons Delpech and Olivier [5] recommended limiting the use of supplementation in wheat straw substrates and testing the addition of low quantities of fungicides. For consumer acceptance and *Trichoderma*'s resistance problems, fungicides are no longer used. In addition rye grain was replaced by millet grain as a spawn support because the number of inoculation points for the same weight of spawn is higher. In the present work the effects of sphagnum peat addition to wheat straw as an alternative cultivation substrate and the effects of an alternative spawn material containing a higher number of inoculation points and adapted

mycelium were assayed as new improvements in Shiitake culture.

2. MATERIALS AND METHODS

Conventional and alternative spawns were prepared for seven strains of *L. edodes* and cultivated on conventional and alternative wheat straw based substrate for measuring competitive colonisation rate by *Trichoderma* spp. and mushroom production.

2.1. Strains

The seven strains studied were maintained on malt extract agar (MEA). Three strains were cultivars obtained from spawn makers: 04 (Le Champion, France), M115 (Lambert Spawn, USA), S610 (France Mycelium, France). Bxc22013 was a hybrid obtained at the Inra Mushroom Research Unit, France. IE40 and IE105 were from the Institute of Ecology, Mexico and one strain (42253) was from ATCC (Canada).

2.2. Spawn

In the conventional spawn support, 100 g millet grain were boiled in 1 L water for 45 min. Excess water was removed and 15 g gypsum were added. The mixture was placed in plastic bags closed with cotton plugs and sterilised by autoclaving twice at 121 °C for 1 h with a rest period at room temperature for 48 h between the two cycles.

The alternative spawn support was prepared by adding 3 g oak bark powder to millet grain before boiling for 45 min. Fifty grams of corn cob beads from 0.25 to 0.56 mm in size (EURAMA, France) were boiled separately in 1 L water for 30 min. Excess water was removed, the ingredients were mixed and 15 g gypsum and 15 g dried powdered sphagnum moss peat were added. The mixture was placed in bags and sterilised by autoclaving twice at 121 °C for 1 h with a rest period of 48 h between the two cycles.

Bags containing 300 g moist grains plus gypsum (65 % H_2O) were inoculated with 1 cm^2 MEA medium with *L. edodes* and placed at 25 °C in darkness for 16 days. They were used to inoculate bags containing the same conventional spawn support or the alternative support tested. After incubation at 25 °C for 28 days both conventional and alternative spawn bags were stored at 4 °C for 7 days and then conditioned at 25 °C for 2 days before use as inoculum for the cultivation substrates.

2.3. Cultivation substrate

For the preparation of cultivation substrates, wheat straw was shredded into pieces from 4 to 6 cm in length and soaked in water for 48 h at room temperature. After leaching, 10 % (w/w) of gypsum was mixed with the straw. In an alternative cultivation substrate, 10 % of sphagnum peat was added. The peat was autoclaved twice, dried and ground before addition to the straw.

The mixtures were placed in trays (15 kg/tray) which were then placed in a fermentor for heat treatment at 65 °C for 24 h with water saturated air. Water content of the substrates after pasteurisation was about 72 %.

2.4. Cultivation

Spawning material was mixed with cultivation substrate (7 %, w/w) under non-axenic conditions. As a consequence *Trichoderma* spp. spores from ambient air could contaminate the substrate during spawning. Blocks of the mixture were formed in lightly perforated transparent polyethylene bags. For each strain, 12 blocks of 5 kg and five blocks of 1 kg were prepared simultaneously. Each strain was studied in a specific trial including the four treatments: conventional spawn plus conventional substrate (cc), conventional spawn plus alternative substrate (ca), alternative spawn plus conventional substrate (ac), alternative spawn plus alternative substrate (aa).

The blocks were incubated at 25 ± 1 °C with a 12 h light/12 h dark cycle for 4 to 7 weeks depending on the strains (spawn running period). The plastic bags were then removed, the blocks were sprinkled with cold water and the temperature decreased to 17 °C with the relative humidity maintained at 90 %, with a 12 h light/12 h dark cycle. These conditions were necessary to induce sporophore production.

2.5. Measurements

During the spawn running period the incidence of *Trichoderma* spp. was assessed on blocks of 1 kg. The zones of the blocks colonised by *Trichoderma* spp. [11] were marked on the bags after incubation for 2 weeks

and at the end of the spawn running period. The areas were measured using a graphic integrator and reported as percentages of the total area of the blocks contaminated. The data were recorded for each strain and were also cumulated to compare the overall effect of each treatment.

At the end of the spawn running period, the area of the 5 kg blocks colonised by *Trichoderma* spp. was estimated visually. When the rate of contamination was higher than 50 % the blocks were not used for the measurement of productivity. The percentage of eliminated blocks was noted. Mushrooms were harvested when the veil had broken and the gills were fully exposed. The productivity of each treatment was estimated by cumulating the yield of sporophores harvested at the beginning of cap opening in each trial. The biological efficiency (B.E.), the ratio of kg of fresh marketable mushrooms (after stem trimming) harvested per kg of dry cultivation substrate spawned was expressed as a percentage.

The objective of the present study was to determine potential positive effects of the treatments under varying conditions of natural attack by *Trichoderma* spp., whatever the strain of shiitake cultivated. Seven experiments were performed successively with the four treatments and with a different strain for each experiment. Because of some great differences between the trials (*figure 1*), the data reported to compare the treatments are the sum of the results for the seven trials.

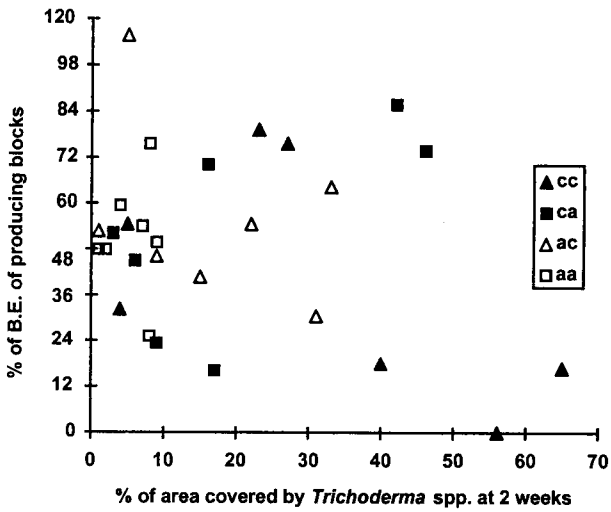

Figure 1. Relationships between the level of contamination by *Trichoderma* spp. at 2 weeks and the biological efficiency of *L. edodes* in seven trials; comparison of four treatments: conventional spawn plus conventional substrate (cc), conventional spawn plus alternative substrate (ca), alternative spawn plus conventional substrate (ac), alternative spawn plus alternative substrate (aa).

3. RESULTS

On conventional substrate the incidence of *Trichoderma* spp. under natural contamination conditions varied with the strains and the assays (*figure 1*) but the lowest rates of contamination by *Trichoderma* spp. were obtained with the addition of peat in the cultivation substrate (treatments ca and aa) and the use of the alternative spawn (treatments ac and aa). Actually, the lowest overall contamination rate measured on blocks of 1 kg was clearly obtained with the alternative spawn inoculated in the alternative substrate where only a few small zones colonised by *Trichoderma* spp. were observed (*table I*). These observations are in agreement with our working hypotheses. However, the percentage of blocks of 5 kg eliminated owing to the importance of the zones colonised by *Trichoderma* spp. was relatively high. This was due to some blocks with abnormal zones of contamination by *Trichoderma* spp. which corresponded to zones without spawning materials, emphasising the need for a good distribution of the spawn.

In two trials, with the strains S610 and M115, the area covered by *Trichoderma* spp. at the end of the spawn running period was less than 6 % of the total area in all the treatments. The observation of the B.E. (*figure 2*) shows that when the incidence of *Trichoderma* spp. attack was low, the addition of peat into the cultivation substrate increased significantly the production of mushrooms. By comparison the effects of the alternative spawn on yield were weak.

4. DISCUSSION

Alternative composition of the spawn and addition of peat to the cultivation substrate were estimated as putative improvement of the defence of *L. edodes* to *Trichoderma* spp. attack in the light of

Table I. Effects of variations in spawn and substrate preparation on the incidence of *Trichoderma* spp. and the yield of mushrooms during cultivation of seven strains of *L. edodes* on pasteurised wheat straw.

	Treatments			
	Conventional spawn Conventional substrate	Conventional spawn Alternative substrate	Alternative spawn Conventional substrate	Alternative spawn Alternative substrate
% of area covered by *Trichoderma* at 2 weeks	31*	20	17	6
% of area covered by *Trichoderma* at 4–7 weeks	34	16	23	8
% B.E. of producing blocks	47**	62	67	61
% of blocks eliminated owing to *Trichoderma*	49	25	12	20
Overall % B.E.	24	47	59	49

* Percentages were calculated on the sum of areas of 35 blocks of 1 kg (five blocks for seven strains).
** B.E., sum of the weight of mushrooms harvested for every strain divided by the sum of the dry weight of substrate inoculated in the seven trials.

previous findings. A high adaptation to sporophore production in wheat straw based substrates by *L. edodes* is dependent on a high biological potential of the spawn and a high metabolic activity during spawn running [6]. *L. edodes* reject the attack of *Trichoderma* spp. by an over-production of laccases induced by extracellular metabolites of *Trichoderma* [13] and the formation of a brown line at the contact between the mycelia. Laccases are also induced by water soluble lignin derivatives after a period of adaptation and favour the mycelial growth of *L. edodes* in the presence of these compounds [7] which limit the growth of *Trichoderma* spp.

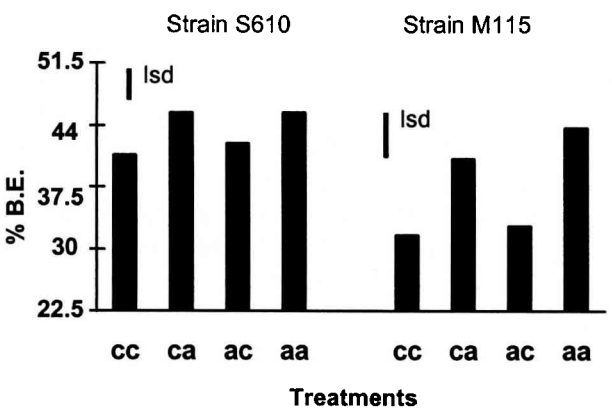

Figure 2. Mean values of percentage of biological efficiency measured on 12 blocks of 5 kg of substrate for two strains of *L. edodes*. Effects of the treatments in absence of significant attack by *Trichoderma* spp. The treatments were conventional spawn plus conventional substrate (cc), conventional spawn plus alternative substrate (ca), alternative spawn plus conventional substrate (ac), alternative spawn plus alternative substrate (aa). lsd is the least significant difference at $P < 0.05$.

Alternative spawn improved the competitive ability of *L. edodes* in the cultivation substrate during the first days after spawning and consequently limited the attacks by *Trichoderma* spp. but did not act directly on yields. During the preparation of the alternative spawn, the mycelium grew quickly and was vigorous at spawning. Higher biomass production was observed in the alternative spawn than in the conventional spawn and consequently there was a risk of bad separation of spawn beads and problems of spawn dispersion. The incubation time and

conditions have to be optimised for alternative spawn.

Addition of peat to the cultivation substrate improved both the defence against *Trichoderma* attack and the yield even in absence of significant contamination. Peat contains cellulose, hemicellulose, lignin, humic acids, phenolic compounds and ash [15] and could partly act as both a slow releasing C source and a protecting component. *L. edodes* is a ligninolytic fungi [8] and is able to degrade different forms of phenolic compounds which could have fungistatic effects for *Trichoderma* spp. Peat is also characterised by a significant ion exchange capacity and adsorbent properties [3]. The adsorption of calcium or another cation in the substrate was associated with increases in later break yields of *Agaricus bisporus* [2]. Otherwise the adsorption of metabolites produced by *Trichoderma* spp., with the release of some toxic phenolic compounds could explain the effect of peat addition on the competition between *L. edodes* and *Trichoderma* spp. A better understanding of the effects of peat addition will eventually lead to the use of other compounds or to the optimisation of the quantity of peat used in the substrate to increase the biological efficiency of *L. edodes* in wheat straw.

Overall positive effects of alternative spawn and addition of peat to the cultivation substrate to reject the attack of *Trichoderma* spp. were determined here. However, it was observed that different strains have different abilities to react to the contact with *Trichoderma* sp. [13]. Selection of fast growing strains able to react to contact with *Trichoderma* spp. is a way to increase the efficiency of these improvements in cultivation conditions proposed here.

Acknowledgement: We thank C. Coldefy, P. Castant and J. Lamarque for their technical assistance. G. Mata was supported by a fellowship of the CONACYT, Mexico.

REFERENCES

[1] Badham E.R., Growth and competition between *Lentinus edodes* and *Trichoderma harzianum* on sawdust substrates, Mycologia 83 (1991) 455–463.

[2] Beyer M.B., The effect of chelating agents on the later break yields of *Agaricus bisporus*, Can. J. Bot. 75 (1997) 402–407.

[3] Beyer M.B., Mutherbaugh H., Nutrient supplements that influence later break yield of *Agaricus bisporus*, Can. J. Plant Sci. 76 (1996) 835–840.

[4] Delpech P., Olivier J.-M., Champignon parfumé (ou shii-take) - Une méthode française pour sa culture, P.H.M.-Rev. Hortic. 305 (1990) 25–30.

[5] Delpech P., Olivier J.-M., Cultivation of shiitake on straw based pasteurised substrates, Mush. Sci. 12 (1991) 523–528.

[6] Mata G., Savoie J.-M., Extracellular enzyme activities in six *Lentinula edodes* strains during cultivation in wheat straw, World J. Microbiol. Biotechnol. 14 (1998) in press.

[7] Mata G., Savoie J.-M., Delpech P., Variability in laccase production by mycelia of *Lentinula boryana* and *Lentinula edodes* in presence of soluble lignin derivatives in solid media, Mater. Organ 31 (1997) 109–122.

[8] Leatham G.F., The ligninolytic activities of *Lentinus edodes* and *Phanerochaete chrysosporium*, Appl. Microbiol. Biotechnol. 24 (1986) 51–58.

[9] Leatham G.F., Griffin T.J., Adapting liquid spawn of *Lentinus edodes* to oak wood, Appl. Microbiol. Biotechnol. 20 (1984) 360–363.

[10] Levanon D., Rothschild N., Danai O., Masaphy S., Bulk treatment of substrate for the cultivation of shiitake mushroom, Bioresour. Technol. 45 (1993) 63–64.

[11] Rifai M.A., A revision of the genus *Trichoderma*, Mycol. Papers 116 (1969) 1–56.

[12] Royse D.J., Bahler B.D., Yield and size of shiitake as influenced by synthetic log diameter and genotype, Mush. J. Trop. 9 (1989) 109–113.

[13] Savoie J.-M., Mata G., Billette C., Extracellular laccase production during hyphal interactions between *Trichoderma* spp. and Shiitake, *Lentinula edodes*, Appl. Microbiol. Biotechnol. 49 (1998) 589–593.

[14] Tokimoto K., Komatsu M., Effect of carbon and nitrogen sources in media on the hyphal interference between *Lentinus edodes* and some species of *Trichoderma*, Ann. Phytopath. Soc. Japan 45 (1979) 261–264.

[15] Yan Y., Li T., Ren Z., Li G., A study on catalytic hydrolysis of peat, Bioresour. Technol. 57 (1996) 269–273.

[16] Zadrazil F., *Lentinula* (= *Lentinus*) *edodes*: physiology and conditions of industrial production, Mush. Information 87 (1993) 5–27.