Pathogenic interactions between *Trichoderma* species and *Agaricus bisporus*

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The interactions of *Trichoderma harzianum*, *T. longibrachatum*, *T. virens* and *Trichoderma* sp. with *Agaricus bisporus* mycelia were studied singly and in combination. Opposing cultures and layered cultures of the *Trichoderma* species with *Agaricus bisporus* mycelium produced a zone of inhibition. had The greatest colonization rates were in the order *Trichoderma longibrachiatum, T. harzianum, T. virens* and *Trichoderma* sp. Mycelial growth of *Agaricus bisporus* ceased upon contact with competitor hyphae all of *Trichoderma* species, after which parasitic growth continued over the *A. bisporus* mycelium in a radial manner. Microscopic observation of growth on 2% and 0.2% MEA showed different trends in interactions between the *Trichoderma* and mushroom mycelium. There was only superficial contact between the *Trichoderma* hyphae and *A. bisporus* in nutrient rich medium, but when the nutrient status of the medium was reduced, some coiling of *A. bisporus* hyphae by *Trichoderma* species was observed and hyphal lysis to occurred. However, the lysis of *A. bisporus* mycelia did not occur in all the species combinations. It is proposed that differences in metabolites produced by *Trichoderma* species have a close relationship with their colonization rate and subsequent mushroom losses. A severe interaction between *T. harzianum* and *T. longibrachiatum* with *A. bisporus* mycelium appears to be due to the production of lytic enzymes by the *Trichoderma* species. Volatile metabolites were either not produced by *Trichoderma* species or, if they were produced they had no inhibitory effects on *A. bisporus* mycelial growth.

Key words: *Agaricus bisporus*, button mushroom, green mould, interaction, parasitism, mushroom production, *Trichoderma*,

Introduction

The white button mushroom (*Agaricus bisporus* (Lange) Singer) is susceptible to many pests and diseases that adversely affect its production. Hyphomycetous fungi such as *Trichoderma* species are the most common (Sinden, 1971). *Trichoderma* is a ubiquitous fungus found in air, soil, plant

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materials and other substrates. Kligman (1950) first reported the presence of *Trichoderma* in mushroom compost. Although there are 4 to 6 species of *Trichoderma* reportedly associated with mushroom compost that can cause green mould (Morris *et al*., 1991; Seaby, 1996), *T. harzianum* biotype-2 (Th-2) has been reported to cause the most severe problem. A serious epidemic of *Trichoderma* green mould occurred in Ireland in 1985-86 and resulted in losses estimated at 3-4 million pounds to the mushroom industries in the UK and Ireland (Fletcher, 1990). Green mould epidemics also have been reported in Asia, Australia Canada, European countries, South America and the USA.

An understanding of the various factors involved in the interaction between *A. bisporus* and *Trichoderma* species may assist in determining methods to eradicate these fungi (Mumpuni *et al*., 1997). The purpose of this study was to determine, *in vitro*, the nature of the interaction between *A. bisporus* mycelium and several species of *Trichoderma* causing green mould that were isolated from mushroom beds.

Materials and methods

Preparation of Agaricus bisporus culture

Spores of *A. bisporus* (512) suspended from spore prints were cultured on a rich medium containing 150 ml aqueous compost extract +20 g malt extract + 3g yeast extract and 20 g agar (MEA). These plates were incubated at 25 ± 1ºC for 4-5 days, and colonies were selected and subcultured.

Comparision of Trichoderma species in colonization of mushroom mycelium

This study was carried out in two phases: In the first phase, 5 mm discs of *A. bisporus* were placed on one side of 2% MEA plates and incubated at 25 ± 1ºC for 3-4 days before placing 5 mm discs of *Trichoderma* spp. mycelium taken from the margins of 4 day old cultures on the other side of the plates. The colonies were examined for a zone of inhibition between the *A. bisporus* mycelium and specific *Trichoderma* species as well as colonization type and rate.

In the second phase, a rich medium including, 250 ml wheat extract, 20 g malt extract, 3 g yeast extract, and 20 g agar inoculated with 5 mm discs of *A. bisporus* mycelium from eight day old cultures and plates were incubated at 25 ± 1ºC. After 10-14 days, 5 mm discs of *Trichoderma* species were placed on the *A. bisporus* mycelium without any contact with the medium surface and

plates were again incubated at 25 ± 1ºC to study the colonization of mushroom mycelium by *Trichoderma.*

Hyphal interactions between Trichoderma species and A. bisporus mycelium

The purpose of this study was to determine the effect of *Trichoderma* spp. on mycelial growth of *A .bisporus* and observe events such as parasitism, coiling, antibiosis, lysis and effect of volatile metabolites.

The dual culture of mushroom and Trichoderma species

A thin layer of either 2% or 0.2% of MEA was spread on a clean and sterile glass microscope slide in the middle of sterile culture plates. On each slide was placed a 5 mm disc of *A. bisporus* from the edge of an actively growing culture. The plates were incubated at 25 ± 1ºC for about 48 hours to allow the *A. bisporus* mycelium to grow before placing a 5 mm disc from the edge of 3-5 day old cultures of *Trichoderma* on the slides 3 cm away. Records of the interactions between the opposing colonies including hyphal contacts or coiling were made after incubating at 25 ± 1ºC for 3-4 days using a stereo microscope. After one week, the slides were observed microscopically for lysis of *A. bisporus* mycelium by *Trichoderma.*

Evaluation of volatile metabolites of Trichoderma species

This study was carried out following the procedure of Dennis and Webster (1971) and Goyal *et al*. (1994) with slight modifications. Firstly, Petri-plates of MEA medium were inoculated with a 5 mm disc of *A. bisporus*. After 7 days, when the mycelium had attained some growth, the bottom Petri-plate (*A. bisporus*) was removed and placed on another plate containing MEA and inoculated with 5 mm discs of *Trichoderma* spp. and taped together by adhesive tape. In the control, *A. bisporus* plates were removed and placed over another MEA plate without *Trichoderma* spp. All of the plates were incubated at 25 ± 1ºC for 7 days and inhibition percent was recorded daily (every 24 hours) by comparing growth of *A. bisporus* mycelium controls with treatment growth using the following equation (Vincent, 1947):

$$\text{Percentage inhibition} = \frac{\text{Colony growth rate in checked plates - colony growth rates in each treatment}}{\text{Colony growth rates in checked plates}} \times 100$$

Results and discussion

Agaricus bisporus mycelial growth with and without *Trichoderma* species showed that no species inhibited the growth of *A. bisporus*. The results did not confirm those of Vijay and Gupta (1992). There was a significant difference in mycelial growth and colonization of host mycelium by different *Trichoderma* spp. *in vitro* which agree with the findings of Gray and Morgan-Jones (1981). In the first experiment, *T. longibrachiatum* had the highest colonization of *A. bisporus* mycelium within 5 days. *Trichoderma harzianum, T. virens* and *Trichoderma* sp. had a colonization rate of 6, 6, and 8 days respectively. In the second experiment, the ratios were similar to the first. At 3, 4, 4, and 7 days colonization by *T. longibrachiatum, T. harzianum, T. virens* and *Trichoderma* sp. respectively occurred. Infection and growth of the *Trichoderma* species with and without *A. bisporus* mycelium was very different.

The interactions of the four *Trichoderma* species, with *A. bisporus* mycelium were very different and influenced by nutrition in 2% or 0.2%. MEA. These results were similar to those reported by Mumpuni *et al*., (1997). In *T. harzianum*, there was only hyphal contact with the *A. bisporus* mycelium on 2% MEA with no symptoms of coiling, penetration or lysis observed. However, with a reduction of nutrients to 0.2% MEA, hyphae coiled around the *A. bisporus* mycelium, as well as penetrating it and lysis of the mycelium occurred after 7 days (Fig. 1).

In *T. longibrachiatum*, hyphal contact between the host and parasite was observed on 2% MEA and some pin-like outgrowths toward *A. bisporus* mycelium was produced, but no symptoms of coiling or lysis of mushroom hyphae was observed. In contrast on 0.2% MEA initial coiling occurred and eventually led to mycelial lysis after one week (Fig. 2). In *T. virens*, hyphal contact and pin-like processes grew towards *A. bisporus* mycelium and hyphal penetration on 2% MEA were observed. There was no evidence of lysis even on 0.2% MEA (Fig. 3). In *Trichoderma* sp. the mechanism was similar to *T. harzianum* but the penetration and lysis of host mycelium were not observed even on 0.2% MEA after 7 days (Fig. 4).

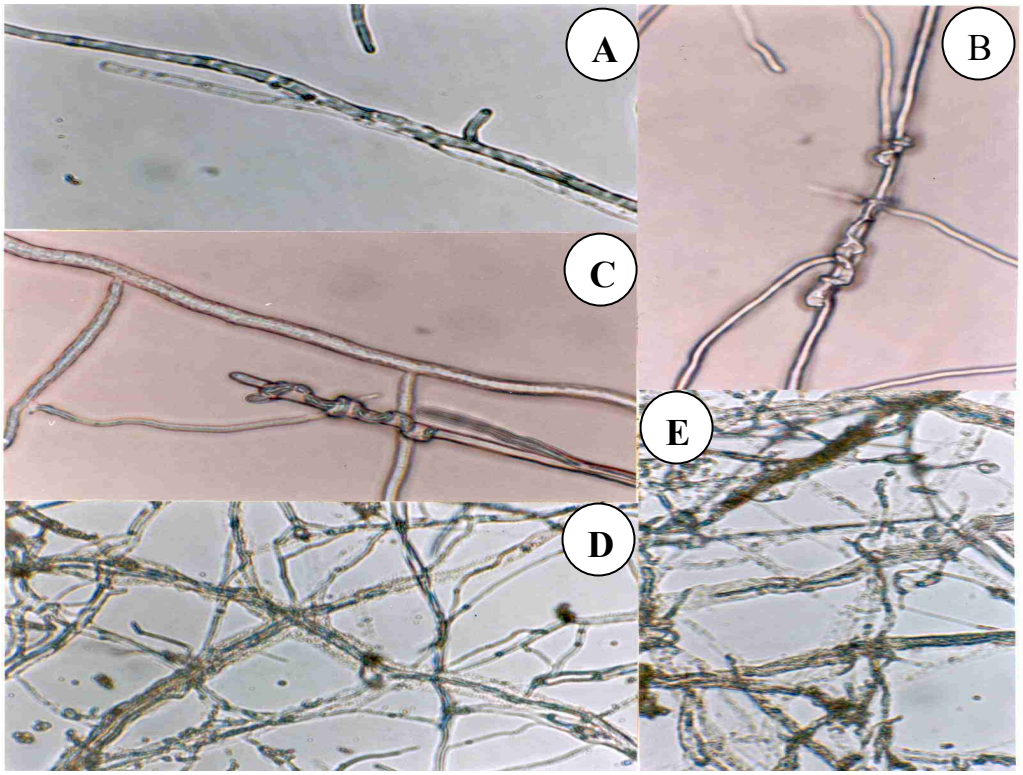

Fig. 1. Interaction between *T. harzianum* and *A. bisporus* mycelium. **A.** Hyphal contact on 2% MEA (40×). **B, C.** Coiling on 0.2% MEA (40×). **D, E.** Hyphal lysis after 1 week on 0.2% MEA (40×).

The coiling of *Trichoderma* hyphae around hyphae of other basidiomycetes such as *Armillaria mellea* and *Polyporus schweinitgii* on 2% malt agar has been reported (Aytoun, 1953) but has not been observed with *A. bisporus*. All hyphomycetes growing on mushrooms cause damage to host tissues by producing antibiotic compounds that cause lysis of host cells and reduce their ability to compete for substrata. The disruption of hyphae such as vacuolation or coagulation of cytoplasm reported for *Fomes annosus* (Risbeth, 1950) and *Lentinula edodes* (Komatsu, 1968) was only seen occasionally. with *Trichoderma* species and *A. bisporus.* The severe interaction of *T. harzianum* and *T. longibrachiatum* with *A. bisporus* mycelium appeared to result from the higher production of lytic enzymes by these species but not the others.

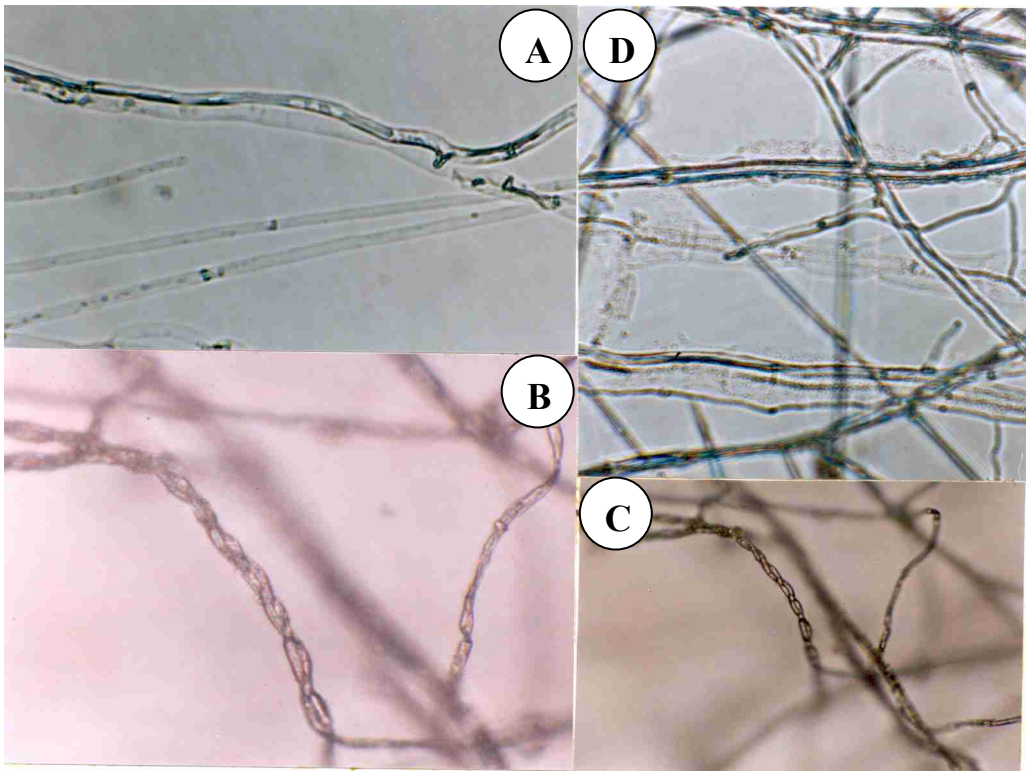

Fig. 2. Interaction between *T. longibrachiatum* and *A. bisporus* mycelium. **A.** Hyphal contact and producing pinlike structures (40×). **B, C.** Coiling on 0.2% MEA (B, 40×; C, 20X). **D.** Hyphal lysis after 1 week on 0.2% MEA (40×).

The outer mucilage and the potassium hydroxide-soluble layer play an important role in protecting hyphal walls against the lytic enzyme activity of parasitic fungi. Most *Trichoderma* species produce proteolytic enzymes, which may digest the protein component of the *A. bisporus* hyphal wall. These proteases have not been determined and it is not known if the enzyme was induced by protein in the *A. bisporus* cell wall or by the *Trichoderma* (Mumpuni *et al*., 1997).

Exo-and endo-β -1, 3 glucanase produced by *T. viride* and *Coniothyrium minitans* acting alone had a limited effect on the degradation of glucan; however, when these enzymes acted together, complete lysis of hyphal walls of *Sclerotinia sclerotinium*.occurred (Jones *et al*., 1974). Therefore, it is possible that the exo-glucanase activities of some *Trichoderma* spp. in conjunction with endo-glucanase are responsible for the observed lysis of *A. bisporus* hyphal walls. Another explanation is that the culture filtrate of *Trichoderma* spp.

contained an unknown substance, which facilitated enzyme hydrolysis of the hyphal walls.

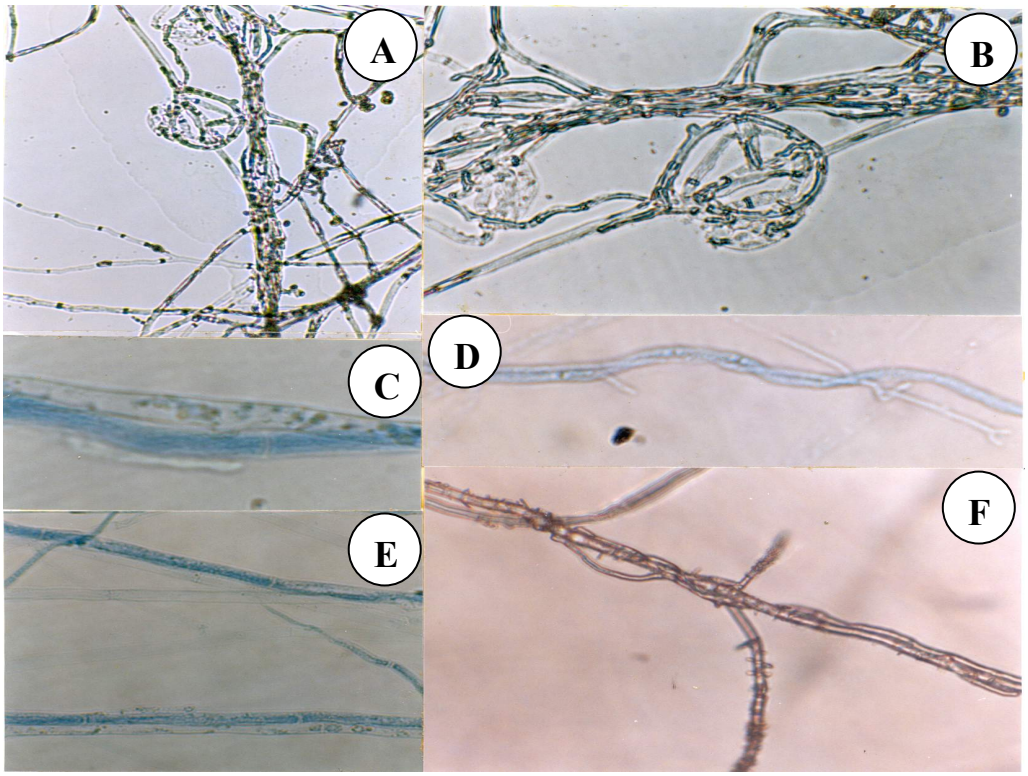

Fig. 3. Interaction between *T. virens* and *A. bisporus* mycelium. **A, B.** Hyphal coiling on 2% MEA (A, 20×; B, 40×). **C, D.** Coiling on 0.2% MEA (C, 100×; D, F, 40×). **E.** Hyphal contact on 2% MEA (40×).

Lysis of *A. bisporus* hyphae by some *Trichoderma* species may explain the aggressive nature of these species or biotypes in compost bearing *A. bisporus* mycelium. None of the *Trichoderma* spp. produced volatile metabolites or if they were produced, the metabolites had no inhibitory effects on *A. bisporus*. In other words, there was no significant difference in growth between the volatile treatments (*Trichoderma* species with *A. bisporus* mycelium) and control plates (only *A. bisporus* mycelium) with the mushroom mycelium covering the plates after 14 days in all cases. Therefore, volatile metabolites do not appear to have a role in aggressive characteristics of *Trichoderma* spp. and their interaction with *A. bisporus* mycelium. This study did not confirm the findings of Dennis and Webster (1971), who had reported that volatile metabolites had inhibitory effects on mycelial growth.

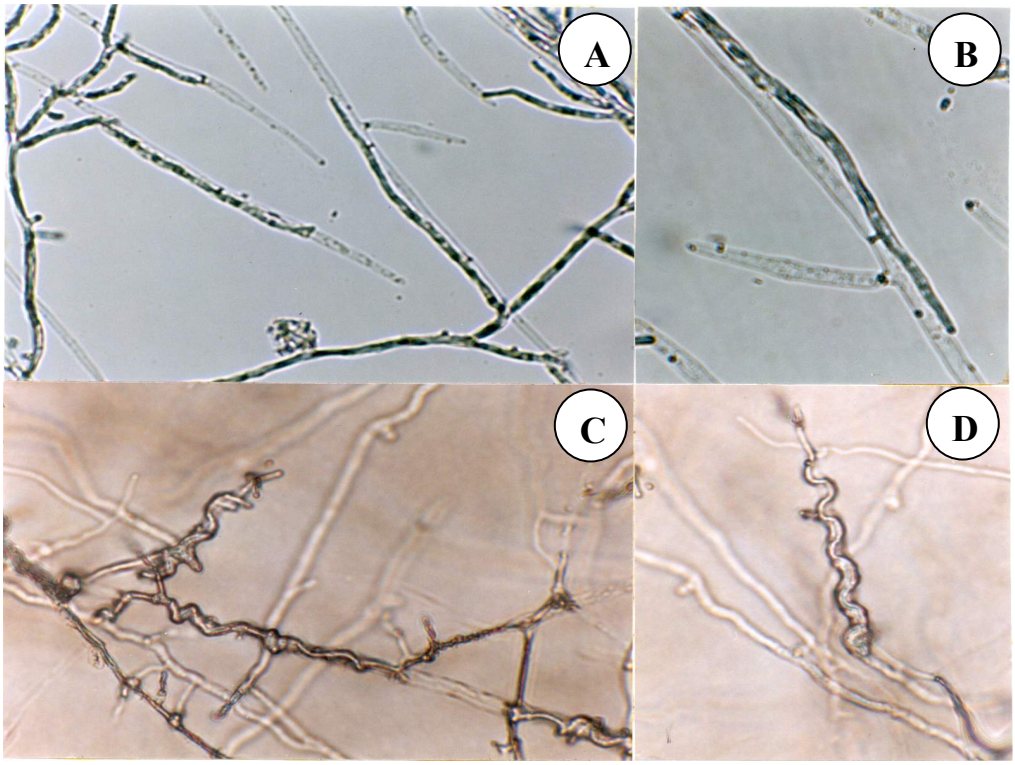

Fig. 4. Interaction between *Trichoderma* sp. and *A. bisporus* mycelium. **A, B.** Hyphal contact on 2% MEA (A, 20×; B, 40×). **C, D.** Coiling on 0.2% MEA (C, 20×; D, 40×).

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