In Vitro Studies on Interactions Between Strains of *Trichoderma* spp. and *Lentinus edodes* (Berk.) Singer Mycelium

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The popularity of shiitake, *Lentinus edodes*, is steadily increasing as more and more people learn about the nutritional value, unique taste, and the medicinal merits of this most valuable edible mushroom.

Although in the Ukraine the establishment of commercial *L. edodes* growing is in its beginning stages, its production is often restricted or hindered by infections through anamorphic fungi of the genus *Trichoderma,* which caused the so-called "green mold epidemics." This green mold disease has resulted in severe crop losses (30–100% in cultures of *Agaricus bisporus* (J.Lge) Imbach) on mushroom farms worldwide in recent years. This is increasingly forcing scientists to elucidate the particular mechanism through which the phenomenon of mycoparasitism and specific host responses might be explained. But data on *Lentinus/Trichoderma* interactions are still rare. Among other reasons concerning culture conditions, it became obvious that the degree of crop damage mainly depends on the species of the pathogenic molds and the varying agressivity of their strains (biotypes) as well as on the individual resistance of the breaded shiitake strain.

Therefore, the purpose of this work was to study the antagonistic actions between different *L. edodes* breeding strains and strains of *Trichoderma* spp., which originated from six shiitake farms in the Ukraine, in dual cultures on solid media. The aim was to observe whether there were characteristic patterns of interactions caused by the individual *Trichoderma* isolates and to identify the latter by morphological and molecular methods.

For the characterization of interaction types, each of six *Lentinus edodes* breeding strains from the Ukrainian culture collections have been confronted with 23 *Trichoderma* strains isolated from sawdust blocks showing green mold symptoms. Dual cultures were prepared by transferring a 5 mm disc of *L. edodes* mycelium from an 8-day-old culture to one side of a 90-mm Petri dish containing 15 mL 2% MEA. Plates were incubated at 25 ± 1 °C. After 5 days, a 5 mm disc of *Trichoderma* mycelium from the margin of a 4-day-old culture was placed in an adverse position, 4 cm apart from the *L. edodes* disc. Then, incubation at 25 °C was continued for 28 days.

The same experimental design was used to study the impact of temperature on interaction patterns. Three replicates were made for each combination of the six *L. edodes* and 23 *Trichoderma* strains. As controls, the growth behavior of each of the *L. edodes* and *Trichoderma* strains was examined in single cultures under the conditions mentioned above (three replicates each).

Based on morphological observations and the results of BLAST searches for the ITS1-5.8S-ITS2 rDNA region, 14 out of the 23 *Trichoderma* strains could be identified as *Trichoderma harzianum* Rifai, which represents the anamorphic state of *Hypocrea lixii* Pat. The similarity to strains recorded in GenBank was between 96 and 100%. Four of the isolates could be assigned to *Trichoderma koningii* Oudem. (GenBank similarity 97–99%). One of the isolates belongs to *T. citrinoviride* Bissett (GenBank similarity 100%) or with a similarity of 99% to *T. reesei* E.G. Simmons. The sequence data for the remaining isolate did not allow a closer determination. All identified *Trichoderma* species are known to be associated with the green mold epidemic of commercially grown *Agaricus bisporus* and/or *Lentinus edodes*.

Reproducible antagonistic interaction types exhibiting a relatively constant appearance of colonies were categorized into three main types (IT 1–3) according to the level of their interaction or the aggressivity of the individual *Trichoderma* species:

- IT 1: a high antagonistic action (mycoparasitism) was characterized by strong growth suppression of the shiitake colony immediately after contact with the opponent, leading to complete overgrowth within 4–5 days in the most unfavourable cases. This antagonistic action finally lead to the lysis of host mycelia. Ninety percent of the tested *Trichoderma* strains caused this type of interaction.

- IT 2: medium antagonistic action (passive antagonism) was characterized by restricted growth of shiitake with a distinct, reddish-brown, on average 6 mm broad inhibition zone between the two colonies.

- IT 3: a low antagonistic action was defined by suspended growth of the shiitake colony within the first days. But after 3-4 days the mycelium of *L. edodes* overgrew the mycelia of the *Trichoderma* strains.

These interaction types strongly varied with the applied incubation temperature: growth rates of the opponents or the aggressivity of individual *Trichoderma* strains distinctly changed at lower and higher temperatures (15 °C and 30 °C). This shows that much more knowledge about antagonistic properties of the involved biotypes (different strains with distinguishing interaction behavior belonging to the same species) of both *L. edodes* and *Trichoderma* spp. is needed.

The results clearly suggest that harvested losses in commercially grown shiitake depend on both the production strain used and the individual properties of *Trichoderma* biotypes. Considering the exploding market for shiitake and other cultivable mushrooms, it is a promising aim to elucidate the biodiversity of potential pathogens and to uncover their mode of action. But reliable and easily applicable tools for an accurate identification of *Trichoderma* species or their biotypes are still missing. Moreover, the establishment of an international databank on green mold epidemics would be highly appreciated by commercial mushroom growers and scientists as well.