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# GREEN MOULD DISEASE OF OYSTER MUSHROOM IN HUNGARY AND ROMANIA: ECOPHYSIOLOGY OF THE CAUSATIVE AGENTS

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**Abstract:**

The green mould disease of oyster mushroom (*Pleurotus ostreatus*) has been recently reported to cause great crop losses in Hungary and Romania. *Trichoderma pleurotum* and *Trichoderma pleuroticola*, the lately described fungal species responsible for the disease are present in both countries. The presented analysis has revealed that the growth of *T. pleurotum* shows a narrower temperature range (15-30°C) than that of *T. pleuroticola* (10-35°C). Acidic and neutral pH values and higher water activities were found to favour the growth of both pathogens. These data provide useful information for mushroom growers to optimize the ecophysiological parameters during oyster mushroom cultivation.

**Key Words:**

ecophysiology, green mould disease, oyster mushroom, *Pleurotus ostreatus*, *Trichoderma pleuroticola*, *Trichoderma pleurotum*

## 1. INTRODUCTION

Following champignon (*Agaricus bisporus*) and shiitake (*Lentinula edodes*), oyster mushroom (*Pleurotus ostreatus*) is the third-most important commercially grown edible basidiomycete worldwide [1]. In the past few years severe green mould infections of cultivated *P. ostreatus* have been reported in South Korea [7], Italy [10], Hungary [4] and Romania [6], which might indicate a worldwide threat.

The two filamentous fungal species responsible for the problem proved to be different from *T. aggressivum* f. *aggressivum* and *T. aggressivum* f. *europaeum*, the causative agents of green mould disease in the case of *A. bisporus* [9]. In 2006 they were described as *Trichoderma pleurotum* S.H. Yu & M.S. Park, sp. nov. and *Trichoderma pleuroticola* S.H. Yu & M.S. Park sp. nov. [8]. Komon-Zelazowska et al. [5] used an integrated approach for the comprehensive characterization of several *T. pleurotum* strains from Hungary, Romania and Italy, as well as *T. pleuroticola* isolates from Canada, the USA, Italy, Hungary, Romania, Iran, The Netherlands, Germany and New Zealand. Both species belong to the *Harzianum* clade of *Hypocrea/Trichoderma*. Morphological studies have revealed that *T. pleuroticola* shows pachybasium-like properties characteristic of the *Harzianum* clade, while *T. pleurotum* possesses gliocladium-like conidiophore morphology. BIOLOG phenotype microarrays were used to determine the carbon source utilization profile of the isolates and the results have shown unequivocal differences between the two species, namely the growth of *T. pleurotum* was slower or impaired on the majority of the carbon sources tested as compared with *T. pleuroticola*, which showed similar growth to that of *T. aggressivum*, indicating a closer relationship. The results suggest that the evolution of *T. pleurotum* was accompanied with the loss of the utilization ability of certain carbon sources. The phylogenetic analysis of a fragment including the internal transcribed spacer (ITS1-5.8S rRNA-ITS2) region of the ribosomal RNA gene cluster, a fragment covering the 4th and 5th introns and the last long exon of the *tef1* gene encoding for translation elongation factor 1α, and a fragment including a portion of the 5th exon of the *chi18-5* (previously named *ech42*) gene encoding a family 18 chitinase confirmed the involvement of the two distinct species in causing the green mould disease of oyster mushroom worldwide [5]. A DNA BarCode for identification of these species based on ITS1 and ITS2 sequences was also provided and integrated in the main database for *Hypocrea/Trichoderma* (www.isth.info, [3]). The aim of this study was to examine the effect of environmental factors (temperature, pH and water activity) on the mycelial growth of *T. pleuroticola* and *T. pleurotum*, the causative agents of oyter mushroom green mould disease, in comparison with related *Trichoderma* species (*T. harzianum*, *T. aggressivum* f. *aggressivum* and *T. aggressivum* f. *europaeum*).

## 2. THE STUDY

The *Trichoderma* strains included in this study are listed in Table 1. Strains were maintained on YEGK medium (0.5% glucose, 0.5% $KH_2PO_4$, 0.1% yeast extract, 2% agar).

Table 1. *Trichoderma* strains involved in the study

| Species | Strain | Isolation source |
|---|---|---|
| *T. harzianum* | C8, C22 | *Agaricus* compost, Hungary |
| *T. aggressivum* f. *aggressivum* | CBS 100527, CBS 450.95 | *Agaricus* compost, Canada |
| *T. aggressivum* f. *europaeum* | CBS 100526, CBS 433.95 | *Agaricus* compost, United Kingdom |
| *T. pleurotum* | A5, A11, A13, A14, A19, A26, A28, C2 | oyster mushroom substrate (wheat straw), Hungary |
| *T. pleuroticola* | CPK 2902, CPK 2894, CPK 2897 | natural substrate of wild-growing oyster mushrom, Hungary |
| | E10 | oyster mushroom substrate (wheat straw), Romania |
| | CPK 230 | decayed *Acer* stump, Canada |
| | CPK 882 | Iran |

The effect of temperature, pH and water activity ($a_w$) on linear mycelial growth was examined on synthetic minimal medium (0.5% $(NH_4)_2SO_4$, 0.5% $KH_2PO_4$, 0.1% $MgSO_4x7H_2O$, 1% glucose, 2% agar) and on a medium containing dried *Pleurotus* powder (0.1% glucose, 0,1% *Pleurotus* powder, 1% $KH_2PO_4$, 2% agar). Strains were inoculated onto the media with mycelial agar plugs cut from the edge of actively growing colonies. For studying the temperature dependence of the growth, plates were incubated at different temperatures (5, 10, 15, 20, 25, 30, 35 and 40°C). The effect of pH was examined at seven different pH values (pH 2,2, 3, 4, 5, 6, 7, 8) adjusted by McIlvain buffer solutions (mixtures of 0.3 M $Na_2HPO_4$ x 2 $H_2O$ and 0.1 M citric acid stock solutions in different proportions). $a_w$ values between 0.997 and 0.922 were adjusted with NaCl according to Chirife and Resnik [2] (0.997: 0% NaCl, 0.991: 1% NaCl, 0.980: 3% NaCl, 0.968: 5% NaCl, 0.962: 6% NaCl, 0.951: 8% NaCl, 0.945: 9% NaCl, 0.922: 12% NaCl). Colony diameters were measured daily along two perpendicular axes. Growth curves were recorded with the aid of Microsoft Excel 2002, the colony diameter extension rates were expressed in mm/day.

## 3. ANALYSES, DISCUSSION, INTERPRETATIONS

The examined *T. pleuroticola* strains showed mycelial growth in the range of 10-35°C (Table 2). The optimum temperature for growth proved to be 30°C, while the growth rate significantly decreased at 35°C and no growth could be observed at 40°C and 5°C. The *T. harzianum* strains showed a temperature dependence similar to that of the *T. pleuroticola* strains. Based on our results, the examined *T. aggressivum* and *T. pleurotum* strains can be characterized with a narrower temperature spectrum. In the case of *T. pleurotum* strains, no growth could be observed at 5, 10, 35 and 40°C, while the temperature optimum proved to be 25-30°C both on synthetic minimal medium and on *Pleurotus* powder medium. No significant differences in the temperature profiles could be observed between the two types of the media.

Although the temperature ideal for the growth of oyster mushroom varies among strains, room temperatures of approximately 25°C, 13-15°C and 18°C are needed for spawn-run, induction of the development of fruit bodies and fruiting, respectively [11]. The substrate is exposed to green mould infection mostly during spawn-run, when the substrate temperature is elevated up to 30°C due to the generation of metabolic heat by mushroom mycelia. Based on our results, the *Pleurotus*-pathogenic *Trichoderma* species show maximal mycelial growth at 25-30°C, while limited, or no growth was observed at 10°C. Woo et al. [10] reported that the temperature optimum for the growth of *Pleurotus* was 28°C, while *Trichoderma* could grow well at a wider range (20–28°C), and exceeded the growth rate of *Pleurotus* by three times at 25°C. These findings suggest that the temperature of the growing room should be maintained between 15 and 18°C after spawn-run in order to minimize the possibility of green mould infection.

In the case of most *T. pleuroticola* and *T. pleurotum* isolates, the highest values of colony diameter extension rates were recorded in the pH range of 5.0-6.0 on synthetic minimal medium, while the pH optimum of the *T. harzianum*, *T. aggressivum* f. *aggressivum* and *T. aggressivum* f. *europaeum* isolates proved to be lower, at pH 4.0 (Table 3). Interestingly, the pH profiles were narrower on *Pleurotus* powder containing medium, with an optimum shifted to pH 4.0 in the case of the *Pleurotus* pathogenic *Trichoderma* species. Only *T. pleuroticola* was capable of growing at all examined pH values on *Pleurotus* powder containing medium. The growth of *T. pleurotum* and *T. aggressivum* f. *aggressivum* ceased at pH values above 5.0, while no growth of *T. harzianum* and *T.*

*aggressivum* f. *europaeum* could be observed at pH values above pH 4.0. Our results suggest that the pH spectrum of mushroom pathogenic *Trichoderma* species can be narrower in the cultivation substrate than it can be expected based on data deriving from *in vitro* studies on synthetic media.

Table 2. Temperature dependence of the examined *Trichoderma* species. Colony diameter extension rates on minimal medium and *Pleurotus* powder containing medium in mm/day

| | Temperature | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Minimal medium | *5°C* | *10°C* | *15°C* | *20°C* | *25°C* | *30°C* | *35°C* | *40°C* |
| *T. harzianum* | – | 0.00-1.72 | 6.35-14.20 | 21.76-26.40 | 27.33-32.50 | 23.10-23.66 | 2.04-6.32 | – |
| *T. aggressivum* f. *aggressivum* | – | 0.00-1.68 | 3.58-10.84 | 9.91-21.42 | 25.66-8.00 | 8.35-25.33 | – | – |
| *T. aggressivum* f. *europaeum* | – | – | 0.00-16.25 | 14.00-0.30 | 18.57-36.50 | 18.35-25.30 | – | – |
| *T. pleurotum* | – | – | 2.50-5.93 | 17.71-21.23 | 22.69-3.46 | 18.42-23.76 | – | – |
| *T. pleuroticola* | – | 3.33-3.66 | 5.89-10.90 | 24.00-6.80 | 22.00-0.00 | 19.60-3.00 | 0.18-1.67 | – |
| *Pleurotus* powder medium | | | | | | | | |
| *T. harzianum* | – | – | 0.00-1.43 | 9.25-13.92 | 21.46-26.00 | 27.33-31.00 | 2.22-10.02 | – |
| *T. aggressivum* f. *aggressivum* | – | – | 0.00-12.28 | 20.10-25.33 | 22.00-4.00 | 6.00-23.66 | – | – |
| *T. aggressivum* f. *europaeum* | – | – | 4.56-18.30 | 17.23-21.30 | 22.69-4.50 | 13.14-22.76 | – | – |
| *T. pleurotum* | – | – | 4.83-8.14 | 10.07-22.53 | 23.46-0.00 | 22.23-0.00 | – | – |
| *T. pleuroticola* | – | 2.36-4.31 | 12.30-0.60 | 21.00-25.10 | 27.50-0.00 | 29.00-0.50 | 0.29-2.07 | – |

Table 3. pH dependence of the examined *Trichoderma* species. Colony diameter extension rates on minimal medium and *Pleurotus* powder containing medium in mm/day

| Minimal medium | pH | | | | | | |
|---|---|---|---|---|---|---|---|
| | *2.2* | *3.0* | *4.0* | *5.0* | *6.0* | *7.0* | *8.0* |
| *T. harzianum* | 4.20-4.68 | 5.40-19.17 | 16.55-20.71 | 19.78-20.25 | 5.80-19.77 | 2.85-14.02 | 4.20-15.21 |
| *T. aggressivum* f. *aggressivum* | 5.10-8.34 | 10.05-18.94 | 10.15-19.10 | 5.60-18.62 | 2.60-16.30 | 2.20-6.41 | 2.40-4.32 |
| *T. aggressivum* f. *europaeum* | 5.52-6.55 | 14.80-25.25 | 18.20-25.55 | 16.25-17.37 | 3.70-12.55 | 3.95-8.05 | 3.15-9.28 |
| *T. pleurotum* | 2.90-5.21 | 14.08-18.78 | 9.50-19.65 | 13.75-20.08 | 15.28-20.27 | 10.27-18.74 | 9.40-14.45 |
| *T. pleuroticola* | 4.00-4.90 | 11.80-22.35 | 8.60-15.65 | 12.40-23.85 | 11.30-24.95 | 3.85-18.40 | 4.05-19.20 |
| *Pleurotus*powder medium | | | | | | | |
| *T. harzianum* | 12.90-15.15 | 10.70-16.9 | 20.70-21.05 | – | – | – | – |
| *T. aggressivum* f. *aggressivum* | 8.20-9.30 | 8.85-23.35 | 8.70-24.75 | 2.45-3.05 | – | – | – |
| *T. aggressivum* f. *europaeum* | 12.35-15.75 | 15.70-18.85 | 13.30-19.25 | – | – | – | – |
| *T. pleurotum* | 9.90-11.70 | 15.05-19.65 | 18.70-23.15 | 2.10-2.70 | – | – | – |
| *T. pleuroticola* | 14.25-19.05 | 21.90-23.20 | 17.35-34.75 | 19.35-29.00 | 0.45-0.80 | 0.35-0.55 | 1.20-1.80 |

Table 4. Water activity dependence of the examined *Trichoderma* species. Colony diameter extension rates on minimal medium and *Pleurotus* powder containing medium in mm/day

| | Water activity | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Minimal medium | *0.997* | *0.991* | *0.980* | *0.968* | *0.962* | *0.951* | *0.945* | *0.922* |
| *T. harzianum* | 19.15-24.20 | 22.45-25.90 | 11.60-12.14 | 7.25-0.13 | 6.62-8.35 | 6.48-6.98 | 2.02-4.80 | – |
| *T. aggressivum* f. *aggressivum* | 23.35-25.80 | 19.30-24.80 | 14.68-20.70 | 8.21-4.77 | 7.07-4.77 | 3.55-7.20 | 1.51-4.37 | – |
| *T. aggressivum* f. *europaeum* | 19.20-34.25 | 19.10-25.20 | 10.77-18.42 | 5.94-9.90 | 5.14-7.88 | 2.97-3.67 | 0.00-2.05 | – |
| *T. pleurotum* | 18.10-19.03 | 9.28-19.00 | 5.14-13.25 | 4.97-6.80 | 2.20-5.85 | 0.00-5.17 | 0.00-1.60 | – |
| *T. pleuroticola* | 17.05-26.75 | 20.90-30.75 | 10.67-16.77 | 7.42-8.80 | 6.68-7.64 | 4.08-5.11 | 0.00-3.94 | – |
| *Pleurotus*powder medium | | | | | | | | |
| *T. harzianum* | 28.50-30.00 | 22.40-35.00 | 15.67-21.20 | 8.95-16.97 | 5.42-7.97 | 3.68-4.14 | 2.42-2.54 | – |
| *T. aggressivum* f. *aggressivum* | 21.00-23.35 | 21.40-24.80 | 14.68-18.20 | 8.21-13.40 | 7.07-13.82 | 3.55-5.82 | 1.51-4.71 | – |
| *T. aggressivum* f. *europaeum* | 22.70-34.25 | 24.40-25.20 | 13.71-18.42 | 9.90-10.54 | 6.65-7.88 | 2.82-3.67 | 0.00-1.82 | – |
| *T. pleurotum* | 21.90-26.50 | 21.30-25.60 | 16.20-18.25 | 7.80-13.49 | 6.86-8.22 | 3.05-4.42 | 1.37-2.82 | – |
| *T. pleuroticola* | 29.25-33.25 | 31.75-34.00 | 16.61-25.55 | 11.78-15.34 | 8.22-9.45 | 3.85-5.74 | 0.00-3.67 | – |

These data are in accordance with the findings of Woo et al. [10], who reported that the pH optimum for the growth of *Pleurotus* was alkaline (pH 8.0-9.0) whereas *Trichoderma* preferred acidic-neutral conditions. Woo et al. [10] suggested that adjusting the pH of the substrate to 8.0-9.0 might slow down the growth of *Trichoderma* resulting in a reduction in the spreading of the infection. However, during the spawn-run period, the pH of the substrate decreases rapidly (within 5-6 days) from 8.0-9.0 to 4.5-5.0 due to the growth of oyster mushroom mycelia. Thus the higher pH can provide protection only in the initial phase, later the oyster mushroom itself changes the circumstances, resulting in a medium with a pH optimal for the pathogen.

Concerning water activity, most of the *Trichoderma* strains showed higher mycelial growth rates on *Pleurotus* powder containing medium with an optimum of $a_w$=0.991, than on synthetic minimal medium, where the optimal water activity for most of the isolates proved to be $a_w$=0.997 (Table 4). In the case of *T. pleurotum*, the presence of *Pleurotus* powder induced mycelial growth also at water activity values where no growth could be observed on minimal medium. All the examined strains showed a lowering mycelial growth rate with the decrease in water activity, while none of the strains were able to grow at $a_w$=0.922.

Yu [11] examined the effect of substrate moisture content (SMC) on the growth of *Pleurotus* and *Trichoderma*. The optimum for oyster mushroom fell into the 60-70% range, and the growth of it was inhibited at 80%. In contrast to this, the mycelial growth of the green mould isolates was proportional to SMC, reaching its maximum at 80%.

## 4. CONCLUSIONS

In the latest years the most severe crop losses in oyster mushroom cultivation have been caused by green mould infections worldwide. The causative agents were identified as new-to-science species of the filamentous fungal genus *Trichoderma*, and they have recently been described as *T. pleurotum* and *T. pleuroticola*. The pieces of information about the ecophysiology of these two green mould species provided in the present study might help oyster mushroom growers to prevent green mould disease of *P. ostreatus*, and thereby reduce crop losses.

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