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# EVALUATION OF THE GROWTH OF *TRICHODERMA PLEUROTUM* AND *TRICHODERMA PLEUROTICOLA* ISOLATES AND THEIR BIOTIC INTERACTION WITH *PLEUROTUS* SP.

*Krzysztof Sobieralski[1], Marek Siwulski[1], Monika Komon-Żelazowska[2], Lidia Błaszczyk[3] Romuald Górski[4], Tomasz Spiżewski[1], Iwona Sas-Golak[1]*

[1] Department of Vegetable Crops, Poznań University of Life Sciences
Dąbrowskiego 159, 60-594 Poznań, Poland
[2] Division Applied Biochemistry and Gene Technology, Institute of Chemical Engineering Vienna University of Technology
Getreidemarkt 9/1665, A-1060, Vienna, Austria
[3] Institute of Plant Genetics, Polish Academy of Science
Strzeszyńska 34, 60-479 Poznań, Poland
[4] Department of Plant Protection Methods, Poznań University of Life Sciences
Zgorzelecka 4, 60-198 Poznań, Poland

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**Abstract:** Growth of *Trichoderma pleurotum* and *T. pleuroticola* isolates on the Potato Dextrose Agar (PDA) medium was investigated. *T. pleuroticola* isolates showed a significantly greater diameter of their mycelium colonies after 5 days of incubation than the *T. pleurotum* isolates. In addition, biotic interactions between *T. pleurotum* and *T. pleuroticola* isolates and species of *Pleurotus* sp. were determined. The following six species of oyster mushroom were used: *P. florida*, *P. cornucopiae*, *P. pulmonarius*, *P. columbinus*, *P. ostreatus* and *P. eryngii*. It was demonstrated that isolates of the *T. pleuroticola* species limited the growth of the examined species of oyster mushroom to a much greater extent than the isolates of the *T. pleurotum* species.

**Key words:** *Trichoderma*, oyster mushroom, mycelium growth, biotic interaction

## INTRODUCTION

Aggressive species of *Trichoderma* fungi may cause green mould diseases – a serious problem in mushroom production. In *Agaricus bisporus* cultivation the pathogenic *Trichoderma* were designated as *T. aggressivum* f. *europaeum* (Th2) and *T. aggressivum* f. *aggressivum* (Th4) (Williams *et al*. 2003). Green moulds occur on a massive scale in oyster mushroom plantations in North America (Sharma and Vijay 1996), South Korea (Park *et al*. 2004a, b, c, 2006), Italy (Woo *et al*. 2004), Hungary (Hatvani *et al*. 2007) as well as in Romania (Kredics *et al*. 2006). Bałaszczyk *et al*. (2011) investigated molecular variability of *Trichoderma* strains occurring in Poland, including those isolated from the substrate used in mushroom cultivations. In the case of oyster mushroom cultivations, the following two closely related genetically *Trichoderma* species were identified as pathogens: *T. pleuroticola* and *T. pleurotum* (Kommon-Żelazowska *et al*. 2007). However, phenotypically, the above species exhibit considerable differences. They were identified in cultivation media in Europe, Iran, and South Korea. Recently, *T. pleuroticola* and *T. pleurotum* species were identified in Spain as well (Gea 2009). In 2010, *T. pleuroticola* and *T. pleurotum* strains were also identified in oyster mushroom cultivations in Poland (Siwulski *et al*. 2011). The wild growing *P. ostreatus* species was accompanied by a number of *Trichoderma* species, most commonly by *T. pleuroticola* but also by *T. harzianum*, *T. longibrachiatum* and *T. atroviride* (Kredics *et al*. 2009).

The first aim of the performed investigations was to determine growth of *T. pleuroticola* and *T. pleurotum* isolates. The second aim was to determine the interactions between these isolates and several species of *Pleurotus* sp. in *in vitro* conditions.

## MATERIALS AND METHODS

The oyster mushroom species used in the experiments were derived from the collection of cultivated and medicinal mushrooms of the Department of Vegetable Crops at Poznań University of Life Sciences, Poland (Table 1). Isolates of the *Trichoderma* genus: four *T. pleurotum* isolates and four *T. pleuroticola* isolates are described in table 2.

*Corresponding address:
sobieralski@up.poznan.pl

Table 1. List of *Pleurotus* sp. strains used in the experiment

| Species | Strain number | Origin |
|---|---|---|
| *P. florida* | Pf149/B | Collection of cultivated and medicinal mushrooms from the Department of Vegetable Crops, Poznań University of Life Sciences, Poland |
| *P. cornucopiae* | Pc74/C | |
| *P. pulmonarius* | Pp47/A | |
| *P. columbinus* | Pc88/F | |
| *P. ostreatus* | Po44/S | |
| *P. eryngii* | Pe132/P | |

Table 2. List of *Trichoderma* isolates used in the experiment

| Isolate | Strain number | Origin |
|---|---|---|
| *T. pleurotum* | E136 | Vienna University of Technology, Institute of Chemical Engineering, Division Applied Biochemistry and Gene Technology, Austria |
| *T. pleurotum* | E139 | |
| *T. pleurotum* | T53/B | Institute of Genetics Polish Academy of Science, Poznań, Poland |
| *T. pleurotum* | T270/C | |
| *T. pleuroticola* | M141 | Vienna University of Technology, Institute of Chemical Engineering, Division Applied Biochemistry and Gene Technology, Austria |
| *T.pleuroticola* | M143 | |
| *T. pleuroticola* | T6/PR | Institute of Genetics Polish Academy of Science, Poznań, Poland |
| *T. pleuroticola* | T52/2D | |

The trial was conducted on Potato Dextrose Agar (PDA) medium (Oxoid Ltd., England) in the biological laboratory of the Department of Vegetable Crops at Poznań University of Life Sciences. During the first stage of the investigations, growth of the examined *T. pleurotum* and *T. pleuroticola* isolates on the above-mentioned PDA medium was determined. Inoculations were performed in a laminar-airflow cabinet by putting mycelia discs (5 mm diameter) of the examined *Trichoderma* isolate in the centre of the medium in a Petri dish. The dish was 9 cm in diameter. Discs were cut out from the PDA medium overgrown with the mycelium of the examined strains. Incubation was carried out in an incubator with no light access, at a temperature of 24–25C and relative air humidity ranging from 80 to 85%. The diameter of the fungus colony was measured after 5 days of incubation.

In the course of the second stage of the experiment, the individual biotic effect (IBE) was estimated with the index of biotic relations developed by Mańka (1974) (Table 3). For this purpose, mycelia discs of *Pleurotus* strains and competitive *Trichoderma* were placed on a Petri dish at a distance of 4 cm from each other. Using a plastic pipe of 5 mm diameter, the discs were cut out from PDA media overgrown with the mycelium of the examined mushroom. The mycelia discs of examined *T. pleurotum* and *T. pleuroticola* isolates, were inoculated 7 days after the inoculation of the tested *Pleurotus* strain. Incubation was carried out in the conditions described above. Mycelium growth measurements of the investigated fungus were taken every 24 hours. Assessments of interactions between the developing mycelia were determined. The observations noted: the degree of one colony surrounding the other, width of the inhibition zone, and growth limitation or infestation of one colony by the other. A precise description of the method of conducting the experiment was given by Frużyńska-Jóźwiak *et al*. (2010).

The experiment was established in six replications in a random design. Two cycles of the experiments were conducted. No significant differences between cycles were found.

Table 3. Score scale for the determination of individual biotic effect (IBE) (acc. to Mańka 1974)

| Type of interaction between colonies | Points |
|---|---|
| Both colonies are in contact along a straight line | 0 |
| Colony A remains in contact with colony B along a slightly curved line so that it surrounds less than 1/3 of colony A | +1 |
| Colony A remains in contact with colony B along a curved line so that it surrounds at least 1/3 but less than 1/2 of colony A | +2 |
| Colony A remains in contact with colony B along a curved line so that it surrounds at least 1/2 but less than 2/3 of colony A | +3 |
| Colony A remains in contact with colony B along a curved line so that it surrounds at least 2/3 or more of colony A | +4 |
| Each millimetre of the inhibition zone is occupied by colony A | +1 |
| Colony B at least by 1/3 but less that ½ smaller than its control colony developed individually on a separate plate | +2 |
| Colony B at least by 2/3 smaller than its control colony developed individually on a separate plate | +3 |
| Colony B completely undeveloped | +4 |

## RESULTS

Figure 1 presents growth of *T. pleurotum* and *T. pleuroticola* isolates on the PDA medium. The diameters of colonies of different strains of *T. pleurotum* after 5 days of incubation were similar and ranged from 72 to 75 mm. *T. pleuroticola* isolates exhibited significantly greater diameters of colonies after the above-mentioned period of incubation. The colony diameter of these isolates ranged from 83 to 88 mm. There were no significant differences between isolates.

When analysing IBE indices for *T. pleurotum* and *T. pleuroticola* isolates, this index assumed a different value depending on the tested *Pleurotus* species. The examined *T. pleurotum* isolates exhibited a very similar IBE index in their interaction with all the species of *Pleurotus*. This index, in the case of all the examined isolates, varied from +3 to +5. No difference was found in the IBE index value for *T. pleurotum* isolates in their interaction with *P. cornucopiae*. In all the cases, the IBE index reached +5. The greatest sensitivity to the effect of *T. pleurotum* was shown by *P. cornucopiae*. A very similar value was also exhibited by the index in the case of the *T. pleurotum* isolate in its interaction with *P. columbinus*. The IBE index amounted to +4 with the exception of the *T. pleurotum* E136 isolate where the value was higher and reached +5. Considerable variation in the IBE index was determined in the case of *T. pleurotum* isolates in their interactions with *P. ostreatus* and *P. eryngii* (Table 4).

Table 4. Individual biotic effect index for *T. pleurotum* and *T. pleuroticola* isolates

| *Trichoderma* species | Isolate designation | *Pleurotus* | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | *florida* | | *cornucopiae* | | *pulmonarius* | | *columbinus* | | *ostreatus* | | *eryngii* | |
| *T. pleurotum* | E136 | +4 | | +5 | | +4 | | +5 | | +4 | | +5 | |
| *T. pleurotum* | E139 | | +4 | | +5 | | +4 | | +4 | | +5 | | +4 |
| *T. pleurotum* | T53/B | +5 | | +5 | | +4 | | +4 | | +4 | | +3 | |
| *T. pleurotum* | T270/C | | +4 | | +5 | | +5 | | +4 | | +3 | | +4 |
| *T. pleuroticola* | M141 | +6 | | +6 | | +6 | | +6 | | +5 | | +6 | |
| *T. pleuroticola* | M143 | | +6 | | +7 | | +6 | | +6 | | +6 | | +6 |
| *T. pleuroticola* | T6/PR | +5 | | +7 | | +7 | | +5 | | +5 | | +6 | |
| *T. pleuroticola* | T52/2D | | +6 | | +7 | | +6 | | +7 | | +7 | | +6 |

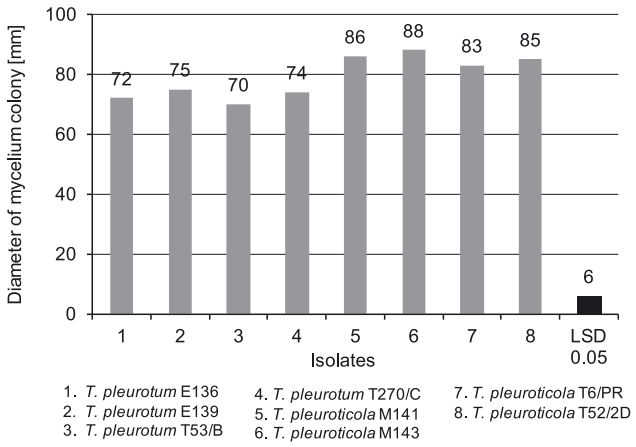


Fig. 1. Diameter of the colonies of *T. pleurotum* and *T. pleuroticola* isolates after 5 days of incubation on PDA medium

The IBE index for the examined *T. pleuroticola* isolates and *Pleurotus* sp. strains was significantly higher and ranged from +5 to +7. For the interaction between the *T. pleuroticola* isolate and *P. florida*, the IBE index was similar and amounted to +6, with the exception of the T6/PR isolate where the IBE index was found to be lower (+5). *T. pleuroticola* isolates in their interaction with *P. cornucopiae* showed a similar IBE index amounting to +7, with the exception of the M141 strain for which the IBE index reached the value of +6. This species of *Pleurotus* showed the greatest sensitivity to *T. pleuroticola* isolates. The IBE index for the examined *T. pleuroticola* isolates and *P. pulmonarius* as well as *P. eryngii* amounted to +6, with the exception of the T6/PR isolate for which the IBE value was higher - reaching a value of +7. The IBE index for interactions between the *T. pleuroticola* isolates and *P. columbinus* as well as *P. ostreatus* fluctuated within wider limits from +5 to +7.

## DISCUSSION

Experiments involving the growth of *T. pleurotum* and *T. pleuroticola* isolates on PDA medium did not show any significant differences in colony diameters of the isolates after 5 days of incubation. All the examined *T. pleuroticola* isolates exhibited a significantly greater colony diameter after the incubation period than *T. pleurotum* isolates. The above results correspond with those reported by Siwulski *et al*. (2011).

In the available literature on the subject, there is no precise information regarding the impact of *T. pleurotum* and *T. pleuroticola* species on the mycelium development of different species of *Pleurotus* sp. In the described investigations, the authors used six different *Pleurotus* species as well as eight isolates of *T. pleurotum* and *T. pleuroticola*. The analysis of IBE indices for individual *T. pleurotum* and *T. pleuroticola* isolates demonstrates that isolates of the *T. pleuroticola* species reduced growth of the examined *Pleurotus* sp. species considerably more than isolates of the *T. pleurotum*. In the case of all examined *T. pleurotum* isolates, the value of the IBE index in their interactions with *Pleurotus* species ranged from +3 to +5. The IBE index for *T. pleuroticola,* on the other hand, reached a value from +5 to +7. When analysing table 3, it can be said that the IBE index of *T. pleurotum* isolates in interaction with individual species of *Pleurotus,* was fairly similar within species and isolates. *T. pleurotum* to the greatest extent inhibited the development of *P. cornucopiae*, which was the most sensitive species to the effect of this fungus. The relatively highest variability of the IBE index for *T. pleurotum* isolates and the *Pleurotus* species occurred in the case of *P. eryngii*. A similar variability in the IBE index was determined for the *T. pleurotum* isolates in interaction with *P. ostreatus*. Greater differences were found in the value of the IBE index for *T. pleuroticola* isolates in interaction with the *Pleurotus* species. The species most sensitive to *T. pleuroticola* was *P. cornucopiae*

The obtained results support the observations of Kommon-Żelazowska *et al*. (2007) regarding faster mycelium growth and the overgrowing of the *P. ostreatus* mycelium by *T. pleuroticola* isolates. The above-mentioned researchers demonstrated that *T. pleuroticola* can overgrow *P. ostreatus* mycelium, whereas the *T. pleurotum* isolates penetrate up to 3 mm into *P. ostreatus* mycelium and then it ceases to overgrow mycelium of the oyster mushroom. The performed investigations indicated that mycelium of the examined oyster mushroom species exhibited a certain antagonistic response in relation to *T. pleurotum* isolates. The above experiments confirmed the fact that none of the experimental mycelia of oyster mushroom exhibited an antagonistic response to *T. pleuroticola* isolates, which were characterized by a very high IBE index in their interactions with nearly all *Pleurotus* species. Only in some cases did the value of the IBE index for the examined *T. pleuroticola* isolates in their interaction with *Pleurotus* sp. species amount to +5. In the majority of the cases, the index reached a value of +6 or +7 indicating the absence of antagonistic responses in relation to *T. pleuroticola* isolates.

Information can be found in the literature about some mushroom species which exhibit a defense response to some aggressive *Trichoderma* isolates. This kind of response was shown by the shiitake (*Lentinula edodes*) as well as oyster mushrooms (*P. ostreatus* and *P. eryngii*) (Savoie *et al*. 2001). *Trichoderma aggressivum* f. *europaeum* is one of the most dangerous pathogens in mushroom production. This species causes large losses in edible and medicinal mushroom cultivation. Earlier investigations by the authors on interaction of edible mushroom species with pathogenic fungi revealed that certain antagonistic responses to *T. aggressivum* f. *europaeum* isolates were shown by some strains of *Coprinus comatus*. (Frużyńska-Jóźwiak *et al*. 2011). Other investigations carried out by the authors (Siwulski *et al*. 2009; Sobieralski *et al*. 2009) correspond with the results obtained in our described experiment. The tested species and strains of *Agaricus* and *Pleurotus* were very sensitive to the effect of *Trichoderma*. They did not exhibit any defensive response in relation to *T. aggressivum* f. *europaeum* isolates. The studies also revealed a significant ability of *Trichoderma* to inhibit mycelium development in the case of some *Pleurotus* species. The most sensitive to *Trichoderma* impact was *P. cornucopiae*. The results clearly indicated greater inhibition of *Pleurotus* growth by *T. pleuroticola* isolates.

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