# BIODECOLORIZATION OF DARK PIGMENTS DERIVED FROM BLUE STAINING FUNGI USING BASIDIAL WHITE-ROT FUNGI

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## 1. INTRODUCTION

Blue stain of sapwood is a serious problem in many countries of the world, and searching for new methods of wood protection which is cost effective and harmless to the environment has been continued. The biological methods of blue stain control used at present are commonly based on the phenomenon of microbial antagonism. Among the antagonists of blue stain fungi (BSF) that have been suggested non-pigmented mutants of *Ophiostomataceae* fungi as well as lignin-degrading basidiomycetes seem to be the most promising as the agents of bio-control in pulp and paper industry. It has been shown that some basidial species are able to inhibit growth of sap BSF by competition for nutrient sources(Croan and Highley, 1991). The promising results were obtained with white-rot fungus (WRF), *Phlebiopsis gigantea* used as an agent of bio-control of *Ophiostoma* and *Leptographium* fungi in pulpwood (Behrendt and Blanchette, 2001; Benko and Henningson, 1986; Croan and Highley, 1991). The fungal wood processing known as biopulping can also improve paper strength properties (Akhtar *et al.*, 1993; Fisher *et al.*, 1994; Martinez-Inigo *et al.*, 1999). This study was performed to understand the interaction between Ophiostomataceae and basidiomycetes fungi during cultures, and whether the basidiomycetes fungi inhibit the growth and decolorize dark pigments of BSF. To study the interaction between basidial WRF and BSF isolates the dual culture technique was used.

## 2. MATERIALS AND METHODS

### 2.1 Fungal strains

Twenty eight isolates belonging to 18 species of basidial WRF were used in this study such as *Abortiporus*, *Agaricus*, *Antrodiella*, *Bjerkandera*, *Daedaliopsis*, *Gleophyllum*, *Fomitopsis*, *Laetiporus*, *Lentinus*, *Lenzites*, *Nematoloma*, *Panus*,

*Piptoporus*, *Pleurotus*, *Pleurotus*, *Trametes*and unknown species. Eighteen isolates of 11 BSF, mainly from Ophiostomataceae family, were also used in this study.

### 2.2 Interaction between basidial WRF and BSF during dual culture

Five basidial WRFs were selected after extended preliminary test as decolorizing agents for further study: *A. hoehnelii* (S28), *B. fumosa*(137), *G. odoratum* (124), *T. versicolor* (B18), and unknown species (528). Besides them, nine isolates of BSF were selected as active producers of dark pigments: *C. laricicola* (l41), *C. polonica* (p24), *L. sibirica* (g1, g9), *O. ips* (i29, i41), *O. minus* (m5), *O. piliferum* (f16), and *O. penicillatum* (n13).

In order to clarify the interaction between basidial WRF and BSF isolates, the dual culture technique was used (Croan and Highley, 1991). Two isolates were cultivated on 2% malt-extract agar (MEA) in Petri dishes (diameter 90 mm). Each agar plate was inoculated on one side with agar disk (7 mm in diameter) cut from the margin of a 7-day colony of a basidial WRF on 2% MEA. The opposite site was inoculated with agar disk of a BSF. After inoculation Petri dishes were sealed with parafilm and incubated at room temperature up to 60 days. To check additionally the ability of selected basidial WRF to decolorize dark pigments, BSF were cultivated on 2% MEA in small Petri dishes (diameter 55 mm) for 10 days, so that dark mycelium covered the all agar plate. Then, inoculum of basidial fungi (7 mm disk cut from the 7-day colony on MEA) was placed on surface of dark colonies. Petri dishes were sealed with parafilm and incubated at room temperature during 2 months. Three replicates were used for each combination basidial culture - blue staining fungus. Interactions between fungi and progress of decolorization were described at 5, 21, 30, 40 and 60 days.

## 3. RESULTS AND DISCUSSION

When cultivated in dual culture on 2% MEA basidial and BSF demonstrated three types of agar interaction as follows:

- **deadlock** after first contact of colonies expansion of both partners stopped, no change was observed during the period of observation (60 days);
- **replacement** after contact of opposite mycelia growth of the both colonies stopped for some time, then one of the fungi began to overgrow the opposite partner, whose growth did not restore;
- **antagonism** growth of one partner were inhibited at a distance, a clear zone of

agar medium (zone of inhibition) forms between the both colonies.

More than half of basidial WRFs were characterized by deadlock interaction with BSF as shown in Table 1. In the dual cultures, where basidial partners were presented by *A. bisporus* (64), *L. sulphureus* (L01/89), *T. versicolor* (09) and unknown fungus (02), antagonism was found at the phase of primary contact of colonies. The deadlock-type of interaction followed by antagonism, and remained constant until the end of experiments(Table 1). Though there was limitation of BSF expansion through the common substrate in this case, deadlock-type of interaction including antagonism seemed to be ineffective in terms of discolorization of wood stain, because contest of two mycelia often stimulated producing additional pigment in their contact area with increasing dark colour of whole substrate.

Table 1. Domination interaction among basidial WRF and BSF

| Type of interaction | |
|---|---|
| (Antagonism) Deadlock | (Deadlock) Replacement |
| *A. bisporus* (64*) | *A. biennis* (123**) |
| *B. adusta* (B04/91) | *A. hoehnelii* (S28/91**) |
| *D. confragosa* (B35/91) | *B. adusta*. (B13/91) |
| *F. pinicola* (B03/91, B15/88) | *B. fumosa* (137**) |
| *L. sulphureus* (L 01/89*) | *G. odoratum* (124**) |
| *L. edodes* (101) | *P. rudis* (S25/91) |
| *L. betulina* (S23/91) | *P. ostreatus* (103) |
| *N. frowardii* Horak (275) | *T. gibbosa* (S20/91) |
| *P. betulinus* (B02/91, B 21/91) | *T. versicolor* (N20, B18/91) |
| *P. eryngii* (102) | unknown species (528) |
| *P. ostreatus* (P15/93) | |
| *T. versicolor* (07, 09*, B08/91) | |
| unknown species (02*) | |

* antagonism was dominating type of interaction after primary contact

** after primary contact of basidial culture began to grow over dark colony of blue staining isolate without deadlock-phase

Replacement interaction resulted usually in decreasing dark colour of substrate was observed for 11 basidial cultures that were belonging mainly to white-rot fungi. Among them *A. biennis* (123), *A. hoehnelii* (S28/91), *B. fumosa* (137), *G. odoratum* (124) were characterized by the absence of deadlock-phase: they began to grow over dark colonies of their partners just after primary contact. Five basidial cultures - *A. hoehnelii* (S28/91), *B. fumosa* (137), *G. odoratum* (124), *T. versicolor* (B18) and unknown species (528) selected for further investigation caused distinct decolorization of dark pigments after 40 days cultivation in dual culture with ophiostomatoid fungi as shown in Table 2. Completed decolorization or trace amounts of dark pigments were characteristic in dual cultures with *O. ips*, *O.*

*minus* and *O. piliferum* isolates, but *O. penicillatum* demonstrated resistance to affecting basidial cultures (Table 2). Intensity of dark colour of *Ceratocystis* sp. and *L. sibirica* isolates was decreased by some of their basidial partners, but not to completed bleaching (Table 2).

Table 2. Dark colour intensity in dual cultures of basidial WRF and BSF

| Incubation, day | | 21 | | | | | 40 | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Code of isolate | | B18 | S28 | 124 | 137 | 528 | B18 | S28 | 124 | 137 | 528 |
| *Ceratocystis* spp. | l41 | 3 | 3 | 3 | 3 | 3 | 2 | 2 | 1 | 1 | 2 |
| | p24 | 1 | 1 | 1 | 1.5 | 1.5 | 1 | 1 | 0 | 0.5 | 0.5 |
| *Leptographium* spp. | g1 | 2.5 | 2 | 3.5 | 2 | 3 | 0.5* | 0.3* | 2.5 | 0,3* | 0.3* |
| | g9 | 2.5 | 2 | 3.5 | 2 | 3.5 | 0.3* | 0.5* | 1 | 0.5* | 0.5* |
| *Ophiostoma* spp. | f16 | 1.5 | 2 | 1 | 1.5 | 2 | 0.3* | 0.3* | 0 | 0.3* | 1 |
| | i29 | 1.5 | 1 | 2 | 1 | 2 | 0 | 0 | 0 | 0 | 0.5* |
| | i41 | 2 | 2 | 2.5 | 1 | 2 | 0 | 0 | 0 | 0 | 0 |
| | m5 | 2 | 2 | 2 | 2 | 2.5 | 0.3* | 0 | 0 | 0 | 0 |
| | n13 | 3 | 3 | 2 | 3 | 3 | 1.5 | 1 | 0 | 1.5 | 1 |

* 0.3-0.5 trace amount of dark pigments in agar

Changing dark colour in colonies of BSF inoculated with basidiomycetes after 10 days growth was in general agreement with this process in dual cultures mentioned above, though pigments in well-developed colonies of BSF seemed to be more resistant to affecting of basidiomycetes (Table 3). As it can be seen from the data of Table 3, basidiomycetes did not affect strongly the pigments of *Ceratocystis* spp. and *L. sibirica* isolates, but completely decolorized colonies of *O. ips* and to a smaller degree O. *minus*. It should be noticed that overgrowing *O. piliferum* mycelium by basidial cultures even stimulated increasing dark pigmentation (Table 3).

Table 3. Decrease in dark pigmentation in the process of overgrowing BSF with basidial WRF

| Incubation, day | | 30 | | | | | 60 | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Code of isolate | | B18 | S28 | 124 | 137 | 528 | B18 | S28 | 124 | 137 | 528 |
| *Ceratocystis* spp. | l41 | 2 | 3 | 1.5 | 2 | 3 | 1.5 | 3 | 3 | 1.5 | 3 |
| | p24 | 1 | 1 | 1 | 1.5 | 1 | 1 | 0.5* | 0 | 1 | 1 |
| *Leptographium* spp. | g1 | 1,5 | 0.5* | 2 | 1.5 | 2 | 1.5 | 0 | 3 | 1 | 2 |
| | g9 | 1 | 0.5* | 2 | 1.5 | 1.5 | 0.5* | 2 | 2 | 0.5* | 1 |
| *Ophiostoma* spp. | f16 | 1.5 | 2 | 1 | 1.5 | 2 | 2 | 2 | 1.5 | 1 | 2 |
| | i29 | 1.5 | 1 | 2 | 1 | 2 | 0 | 0 | 0 | 0 | 0 |
| | i41 | 2 | 2 | 2.5 | 1 | 2 | 0 | 0 | 0.5* | 0 | 0 |
| | m5 | 2 | 2 | 2 | 2 | 2.5 | 1.5 | 0 | 1.5 | 0 | 0.5* |
| | n13 | 3 | 3 | 2 | 3 | 3 | 1.5 | 1 | 1.5 | 0 | 1 |

* 0.3-0.5 trace amount of dark pigments in agar

For interspecific variability it was hard to distinguish the white-rot fungi which showed the most active decolorizing ability. However, *A. hoehnelii* (S28/91) and *B. fumosa* (137) were active towards *Ophiostoma* and partially *L. sibirica* isolates during all experiments. Additionally, *T. versicolor* (B18), *G. odoratum* (124) and unknown cultures (528) also demonstrated high level of decolorizing activity when grew together with some *Ophiostoma* species. Moreover, only *G. odoratum* (124) was able to remove dark pigments of *O. penicilltum*.

It can be suggested from our results that white-rot fungi decolorize dark pigments of *Ophiostoma* species easier than those of *Ceratocystis* genus (Table 2 and Table 3). Such resistance to decolorization may be connected with differences in chemical compositions, mainly melanin pigmint, of these two genera. Despite of morphological similarity *Ceratocystis* genus has been confirmed to be not in genetical affinity with *Ophiostoma* species (Samuels, 1993) that allows chemical differences in their metabolites. By contrast, *Leptographium* species are closely related to ophiostomoid fungi, for many of them are anamorphs of *Ophiostoma* spp. (Jacobs and Wingfield, 2001).

During this experiment, many cases of inter- and intra-specific variabilities are observed, and the necessity to select basidial fungi for controling blue stain is required through the screening many cultures in laboratory. Lack of information about types of interaction between basidial WRF and BSF makes it impossible to designate some taxon or ecological group as the most promising bio-controlling agents. It is obviously expected that bio-decolorization control of wood stain using white-rot fungi depends on the progress of the study of biology and ecology of wood-inhabiting BSF.

## 4. CONCLUSIONS

More than half of basidial cultures were characterized by deadlock interaction with BSF. In the dual cultures, where basidial partners were presented by *A. bisporus* (64), *L. sulphureus* (L01/89), *T. versicolor* (09) and unknown fungus (02), antagonism was found at the phase of primary contact of colonies. Replacement interaction resulted usually in decreasing dark colour of substrate was observed for 11 basidial cultures that were belonging mainly to white-rot fungi. Among them *A. biennis* (123), *A. hoehnelii* (S28/91), *B. fumosa* (137), and *G. odoratum* (124) were characterized by the absence of deadlock-phase: they began to grow over dark colonies of their partners just after primary contact. Basidiomycetes did not affect strongly the pigments of *Ceratocystis* spp. and *L. sibirica* isolates, but completely

decolorized colonies of *O. ips* and to a smaller degree *O. minus*. The basidial white-rot fungi, *A. hoehnelii* (S28/91), *B. fumosa* (137) and *T. versicolor* (18), which were the most active cultures in decolorizing dark pigments produced by ophiostomatoid species during cultivation on 2% MEA, could be selected as the promising basidial fungi for biopulping of wood chips and bio-control of blue stain in woods.

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