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Decolorization of Blue-Stain by Dual Culture of Blue Staining and Basidial Fungi*1

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ABSTRACT

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 blue staining fungi. The conjoint cultivation was studied on 2% malt extract agar. The ability of basidial cultures to decolorize dark pigments of ophiostomatoid fungi was the main characteristics estimated during this study. More than half of basidial cultures were characterized by deadlock interaction with blue staining fungi. In the dual cultures, where basidial partners were presented by *Agaricus bisporus*(64), *Laetiporus sulphureus*(L01/89), *Trametes 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 *Abortiporus biennis*(123), *Antrodiella hoehnelii*(S28/91), *Bjerkandera fumosa* (137), and *Gleophyllum 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 *Leptographium sibirica* isolates, but completely decolorized colonies of *Ophiostoma ips* and to a smaller degree *Ophiostoma minus*. *Antrodiella hoehnelii*(S28/91), *Bjerkandera fumosa*(137), *Gleophyllum odoratum*(124) and *Trametes versicolor*(B18/91) cultures were found to be the most active in decreasing dark color of blue staining fungi colonies. The cultures were recommended for further development as agents of biopulping of wood chips and bio-control of blue stain in woods.

Keywords : white-rot fungi, blue staining fungi, biopulping, ophiostomatoid fungi, decolorization

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. Methods of biological control that are in progress currently for wood protec-

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tion from blue stain are observed. The increasing interests in natural plant extractives that are able to inhibit both blue stain and destructive fungi are noted. 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 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.

Fungal antagonism is considered to be a promising direction of wood protection, which corresponds to ecological safety. Until the recent time wood-inhabiting basidiomycetes did not pay special attention as antagonists of blue staining fungi, because of their own destructive activities to wood. Nevertheless, a few recent papers were devoted to study interactions between blue staining and basidial fungi. It was admitted that the latter could be used as agents of controlling blue stain in pulp and paper industry. It has been shown that some basidial species are able to inhibit growth of sap staining fungi by competition for nutrient sources, through mycoparasitism and releasing the antibiotic-like substances (Croan and Highley, 1991). The promising results were obtained with white-rot fungus, *Phlebiopsis gigantea* used as an agent of bio-control of *Ophiostoma* and *Leptographium* fungi in pulpwood (Behrendt and Blanchette, 2001). It has been also revealed that white-rot fungi can be parasites of *Ceratocystis coerulescens*. *Bjerkandera adusta* metabolites have been reported to decolorize pine veneer discs colonized by *C. coerulescens*. The latter phenomenon may be connected with peroxidase activity of basidiomycetes (Benko and Henningson, 1986; Croan and Highley, 1991).

Apart from the ability to inhibit the growth of blue staining fungi, some white-rot fungi have been reported to reduce the extractive contents in wood chips (Fisher *et al.*, 1994; Bechrendt and Blanchette, 1997; Martinez-Inigo *et al.*, 1999). They seem to utilize easily the extractives of heartwood of conifer species that are differentiated from those of sapwood in compositions and contents (Zheng *et al.*, 1995; Martinez-Inigo, 1999). Pretreatment of wood chips by white-rot fungi that are well known as the selective lignin destructors is enable significant saving of energy required for mechanical pulp refining. 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 blue staining fungi.

2. MATERIALS and METHODS

2.1. Fungal Strains

Twenty eight isolates belonging to 18 species of basidiomycetes were used in this study as follows (codes of isolates are enclosed in parentheses): *Abortiporus biennis* (Bull. Ex Fr.) Sing (123), *Agaricus bisporus* (Lge.) Sing (64), *Antrodiella hoehnelii* (Bres.) Niemela (S28/91), *Bjerkandera adusta* (Willd.: Fr.) P. Karst (B04/91, B13/91), *Bjerkandera fumosa* (Pers. Ex Fr.) Karst (137), *Daedaliopsis confragosa* (Bolt.: Fr.) Schroet (B35/91), *Gleophyllum odoratum* (Wulf. Ex Fr.) Imaz. (124), *Fomitopsis pinicola* (Swartz.: Fr.) P. Karst. (B03/91, B15/88), *Laetiporus sulphureus* (Bull.: Fr.) Murril. (L 01/89), *Lentinus edodes* (Berk.) Sing. (101), *Lenzites betulina* (Fr.) Fr. (S23/91), *Nematoloma frowardii* Horak (275), *Panus rudis* Fr. (S25/91), *Piptoporus betulinus* (Bull.: Fr.) P. Karst. (B02/91, B

21/91), *Pleurotus eryngii* (Jacq. Ex Fr.) Quel. (102), *Pleurotus ostreatus* (Jacq. Ex Fr.) Kumm (103, P15/93), *Trametes gibbosa* (Pers.: Fr.) Fr. (S20/91), *Trametes versicolor* (L. Ex Fr.) Pil. (07, 09, N20, B08/91, B18/91), and unknown species (02, 528).

Eighteen isolates of 11 blue staining fungi, mainly from Ophiostomataceae family, were also used in this study as follows (codes of isolates are enclosed in parentheses): *Aureobasidium pullulans* (de Bary) Arnaud (Dap), *Ceratocystis laricicola* Redfern & Minter (lrc 41/98, lrc 52/98, lrc 53/98), *Ceratocystis polonica* (Siem.) C. Moreau (pln 01/95, pln 24/96), *Leptographium sibirica* Jacobs & Wingfield (deg 01/02, deg 09/99), *Ophiostoma ainoae* H. Solheim (ain 14/95), *O. brunneo-ciliatum* Mathiesen-Kaarik (ain 37/98), *O. europhioides* (Wright & Cain) (eur 09/95), *O. ips* (Rumb.) Nannf. (ips 29/94, ips 41/98), *O. minus* (Hedgc) H. & P. Syd. (mns 05/99), *O. piliferum* (Fries) H. & P. Syd (plf 14/94, plf 16/94), and *O. penicillatum* (Grosm.) Siem. (pnc 05/97, 13/97).

All the cultures were obtained from fungal collections of School of Forest Resources (Chungbuk National University, Cheongju, Korea) and Department of Physical and Chemical Biology and Biotechnology of Woody Plants (V.N. Sukachev Institute of Forest SB RAS, Krasnoyarsk, Russia). Cultures were maintained on 2% malt extract agar (MEA) at 4°C.

2.2. Interaction between Basidial and Blue Staining Fungi During Dual Culture

Five basidial cultures 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) (in parentheses are listed short codes of isolates used in this paper for tables and the diagram). Besides them, nine isolates of blue staining fungi 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 and blue staining fungi 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 fungus on 2% MEA. The opposite site was inoculated with agar disk of a blue staining fungus. 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 fungi to decolorize dark pigments, blue staining fungi 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. Terms proposed by A.D.M. Rayner and L. Boddy (1988) were used for describing interaction types. Intensity of dark colour in cultures estimated visually from 0 (dark pigments absent) till 4 (very dark colour).

3. RESULTS and DISCUSSION

When cultivated in dual culture on 2% MEA basidial and blue staining fungi demonstrated three types of agar interaction:

Table 1. Distribution of basidial cultures among types of dominating interaction with blue staining fungi

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

Table 2. Changing intensity of dark colour in dual cultures of basidial and blue staining fungi growing on 2% MEA

Incubation, day		5					21					40				
Code of isolate		B18	S28	124	137	528	B18	S28	124	137	528	B18	S28	124	137	528
Ceratocystis spp.	l41	4	4	4	4	4	3	3	3	3	3	2	2	1	1	2
	p24	2	2	2	2	2	1	1	1	1.5	1.5	1	1	0	0.5	0.5
Leptographium spp.	g1	3	3	3	3	3	2.5	2	3.5	2	3	0.5*	0.3*	2.5	0.3*	0.3*
	g9	3	3	3	3	3	2.5	2	3.5	2	3.5	0.3*	0.5*	1	0.5*	0.5*
Ophiostoma spp.	f16	1	1	1	1	1	1.5	2	1	1.5	2	0.3*	0.3*	0	0.3*	1
	i29	3	3	3	3	3	1.5	1	2	1	2	0	0	0	0	0.5*
	i41	3	3	3	3	3	2	2	2.5	1	2	0	0	0	0	0
	m5	3	3	3	3	3	2	2	2	2	2.5	0.3*	0	0	0	0
	n13	3	3	3	3	3	3	3	2	3	3	1.5	1	0	1.5	1

* 0.3~0.5 trace amount of dark pigments in agar

– **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 cultures were char-

Table 3. Decrease in dark pigmentation in the process of overgrowing blue staining fungi colonies with basidial cultures

Incubation, day		0					30					60				
Code of isolate		B18	S28	124	137	528	B18	S28	124	137	528	B18	S28	124	137	528
Ceratocystis spp.	l41	3	3	2	2	3	2	3	1.5	2	3	1.5	3	3	1.5	3
	p24	2	2	2	2	2	1	1	1	1.5	1	1	0.5*	0	1	1
Leptographium spp.	g1	2	2	2	2	2	1.5	0.5*	2	1.5	2	1.5	0	3	1	2
	g9	2	2	2	2	2	1	0.5*	2	1.5	1.5	0.5*	2	2	0.5*	1
Ophiostoma spp.	f16	1.5	1.5	1.5	1.5	1.5	1.5	2	1	1.5	2	2	2	1.5	1	2
	i29	2	2	2	2	2	1.5	1	2	1	2	0	0	0	0	0
	i41	3	3	3	3	3	2	2	2.5	1	2	0	0	0.5*	0	0
	m5	4	4	4	4	4	2	2	2	2	2.5	1.5	0	1.5	0	0.5*
	n13	4	4	4	4	4	3	3	2	3	3	1.5	1	1.5	0	1

* 0.3~0.5 trace amount of dark pigments in agar

acterized by deadlock interaction with blue staining fungi 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 blue staining fungi 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.

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).

Changing dark colour in colonies of blue staining fungi 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 blue staining fungi 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).

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 controlling blue stain is required through the screening many cultures in laboratory. Lack of information about types of interaction between basidial and blue staining fungi makes it impossible to designate some taxon or ecological group as the most promising bio-controlling agents. Additionally, process of decolorization may be influenced by many factors including aeration and humidity conditions in substrates, pH and temperature etc. 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 blue staining fungi.

4. CONCLUSIONS

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 blue staining fungi. To study the interaction between basidial and blue staining fungi isolates the dual culture technique was used.

More than half of basidial cultures were characterized by deadlock interaction with blue staining fungi. 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 biocontrol of blue stain in woods.

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