Preliminary Approaches On Decolorization of Blue-Stained Wood Chips By Basidial Fungi

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SUMMARY

Interaction between wood-destroying basidiomycetes and blue stain fungi were studied during conjoint cultivation on 2% malt extract agar. The ability of basidial cultures to decolourise dark pigments of ophiostomatoid fungi was the main characteristics estimated during this investigation. *Antrodiella hoehnelii* (S28/91), *Bjerkandera fumosa* (137), *Gleophyllum odoratum* (124), *Trametes versicolor* (B18/91) cultures were found to be the most active in decreasing dark colour of blue stain fungi colonies. The cultures were recommended for further development as agents of biopulping and control of blue stain fungi in wood chips.

Key words: white rot fungi, blue stain fungi, biopulping.

INTRODUCTION

Blue stain of sapwood is a serious problem in many countries of the world, and search for new methods of wood protection that are cost effective and harmless to

environment is continued at present. Fungal antagonism is considered as a promising direction of wood protection, which corresponds to ecological safety. Until the recent time wood-inhabiting basidiomycetes did not draw special attention as *antagonists of blue stain fungi* because of their own destructive activity. Nevertheless, a few recent papers were devoted to studying interactions between blue stain and basidial fungi, for 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 sapstain fungi by competition for nutrient sources, through mycoparasitism and releasing the antibiotic-like substances (Croan, Highley, 1991). The promising results were obtained with white-rot fungus *Phlebiopsis gigantea* used as an agent of biocontrol of *Ophiostoma* and *Leptographium* fungi in pulpwood (Behrendt, Blanchette, 2001). It has been also revealed that white-rot fungi can be parasites of *Ceratocystis coerulescens*, and *Bjerkandera adusta* metabolites have been reported to decolourise pine veneer discs colonised by *C. coerulescens*. The latter phenomenon may be connected with peroxidases activity of basidiomycetes (Benko and Henningson, 1986; Croan and Highley, 1991).

In this work we proceeded from the assumption that agents of biopulping with ability to inhibit growth of blue stain fungi and to decolourise their dark pigments could provide more effective utilization of wood in pulp- and paper-making industry. The aim was to study the interactions between basidiomycetes and *Ophiostomataceae* fungi cultivated on nutritive agar for selection of cultures that were able to inhibit growth and to decolourise dark pigments of blue stain fungi at the same time.

MATERIALS AND METHODS

28 isolates belonging to 18 species of basidiomycetes were used in present study as follows: *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), unknown species(02, 528).

18 isolates of 11 blue stain 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 (ain37/98), *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), *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 V.N. Sukachev Institute of Forest SB RAS, Krasnoyarsk, Russia. Cultures were maintained on 2% malt extract agar (MEA) at 4℃. Five basidial cultures were selected after extended preliminary test as agents of decolouration for further study: *A. hoehnelii* (S28), *B. fumosa* (137), *G. odoratum* (124), *T. versicolor* (B18), unknown species (528). Besides them, nine isolates of blue stain 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), *O. penicillatum* (n13).

To study the interaction between basidial and blue stain fungi isolates the dual culture technique was used (Croan, Highley, 1991). Three replicates were used for each combination basidial culture blue stain fungus . Interactions between fungi and progress of decolouration 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).

RESULTS AND DISCUSSION

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

- **deadlock** after first contact of colonies expansion of the 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 characterized by deadlock interaction with blue stain 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. The deadlock-type of interaction followed antagonism and remained constant until the end of experiments,. Though there was limitation of blue stain fungi expansion through the common substrate in this case, deadlock-type of interaction including antagonism seemed to be not of interest for wood protection, because contest of

two mycelia often stimulated producing additional pigment in their contact area with increasing dark colour of whole substrate.

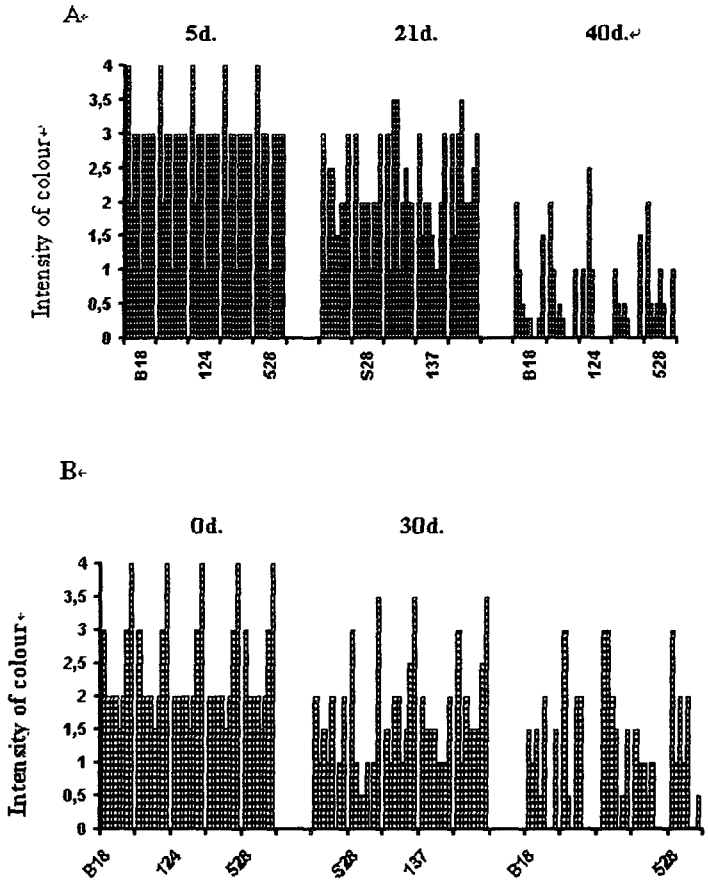

Fig. 1. Decrease of dark colour in dual cultures of basidial and blue stain fungi (A) and in the process of overgrowing blue stain fungi colonies with basidiomycetes (B). Basidial cultures are presented as follows: *A. hoehnelii* (S28), *B. fumosa* (137), *G. odoratum* (124), *T. versicolor* (B18), unknown species (528).

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 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 decolouration of dark pigments after 40 days cultivation in dual culture with ophiostomatoid fungi Fig. 1). Completed decolouration or trace amount 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. Intensity of dark colour of *Ceratocystis* sp. and *L.sibirica* isolates was decreased by some of their basidial partners though not to completed bleaching.

Changing dark colour in colonies of blue stain 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 stain fungi seemed to be more resistant to affecting of basidiomycetes (Fig. 1). In this case basidiomycetes did not affect strongly the pigments of *Ceratocystis* spp. and *L. sibirica* isolates, but completely decolourised 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.

It can be suggested from our results that white-rot fungi decolourise dark pigments of *Ophiostoma* species easier than those of *Ceratocystis* genus. Such resistance to decolouration may be connected with differences of melanins chemical compositions of this 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 of their metabolites. By contrast, *Leptographium* species closely relates to ophiostomoid fungi, for many of them are anamorphs of *Ophiostoma* spp. (Jacobs and Wingfield, 2001).

During the experiments, many cases of inter- and intraspecific variability were observed that showed the necessity to select basidial fungi for controlling blue stain through screening many cultures in laboratory. Additionally, process of decolouration may be influenced by many factors including aeration and humidity conditions in substrate, pH, temperature etc. So, it is obvious that successful using white-rot fungi for stain control of wood in pulp and paper-making industry depends on the progress of our study of biology and ecology of wood-inhabiting fungi.

Finally, the results of this study showed that the basidial white-rot fungi *A. hoehnelii* (S28/91), *B. fumosa* (137) and *T. versicolor* (18), which were the most active cultures in decolourising the dark pigments produced by ophiostomatoid species during cultivation on 2% MEA, could be selected as the promising agents for treatment of stained wood chips in papermaking. These cultures will be used for further investigations.

ACKNOWLEDGEMENTS

This work was carried out with the support of KOSEF Grant (R01-2001-000519). In addition authors are very much appreciated for the Russian Scientist Invitation Program (02-4-21-1) by KISTEP.

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