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Assessment of selected decay Basidiomycetes for selective biodefibrillation of **Picea abies** wood

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We studied the capacity of selected Basidiomycetes (72 species; 109 strains) to defibrillate *Picea abies* wood blocks, and determined the remaining cellular cohesion and the lignin content in the wood after treatment. Forty strains were sufficiently aggressive to invade non-sterile wood blocks in laboratory conditions; but only seven of them – *Gloeophyllum trabeum, Gloeoporus taxicola,* two strains of *Phanerochaete velutina, Polyporus badius, Resinicium bicolor* and *Trametes versicolor* produced an significant biodefibrillation. A combination of *Gloeophyllum trabeum* and *Gloeoporus taxicola* or *Gloeophyllum trabeum* and *Resinicium bicolor* created a synergetic effect and a nearly 70 % loss of the cellular cohesion. The use of selected rot fungi as pre-treatment to save wood pulping energy in several manufacturing processes is discussed.

Industrial delignification of wood is traditionally carried out by various mechanical, chemical or thermal treatments. However, in natural environments, filamentous fungi are the dominant decomposer of wood fiber (CARREIRO & KOSKE 1992, NIEMELÄ, RENVALL & PENTTILÄ 1995) and their use for modifying lignocellulose is a recent innovation.

At present, an economically important application of fungi is delignification of chipped wood as a pre-treatment for pulping paper industry (AKHTAR et al. 1993, SAYADI & ELLOUZ 1995, MESSNER 1998). The objective of this pulping process is to separate wood into its constituent fibers and to remove appreciable amounts of lignin. However, lignin is an essential humic precursor substance needed to generate soil amendments.

The main component of rooting media for vegetable transplant production is peat. The advantage of peat is its physical properties that allow an adequate air to water ratio in the root zone and a high cation exchange capacity (RAVIV et al. 1986). However in Switzerland, the peat bogs are protected and it is forbidden to exploit them since 1991 (protection of swamp's law, RS n°451). For that reason, several Swiss industries have been recently using *Picea* wood fibres as an ingredient of different horticultural products as a peat substitute. The wood fibres present a large content of easily available water, a high fraction of large pores which facilitate the oxygen interchange, and up to 25% lignin (FENGEL & WEGENER 1989). Nevertheless, this industrial pulping is accomplished in several countries by mechanical and thermal treatments that are energy consuming.

Composting is also an industrial method usually used to produce wood fibers and soil amendments (RAVIV, ZAIDMAN & KAPULNIK 1998). In this biological process, mixed microbial communities partially decompose plant material into an agricultural product. The disadvantage of this natural non-energy consuming process, is the prolonged time required for degrading wood. Potentially, these disadvantages could be reduced by substituting the natural microbial communities by more efficient wood-rot fungi.

In a previous study (JOB, KELLER & JOB 1991, 1996), we observed that some of the most frequently appearing rot fungi in Swiss forest, degrade selectively the external layer of the sulphite pulp paper fibers, without loss of appreciable amounts of lignin in the inner walls. Henceforth, we decided to study the capacity of 109 wood rot strains, preserved in our laboratory, to defibrillate *Picea abies* wood blocks, and to determinate, in comparison with a traditional compost process, the remaining cellular cohesion in the wood blocks after treatment by a selection of the most active fungi.

Materials and methods

Wood: *Picea abies* hard wood blocks without imperfections or knots were used. A 15.6 cm^3 (25 x 25 x 25 mm each side) blocks were used for the selection of the most active strains and 85.2 cm^3 (44 x 44 x 44 mm each side) blocks were used for analyzing the wood defibrillation process by the selected strains in function of time.

Organisms: the 109 strains of wood rotting Basidiomycetes (72 species) tested in this study, are listed in Table 1 and maintained in the culture collection of the Laboratory of Microbiology at the University of Neuchâtel (Switzerland).

Tab. 1. Capacity of different species of Basidiomycetes (strain collection number in brackets) to invade the 15.6 cm^3 blocks of *Picea abies* in non-sterile conditions

Strains able to completely colonize the *Picea abies* blocks after 7 weeks	**Strains not able to colonize the *Picea abies* blocks after 7 weeks**
Basidioradulum radula (4)	*Antrodia mollis* (76, 88) - *A. serialis* (9)
Gloeophyllum sepiarium (54, 55, 58)	*Auricularia auricula-judae* (65, 93)
Gloeoporus taxicola (23)	*Basidiodendron caesiocinereum* (20)
G. trabeum (56)	*Bjerkandera adusta* (25)
Grifola frondosa (64, 104, 107)	*Cerrena unicolor* (61)
Lyophyllum ulmarium (1)	*Coniophora puteana* (36)
Phanerochaete velutina (5, 17)	*Daedalea quercina* (77)
Phlebia lilascens (19, 90)	*Daedaleopsis confragosa* (21, 57)
P. livida (103)	*Exidia thuretiana* (15)
P. radiata (39, 112)	*Funalia trogii* (12)
Pleurotus ostreatus (108, 113)	*Ganoderma applanatum* (8, 82) - *G. carnosum* (40, 95)
Polyporus badius (46)	*Gloeophyllum abietinum* (6) - *G. protractum* (62)
P. brumalis (22)	*Hapalopilus rutilans* (49)
P. ciliatus (42, 101)	*Heterobasidion annosum* (16)
P. tuberaster (53)	*Hymenochaete tabacina* (29)
P. varius (37)	*Hyphoderma radula* (100)
Pycnoporus cinnabarinus (71)	*Laetiporus sulphureus* (41)
Resinicium bicolor (30, 32, 35)	*Laricifomes officinalis* (70, 105)
Sparassis crispa (68, 89, 106)	*Meruliopsis corium* (14)
S. laminosa (69, 109, 111)	*Peniophora cinerea* (43, 96, 97) - *P. incarnata* (18, 92)
Trametes gibbosa (27)	*P. limitata* (33) - *P. picea* (38) - *P. quercina* (44)
T. hirsuta (11, 110)	*Phaeolus schweinitzii* (68)
T. versicolor (2, 78)	*Phellinus conchatus* (13 , 102) - *P. hartigii* (75, 99)
	Phlebia lilascens (80) - *P. livida* (3) - *P. rufa* (47, 51)
	Piptoporus betulinus (52, 83, 85)
	Polyporus mori (45, 91) – *P. umbellatus* (60)
	Porpomyces mucidus (50)
	Radulomyces confluens (7)
	Schizopora paradoxa (10, 81)
	Sistotrema brinkmannii (24)
	Skeletocutis nivea (72, 86)
	Steccherinum laeticolor (63)
	Stereum hirsutum (48, 94, 98) *S. ochraceo-flavum* (31)
	Trametes gibbosa (28) - *T. subaveolens* (73)
	Trichaptum abietinum (34)
	Tubulicrinis subulatus (26)
	Tyromyces chioneus (74, 79)

Inoculation: three non sterilized wood blocks (humidified by immersion at aw: 0.66 to 0.69) were used for each strain. The inoculation was made simultaneously above and below the longitudinal wood block sides with 15–20 wheat grains completely invaded by the fungus. In the experiment of synergy between two species, one strain was inoculated above the longitudinal wood block side an the other below.

Cultural conditions: for the selection of the most active strains the blocks were incubated 7 weeks in controlled conditions (non-sterile environment, temperature: 25 °C ± 2 °C, relative humidity : 85 % ± 4 %, CO_2 600–800 ppm, obscurity). For analyzing the wood defibrillation process with the selected most active strains the blocks were incubated 16 weeks in the same experimental conditions.

Growth rate: to study the growth rate, the mycelium front was measured every week, in the longitudinal and transversal external sides.

Natural compost degradation: six wood blocks (85.2 cm^3) of *Picea abies* were placed in an external 1 m^3 young compost in accordance with Nusbaumer, Aragno & Job (1996). At 12, 24 and 36 weeks two blocks were taken and their cellular cohesion analyzed.

Analytical methods

Cellular cohesion: the cellular cohesion was measured by a non-destructive method as described by Friis-Hansen (1980) modified to obtain a better reproducibility: instead of a resort

Tab. 2. Remaining wood resistance, wood density and lignin ratio after 7 weeks degradation and time for complete invasion with the selected active strains (* the needle of the penetrometer completely passed through the wood block)

Strain	Average Wood Resistance (%)	Minimal Wood Resistance (%)	Maximal Wood Resistance (%)	Wood Density g/cm^3	Total Lignin (%)	Time for Complete Invasion (weeks)
Control	100	76	126	0.40	21	
Gloeophyllum trabeum (56)	43	*	63	0.23	56	3
Gloeoporus taxicola (23)	61	59	73	0.33	35	2
Phanerochaete velutina (5)	54	37	66	0.28	39	1
Phanerochaete velutina (17)	59	49	68	0.32	37	1
Polyporus badius (46)	53	37	80	0.30	24	3
Resinicium bicolor (30)	59	46	78	0.40	24	3
Trametes versicolor (2)	46	37	54	0.36	34	3

Pylodin (LEIGHTLEY 1981), a PNR 10 penetrometer of Sommer & Runge, with a fixed weight of 2 K and a steel needle of 1 mm diameter and 2.5 g (norm ASTM D5) was used.

Each value of wood block penetration is the mean of three measurements carried out over the transversal side in the summer rings.

The remaining cellular cohesion in the wood block, RC (%) is defined as: RC = (P non-degraded wood block / P degraded wood block) X 100, given that P = penetration.

Holocellulose determination: chlorite holocellulose was determined as described by SEIFERT (1983).

Residual Klason lignin was determined in accordance with EFFLANED (1977).

The total fungal biomass in the wood block was measured as described by KIRPATRICK et al. (1989).

Results

Selection of the most active strains

Of 109 fungal strains tested, only 40 were able to colonize completely the *Picea abies* blocks after seven weeks (Table 1). The growth of the other 69 strains was very slow or clearly stopped several days after the inoculation by environmental widespread fungal contaminants (i.e. *Aspergillus* spp., *Mucor* spp., *Penicillium* spp., *Trichocladium* spp., and *Trichoderma* spp.).

With the aim to select the best strains for the future tests, we analyzed the remaining cellular cohesion in the wood blocks degraded by the 40 invasive strains. The seven most active strains are presented in Table 2. All of these strains were able to damage the blocks without an important loss of lignin.

Defibrillation process with the selected strains

Significant differences were found in the development rate among the species tested. *Gloeophyllum trabeum* and *Gloeoporus taxicola* invaded completely the transversal external side of the wood block in only four weeks, while *Trametes versicolor* and *Phanerochaete velutina* (5), grew very slowly, at nearly half rate of the former (Table 3). However, this speed rate is not entirely correlated with the degradation capacity and an important dissimilarity among the strains was also found.

Although *Gloeoporus taxicola* is the only strain that produced a loss of resistance in the wood blocks 4 weeks after the beginning of the test, the degradation rate was slow and the wood blocks had conserved 70% of their initial resistance at the end of the experiment (16 weeks). On the other hand, the brown-rot fungus *Gloeophyllum trabeum*, had a similar invasion rate than the former but produced a loss of resistance near 50% after only eight weeks (Fig. 1). The degradation process of this fungus made a destruction of the summer rings and a clear change in the wood color. The final loss of resistance was also important in the blocks inoculated with the white-rot fungi *Phanerochaete velutina* (5) and *Trametes versicolor* strains: the destruction of the summer rings of the wood by these white-rots was less important than by *Gloeophyllum trabeum* but the enzymatic activity produce, principally in *T. versicolor*, a net separation of the rings.

At the end of the test, the percent of lignin remained stable in all the white-rot fungi indicating a non-selective degradation, but went up to 40 % in the wood degraded by the brown-rot fungus *Gloeophyllum trabeum*.

Study of the synergetic effect on biodefibrillation

With the aim to study the influence of an eventual biodefibrillation synergetic effect between a brown-rot fungus and a white-rot fungus we inoculated one side of the wood blocks with *Gloeophyllum trabeum* (56) and the other side with one of the six other selected strains.

We observed that in the contact zone only two combinations produced a more rapid and important biodefibrillation

Tab. 3. Time for complete invasion of the transversal, longitudinal or inner wood by the most active strains, and total wood loss and weight of the mycelium for each selected species measured after the complete wood block colonization

Strain	transversal side invasion (weeks)	longitudinal side invasion (weeks)	Inner wood invasion (weeks)	Weight wood loss (%)	Fungal biomass (mg of dry weight)
Gloeophyllum trabeum (56)	4	5	6	21.6	276
Gloeoporus taxicola (23)	4	5	7	14.3	119
Phanerochaete velutina (5)	9	10	14	13.9	148
Phanerochaete velutina (17)	7	8	11	11.1	190
Polyporus badius (46)	9	9	11	9.6	179
Resinicium bicolor (30)	5	7	9	7.9	204
Trametes versicolor (2)	9	10	14	10.8	225

that each strains separately. The first combination was *Gloeophyllum trabeum* with *Gloeoporus taxicola* (Fig. 2). The advance mycelium fronts of these two strains were in contact three weeks after the inoculation. At the end of the experiment the remaining cohesion in this contact zone was only 38 %, versus 52 % for *G. trabeum* and 68 % for *G. taxicola* in the margin of the wood (degraded only by one strain).

The second combination was *Gloeophillum trabeum* and *Resinicium bicolor*. In this experiment the mycelium fronts of these strains were also in contact three weeks after inoculation. Nine weeks later this zone presented a remaining cohesion of only 28 % whereas in the margin (non-double contact zone) the remaining cohesion was significantly less important (Fig. 3).

Analysis of the defibrillation in an external compost

Table 4 shows the biodefibrillation (remaining resistance) obtained after 12, 24 and 36 week in a composting process with a natural mycoflora. We observed that, in these conditions, the wood defibrillation process was very slow. After nearly nine months of composting the wood blocks had a residual cohesion of 80%. No macroscopic signs of white or brown rot were observed, and the microscopic observation of the wood showed a superficial invasion of the wood by several molds.

Discussion

In vitro degradation studies must not be taken as an absolute indication of the behavior of wood-rot fungi. The use of different isolates or different cultural conditions may change the specificity of the organism. Nevertheless, these models enable us to evaluate the decomposing capacities of different species in a comparative way (JOB & WRIGHT 1986, JOB & RAJCHENBERG 1988).

Of the 109 strains tested, 40 were able to invade a non-sterile wood block in laboratory conditions but only seven of them produced significant defibrillation. Surprisingly the most active strain was *Gloeophyllum trabeum*, a brown-rot fungus, which has not the capacity to degrade lignin or produce extracellular phenol oxidase (PASZCZYNSKI et al. 1999). This capacity of defibrillation may be correlated with the strong ability of this fungus to depolymerize the cellulose of the wood fibers (JOB, KELLER & JOB 1996). As a matter of fact the microscopic analysis of the damaged rings showed an important destruction of the cellulose structures in the attacked fibers. In the wood blocks degraded by the other selected six white-rot fungi the microscopic analysis showed principally a destruction of the middle lamella complex and the external secondary wall, which explains the observed loss in the cellular fibers' cohesion. In this group of white-rot fungi we found no apparent relationship between wood weight loss and defibrillation. For example *Gloeoporus taxicola* produced a wood weight loss of 14.3 % and a 30 % loss of cellular cohesion, but *Polyporus badius* which produced a 40 % loss of the cellular co-

Tab. 4. Remaining resistance of the *Picea* wood blocks after 12, 24 and 36 weeks of degradation by a natural mycoflora in an external compost

	0 weeks	12 weeks	24 weeks	36 weeks
Control	100 %			
Block 1	100 %	89 %	87 %	82 %
Block 2	100 %	86 %	85 %	80 %

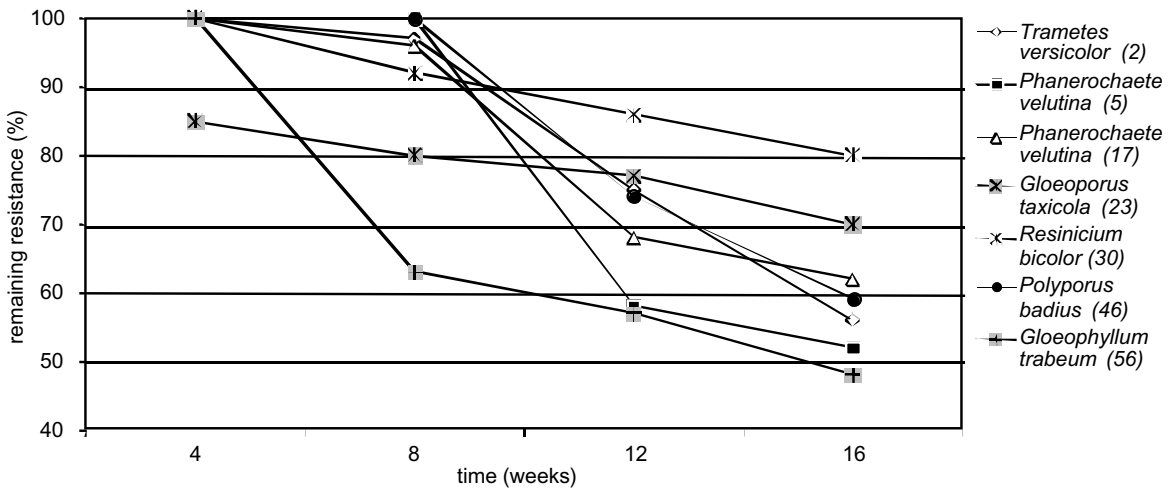

Fig. 1. remaining wood blocks resistance (in percent) after degradation by the seven selected strain of wood-rotting Basidiomycetes.

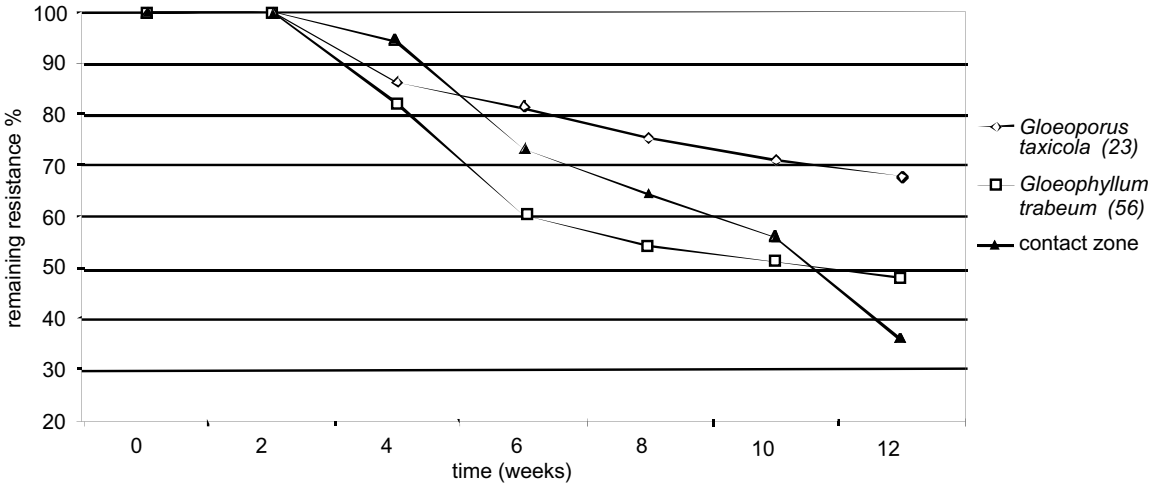

Fig. 2. Remaining cohesion in the extremity of the wood blocks degraded by only one strain (*Gloeoporus taxicola* or *Gloeophyllum trabeum*) and in the contact zone degraded at the same time by the two strains.

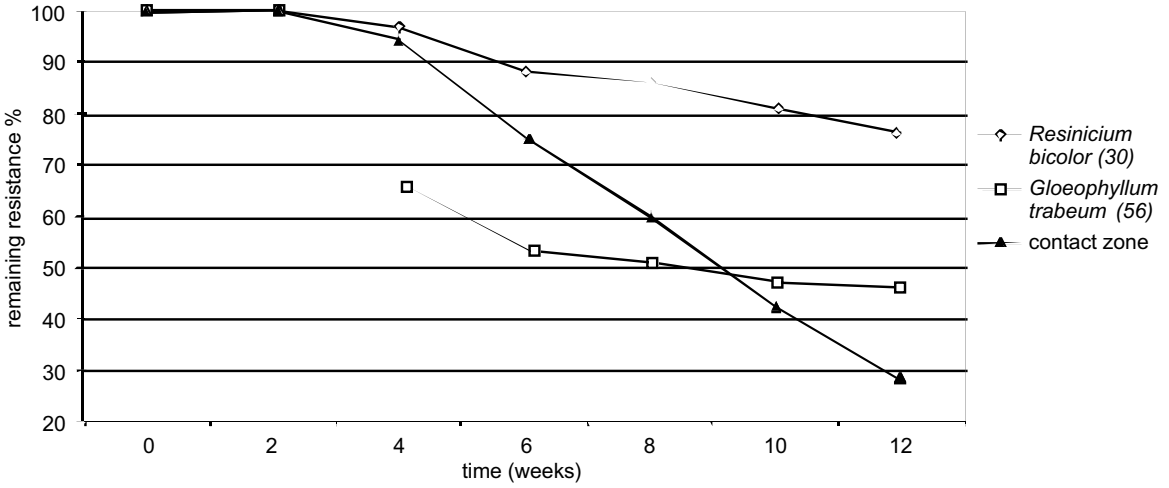

Fig. 3. Remaining cohesion in the extremity of the wood blocks degraded by *Resinicium bicolor* or *Gloeophyllum trabeum* alone one strain and in the contact zone degraded simultaneously by the two strains.

hesion engendered only 9.6 % of wood weight loss. This lack of correlation is in agreement with the results obtained by AKHTAR et al. (1993) in biopulping pine chips by white-rot fungi and revealed a difference in the behavior of the degradation. *Gloeoporus taxicola* probably produces a cellulose degradation of the cellular walls while *Polyporus badius* causes a simultaneous degradation (AKHTAR & BLANCHETTE 1997).

Several studies examining the penetrability of wood decay enzymes into the cell wall concluded that lignin peroxidases are unable to penetrate the walls of several sound wood (DANIEL et al. 1990, 1991). FLOURNOY and co-workers (1993) mentioned the infiltration of lignin peroxidases only into areas where the cell walls were disintegrated. Viewing this results a whole synergic effect in the confrontation experiment between a brown-rot fungus and a white-rot fungus was expected. However the analysis of this experiment surprisingly showed that the two most effective combinations found correspond to the simultaneous attack by *Gloeophyllum trabeum* and the less effective defibrillating white-rot fungi *Resinicium bicolor* and *Gloeoporus taxicola* The former was already found in field in a degradation association with species of the genus *Gloeophyllum* (KRIEGLSTEINER 2000) but no data were found for a natural association between *Gloeophillum trabeum* and *Gloeoporus taxicola*.

For the other four strains the competitive interaction between the strains was probably stronger and partially inhibited the degradation process. Indeed the most active white-rot strains were the two of *Phanerochaete velutina*. WELLS, HARRIS & BODDY (1998) and BODDY (1999) showed that this species is very aggressive against other superior fungi in field studies.

Important differences were also observed when comparing our laboratory experience with the defibrillation process developed in the compost. During the 36 week composting process only 20% of the cellular cohesion of the wood blocks were lost versus 70% obtained in a one third of the time in the laboratory with the combination of strains *Gloeophyllum trabeum* + *Gloeoporus taxicola* or *Gloeophyllum trabeum* + *Resinicium bicolor*. However, the experimental conditions were not the same and the fluctuations of temperature and humidity in the outdoor compost process could have delayed the natural degradation.

Finally, this work demonstrates that we can drastically reduced wood resistance with selected wood rot strains in experimental conditions. However wood is biologically degraded by the fungi only under aerobic conditions and in a restricting niche defined by axes of pH, temperature, moisture, nutrient availability and atmosphere composition.

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