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Optimized fungal defibrillation of wood using selected strains of *Gloeophyllum trabeum* and *Resinicium bicolor*

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We studied the capacity of a selected strain of *Gloeophyllum trabeum*, alone or in combination with *Resinicium bicolor*, to defibrillate non treated deciduous wood at a semi industrial composting scale. Inoculum amount, aeration of the composted wood, type (freshly cut wood and wood stored since several years) and the quantity of wood used were analysed. The remaining cellular cohesion, lignin and holocellulose, as well as fungal biomass content in the wood after various treatments were determined. Results showed that *G. trabeum* rapidly colonised the non-sterile substrate and caused greater biodefibrillation compared with the control (non inoculated wood). Effects of the various treatments on biodefibrillation were compared and are discussed.

Wood fibers are largely used in different industrial products, like in rooting media for vegetable transplant production where they replace peat as the main component. 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, CHEN & INBAR 1986, 1988). However, in Switzerland, peat bogs are protected and it has been legally forbidden to exploit them since 1991 (Protection of Swamp law, RS n°451). For that reason, several Swiss industries have been recently using wood fibers as an ingredient for different horticultural products such as a peat substitute. The wood fibers provide 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.

In the industry of edible fungi, the selection of substrate components is critical for growing gourmet mushrooms indoors (JOB & GIOVANNINI 2001), and so several processes depend on defibrillate wood debris as primary substrate components (STAMETS 1993), carried out by various energy consuming mechanical treatments.

In natural environments, filamentous fungi are the dominant decomposers of wood fibers (CARREIRO & KOSKE 1992, NIEMELÄ, RENVALL & PENTTILÄ 1995), however their use for modifying wood is a recent innovation (AKHTAR et al. 1993, SAYADI & ELLOUZ 1995, MESSNER 1998).

In a previous study (JOB 2002) we analyzed the capacity of 72 selected species of Basidiomycetes to defibrillate wood blocks at laboratory scale and showed that *Gloeophyllum trabeum* (Pers.: Fr.) Murrill headily colonised non-sterile wood and induced biodefibrillation. We also showed that a combination of *G. trabeum* and *Resinicium bicolor* (Alb. & Schwein.: Fr.) created a synergetic effect and a nearly 70 % loss of the cellular cohesion. In the present work, we investigate the ability of the previously selected strains to biodefibrillate different types of wood in semi industrial composting conditions, with the aim to verify whether the defibrillation process developed at a laboratory scale is also effective at a semi industrial scale. We also analysed the influence of several factors on wood degradation with the final objective to produce a non energy consuming defibrillation process for large-scale industrial applications.

Material and methods

Wood: two types of wood wastes were tested:

a- "Green wood": 30 to 120 cm^3 debris of branches from non-treated urban deciduous trees (principally *Alnus incarnata*, *Populus alba*, *P. nigra* and *Quercus pubescens*), cut 4 to 6 months before the experiment's start.

b- "Old wood": same type of debris as the "green wood" but exposed to natural degradation during the 4 to 5 years before the experiment in non protected outdoor conditions.

Organisms: the 2 strains of wood rotting basidiomycetes tested in this study, are *Gloeophyllum trabeum* (Gt56) and *Resinicium bicolor* (T77), both strains were isolated for wild fructifications in our laboratory and maintained in the culture collection of the Laboratory of Microbiology at the University of Neuchâtel (Switzerland).

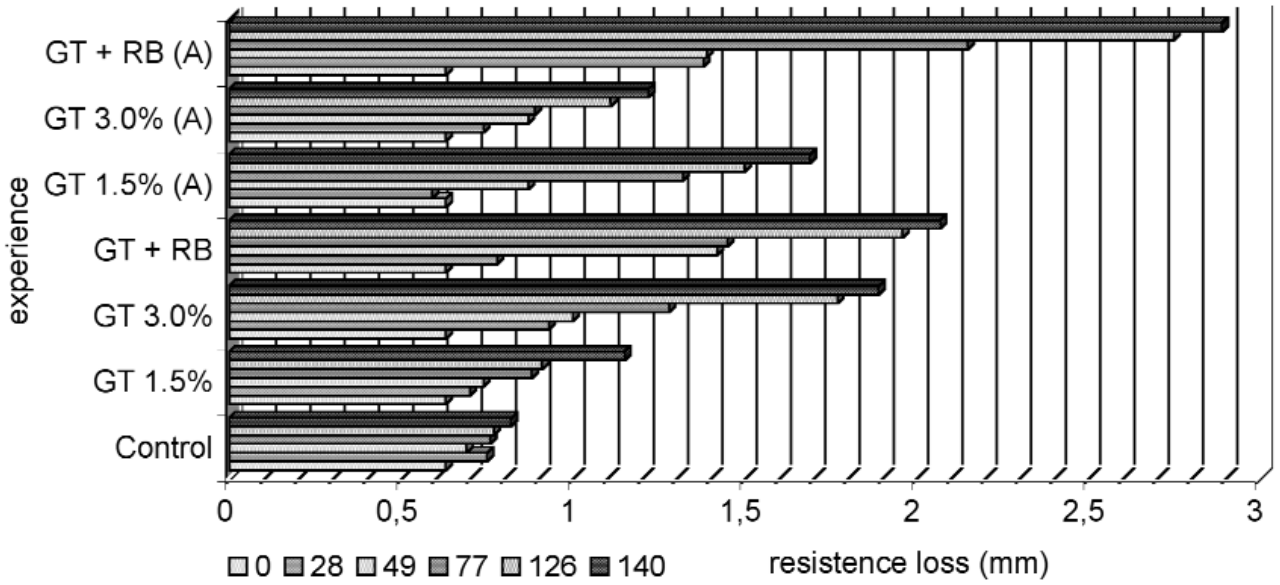

Fig. 1: Resistance loss in "Old wood" (measured in mm) in function of time in the control and the experiments carried out with the selected strains. (A) = container with aeration chimney, (GT) = *Gloeophyllum trabeum,* (RB) = *Resinicium bicolor.*

Inoculation: wood substrates were inoculated with mycelium grown on sterile birdseed. Depending on the experiments, wood substrates were inoculated either with 1.5 % (w/w) or 3 % (w/w) *G. trabeum* inoculum, or with 1.5 % (w/w) *G. trabeum* and 1.5 % (w/w) *R. bicolor* inoculum.

Containers: two sizes of boxes with metallic walls were used. 336 litre boxes (bottom area 60 × 80, height 70 cm), containing 75 kg of wood debris, and 155 litre boxes (bottom area 55 × 47, height 60 cm), containing 30 kg of wood debris, both with a grilled floor (to facilitate percolation) and a linen canvas. The experiments were run in two series: the first one with or without a central 10 cm lateral holed compost chimney to facilitate air exchange in the 155 litre boxes, and the second one with a central chimney in the 336 litre boxes.

Environmental conditions: the experiments lasted 5 months (until May to September) in a non heated shed with natural light. Temperature in the centre of the wood boxes at 30 cm depth was measured continually (minimum of 12.5 °C in the beginning of the test, to a maximum of 37.3 °C). The wood boxes were watered with cold water when the temperature exceeded 37 °C in order to stop temperature augmentation.

Sampling: 3 pieces of wood were taken both at 10 cm of the box margin and in the centre and both at 10 cm and 40 cm depth, according to NUSBAUMER, ARAGNO & JOB (1996), to be used for different analyses. The values are the average of the 12 samples collected at each time 0, 28, 49, 77, 126, and 140 days after the experiment commenced. Fungal biomass was only measured in the beginning and at the end of the experiments.

Analytical methods

Cellular cohesion: the remaining cellular cohesion in the wood was measured by a non-destructive method as described by FRIIS-HANSEN (1980) and modified to obtain a better reproducibility by JOB (2002): instead of a resort Pyloidin (LEIGHTLEY 1981), a PNR 10 penetrometer of Sommer & Runge, with a fixed weight of 2 K and a steel needle of 1mm diameter and 2.5 g (norm ASTM D5) was used.

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

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

The total fungal biomass in the wood block was measured as described by KIRPATRICK et al. (1989), and the weight of fungus (mg) was estimated according to JOB-CEI et al. (1996).

Results

Effect of strain combination, inoculum amount and aeration in the "old wood" on biodefibrillation

With the aim to select the best conditions for the biodefibrillation process, we analyzed the loss of the cellular cohesion in the wood blocks degraded under different conditions. Figure 1 shows the biodefibrillation results (in function of time) of the "old wood" degraded by natural microflora (control), of the wood inoculated seperately with *G. trabeum* or in combination with *R. bicolor*, and with or without a central compost chimney to enhance air exchange.

Substantial differences were found in the loss of wood resistance among the control and the others experiments. The final loss of resistance was more important in the containers

Tab. 1: Physical and chemical values of the "old wood" obtained in the different containers at the end of the experiments (140 days). (A) = container with aeration chimney, (GT) = *Gloeophyllum trabeum,* (RB) = *Resinicium bicolor.*

	Lignin (percent)	Holocellulose (percent)	Humidity (percent)	pH	Fungal biomass (mg/g wood)
Control	30	71	65	6.47	112
GT 1.5 %	36	66	61	6.45	194
GT 3.0 %	38	64	66	7.02	316
GT + RB	36	67	58	6.38	271
GT 1.5% (A)	35	64	59	6.66	286
GT 3.0% (A)	39	59	62	6.12	140
GT + RB (A)	34	66	55	6.94	303

Tab. 2: Physical and chemical values of the "green wood" obtained in the different containers at the end of the experiments (140 days). (A) = container with aeration chimney, (GT) = *Gloeophyllum trabeum,* (RB) = *Resinicium bicolor.*

	Lignin (percent)	Holocellulose (percent)	Humidity (percent)	pH	Fungal biomass (mg/g wood)
Control	28	72	69	5.98	96
GT 1.5 %	36	67	64	5.68	129
GT 3.0 %	38	65	66	7.02	176
GT + RB	34	69	59	6.09	214
GT 1.5 % (A)	36	66	61	5.75	183
GT 3.0 % (A)	37	69	69	5.61	165
GT + RB (A)	30	71	61	5.68	191

inoculated with the two rot fungi, with or without aeration. We could also observe that, in the synergy experiments (*G. trabeum* + *R. bicolor*), aeration was favourably influenced the biodefibrillation process, like in a normal composting process. Moreover, defibrillation was markedly better in the aerated container inoculated with 1.5 % *G. trabeum* than in the one inoculated with 3 % of the same strain. However, Table 1 shows that the final fungal biomass in this later container (140 mg/g wood) was lower than that obtained with 1.5 % inoculum (286 mg/g wood), which may explain the weak biodefibrillation value. It is possible that several natural contaminants competed with installation and growth of *G. trabeum*. However the final total amount of mycelia cannot be taken as an absolute evidence for fungal defibrillation.

Effect of strain combinations, inoculum amount and aeration in the "green wood" on biodefibrillation

Figure 2 presents the results of the biodefibrillation (according to time), of the "green wood" degraded by a natural microflora (control), of the wood inoculated with *G. trabeum* alone or in combination with *R. bicolor*, and with or without a central compost chimney. The final loss of resistance was more greater in the containers inoculated with the selected fungi, principally with the synergic combination of *G. trabeum* and *R. bicolor*, than in the control. However, we can observe that the maximum final loss of wood resistance (*G. trabeum* + *R. bicolor*, without chimney) was only half of the maximum values obtained with the same fungal combination in the "old wood" experiments. Although, in both the types of wood studied, the final defibrillation values of the control were very similar. This may indicate that our two selected species did not degrade "green wood" to the same extent as "old wood", or that they grew and colonised this substrate more slowly. The weak amounts of the total biomass estimated at the end of these experiments (Table 2), compared with the "old wood", seems to confirm this hypothesis.

Effects of the scaling up in the biodefibrillation process

With the aim to analyze, whether the biodefibrillation observed in the previous experiments may also be obtained when augmenting the amount of wood debris, we measured biodefibrillation of the two wood types in 336 litre containers, inoculated with 1.5 % *G. trabeum* and 1.5 % *R. bicolor*. Figure 3 shows that the kinetic of the biodefibrillation was similar in the two amounts analysed, and depended principally on the type of wood or the strains applied, but not on the wood quantity. We can also show, that biodefibrillation commenced in the green wood at the beginning of the process but progressed very slowly after the first 49 days. It is possible that the mycela of *G. trabeum* and *R. bicolor* use the nutrients available in birdseed inoculum to maintain a certain speed growth in the beginning of the experiments. Thesafter growth slow down after 49 days, affected by nutritional or competition factors.

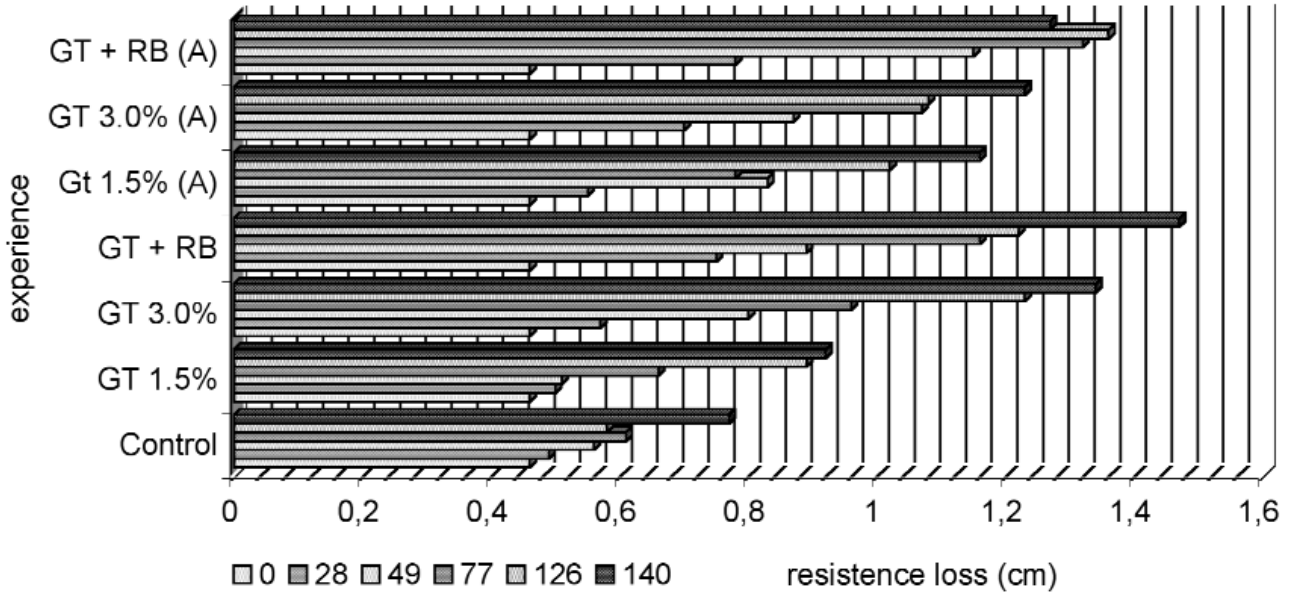

Fig. 2: Resistance loss in "Green wood" (measured in mm) in function of time in the control and the experiments carried out with the selected strains. (A) = container with aeration chimney, (GT) = *Gloeophyllum trabeum,* (RB) = *Resinicium bicolor.*

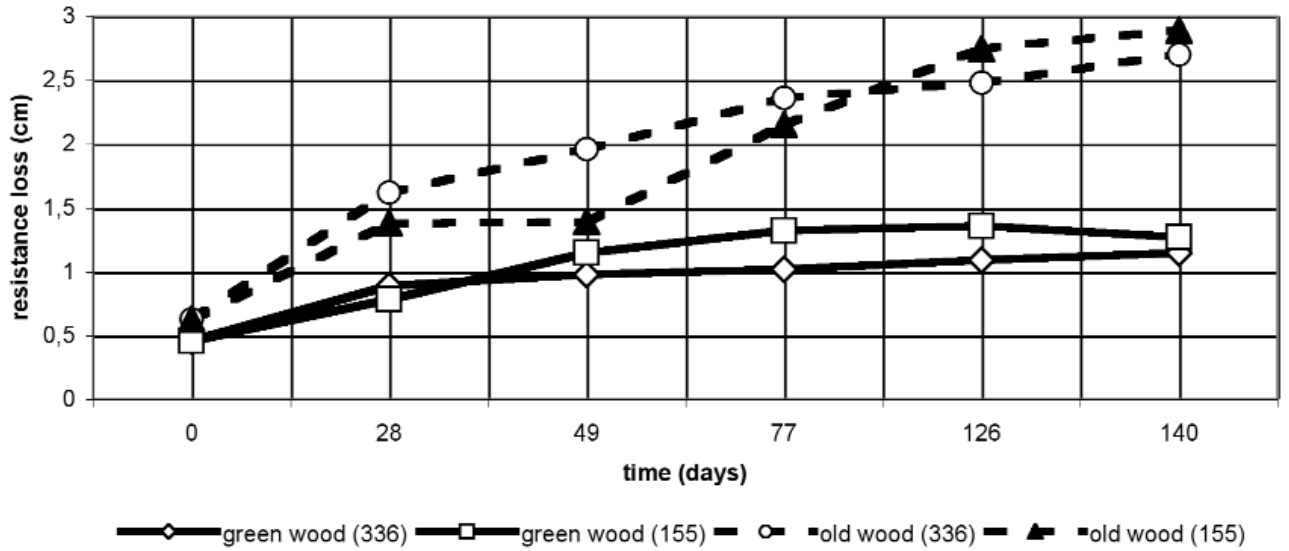

Fig. 3: Kinetic of the "old" and "green" wood resistance loss in the two aerated containers analyzed (336 and 155 litre), inoculated with *Gloeophyllum trabeum* and *Resinicium bicolor*.

Discussion

In a previous study, we showed that under laboratory conditions, a combination of *G. trabeum* and *R. bicolor* was able to biodefibrillate wood blocks of *Picea abies* resulting in a nearly 70 % loss of cellular cohesion (JOB 2002). However *in vitro* studies on degradation must not be taken as an absolute indication of the behaviour of wood-rot fungi, and different cultural conditions may alter the specificity of the organism (JOB & WRIGHT 1986, JOB & RAJCHENBERG 1988). Nevertheless, in this study, we demonstrated that these two selected strains were also able to biodefibrillate wood very strongly irrespective the different wood types (deciduous wood) and different environmental and experimental conditions. The main fungus applied, the brown-rot species *G. trabeum*, was able to colonise and degrade the wood faster, even though it das not the capacity to degrade lignin or produce extracellular phenol oxidases (PASZCZYNSKI et al. 1999). The defibrillation capacity may be correlated with the strong ability of this fungus to depolymerize cellulose of the wood fibers (JOB, KELLER & JOB 1996) and recent data suggest that *Gloeophyllum* uses an extracellular Fenton system (Fe_2+ H_2O_2) to generate hydroxyl-radical oxidants that degrade wood (DIOUF 2002). In our test we could observe its "brown-rot" activity, not only because of the drastic wood resistance loss in function of time, but also because we obtained a greater accumulation of lignin in the wood at the end of the experiments (Tables 1 and 2).

As for me observed synergic action of *G. trabeum* and *R. bicolor*, several studies examining the penetrability of wood decay enzymes into the cell wall conclude that the white rot lignin peroxidases (present in the *Resinicium bicolor* species) are unable to penetrate the walls of sound wood in several tree genera (DANIEL et al. 1990, 1991). FLOURNOY and co-workers (1993) mention the infiltration of lignin peroxidases only into areas where the cell walls were disintegrated. In this context a synergic effect in the confrontation experiment between the two degrading systems (oxidative) in the brown-rot fungus *G. trabeum* and enzymatic in the white-rot fungus *R. bicolor* may be explained. An interesting fact is that these two species were already found as a degradation association in the field (KRIEGLSTEINER 2000).

Finally, this work demonstrates that we can drastically reduce wood resistance with selected wood rot strains not only at a laboratory scale but also under semi industrial composting conditions. The capacity of *G. trabeum* to degrade treated wood (YANG & ILLMAN 1999), and to tolerate temperatures of 40 to 42.5 °C for several weeks (SCHMIDT et al. 2002), may indicate the potential of this species as a very promising defibrillator for a wide range of industrial conditions.

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