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Influence of a nitrogen supplement on the growth of wood decay fungi and decay of wood

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Abstract

Bioremediation processes require cheap and effective nutrient sources which contain significant amounts of nitrogen, e.g. corn steep liquor (CSL). In order to elucidate fungal copper tolerance in a nitrogen-rich environment, experiments were performed on a nutrient medium and with wood. CSL was added to nutrient medium containing different copper concentrations and to Norway spruce (*Picea abies*) wood specimens impregnated with a commercial copper-based preservative (CCB). Sterilized CCB-impregnated and control CSL-supplemented specimens were exposed to copper-tolerant (*Antrodia vaillantii*, *Leucogyrophana pinastri*) and copper-sensitive (*Postia placenta*, *Gloeophyllum trabeum, Trametes versicolor* and *Hypoxylon fragiforme*) fungal species according to the mini-block procedure. Additionally, nutrient media containing CSL and copper(II) sulphate of different concentrations were inoculated with the same fungi and the growth of the fungal hyphae was visually estimated. The results of both experiments showed that CSL increases the ability of the copper-sensitive brown- and white-rot fungi to grow on copper-containing substrates. CSL inhibited growth of the copper-tolerant fungi on nutrient medium containing copper and decreased decay of CCB-preserved wood. It is believed that the reason for changed copper tolerance originates in copper-tolerant fungi producing less oxalic acid in the presence of high concentrations of nitrogen in the growth environment.

Keywords: Copper-tolerant fungi; Wood decay fungi; Bioremediation; Waste-treated wood; Corn steep liquor; Nitrogen-rich substrate

1. Introduction

Copper compounds were one of the most important biocides in wood preservatives for almost 200 yr. In this period, wood decay fungi developed mechanisms that allowed them to survive on copper-treated wood. Fungi that are able to grow on the substrate of copper concentrations higher than 1.6 mmol L^{-1} are considered to be copper tolerant (Gadd, 1993). The phenomenon of copper tolerance has been known for more than 55 yr (Hirt, 1949), but has become more important during the past 20 yr. Despite this fact, the exact mechanism of copper tolerance and copper toxicity are not completely understood. Fungal Cu-tolerance is linked to oxalic acid excretion (Clausen and Green, 2003). This acid is involved in wood-rotting fungal-mediated processes of ligno-cellulose degradation, particularly in the initial phases of wood colonization. Predominantly brown-rot fungi excrete significant amounts of oxalic acid that react with copper in wood to form copper oxalate (Jarosz-Wilkolazka and Gadd, 2003; Humar et al., 2004). As copper oxalate is insoluble, it is less toxic to wood decay fungi. However, the phenomenon of copper tolerance cannot be ascribed to the formation of insoluble copper oxalate alone, as it was proved that even soluble copper is less fungitoxic in acidic substrates (pH 1–3) than in slightly acidic or even neutral conditions (Humar et al., 2005).

Copper-tolerant organisms are of scientific interest from two different points of view. Firstly, if we know the exact mechanisms of tolerance, we could develop new

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preservatives that are more efficient. On the other hand, we could use copper-tolerant organisms for biorecycling of copper-containing waste wood through bioremediation and bioconversion (Humar et al., 2004).

Biotechnological processes, such as bioremediation or bioconversion of waste CCA-treated wood, usually require an inexpensive nutrient source. In this experiment, relatively inexpensive and readily available corn steep liquor (CSL) was used. It comes from the corn wet milling process; it is already used in several biotechnological processes (Akhtar et al., 1997). CSL is a viscous concentrate of corn solubles, rich in proteins and peptides (20–25%), amino acids, lactic acid (7–9%), minerals, vitamins and other growth stimulants, with pH approx. 4. It contains 50–60% solids. Approx. 90% of the nitrogen present in CSL is amino nitrogen, >95% being present in peptides and <5% in free amino acids. The total nitrogen content of CSL, which varies from batch to batch, is 3–5% (Schroeder, 1997).

Fungi, like other organisms, require substantial amounts of nitrogen for synthesis of proteins and other cell constituents (Zabel and Morrell, 1992). However, we were interested in how the presence of a rich nitrogen environment influences the growth and decay abilities of copper-tolerant and copper-sensitive fungi in the presence of copper-based wood preservatives. The influence of nitrogen on fungal growth is interesting from a wood preservation perspective as well, since several new wood preservatives contain amines (Cao and Kamdem, 2004).

2. Materials and methods

2.1. Test on nutrient medium

Diluted solutions of copper (II) sulphate were added to 10 ml cooling sterilized potato dextrose agar (PDA, Difco) to achieve the following final copper concentrations of 1×10^{-3}, 5×10^{-3}, 1×10^{-2} and 2.5×10^{-2} mol L^{-1}. Medium without copper was used for controls. In order to determine the influence of CSL on copper toxicity, appropriate amounts of CSL were added to selected tubes to achieve a final concentration of 1 or 5%. The solidified growth media were inoculated with 0.7 cm diameter pieces of mycelium of the wood decay fungi listed in Table 1. *A. vaillantii* and *L. pinastri* strains used were shown to be copper-tolerant previously (Humar et al., 2001; Pohleven et al., 2002). The *L. pinastri* culture was kindly provided by Sam Amartey of the Forest Products Research Centre, Buckinghamshire Chilterns University College, High Wycombe, United Kingdom. The other cultures were obtained from our fungal culture collection. The tubes were then incubated in a growth chamber at 25 °C and fungal growth was assessed visually after one week of exposure and compared with growth of controls. The experiment was performed with five replicate tubes per treatment.

Fungicidal activity was estimated by fungal growth retardation, using the following visually determined marks:

0 mycelium growth more intense than control,
1 normal growth, insignificant retardation (area of colony ⩾90% of area of controls),
2 visible signs of retardation (colony <90% and ⩾60% of controls),
3 pronounced retardation (colony <60% and ⩾25% of controls),
4 very marked retardation (colony <25% of controls),
5 no growth.

2.2. Mini-block procedure

Norway spruce (*Picea abies*) samples ($0.5\times10\times30$ mm) were vacuum impregnated with 1% or 5% CCB solution according to the EN 113 procedure (European Committee for Standardization (ECS), 1989). The treatment resulted in a preservative uptake of about 4 kg/m^3 for specimens impregnated with 1% CCB solution and 19 kg m^{-3} for those impregnated with 5% CCB. The samples were then conditioned for four weeks: the first two weeks in closed chambers, the third week in half closed chambers and the fourth week in open chambers. The conditioned samples were then oven dried (75 °C) for three days in order to ensure complete reduction of chromium. Following

Table 1
Characteristics of wood decay fungi used in tests

Fungi	Origin	Estimated Cu tolerance	Type of rot
Antrodia vaillantii	University of Ljubljana ZIM L037	Cu-tolerant, 1	Brown
Leucogyrophana pinastri	Buckinghamshire Chilterns University College UK	Cu-tolerant, 2	Brown
Postia placenta	University of Ljubljana ZIM L033	Cu-sensitive/Cu-tolerant, 3	Brown
Gloeophyllum trabeum	University of Ljubljana ZIM L018	Cu-sensitive, 5	Brown
Trametes versicolor	University of Ljubljana ZIM L057	Cu-sensitive, 5	White
Hypoxylon fragiforme	University of Ljubljana ZIM L108	Cu-sensitive, 5	White

Copper tolerance is rated according to Pohleven et al. (2002): 1 = greatest copper tolerance; 5 = greatest copper sensitivity.