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THE IDENTIFICATION OF ORGANIC ACIDS IN *TRAMETES VERSICOLOR* CULTURE, GROWING ON A MEDIUM WITH CuHDO COMPLEX

Izabela Betlej*, Marcin Grąz**

*Department of Wood Protection
Warsaw Agricultural University,
**Department of Biochemistry
Maria Curie-Skłodowska University in Lublin

SYNOPSIS. In this study three organic acids produced, by white rot fungi – *Trametes versicolor* were identified. The fungi were cultivated on a liquid, mineral medium containing various concentrations of the CuHDO complex. The highest level of acids was observed on the 13th day of the growth cycle. It must be noticed that, in the presence of the copper preservative, the production of organic acids, especially oxalic acid was stimulated.

KEY WORDS: copper based preservatives, white rot

INTRODUCTION

Copper based preservatives are currently the most popular protection against wood degrading fungi. Copper, as a biocyde, is an essential constituent of impregnated wood such as CCA, (chromated copper arsenate), CCB (chromated copper boron), CC (ammoniacal copper citrate) CuHDO compositions and others (Ważny et al. 2001). Regardless, fungi can be tolerant to copper and other toxic metals (Clausen and Green 2003, Clausen et al. 2000). Brown rot fungi such as *Postia* or *Wolfiporia* genus, especially, are known to be resistant to copper treated wood (Clausen et al. 2000, Young 1961). In 1933 Rabanus proposed that fungi resistance on copper is caused by the transformation of copper in the presence of oxalic acids to insoluble, less toxic copper oxalate. Oxalic acid is produced by the fungi in response to certain levels of metal ions in a medium. On the other hand, it is also involved in xenobiotic bioremediation, metal detoxification, carbon and nitrogen biogeochemical cycles, mineral formation, ligninocellulose decay and other processes (Green and Clausen 2003, Shimada et al. 1997). The corelation between the levels of oxalic acid production and copper tolerance was

tested by several authors (Green and Clausen 2003, Clausen et al. 2000, Clausen and Smith 1998). Clausen and Green (2003) in their study on copper tolerant brown rot fungi on southern yellow pine treated with copper based preservatives, noted an accumulation of oxalic acid and copper oxalate production. Kartal et al. (2004) analyzed bioremediation of metals in the presence of oxalic acid, which was formed by *Fomitopsis palustris*, *Coniophora puteana* and *Leatiporus sulphureus*. Leaching the metals from treated wood using microorganisms has been intensivly researched. Clausen and Smith (1998) demonstrated that in the presence of oxalic acid, 81 of copper is removed from CCA treated wood. Using oxalic acid Kartal et al. (2004) proposed two possible steps to the remediation of copper from CCA treated wood. It is well known that fungi tolerant to copper intensively produce oxalic acid in presence of copper treated wood or copper preservatives. Except for *Poria placenta* which is known to produce high levels of oxalic acid in presence of copper being less tolerant to copper preservatives (Sutter and Jones 1985). An understanding of the mechanism of this tolerance could be a basis for biotechnological bioremediation of treated wood waste and also a basis for creating better wood preservatives. In this study influence of copper with CuHDO complex on levels of organic acids synthesis by white rot decay fungi – *Trametes versicolor* was examined.

MATERIALS AND METHODS

The fungus *Trametes versicolor* (L. Ex Fr.) Pil. used in the experiment was obtained from The Fungal Collection of the Department of Wood Protection SGGW. The fungus was cultivated on a liquid medium according to Fahreus. The medium contained (gl^{-1}): 20 g glucose, 2.5 g L-asparagine, 0.075 g phenylalanine, 0.027 g adenine, 1 g KH_2PO_4, 0.5 g $MgSO_4 \cdot 7H_2O$, 0.1 g Na_2HPO_4 and 50 µg of thiamine, $CaCl_2$, $MnSO_4 \cdot 4H_2O$, $ZnSO_4 \cdot 7H_2O$, $CuSO_4 \cdot 5H_2O$, $FeSO_4 \cdot 7H_2O$. The sterile medium was inoculated with 1 ml of mycelial suspension. Cultures of fungi were conditioned at 28°C for 10 days. After that CuHDO complex was added at concentrations of 0.1 and 0.05%. On the 11th, 13th, 14th, 15th and 17th day of breeding (that mean 1st, 3rd, 4th, 5th and 7th day after induction) the medium was colected and the levels of organic acids were measured. Sample without CuHDO complex was used as a control. Identification of organic acids produced by *Trametes versicolor* growing on liquid medium was detected by capillary electrophoresis (CE) on Thermo Capillary Electrophoresis model Crystal 100 (Thermo Separation Products, San Jose USA). The capillary contained an electrolyte buffer solution: 5 mM phtalic acid, 0.26 mM CTAB, 0.5% v/v methanol. Before the assay the medium was filtred on Amicon filters (10 kDa). The capillary 50 µm ID was used, the detection windows were at 50 cm. Indirect detection was used at 210 nm, applied voltage was −25 kV, capillary temperature was maintained at 25°C, injections of samples were pressured for 0.5 s. All the solutions were filtered through 0.22 µm membrane filters, before being used (Mäkelä et al. 2002). CuHDO complex (LP 15731) was

obtained from Dr Wolman GMBH (Germany). Each result is an arithmetic mean of six measurements.

RESULTS AND DISCUSSION

In this study the positive influence of the CuHDO complex on organic acids production by white rot fungi – *Trametes versicolor* was noticed. Three kinds of organic acids were identified – oxalic acid, formic acid and malic acid. Their concentration varied according to culture conditions, such as carbon and nitrogen sources in the culture medium and the pH of the environment (Dutton and Evans 1996). It is supposed that production of organic acids especially oxalic acids is one of the mechanisms of copper tolerance (Clausen and Green 2003, Gadd 1999). The concentration of the compound varied according to day of cultivation. The amount of oxalate, was clearly higher than others acids. In the presence of CuHDO complex in Trametes versicolor culture the production of organic acids clearly increased, especially on the 13th day of fungal cultivation. After this success the pool of acids was observed to have decreased and that state was more or less maintained in successive days of the cultivated cycles (Fig. 1, 2). The levels of oxalate on the 3rd day were 2.4 mM for 0.1% of the amount of CuHDO complex (Fig. 1) and 0.6 mM for 0.05% of the amount of CuHDO complex respectively (Fig. 2).

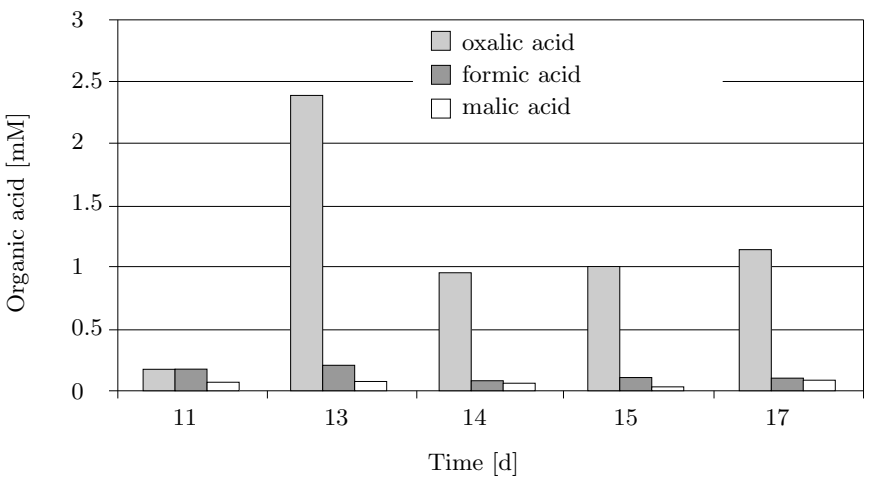

Fig. 1. Change in levels of organic acids in *Trametes versicolor* cultures, growing on a liquid medium containing 0.1% of the amount of CuHDO complex

No acids obtained in the experimental cultures were detected in the controls. Similar observations were made in other studies regarding the influence of copper preservatives on the increase in organic acids production in fungal decay wood cultures (Clausen and Green 2003, Clausen et al. 2000).

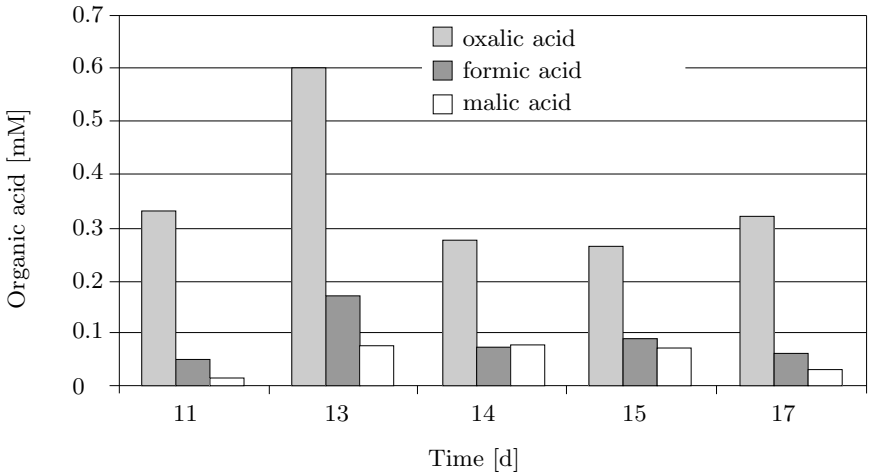

Fig. 2. Change in levels of organic acids in *Trametes versicolor* cultures, growing on a liquid medium containing 0.05% of the amount of CuHDO complex

CONCLUSION

1. In this study it was verified that the synthesis of organic acids (oxalic, formic, malic) by the white rot fungus *Trametes versicolor* was stimulated by CuHDO complex.
2. The highest levels of acids (oxalic, formic, malic) were observed on the 3rd day of fungus *Trametes versicolor* growth.

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Authors' addresses:
Izabela Betlej
Department of Wood Protection
Warsaw Agricultural University
ul. Nowoursynowska 159
02-776 Warszawa

Dr. Marcin Grąz
Department of Biochemistry
Maria Curie-Skłodowska University in Lublin
ul. Plac Skłodowskiej 3
20-031 Lublin