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Rapid copper transfer and precipitation by wood-rotting fungi can effect copper removal from copper sulfate-treated wood blocks during solid-state fungal treatment

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ABSTRACT

Copper sulfate ($CuSO_4$)-treated Japanese cedar (*Cryptomeria japonica*) blocks were cultivated with copper-tolerant wood-rotting fungi, either *Fomitopsis palustris* TYP-0507 or *Antrodia xantha* Shiga-1F. After 2 weeks, mycelia of both species had covered the blocks, but wood weight loss was not observed. At that time, oxalate accumulations were 21% (*F. palustris*) and 47% (*A. xantha*) of their maxima after 6 weeks. Within 2 weeks, the natural copper oxalate complex moolooite appeared at the interface between the wood surface and fungal mat of both species. In addition, the copper content in *F. palustris* mycelia located far from the $CuSO_4$-treated wood block was at least 5.5 times greater than that in mycelia on untreated controls. By brushing off the moolooite and mycelia, 42.9% (*F. palustris*) and 34.7% (*A. xantha*) of the original copper was removed within 2 weeks. The results showed that both species transferred copper from inside the wood blocks and precipitated some of it as moolooite before significant wood decay was observed. Furthermore, *F. palustris* transferred copper far from the wood blocks, probably through the hyphae. This rapid fungal transfer and precipitation of copper could provide a practical method for the bioremediation of CCA-treated wood.

Introduction

Copper-containing wood preservatives have been widely used to protect timber from fungal decay. However, as these wood materials reach the end of their useful life spans, copper-containing waste accumulates in large amounts. For example, by 2020, chromate copper arsenate (CCA)-treated wood is predicted to be discarded at the approximate rates of 0.3 million m^3/year in Japan (Kakitani et al., 2004) and 19 million m^3/year in the USA (Felton and De Groot, 1996).

In the USA, CCA-treated wood has been traditionally discarded through construction and demolition debris facilities, or through municipal solid waste landfills (Khan et al., 2006). However, the risk of environmental contamination during the disposal of the preservative-treated wood is an increasing concern. These materials could be inadvertently incorporated into wood fuels (Khan et al., 2006). In addition, copper-containing wood in microbially-active non-lined landfills may contaminate soil and groundwater with toxic pollutants, because a wide variety of microorganisms can leach metals from such materials (Clausen and Smith, 1998; Kartal et al., 2004; Moghaddam and Mulligan, 2008; Dubey et al., 2009, 2010). In Japan CCA-treated wood must be separated from untreated wood and incinerated or appropriately disposed of in a landfill (Ministry of Agriculture, 2001); most treated wood waste is incinerated because of limited landfill space (Hata et al., 2006). However, in a co-combustion or high temperature gasification treatment for CCA-treated wood, 11–14% arsenates are emitted during an air emission (Sasson et al., 2005; Nizihou and Stanmore, 2013). Therefore, researchers have been urged to develop systems to recover the heavy metals.

In this context, a solvent extraction and a bioremediation have been receiving much attention as alternative methods (Clausen and

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Lebow, 2011). For example, by solvent extraction using synthetic chelating agent [*S*,*S*]-ethylenediaminedisuccinic acid (EDDS), maximally about 90% copper was extracted from CCA-treated wood chip (Chang et al., 2013). Natural chelating agent, sodium bioxalate at pH 3.2, extracted about 90% copper from CCA-treated wood chip (Kakitani et al., 2009). In addition, by using wood vinegar containing an acetic acid as a main component, copper was extracted from CCA-, alkaline copper quats (ACQ)-, copper azole (CuAz)-treated sawdust with 95.7%, 97.6% and 95.7% removal (Choi et al., 2012a). Furthermore, H_2SO_4 extracted greater than 90% copper from ACQ-, CuAz-, micronized copper quaternary (MCA)-treated wood mulch (0–1.2 cm in size) (Coudert et al., 2013), although from CCA-treated wood the yield ranged 76.4%–98.5% (Coudert et al., 2014). As described above, the solvent extraction achieved an excellent copper removal. However, as a disadvantage, huge amounts of chemicals are needed for the treatment (Helsen and Bulck, 2005).

On the other hand, copper-tolerant wood-rotting fungi have been investigated to remediate heavy metal-containing wood wastes. In this procedure, these fungal mycelia or their culture media are treated with the copper-containing wood wastes (Humar et al., 2002, 2004; Kartal et al., 2004; Sierra-Alvarez, 2007; Kim et al., 2009; Choi et al., 2012b, 2013). Then, to complete copper removal, fungal mycelia on the wood surfaces are brushed off, or the treated wood sample is further extracted with an appropriate solvent. In some cases, the fungal treatment is carried out after solvent extraction (Sierra-Alvarez, 2009).

In the present study, a solid-state stationary culture of a copper-tolerant wood-rotting fungus on a heavy metal-containing wood block was termed a “solid fungal treatment”. Compared to a liquid shaking culture for the treatment with bacteria (Clausen, 2000), no energy for the shaking is required for the solid fungal treatment, which is one of the advantages. Such solid fungal treatment followed by mycelia removal or solvent extraction has been shown to remove copper, chromium, and arsenate. However, the copper yield was lower than those of chromium and arsenate. For example, 52.4% of chromium but only 15.6% of copper was removed from copper and chromium-containing Scots pine (*Pinus sylvestris* L.) sapwood blocks using the fungi *Antrodia vaillantii* and *Poria placenta* separately (Sierra-Alvarez, 2007). Solid fungal treatment using *A. vaillantii* removed 84.9% of chromium, 66.0% of arsenate, and 18.3% of copper from CCA-treated Scots pine sapwood (Sierra-Alvarez, 2009). Humar et al. showed that copper was not significantly removed from $CuSO_4$-treated Norway spruce (*Picea abies*) chips by treatment with *Gloeophyllum trabeum*, *A. vaillantii*, *Poria monticola*, or *Leucogyrophana pinastri* (Humar et al., 2002, 2004).

The low copper removal rates are due to the conversion of copper to a copper oxalate that is immobilized inside the wood (Humar et al., 2002, 2004; Kim et al., 2009). Therefore, to enhance copper removal, copper migration and precipitation during solid fungal treatment must be elucidated. Recently, Choi et al. reported that *Fomitopsis palustris* TYP-0507 and TYP-6137 removed copper oxide (CuO) from CCA-treated Japanese cedar (*Cryptomeria japonica*) blocks by the solid fungal treatment followed by removal of mycelia (Choi et al., 2012b). After 12 weeks, 50% of copper was removed, in contrast to 79% chromium trioxide and 87% diaresenic pentoxide. Importantly, Choi et al. showed that during solid fungal treatment using *F. palustris* TYP-0507 or *Crustoderma* sp. KUC8611, copper was distributed to the soil, feeder strips for fungal hyphae, and the decayed block. However, the mechanisms of the copper migration are not clear.

The purpose of this study was to elucidate the mechanisms of copper migration in relation to wood decay and oxalate production under a similar solid fungal treatment. From a view point of fungal physiology, the mechanism would be interested. We investigated the manner of copper removal during 18-week solid fungal treatments of $CuSO_4$-treated Japanese cedar (*C. japonica*) blocks using either *F. palustris* TYP-0507 or *Antrodia xantha* Shiga-1F. Within 2 weeks, both fungi transferred copper from inside the wood blocks and precipitated some as a natural copper oxalate complex, moolooite, on an interface between the wood surface and mycelial mat before the wood lost significant weight. Additionally, *F. palustris* TYP-0507 transferred copper to mycelia located far from the wood block, probably through the mycelia themselves. The importance of copper transfer and its precipitation at the interface is discussed in relation to copper removal.

Materials and methods

Chemicals and organisms

All chemicals were purchased from Nacalai Tesque (Kyoto, Japan) and Wako Pure Chemical Industries (Osaka, Japan). *Fomitopsis palustris* strain TYP-0507, a Japanese industrial standard fungus for wood-preservative efficiency tests, and *A. xantha* Shiga-1F were used from stock cultures on a potato dextrose agar (PDA) medium at Nara Forest Research Institute.

Copper sulfate-treated wood specimens

Wood specimens (20 × 20 × 20 mm) were prepared from sapwood portions of Japanese cedar (*C. japonica*). All specimens were dried at 60 °C for 48 h and weighed. Specimens were then vacuum-treated with $CuSO_4$ solution (0.127% Cu^{2+}, w/w). After treatment, specimens were reweighed, dried, and weighed again in the same way. In average, 0.4% Cu^{2+} (w/w) was contained.

Solid fungal treatment of wood specimens

Mycelial disks of *F. palustris* TYP-0507 were added to a 500-mL flask holding 300 mL of liquid medium containing 3 g glucose, 0.9 g peptone, and 6 g malt extract. The culture was incubated with shaking at 110 rpm at 27 °C for 5 days. Mycelia of *A. xantha* Shiga-1F were prepared in the same way except that the culture time was 20 d to obtain enough mycelia.

Sea sand (150 mL, size 5) was placed in a 900-mL test bottle, and a plastic net was spread on the sand. Medium (50 mL) containing 1 g glucose, 0.15 g peptone, 1 g malt extract was added and the bottle was autoclaved (121 °C, 40 min). After cooling, approximately 10 mL of shaken mycelial suspension of *F. palustris* TYP-0507 was added to the test bottle. These test bottles were cultured at 27 °C and 75% humidity for about 6 days. In case of *A. xantha* Shiga-1F, the cultivation was done for 12 days. Once the sand surface was covered with mycelia, three wood specimens were placed in each test bottle and cultured under the same conditions. Non-inoculated controls were run in parallel to determine the losses of weight and copper. Five bottle replicates were prepared for each of the test and control treatments for both fungi.

The solid fungal treatment was done twice for different durations, 18 weeks and then 6 weeks. The wood specimens were removed and the mycelia on their surfaces were brushed off after 6, 12, and 18 weeks for the 18-week treatment and after 2, 4, and 6 weeks for the 6-week treatment. The specimens were dried at 60 °C for 48 h and weighed.

The weight loss (%) was determined by the following equation: