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Differential Expression of a Putative Copper Homeostasis CutC Gene in *Fibroporia radiculosa* During Initial Decay of Copper-Treated Wood

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Abstract

Living organisms require copper for several cellular processes. Yet intracellular concentrations of copper must be regulated to avoid toxicity. Not much is known about mechanisms of copper regulation in wood decay fungi. However, one putative annotation for a copper homeostasis CutC gene (FIBRA_00129), found in other brown-rot wood decay fungi, has been annotated in *Fibroporia radiculosa*. The aim of this study was to evaluate wood mass loss and differential expression of FIBRA_00129 during initial decay of untreated and copper-treated wood by two copper-tolerant *F. radiculosa* isolates (FP-90848-T and L-9414-SP) compared with copper-sensitive *Gloeophyllum trabeum*. Untreated southern pine (*Pinus* spp.) and ammoniacal copper citrate treated southern pine at three concentrations (0.6%, 1.2%, and 2.4%) were used in a 4-week-long standard decay test. Results showed *G. trabeum* was unable to decay copper-treated wood while both *F. radiculosa* isolates successfully decayed southern pine at all copper concentrations. *G. trabeum* and *F. radiculosa* L-9414-SP showed no detectable FIBRA_00129 expression over the course of this study. *F. radiculosa* FP-90848-T showed greater FIBRA_00129 downregulation on copper-treated wood than on untreated wood ($P = 0.003$). Additionally, there was greater FIBRA_00129 downregulation in *F. radiculosa* FP-90848-T at week 3 compared with other weeks ($P = 0.015$). Future studies are needed to further evaluate FIBRA_00129 during the decay process to determine its potential role in copper-tolerance.

Metal homeostasis is critical in living organisms because metal ions are essential for certain biochemical processes. Typically, transition metals (copper, iron, zinc, etc.) are required by enzymes to catalyze various processes in cells (e.g., respiration, detoxification, acquisition, electron transfer, maintaining protein structure). Copper cycles between two oxidation states, Cu^{+} and Cu^{2+} (Lattore et al. 2011, Smith et al. 2017, Raffa et al. 2019). Copper is involved in a wide variety of functions, including generating energy, mobilizing iron, inactivating enzymes by metal displacement, interacting with and stabilizing metallophilic ligands, driving reactive oxygen species (ROS) generation through Fenton chemistry, and subsequent detoxification of ROS (Li et al. 2005, Smith et al. 2017, Raffa et al. 2019). Living organisms must strictly regulate copper levels by minimizing toxicity through acquisition, distribution, storage, and efflux (Smith et al. 2017).

The majority of research addressing metal homeostasis has been conducted in prokaryotes. In bacteria, there are two gene families associated with the uptake, storage, intracellular transport, and efflux of copper. These are *cop* genes, classified as copper efflux ATPase pumps, and the cut genes, generally classified as delivery proteins (Burkhead et al. 2009, Lattore et al. 2011, Djoko et al. 2017). In *Escherichia coli* ((Migula 1895) Castellani and Chalmers 1919), the cut genes consist of *cutA* (uptake), *cutB* (uptake), *cutC* (storage and transport), *cutD* (storage and transport), *cutE* (efflux), and *cutF* (efflux). Mutation in any of these genes can lead to an increase in copper sensitivity or copper dependency (Rouch et al. 1989; Li et al. 2005, 2010; Lattore et al. 2011). In *E. coli,* many cut family proteins are classified as

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cytoplasmic copper-binding proteins (Rogers et al. 1991, Gupta et al. 1995). Other authors have hypothesized that the cut genes could function in intracellular copper (I) trafficking (Kimura and Nishioka 1997, Rensing and Grass 2003). In bacteria, the role of *cutC* in copper homeostasis is not fully understood at this time (Zhu et al. 2005, Calafato et al. 2008, Rodrigues et al. 2008). However, differential expression in response to elevated copper concentrations indicates a connection in protection against excess copper in bacteria (Rouch et al. 1989; Gupta et al. 1995; Li et al. 2005, 2010; Zhu et al. 2005; Calafato et al. 2008; Rodrigues et al. 2008; Lattore et al. 2011).

Brown-rot fungi are the most important threat to the destruction of coniferous wood used in service (Goodell 2003). Pressure-treatment with chemical preservatives is the most common form of protection for wood products in service. Chemical preservative treatment significantly extends the service life; however, adequate protection is dependent upon chemical penetration and retention within wood (Lebow 2004, Shupe et al. 2008). Copper treatment has efficacy against a wide range of wood-inhabiting organisms and is used in most preservative formulations for residential applications and outdoor exposure (Freeman and McIntyre 2008). However, the use of copper can be problematic because of greater tolerance by certain fungi, corrosivity, and aquatic toxicity (Shupe et al. 2008, Lebow 2010). Some brown-rot decay fungi have been shown to be extremely tolerant of toxic metals in high concentrations (DeGroot and Woodward 1999, Hall 2002, Green and Clausen 2003, Hastrup et al. 2005, Freeman and McIntyre 2008). Copper-tolerant brown-rot decay fungi have shown the capability to decay copper-treated wood with copper concentrations exceeding 1.6mM l^{-1}; however, tolerance is variable with respect to fungal species and preservative formulations (Gadd 1993, Hastrup et al. 2005). The species *Fibroporia radiculosa* ((Peck) Glib & Ryvarden) has been documented to cause 30 to 49 percent mass losses in wood treated with ammoniacal copper citrate, alkaline copper quaternary, and micronized copper quaternary (Green and Clausen 2003, Jenkins et al. 2014, Ohno et al. 2017).

There is minimal information about copper homeostasis in decay fungi. Recently, four proteins with function related to copper regulation were characterized in *F. radiculosa* (Tang et al. 2012). Three of these proteins were categorized as copper-transporting ATPase pumps and one protein as a copper homeostasis CutC, FIBRA_00129 (Tang et al. 2012). Information regarding FIBRA_00129 in decay fungi is extremely limited. Currently, this gene has been annotated to have a role in regulating intracellular copper concentrations in only a few fungal genomes. *F. radiculosa, Postia placenta* ((Fr.) M.J. Larsen & Lombard), and *Serpula lacrymans* ((Wulfren) J. Schröt.; all copper-tolerant fungi) share homologous gene sequences (Tang et al. 2012). There have been no additional studies in determining expression of FIBRA_00129 during decay exposure to elevated copper levels. A baseline of data is greatly needed to understand whether this gene is a necessary component in the breakdown of copper-treated wood products.

The overall objective of this study was to compare FIBRA_00129 gene expression between copper-sensitive and copper-tolerant decay fungi. Typically, copper-tolerance necessitates the addition of co-biocides (i.e., quaternary ammonium compounds, azoles) in commercially available wood preservatives, in which the co-biocide serves as further protection against copper-tolerant fungi (Illman et al. 2000). However, to better understand how *F. radiculosa* responds to copper without co-biocides, we used the model copper preservative, copper citrate, for this study (Clausen et al. 2000, Clausen and Green 2003, Hastrup et al. 2005). In addition, we were interested in how *F. radiculosa* initiates decay; therefore, we chose to determine mass loss during early decay (weeks 1 through 4). Two copper-tolerant *F. radiculosa* isolates (FP-90848-T and L-9414-SP) were used as well as copper-sensitive *Gloeophyllum trabeum* (Pers.) Murrill (MAD 617). Expression of FIBRA_00129 was compared on both untreated and copper-treated southern pine wood. Implications from this study could elucidate components in the mechanism of copper-tolerance.

Material and Methods

Fungal cultures

Copper-tolerant *F. radiculosa* isolates (FP-98048-T and L-9414-SP; USDA-NRS-FMHC, Forest Products Laboratory, Madison, Wisconsin) were used for this study (Ortiz-Santana 2018). A copper-sensitive *G. trabeum* (MAD 617; USDA-NRS-FMHC, Forest Products Laboratory) was also used. Fungal cultures were maintained on malt extract agar (BD, ThermoFisher Scientific, Waltham, Massachusetts) at 27°C and 70 percent relative humidity (RH).

Preservative treatment and decay tests

Accelerated decay tests were set up according to the American Wood Protection Association (AWPA) Standard E10-16 (AWPA 2019). In each decay test, a southern pine (*Pinus* spp.) sapwood wafer (40 by 30 by 3 mm) was colonized by fungal mycelial plugs (cut from malt extract agar plates of a 2-wk-old culture) of the three test fungi, respectively (one fungus/wafer/jar) until sufficient colonization was achieved (about 3 wk). Southern pine test blocks (20-mm cubes) were vacuum-treated with three concentrations of ammoniacal copper citrate (composition of 62% copper oxide, 38% citric acid): 0.6 percent (3.350 kg Cu/m^3), 1.2 percent (6.888 kg Cu/m^3), and 2.4 percent (13.642 kg Cu/m^3). Vacuum-treatment followed the AWPA E10-16 Standard consisting of the following: submerging test blocks in ammoniacal copper citrate solutions respectively, applying vacuum (30 min at 100 mm Hg) followed by holding pressure at 700 kPa (100 psi) for 60 minutes, and finally holding at atmospheric pressure for 30 minutes (AWPA 2019). Untreated southern pine test blocks were also included (conditioned to 27°C and 30% RH for 2 wk). Following vacuum-treatment, test blocks were dried under a hood overnight and conditioned to 27°C and 30 percent RH for 2 weeks. Following conditioning, test blocks were steam-sterilized (20 min, 122°C) and cooled to room temperature. Two test blocks were aseptically placed in each jar on the colonized wood wafer and incubated at 27°C and 70 percent RH for up to 4 weeks. Each week, one test block from each of three jars for each treatment was brushed free of mycelia and used to measure wood mass loss ($n = 3$). Blocks were oven-dried overnight (60°C) to arrest mycelial growth then reconditioned (27°C and 30% RH) for 2 weeks prior to weighing and calculating mass loss (%). The second test block from the same jar was used for RNA extractions ($n = 3$).

Sample preparation and RNA extraction

Test blocks were ground into sawdust using a sterilized drill bit. Approximately 0.2 g of test block sawdust from each sample was placed into individual microtubes and RNA was immediately extracted. The Ambion RNAqueous Kit (ThermoFisher Scientific) was used to isolate RNA following the manufacturer's specifications with an added DNaseI (Promega Corporation, Madison, Wisconsin) digestion to removed unwanted genomic DNA contamination. DNaseI digestion was prepared according to the manufacturer's specifications and was included between the Wash Solution 1 step and Wash Solution 2 step of the RNAqueous Kit. RNA for all samples was quantified by a NanoDrop 2000 spectrophotometer (ThermoFisher Scientific). RNA yields ranged from 2 to 12 ng g^{-1} sawdust. Following extraction, all RNA samples were stored at –80°C.

RT-qPCR

Primers were designed using gene and coding sequences for FIBRA_00129 in *F. radiculosa* through National Center for Biotechnology Information GenBank. The FIBRA_00129 forward primer (5′-TTAGCTAGCCGTTGCTGGAT) and reverse primer (5′-CTCTCATGATGGCGAACTCA) spanned at least one intron. Amplicon length was approximately 250 basepairs (bp). Additionally, β-actin was used as the housekeeping gene. The β-actin forward primer (5′-GTGATGGTTGGTATGGGTCAGAAGG) and reverse primer (5′-GAAGCTCGTTGTAGAAAGTGTGATGC) were used. A preliminary electrophoresis analysis was performed from reverse transcription polymerase chain reaction (RT-PCR) products for each gene and nontemplate and reverse-transcriptase-free controls on a 2 percent agarose gel in TAE (1×) buffer. Target bands were absent from controls but present in samples for the respective amplicon lengths.

RNA (20 ng/20 μL reaction mixture) was synthesized to first strand cDNA using the SuperScript II Reverse Transcriptase Kit (Invitrogen, Carlsbad, California) following the manufacturer's specifications. cDNA synthesis was carried out on an MJ Research PTC-225 Thermal Cycler (Bio-Rad Laboratories, Carlsbad, California) with the following settings: incubation at 42°C for 50 minutes and inactivation at 70°C for 15 minutes. Both nontemplate controls and reverse transcriptase free controls were included. cDNA samples were stored at –20°C until they were processed for gene expression.

Expression profiles of FIBRA_00129 were monitored in real time using a StepOne detection system (Applied Biosystems, Waltham, Massachusetts). Samples were prepared using the PowerUp SYBR Green Master Mix (ThermoFisher Scientific) according to manufacturer's specifications. FIBRA_00129 was analyzed in duplicate (two technical replicates) for the three biological replicates ($n = 3$) for each expression plate. Nontemplate controls and reverse transcriptase free controls were included on each expression plate. The reaction protocol included an uracil-DNA glycosylase activation at 50°C for 2 minutes, an initial denaturation at 95°C for 2 minutes, followed by 40 cycles of a 15-second 95°C denaturation, 30-second annealing at 60°C, and a 45-second 72°C extension. A melt curve analysis of the resulting PCR product was performed immediately following amplification to check for primer specificity and primer dimer formation (1 min incubation at 95°C, followed by a 1-min annealing at 55°C prior to 81 cycles of 10 s with temperatures increasing by 0.5°C each cycle).

Using the housekeeping gene (β-actin; 270-bp amplicon) mean C_T values, average replicate C_T values were normalized for all plates. Normalized C_T values were used to calculate the mean fold change ($2^{-\Delta\Delta C_T}$) in CutC expression using the comparative C_T method (Livak and Schmittgen 2001). The endogenous control was set using untreated wood at week 2 for fold change calculations. Mean fold change (expression ratio) was then converted to the $\log_2$ scale to equally represent CutC upregulation (+ values) and downregulation (– values). Values presented reflect the differences between the endogenous control and each treatment in the subsequent weeks. If no value is shown in the figure, the gene was not expressed for that particular variable.

Statistical analysis

A three-way analysis of variance (ANOVA) was performed using the general linear model (GLM) to determine effects of fungi, treatment, and time on mass loss. GLM describes statistical relationships between multiple factors using mean values to find significant differences. Mass loss data was reported in percentages and therefore did not meet the assumptions of a parametric statistical analysis. Data were analyzed after applying the arcsine of the square root transformations to proportions.

Nonparametric statistical analysis using the Kruskal-Wallis test ($P = 0.05$) was conducted to determine differences among treatment and time for FIBRA_00129 gene expression (Goni et al. 2009). Treatments were grouped for each fungus prior to statistical analysis. Additionally, time points were grouped for each fungus prior to statistical analysis. Statistical analyses were performed using Minitab 17.2.1 (Minitab, LLC, State College, Pennsylvania).

Results and Discussion

Decay analysis

Figure 1 shows differences in mass loss (%) by the three test fungi on untreated and treated wood over time. ANOVA indicated significant effects of fungus, treatment, time, and all interactions (values of $P \leq 0.01$; Table 1). *G. trabeum* exhibited copper sensitivity on copper-treated wood, with mass losses below 5 percent through 4 weeks (Fig. 1A). On untreated wood, *G. trabeum* mass loss increased from 1.2 to 11.1 percent over the 4-week test (Fig. 1A). In contrast, *F.*

Table 1.—Three-way analysis of variance using the general linear model showing effects of fungi, treatment, and time on mass loss.

Interactions	dF	*F* value	*P* value
Organism (Org)	2	44.79	0.000
Treatment (Trt)	3	6.73	0.000
Time (Ti)	3	61.22	0.000
Org × Trt	6	15.54	0.000
Org × Ti	6	12.16	0.000
Trt × Ti	9	3.62	0.001
Org × Trt × Ti	18	3.99	0.000

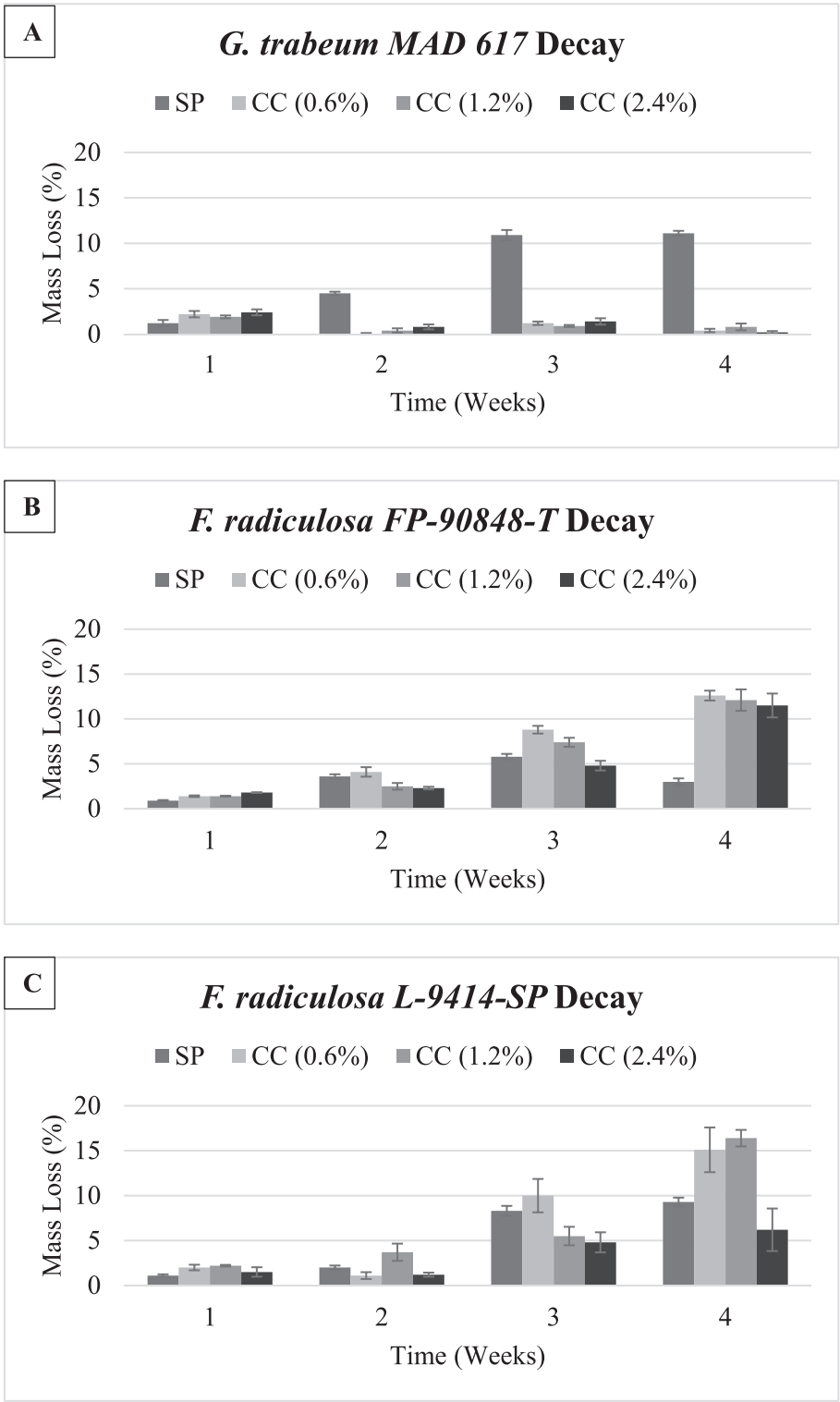

Figure 1.—Mass loss (%) ± standard error of untreated (SP), 0.6 percent ammoniacal copper citrate–treated (0.6% CC), 1.2 percent ammoniacal copper citrate–treated (1.2% CC), and 2.4 percent ammoniacal copper citrate–treated (2.4% CC) southern pine test blocks exposed to (A) copper-sensitive Gloeophyllum trabeum *(MAD 617), (B) copper-tolerant* Fibroporia radiculosa *(FP-90848-T), and (C) copper-tolerant* Fibroporia radiculosa *(L-9414-SP).*

radiculosa FP-90848-T (Fig. 1B) and L-9414-SP (Fig. 1C) were not inhibited by copper-treated wood through 4 weeks. Results of this study are consistent with previous studies, which determined that *G. trabeum* is significantly inhibited by copper-treated wood (Green and Clausen 2003; Hastrup et al. 2005, 2006; Tang et al. 2013; Jenkins et al. 2014; Ohno et al. 2017). *G. trabeum* showed inhibition on Norway spruce (*Picea abies*) treated with copper sulfate (3.9% mass loss) and copper octanoate with ethanolamine (0% mass loss) by 4 weeks (Humar et al. 2002). When grown on

Norway spruce treated with copper sulfate with potassium dichromate (CuCr) and copper sulfate with ethanolamine (CuE), *G. trabeum* had only 0.3 percent mass loss on CuCr and 0.4 percent mass loss on CuE by week 4 (Humar et al. 2006). Liew and Schilling (2012) documented similar effects of *G. trabeum* on CuE-treated wood (4.9% mass loss) after 12 weeks. In addition, these researchers reported *G. trabeum* produced 2.1 percent mass loss of micronized copper quaternary-treated wood by 12 weeks (Liew and Schilling 2012). When challenged with 1.2 percent CC-treated SP for 10 weeks, *G. trabeum* was unable to cause mass loss (Green and Clausen 2003). In a separate study, *G. trabeum* exhibited 4.8 percent mass loss on 1.2 percent CC-treated SP after 10 weeks (Hastrup et al. 2006). Jenkins et al. (2014) also showed copper sensitivity of *G. trabeum* on 1.2 percent CC-treated wood at weeks 2 and 4; however, there was no copper sensitivity when SP was not pressure-treated (dip-treated or adjacent) with copper.

Mass losses of untreated wood exposed to both *F. radiculosa* isolates were significantly less than mass losses on copper-treated wood. *F. radiculosa* FP-90848-T caused mass losses from 11.5 to 12.6 percent on all concentrations of copper-treated wood. *F. radiculosa* L-9414-SP caused mass losses from 6.2 to 16.4 percent on all concentrations of copper-treated wood. However, *F. radiculosa* L-9414-SP exhibited lower mass loss on 2.4 percent CC by week 4 compared with FP-90848-T. Results of this study are also consistent with previous studies of decay of copper-treated wood by *F. radiculosa*. Similar mass loss values were reported by Clausen and Green (2003) for another *F. radiculosa* (formerly *Meruliporia incrassata*) isolate TFFH 294 on CC-treated wood for weeks 1 through 4 (1.6%, 3.5%, 3.6%, and 8%, respectively). These researchers also documented 49 percent mass loss by *F. radiculosa* (formerly *Antrodia radiculosa*) FP-90848-T on 1.2 percent CC-treated wood by 10 weeks, and found mass loss to be significantly greater on copper-treated wood compared with untreated wood for isolates FP-90848-T and TFFH 294 (Clausen and Green 2003). Over the course of this study, the two *F. radiculosa* isolates responded differently from one another. These differences in mass loss are consistent with previous work suggesting *F. radiculosa* L-9414-SP has a delayed response in copper adaptation when compared with other *F. radiculosa* isolates (Jenkins 2012, Ohno et al. 2015). In addition, other *F. radiculosa* isolates (TFFH 294 and L-11659-SP) have been documented to have differences in oxalic acid accumulation and differential expression of genes encoding citrate synthase, isocitrate lyase, glyoxylate dehydrogenase, a succinate/fumarate antiporter and a copper-transporting ATPase pump through 8 weeks (Ohno et al. 2015).

FIBRA_00129 expression analysis

Currently, the CutC gene has been annotated in only three decay fungi: *F. radiculosa*, *P. placenta,* and *S. lacrymans* (Tang et al. 2012). This study is the first to analyze FIBRA_00129 gene expression in any of these decay fungi during the decay process. FIBRA_00129 data indicated differences in expression by the three test fungi on untreated and copper-treated wood over time. *G. trabeum* had no detectable FIBRA_00129 gene expression when exposed to either untreated or copper-treated wood over the course of this study. *F. radiculosa* FP-90848-T had no detectable FIBRA_00129 gene expression at week 1 when exposed to untreated or copper-treated wood. For weeks 2 through 4, *F. radiculosa* FP-90848-T downregulated FIBRA_00129 when exposed to untreated and copper-treated wood (Fig. 2). *F. radiculosa* FP-90848-T showed greater FIBRA_00129 downregulation on copper-treated wood compared with untreated wood ($P = 0.003$; Table 2). *F. radiculosa* L-9414-SP had no detectable FIBRA_00129 gene expression when exposed to untreated or copper-treated wood over the course of this study.

The results of this study do not support a potential role of FIBRA_00129 in managing intracellular copper levels in copper-tolerant decay fungi, at least early in the decay process. There was no detectable FIBRA_00129 gene expression in *G. trabeum* or *F. radiculosa* L-9414-SP during this study. *F. radiculosa* FP-90848-T downregulated FIBRA_00129 over the course of this study and exhibited greater downregulation of FIBRA_00129 on copper-treated wood than untreated wood ($P = 0.003$). It is thought that *F. radiculosa* L-9414-SP has a delayed response in adapting to copper environments, and that could explain why there was no detectable FIBRA_00129 gene expression over the course of the current study (Jenkins 2012, Ohno et al. 2015).

Studies of copper homeostasis in *Enterococcus faecalis* ((Andrewes and Horder 1906) Schleifer and Kilpper-Bälz 1984), and *Xylella fastidiosa* Wells, Raju, Hung, Weisburg, Parl & Beemer documented significant increases in CutC expression during prolonged copper exposure (Rodrigues et al. 2008, Lattore et al. 2011). Lattore et al. (2011) showed that there are differences in expression during copper exposure between the *cop* gene family (copper efflux ATPase pumps) and the *cut* gene family in *E. faecalis*. They suggested that *E. faecalis* has a primary response (upregulation of the *cop* genes) followed by a secondary response (upregulation of the *cut* gene) when exposed to an increased availability of copper (Lattore et al. 2011). They concluded the primary response is most likely linked to the regulation of intracellular copper, while the secondary response could be linked to changes in the redox state of the cell (Lattore et al. 2011). It is possible that measuring FIBRA_00129 gene expression in *F. radiculosa* during later decay (weeks 8 through12) could give insight as to the role FIBRA_00129 plays in managing copper environments by decay fungi.

Lattore et al. (2011) also analyzed the effects of oxidative stress by measuring thioredoxin, superoxide dismutase and catalase expression in *E. faecalis* during prolonged copper

Table 2.—Nonparametric statistical analysis using the Kruskal-Wallis test (P = 0.05) showed significant differences among treatment and time for FIBRA_00129 gene expression.

Parameter	Median	*Z* value
Treatment[a]		
0.6% CC	−0.595	2.52
1.2% CC	−1.620	−1.49
2.4% CC	−2.375	−2.71
SP	−0.585	1.65
Time[b]		
2	−0.86	1.93
3	−1.965	−2.81
4	−0.84	1.07

[a] $P = 0.003$.
[b] $P = 0.015$.

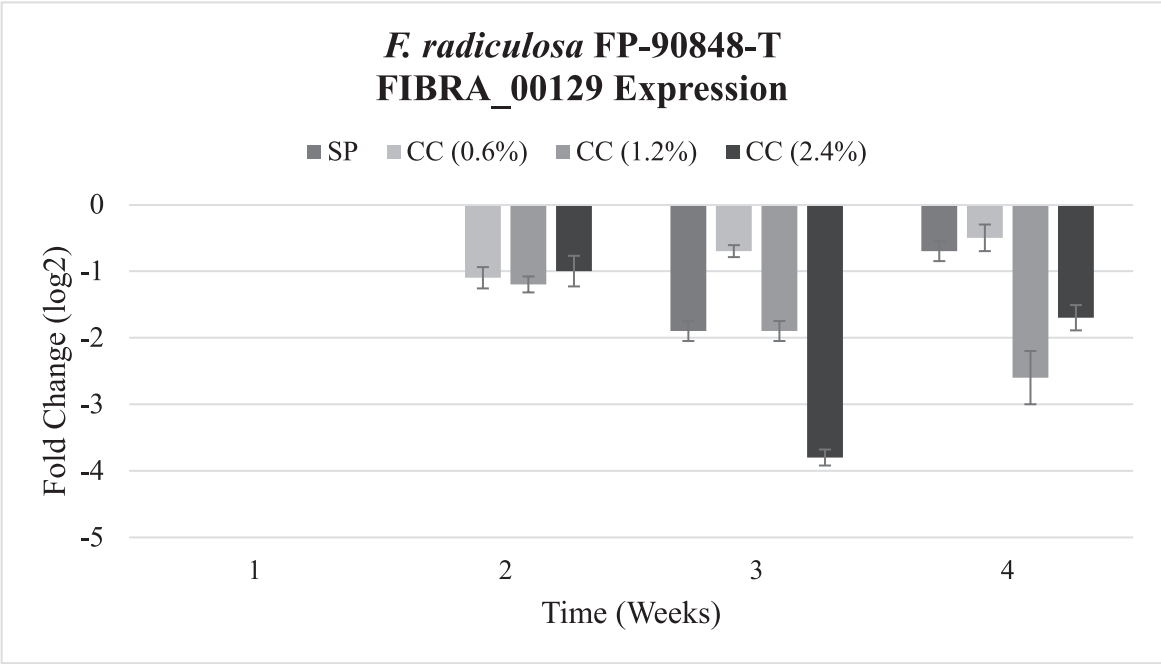

Figure 2.—Gene expression values (fold change) ± standard error of copper homeostasis CutC encoded gene (FIBRA_00129) in copper-tolerant Fibroporia radiculosa *(FP-90848-T) on untreated (SP), 0.6 percent ammoniacal copper citrate–treated (0.6% CC), 1.2 percent ammoniacal copper citrate–treated (1.2% CC), and 2.4 percent ammoniacal copper citrate–treated (2.4% CC) southern pine test blocks over the course of 4 weeks (*n = 3*). Values <0 indicate downregulation.*

exposure. They found thioredoxin expression increased significantly during prolonged copper exposure and hypothesized that increases in expression of CutC and thioredoxin are stimulated by copper (Lattore et al. 2011). Thioredoxins reduce thiol groups and are linked to a response against reactive oxygen species generated by copper (Lattore et al. 2011). In addition, several other studies have shown correlation of increased expression in oxidative stress genes (catalase and superoxide dismutase) when exposed to various metals (Schellhorn 1995, Ernst et al. 2005, Ward et al. 2008, Hoopman et al. 2011), which is logical because of the role of copper (and other metal ions) in generating reactive oxygen species (Li et al. 2005, Smith et al. 2017, Raffa et al. 2019). Future research is needed to determine whether these oxidative stress genes play a role in copper-tolerance. Tang et al. (2012) annotated 13 genes with functions related to thioredoxins in *F. radiculosa*. Future studies should determine whether thioredoxin (and other oxidative stress enzymes) encoded gene expression is increased in decay fungi.

Conclusions

The goal of this research was to contribute to the understanding of the mechanism of copper-tolerance in *F. radiculosa*. Copper sensitivity exhibited by *G. trabeum* and copper-tolerance showed by *F. radiculosa* supports previous findings. The results from this study suggest different isolates of *F. radiculosa* employ different mechanisms when decaying copper-treated wood. During this research FIBRA_00129 gene expression was measured for the first time in a wood decay fungus. However, the results from this study do not support the involvement of FIBRA-00129 in the mechanism of copper-tolerance in *F. radiculosa* during initial decay. The mechanism of copper-tolerance in *F. radiculosa* remains undefined. Additional research is needed to elucidate FIBRA_00129 function in managing toxic copper environments in decay fungi. Future research should measure CutC expression that includes longer decay periods and comparative studies in other copper-tolerant decay fungi including *P. placenta, S. lacrymans,* and *Fomitopsis palustris* ((Berk. & M.A. Curtis) Gilb. & Ryvarden).

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