Functional characterization of the manganese transporter smf2 homologue gene, PsMnt, of Phanerochaete sordida YK-624 via homologous overexpression

Research Letter

Title:

Functional characterization of the manganese transporter *smf2* homologue gene, *PsMnt*, of *Phanerochaete sordida* YK-624 via homologous overexpression

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Keyword

homologous expression, manganese transporter, manganese peroxidase, white rot fungi

Abstract

A homologue gene of the yeast natural resistance–associated macrophage protein (Nramp) family transporter *smf2* was identified in the white-rot fungus *Phanerochaete sordida* YK-624. Relative expression levels of the homologue, designated *PsMnt*, were roughly equivalent in cultures containing 0 to 1,000 μM Mn(II), a concentration nontoxic to the fungus. In the *PsMnt*-overexpressing mutant, cellular Mn accumulation and manganese peroxidase (MnP) activity increased significantly in 4-day cultures containing 10 μM $MnSO_4$. Compared with the wild-type strain, MnP activity in the overexpressing mutants was higher at lower Mn concentrations (specifically 10-15 times higher). These results suggest that PsMnt is a high-affinity Mn transporter involved in cellular Mn accumulation under Mn-deficient conditions. This is the first report of an *smf2* homologue in wood rot fungi.

11 **Introduction**

12 Filamentous white-rot fungi can degrade recalcitrant woody polymer components such as
13 cellulose, hemicellulose, and lignin. The name ‘white rot’ derives from the tendency of these fungi to
14 degrade brown lignin prior to white polysaccharides during wood degradation. As other microorganisms
15 generally do not degrade lignin, this fungal group plays a very important role in carbon cycling in forest
16 ecosystems. Lignin, a heterogeneous aromatic biopolymer gives mechanical strength and highly resistance
17 for chemical/biological degradation to wood. Lignin form amorphous matrix together with hemicellulose
18 in cell wall to protect cellulose and other polysaccharides by preventing penetration of enzymes. Although
19 white rot fungi secrete various enzymes for lignin degradation, it is said that ligninolytic enzymes (e.g.
20 lignin peroxidase (LiP), manganese peroxidase (MnP), and laccase) take most important part of lignin
21 degradation (Dashtban *et al.* 2010; Martínez *et al.* 2005).

22 It is well known that metal ions are important for lignin degradation as well as polysaccharide
23 decomposition. Manganese (Mn), in particular, are directly involved in lignin degradation by white-rot
24 fungi. Mn is a well-known inducer of MnP (Bonnarme and Jeffries 1990), and Mn(II)-chelator is oxidized
25 as a substrate of MnP; the resulting Mn(III)-chelator functions as a radical mediator to oxidize phenolic
26 residues contained in lignin. The Mn(III)-chelator also oxidizes other organic compounds, such as
27 derivatives of unsaturated fatty acids and sulfur compounds to produce respective reactive radicals that
28 attack the recalcitrant "condensed structures" of lignin (Hofrichter 2002). Addition of Mn at an optimum
29 concentration enhances lignin degradation (Kerem and Hadar 1995). *Phanerochaete sordida* YK-624, a
30 typical white-rot fungus with excellent lignin-degradation capability, secretes MnP and LiP as major
31 ligninolytic enzymes. (Hirai, Kondo and Sakai 1994, Sugiura, Hirai and Nishida 2003). Although this
32 fungus usually produces MnP, LiP is produced under Mn-deficient conditions (Hirai *et al.* 2005). Generally,

Mn is abundant in trees; thus, Mn deficiency in trees is rare. Stone (1968) well summarized about the concentration of Mn contained in various tree species. Mn concentrations in trees had various values depending on tree species, habitat, site, season, but the deficiency level (less than 20 ppm) was hardly observed except in orchard trees. Therefore, Mn is considered a critical micronutrient in lignin degradation by *P. sordida* YK-624 in natural environment. Based on these facts and a speculation, it is expected that *P. sordida* YK-624 retains the efficient Mn uptake systems and MnP expression mechanisms.

Diss *et al*. (2011) identified multiple candidate Mn transporter genes in fungal genomes. In this report, 11 transporters belonging to several Mn transporter families were identified in the genome of a typical white-rot fungus, *P. chrysosporium*. Some of these candidates are predicted to function in Mn homeostasis based on the results of heterologous expression in yeast, and PcPHO84 (plasma membrane phosphate transporter family) is predicted to be involved in Mn uptake under excess Mn conditions. Yeast phosphate transporter PHO84p is involved in cellular phosphate accumulation, and it prefers of a divalent metal complex of phosphate as a substrate. PHO84p transports Mn-phosphate complex under Mn surplus conditions, but the expression level is strongly regulated by phosphate (Reddi, Jensen and Culotta 2009). On the other hand, functional characterization of SMF homologues has been not carried out in white rot fungi. *Saccharomyces cerevisiae* SMF1 and SMF2 are members of the Nramp (natural resistance–associated macrophage protein) family of metal transporters that are widely conserved in bacteria, plants, fungi, and animals. It is considered that SMFs are the significant important transporter on cellular manganese homeostasis, and are involved in activation of Mn-dependent enzymes, resistance in oxidative stress (Reddi, Jensen and Culotta 2009). These metal-proton symporters act on a broad range of divalent metals (Culotta, Yang and Hall 2005). In yeasts, SMF transporters are localized at the cellular surface or intercellular vesicles, where they play a role in Mn uptake or distribution under Mn-starvation conditions.

Under high Mn conditions, by contrast, SFM transporters are moved into vacuoles and degraded to prevent toxic effects caused from high Mn concentrations (Reddi, Jensen and Culotta 2009). SFM2 significantly affects Mn accumulation and the activities of numerous Mn-dependent enzymes other than SFM1.

As mentioned above, there is no report on the function of the *smf* homolog in the white rot fungi. However, we speculated that *smf* homologues in white rot fungi are strongly involved in the uptake of Mn and the expression of the Mn dependent enzyme including MnP, as with yeast SMF. Hence, we searched for *smf* homologues retained by *P. sordida* YK-624, a strong ligninolytic white rot fungus. In this study, we identified the gene encoding an *smf2* homologue in *P. sordida* (*PsMnt*). And the function of *PsMnt* was then characterized, particularly with respect to its involvement in MnP activity, using a homologous overexpression strategy.

Materials and methods

Strains and cultivation conditions

White-rot fungus *P. sordida* YK-624 (ATCC 90872), the isogenic uracil auxotrophic strain UV-64, and the prototrophic transformant strain U2 were used in this study (Mori *et al.* 2016a). Strain U2 was used as control strain, because it had best ligninolytic properties (ligninolytic activity and selectivity) among all 7 prototrophic transformants (Mori *et al.* 2016a). All strains were maintained on potato dextrose agar (PDA) medium at 4ºC. And strains were pre-cultured on PDA medium (9-cm inner diameter) at 30ºC for 3 days, and then two mycelial pellets (10 mm i.d.) were inoculated into the liquid medium described below.

PsMnt sequence determination and *PsMnt* expression plasmid construction

P. sordida YK-624 genomic DNA and total RNA were obtained from mycelia grown on potato dextrose broth (PDB) medium and Kirk's low-nitrogen (LN, ligninolytic condition) medium (Fenn and Kirk 1981; Tien and Kirk 1988), respectively. DNA extraction (from PDB culture), RNA extraction (from LN culture), and cDNA synthesis were performed as previously reported (Mori *et al*. 2016b). Full-length DNA and cDNA sequences of *PsMnt* were determined using PCR techniques. The primers were designed from a partial cDNA sequence of putative *PsMnt* obtained from unpublished RNA-seq data (see Supplementary Figure 1). Downstream of 3'-sequence of cDNA was determined by 3'-RACE (rapid amplification of cDNA ends) method and genomic PCR for determination of full-length PsMnt gene was performed by thermal asymmetric interlaced (TAIL)-PCR using degenerate primers (Liu and Whittier 1995)(Yamagishi *et al.* 2007).

Restriction sites (*Kpn*I and *Xba*I) were incorporated into the *PsMnt* gDNA sequence using the following primers: 5'-GGTACCGTGTGGTACCATGCCCCCTGAGCCTC-3' and 5'-AAGAGTCTAGACTGCTGGACATGGTTGAGAG-3'. The amplified product was digested with *Kpn*I and *Xba*I and cloned into *Kpn*I-*Xba*I–digested p*GPDpro* (Suzuki *et al.* 2014), yielding p*PsMnt*.

Genetic transformation

The p*PsMnt* expression plasmid was transformed into UV-64 protoplasts using a co-transformation method with p*PsURA5* (Yamagishi *et al.* 2007). Regenerated Ura^+-prototrophic transformants were screened by genomic PCR for verification of target gene transformation. Mycelia were boiled in 50 mM NaOH solution for 5 min, then neutralized with 1 M Tris-HCl (pH 8.0). The resulting supernatant was used as crude DNA solution. The primer pair 5'-AAGCAGCGAGGATTGTACC-3' and 5'-CAGACAGAGGAAGGCATACG-3' was used to amplify the transformed gene.

Transcriptional analysis

Fungal strains were cultivated in 10 ml of LN medium with 0, 1, 10, 100, or 1000 μM $MnSO_4$ for 4 days, at 30 ºC. Mycelia were separated from the culture broth by filtration. The filtrate was used for MnP activity testing, as described below. Total RNA was extracted from mycelia using an RNeasy Mini kit (Qiagen, Hilden, Germany) according to the instruction manual; cDNA was synthesized from 200 ng of total RNA using a PrimeScript RT-PCR kit (TaKaRa Bio Inc., Shiga, Japan). Synthesized cDNA was used in quantitative reverse transcription (qRT)-PCR analyses to evaluate the *PsMnt* expression level (primers: 5'-GACGGGTCTCGATCTTGC-3' and 5'-CCATACAGACTAGCACGGTGAA-3'; standard curve was illustrated in Supplementary Figure 2). Actin was used as a reference gene (primers: 5'-CCCTCAAGCATGAAGGTCAAG-3' and 5'-TAGAAGCACTTGCGGTGGAC-3') (Hirabayashi *et al.* 2015). SYBR Premix Ex Taq II (Tli RNaseH Plus, TaKaRa Bio Inc.) was used as qRT-PCR reagents following manufactured protocol and qRT-PCR conditions were as follows: 95 °C for 3 min; 44 cycles of 95 °C for 10 sec, 58 °C for 30 sec, and 72 °C for 30 sec.

MnP activity

Two mycelial discs on PDA medium (i.d. 10 mm) were inoculated into 10 ml of LN medium (containing 10 or 30 μM $MnSO_4$) or Mn-deficient LN medium. In the case of Mn-deficient cultures, $MnSO_4$ solution was added (final concentration 0, 1.0, 5.0, or 10 μM) after 3 days incubation, and the cultures were incubated for an additional 24 h. After incubation for a total of 96 h, the culture broth was collected and filtered through a 0.22-μm membrane filter. In the case of Mn-supplemented LN medium, filtrate was collected every 2 days. MnP activity in the filtrate was measured according to a method described

121 previously (Hirai, Kondo and Sakai 1994).

122

123 **Mn uptake**

124 Fungal strains were cultivated in Mn-deficient (10 μM) LN medium (50 ml) for 4 days at 30ºC.
125 Mycelia were collected by centrifugation and washed 3 times with Tris-EDTA buffer (10 mM Tris-HCl, 1
126 mM EDTA, pH 7.4), freeze-dried, then weighed. Dried mycelia (15-30 mg) were mineralized with 8%
127 nitric acid and 6% hydrogen peroxide at 120ºC for 8 h. After dilution to 10 ml with milli-Q water, the Mn
128 content was determined using inductively coupled plasma optical emission spectrometry on an SPS7800
129 instrument (Seiko Instruments Inc., Chiba, Japan).

130

131 **Statistical analysis**

132 Data presented are the average of three of more replicates in each experiment. One-way analysis
133 of variance (ANOVA) was carried out to determine the significance of differences in *PsMnt* expression and
134 $MnSO_4$ concentration. For other statistical analyses, the Student's *t*-test was performed to determine the
135 significance of differences compared with control strain U2. Differences between means at a 5% confidence
136 level ($P<0.05$) were considered statistically significant.

137

138 **Results and discussion**

139 We initially sought to determine the DNA and mRNA sequences of the putative *P. sordida*
140 YK-624 manganese transporter. From RNA-seq analysis (unpublished), a partial SMF-like sequence
141 exhibiting low expression levels on beech wood meal culture was obtained. Based on this sequence data,

142 *PsMnt* full-length DNA and cDNA sequences were determined using PCR techniques. The open reading
143 frame of *PsMnt* (accession number: LC326251) has 2,141 bp and six introns, with an 1,824-bp coding
144 sequence. Blast search (Altschul *et al.* 1997) results indicated that *PsMn*t homologues are preserved with
145 high homology among Agricomycetes (especially Polyporales) at the amino acid sequence level
146 (Supplementary Table 1). The translated amino acid sequence showed high similarity (identity 32~41 %)
147 to the Nramp family of divalent ion transporters, according to SmartBlast analysis (Table 1). Therefore,
148 *PsMnt* was presumed to be an *SMF* homologue. In yeast, it is thought that SMF2 (for which evidence of
149 cell surface localization is still lacking) is the most important proton-coupled transporter of Mn ion into the
150 cell. Reportedly, Mn homeostasis is significantly affected in *smf2*Δ deletion yeast, reducing Mn
151 accumulation and the activity of Mn-dependent enzymes (Reddi, Jensen and Culotta 2009; Cyert and
152 Philpott 2013). The relative *PsMnt* expression level and dry mycelial weight of *P. sordida* YK-624 in
153 cultures containing various Mn concentrations are shown in Figure 1. As no significant difference was
154 observed in mycelial dry weight between cultures at any $MnSO_4$ concentration, it can be said that $MnSO_4$
155 concentrations in the range used in this experiment are physiologic for *P. sordida* YK-624 (except 0 μM).
156 Because *P. sordida* YK-624 produces LiP instead of MnP as a ligninolytic enzyme under Mn(II)-deficient
157 conditions (Hirai *et al.* 2005), it is likely that a lack of Mn in the culture medium does not critically affect
158 the growth of this fungus. *PsMnt* was constitutively expressed; no significant difference was observed in
159 relative *PsMnt* expression level.

160 A previous review described Mn transporters in *S. cerevisiae* (Culotta, Yang and Hall 2005).
161 According to this report, transcription of yeast *smf1* and *smf2* is not regulated by the Mn concentration; Mn
162 starvation just increases the stability of SFM proteins. Portony et al. have reported post-translational
163 regulation of SMF1 and SMF2, these transporter protein levels have been repressed under culture condition

supplemented10 μM Mn (Portnoy, Liu and Culotta 2000). As other transporters also function in controlling the cellular Mn concentration under physiologic conditions, it is difficult to characterize the function of *PsMnt* from the transcription level. Therefore, we investigated the involvement of *PsMnt* in Mn(II) uptake using homologous overexpression. By genomic PCR screening, 15 clones (MT-1 to -15) of *PsMnt* co-transformants were obtained from 180 uracil prototrophic clones. Bonnarme and Jeffries (1990) reported that the production of MnP is regulated by Mn(II) in several white-rot fungi. In addition, MnP activity in *P. sordida* is reportedly regulated by the Mn(II) concentration in liquid culture (Rüttimann-Johnson, Cullen and Lamar 1994). Therefore, Mn(II) uptake driven by PsMnt was indirectly evaluated by measuring MnP activity. MnP activity in the culture filtrates obtained from 4-day LN cultures (30 μM Mn) incubated with the wild-type strain, control strain U2, and the 15 MT strains was measured. Although 80% of all transformants showed higher MnP activity than the wild type, only two co-transformants (MT-20 and -40) showed significantly higher activity than control strain U2 (Supplementary Figure 3).

To evaluate the Mn uptake activity in more detail, MnP activity was measured after 24 h of induction by the addition of Mn(II) to a Mn-deficient LN culture. As shown in Figure 2A, the MnP activity of all strains was correlated with the addition of Mn. However, *PsMnt* transformants MT-20 and -40 showed significantly higher MnP activity than the control strain in all tested ranges. Furthermore, the reduction rate of MnP activity in the co-transformants (especially MT-20) due to the decrease in Mn(II) concentration was lower than that of control strains. MnP activity relative to the wild-type strain is shown in Figure 2B. Although control strain U2 showed almost constant ratios in the tested range, the co-transformants showed much higher relative MnP activity at lower Mn(II) concentrations. Time-course analysis of the MnP activity of U2 and MT-20 in cultures containing 30 or 10 μM $MgSO_4$ are shown in Figure 3A and B, respectively. At both Mn(II) concentrations, both strains secreted MnP 2 days after inoculation and showed maximum

activity at 4 days of incubation. Although MT-20 consistently showed higher MnP activity (except for day 2), these strains exhibited similar MnP expression patterns in both cultures. From these results, we hypothesized that co-transformants exhibit higher Mn uptake activity and higher resulting intracellular Mn(II) concentrations.

The intracellular Mn concentration was compared in strains MT-20 and U2 cultured in 10 μM Mn(II). Because the amount of Mn accumulated in mycelia obtained after Mn(II) addition to Mn-deficient cultures did not reach the lower detection limit, the cultures were scaled up (10 to 50 ml), and strains were cultivated at constant Mn concentrations. As shown in Table 2, the amount of Mn accumulated in MT-20 mycelia was 1.36 times higher than that in U2 mycelia at 4 days of incubation. At 4 days of culture, the MnP activity of MT-20 was 2.8 and 3.0 times higher per unit volume and per unit mycelium dry weight, respectively, than the activity of U2. The relative level of *PsMnt* expression in MT-20 was also much higher. These results indicate that overexpression of *PsMnt* leads to incremental increases in the amount of Mn taken up and a resulting increase in MnP activity. Although the increase in MnP activity for Mn uptake seems reasonable, the values for the increment of the activity and Mn uptake with respect to the fold change in relative *PsMnt* expression appear too small. In *S. cerevisiae*, most Nramp-type Mn transporters (SMF1 and SMF2) are quickly degraded under physiologic conditions (Reddi, Jensen and Culotta 2009); therefore, it is assumed that the product of *PsMnt* overexpression is also degraded under these experimental conditions. It was reported that yeast SMFs are regulated by manganese and metal homeostatic protein BSD2. SMF proteins are delivered for the degradation to vacuole through the function of BSD2. On the other hand, there is no knowledge about the mechanism of degradation of *smf* homologue proteins and the presence of *bsd*2-like gene of white rot fungi. In addition to these question, the investigation of the function of PsMnt during wood decay will be a future research subject.

Conclusion

Several transporter proteins mediate import and distribution of cellular Mn in all organisms, including white-rot fungi. Nevertheless, the expression and function of SMF Nramp-family high-affinity Mn transporters in the white-rot fungi had not been explored. In this study, we found an *smf2* homologue, *PsMnt*, in a *P. sordida* YK-624 cDNA library. Overexpression of *PsMnt* led to significantly enhanced Mn uptake and activity of MnP (a Mn-dependent enzyme). These results indicate that PsMnt is a high-affinity Mn transporter exhibiting significant Mn uptake activity under Mn-deficient conditions. By clarifying the action of PsMnt on ligninolysis in future research, some useful information will be obtained to elucidate the details of lignin degradation mechanism of white rot fungi.

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Figure captions

Fig. 1. *PsMnt* expression (gray bars) and dry mycelial weight (white circles) in wild-type *P. sordida* YK-624 in cultures containing various $MnSO_4$ concentrations. Data were analyzed using one-way ANOVA to determine the significance of differences. Actin was used as reference gene for expression normalization.

Fig. 2. Enhancement of MnP activity in *PsMnt* recombinant strains at various $MnSO_4$ concentrations. A) MnP activity in culture supernatants of wild type, control strain U2, and *PsMnt* transformants (MT-20 and MT-40) incubated for 4 days. Asterisks indicate significant difference from control strain U2 as determined using the Student's *t*-test ($P<0.05$). B) Rate of increase in MnP activity in U2, MT-20, and MT-40 at each $MnSO_4$ concentration, compared with the wild-type strain.

Fig. 3. Activity of total MnP secreted from control strain U2 and *PsMnt* recombinant MT-20 over time in cultures containing 30 μM (A) or 10 μM (B) $MnSO_4$. Asterisks indicate significant differences from control strain U2, as determined using the Student's *t*-test ($P<0.05$).

Table 1. Function and accession number (AN) of proteins showing high similarity to the *PsMnt* translated amino acid sequence.

Function	Organism	AN	Identity	Coverage
Divalent metal ion transporter SMF2	*Saccharomyces cerevisiae*	NP_011917	34%	96%
Nramp family manganese ion transporter	*Schizosaccharomyces pombe*	NP_594537	41%	74%
Nramp	*Dictyostelium discoideum*	XP_643409	32%	80%

Table 2. Cellular accumulation of Mn, MnP activity, and *PsMnt* expression by co-transformant MT-20 and control strain U2.

Strain	Mn accumulation (ng/mg[a])	MnP activity		Relative PsMnt expression
		(nkat/ml)	(nkat/mg)	
U2	6.85 ± 0.12	0.35 ± 0.36	205.2 ± 16.4	1.0 ± 0.5
MT-20	9.33 ± 0.32*	9.71 ± 0.38*	613.0 ± 38.2*	10.2 ± 0.7*

[a]Dry mycelial weight.

*Asterisks indicate significant difference from control strain U2, as determined using the Student's *t*-test ($P<0.05$).

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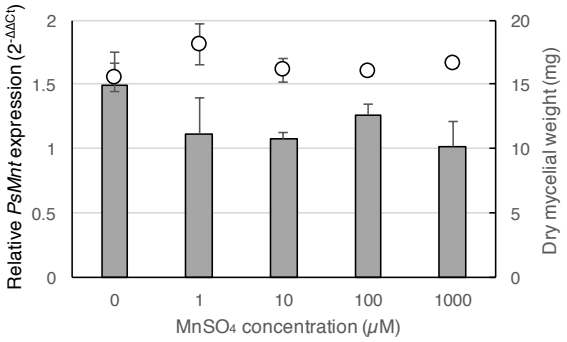

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301

302 Fig. 1

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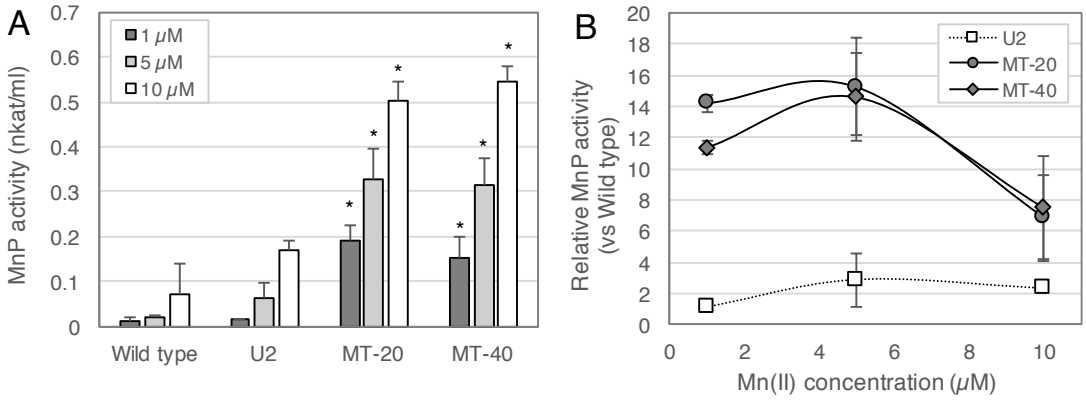

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307 Fig. 2

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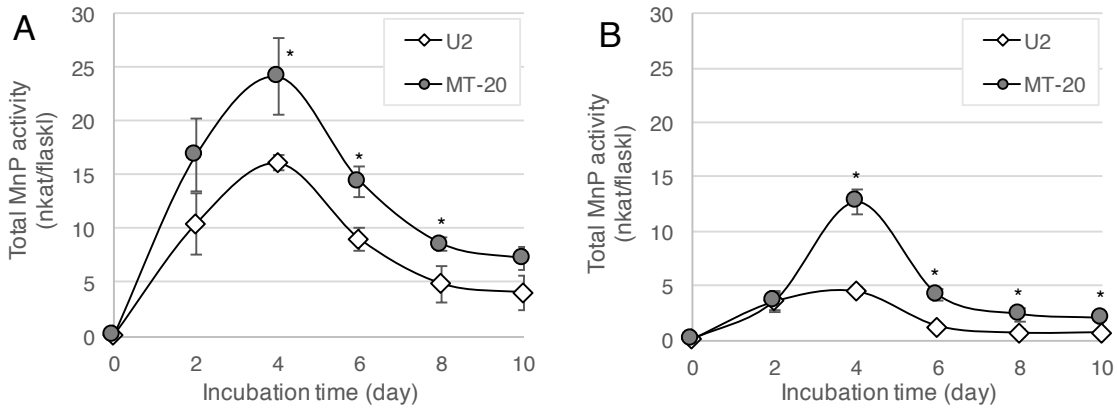

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312 Fig. 3

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CCTCCTCGTCGTCTTTTTACCTCGAGCCCCCATGGCCGTCGCTCTCCAGCCTCGCTCTCCGACCACCCGC
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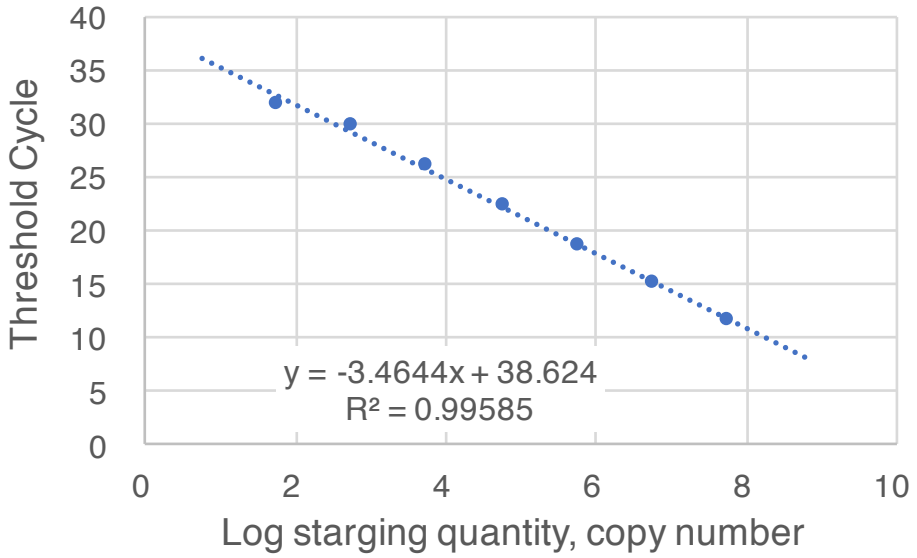
GTCTTCTACTACGGTGCGGGGCGGGGCTCGGGCGGGCCGGCGAGCCTCTTCGATGCGTACGACCTGCTGA
AGCAGATCGTGGGGCAGGGTGCAGCGACGCTCTTCGCGCTCGCGCTGCTCGCGTCGGGGCAGAGCTCGTC
GATCATCGCGACGATGGCAGGCCAGGTCGTGTCCGAGGGCTTCCTGCGCTGGCGCGTGTCGCCCGTGTTC
CGGCGCGGGCTCACGCGCGCGATCGGGCTCGTGCCGTCGATGGCCGTCGCGCTCGCGGCGGGCCGCAGCG
GCGTCGACACGATGCTCGTCGCGAGCCAGGTTGTGCTCAGCATCGTCCTCCCGTTCATCGTCTTCCCGCT
GCTGTACCTCACCAACTCGCGCGCGGTCATGACCGTCAAGAAGCTCCCGCCAGAGGACACCGTCGATATG
CTCGGGCTCGACAAGGACGCGGACGAGGACCGCGAGCAGGCCCCGGACGAGGACGAAGACCTGCAGGAGG
AGGAGCCGGACAA.....AAAAAAAAAAAAA

Supplementary Figure 1

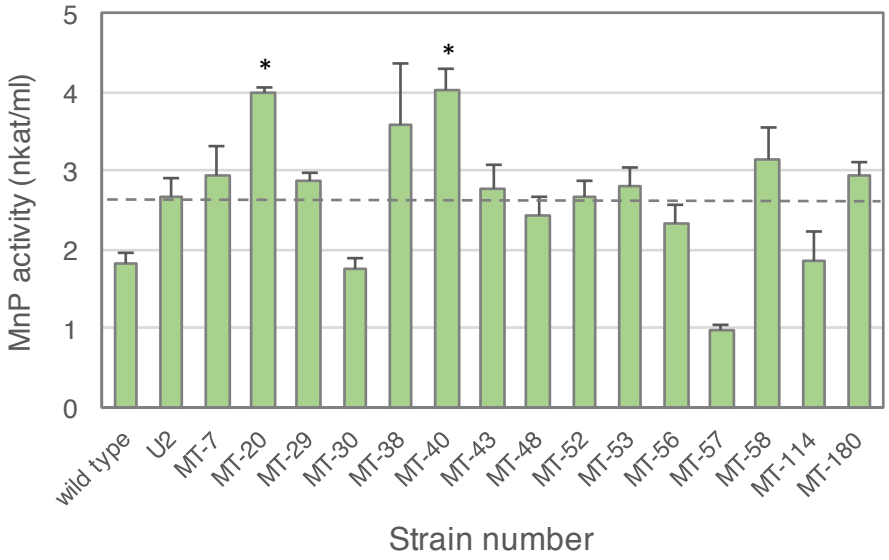
A partial cDNA sequence of putative *PsMnt* obtained from RNA-seq data.

Enclosed sequences were used as RT-PCR primers. The PCR conditions were as follows: 98 ºC for 3 min, 30 cycles at 98 ° C for 10 sec, 54 ° C for 30 sec, and 72 °C for 2 min, 72 ºC for 5min.



Supplementary Figure 2

The qRT-PCR standard curve made by gradient dilution of PsMnt RT-PCR product.



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358 **Supplementary Figure 3**

359 MnP activities in culture filtrate of MT strains and control strains. The filtrate was obtained from Mn
360 supplemented culture incubated with fungal strains for 2 days, at 30 ºC. Asterisks indicate significant
361 difference from control strain U2 as determined using the Student's t-test ($P<0.05$).
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364 **Supplementary Table 1**

365 Function and accession number (AN) of proteins showing high homology to the *PsMnt*
366 translated amino acid sequence

Putative function	Organism	AN	Identity	Coverage
hypothetical protein	*Phanerochaete carnosa*	XP_007391169	76%	100%
hypothetical protein	*Phlebiopsis gigantia*	KIP03915	68%	97%
Manganese transporter pdt1	*Trametes pubescens*	OJT03088	60%	95%
Manganese transporter pdt1	*Hypsizygus marmoreus*	KYQ32377	58%	99%
Nramp-domain-containing protein	*Dichomitus squalens*	XP_007360725	59%	94%
smf Mn^{2+} and Fe^{2+} transporter	*Wolfiporia cocos*	PCH34295	58%	96%
hypothetical protein	*Trametes cinnabaria*	CDO73418	57%	99%
hypothetical protein	*Gelatopria subvermispora*	EMD42264	58%	97%
Nramp protein	*Pycnoporus coccineus*	OSD00839	59%	95%
Nramp family transporter	*Heterobasidion irregulare*	XP_009540380	59%	95%
Nramp protein	*Trametes versicolor*	XP_008032913	58%	99%

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