

Supporting Information

Decolorization and detoxification of textile wastewaters by recombinant *Myceliophthora thermophila* and *Trametes trogii* laccases

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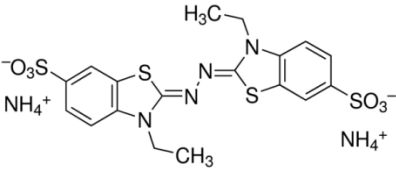
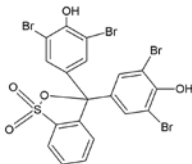
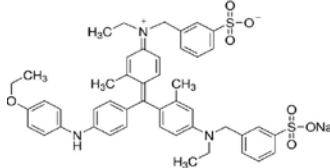
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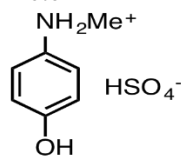
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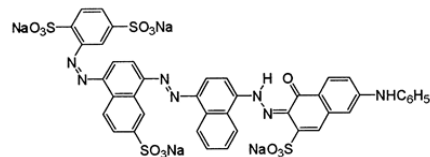
Table S1 Chemical formulas of substrates and synthetic dyes used in this study. Non-phenolic ABTS substrate and two phenolic substrates metol and syringaldazine were selected for the substrate specificity measurement of purified laccases. Two triphenylmethane dyes Bromophenol Blue and Coomassie Brilliant Blue, one azo dye Saturn Blue and one anthraquinonic dye Remazol Brilliant Blue R were used for testing of decolorization abilities of purified laccases.

Substrates	Synthetic dyes	
ABTS 	Bromophenol Blue 	Coomassie Brilliant Blue 

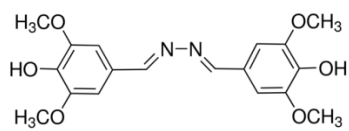
Metol



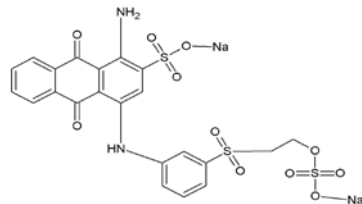
Saturn Blue



Syringaldazine



Remazol Brilliant Blue



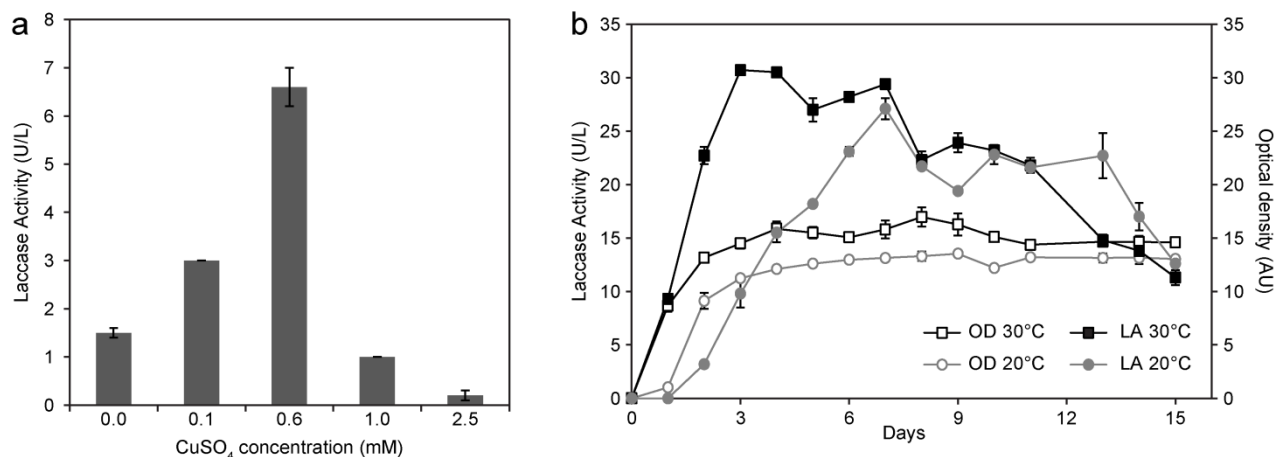


Fig. S1 (a) Effect of copper in cultivation medium on the production of MtL to supernatant. The yeast strain *S. cerevisiae* BW31a with plasmid [pVT-100U-MtL] was cultivated in YNB medium with 0.8 % alanine at 30 °C for 24 h. **(b)** Effect of cultivation temperature on the production of MtL to supernatant. Growth of *S. cerevisiae* cells (measured as optical density) with plasmid [pVT-100U-MtL] cultivated in YNB medium with 0.8 % alanine and 0.6 mM copper was determined at 20 °C (○) and 30 °C (□). The extracellular production of laccases (measured as laccase activity - U/l in culture supernatant) was determined at 20 °C (●) and 30 °C (■). All experiments were performed in triplicate and data are shown as averages ± standard deviation

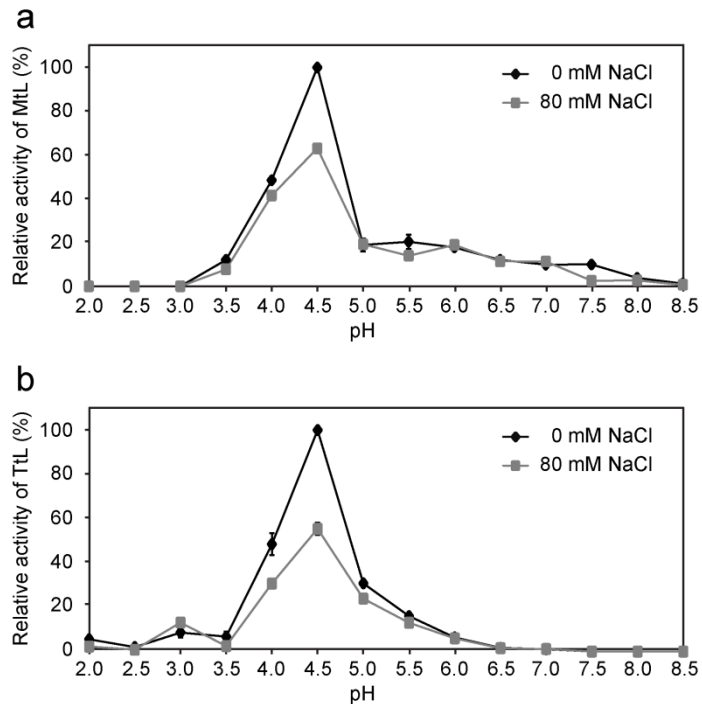


Fig. S2 (a) Comparison of pH dependence on purified MtL and **(b)** TtL activity in 100 mM buffers (NaAc buffer for pH 2,3,4; NaCit buffer for pH 2.5, 3.5, 4.5, 5.5; Kphos buffer for pH 5, 6, 7; NaPhos buffer for pH 6.5 and Tris buffer for pH 7.5, 8, 8.5) supplemented (■) or without addition of 80 mM NaCl (◆) by measuring laccase activity using 100 mM ABTS (in 100 mM citrate buffer, pH 4.5) as a substrate. The experiments were performed in triplicate and data are shown as averages \pm standard deviation

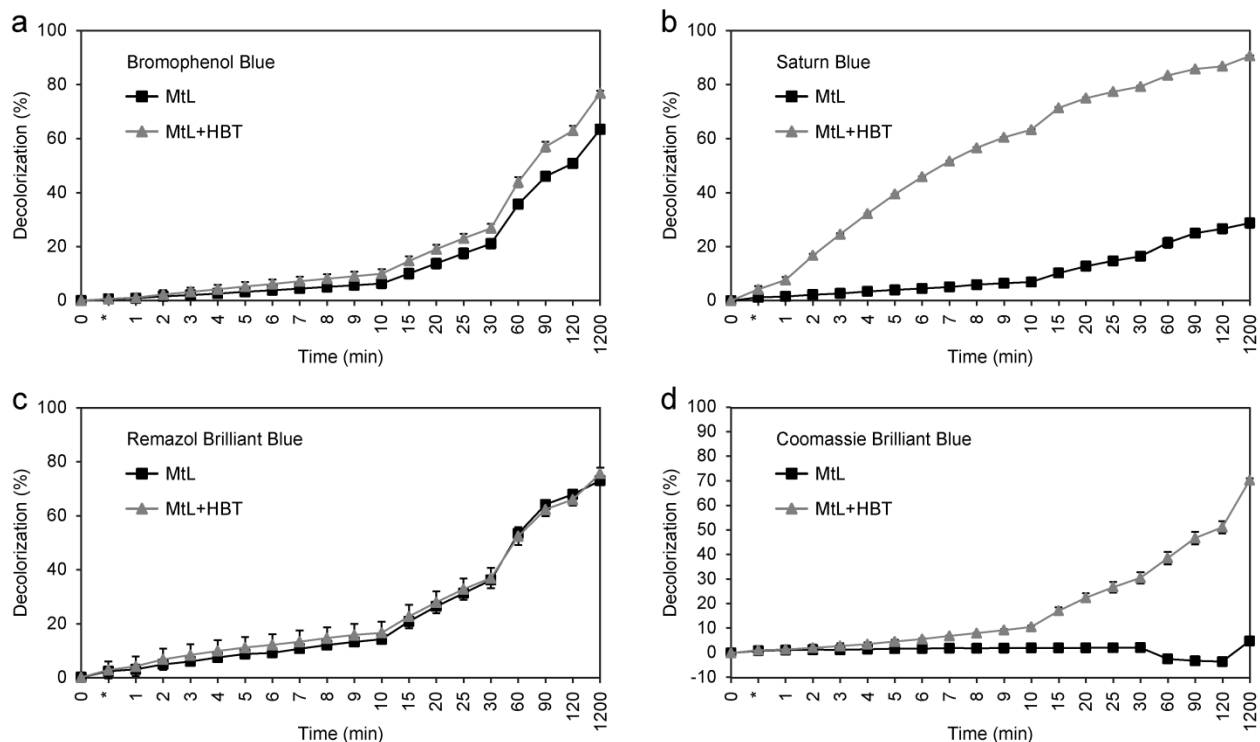


Fig. S3 Decolorization of (a) Bromophenol Blue, (b) Saturn Blue, (c) Remazol Brilliant Blue, (d) Coomassie Brilliant Blue over 20 h by purified MtL (■) and MtL with 1 mM HBT (as a mediator) (▲). The activity of MtL in the reaction was 0.2 U/mL and the reaction was carried out in citrate buffer (pH 4.5) at 40 °C. The level of decolorization was measured continuously within 0 - 30 min (* 40 sec) and also after 60 min, 90 min, 120 min and 20 h. The experiments were performed in triplicate and data are shown as averages ± standard deviation

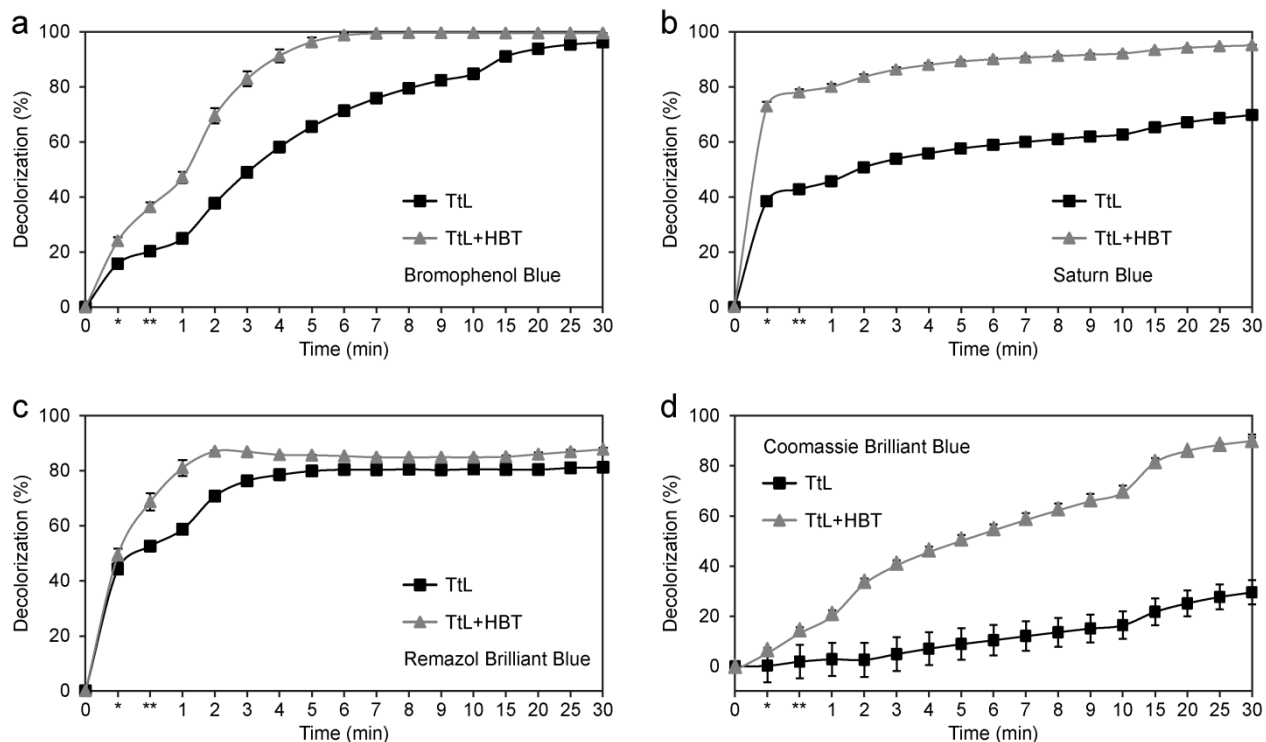


Fig. S4 Decolorization of (a) Bromophenol Blue, (b) Saturn Blue, (c) Remazol Brilliant Blue, (d) Coomassie Brilliant Blue over 30 min by purified TtL (■) and TtL with 1 mM HBT (as a mediator) (▲). The activity of TtL in the reaction was 0.2 U/mL and the reaction was carried out in citrate buffer (pH 4.5) at 40 °C. The level of decolorization was measured continuously within 0 - 30 min (* 20sec, ** 40 sec). The experiments were performed in triplicate and data are shown as averages \pm standard deviation

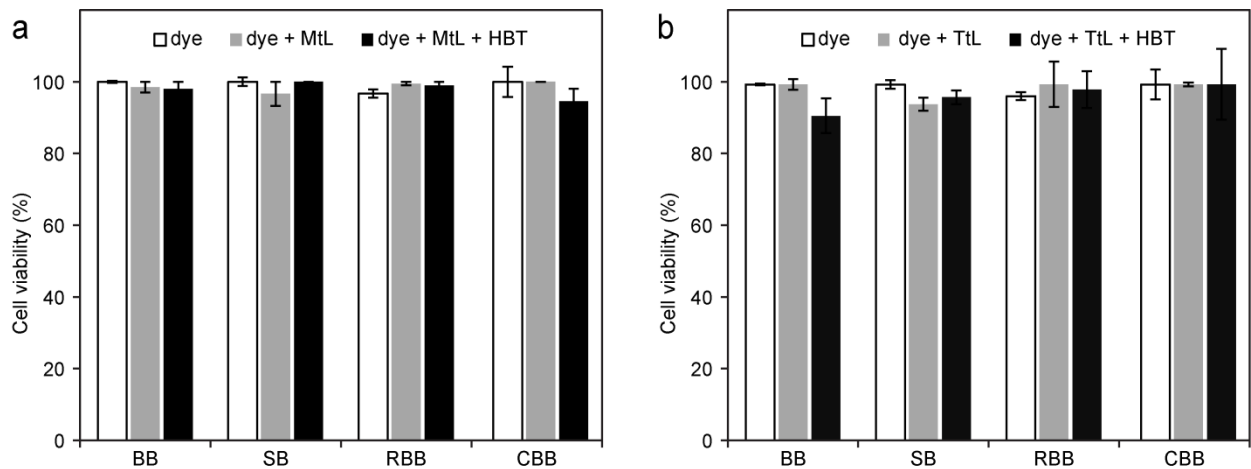


Fig. S5 (a) Cell viability in the presence of untreated dyes and decolorized dyes treated with purified MtL in the presence or absence of HBT (as a mediator). **(b)** Cell viability in the presence of untreated dyes and decolorized dyes treated with purified TtL in the presence or absence of HBT. Tests were carried out using PBMCs and CellTiter-Glo[®] Luminescent Cell Viability Assay determining the number of viable cells based on the cellular ATP level. The experiments were performed in triplicate and data are shown as averages \pm standard deviation