3.1.1. Supplementary Materials

Plasmid used for OB-1 expression

pVTU Expression Vector (6.9 kb): this vector is used for the constitutive expression of recombinant protein in *S. cerevisiae*. It contains the elements for the manipulation in *E. coli* (ColE1 ORI and Amp^r) and for the replication and selection in yeast (2µ ORI, URA3). The expression is under the control of alcohol dehydrogenase promoter (ADH1).

pEMBLyex4 Expression Vector (8.8 kb): this vector is used for the inducible expression of recombinant protein in *S. cerevisiae*. It contains the elements for the manipulation in *E. coli* (ColE1 ORI and Amp^r) and for the replication and selection in yeast (2µ ORI, URA3). The expression is under the control of galactokinase promoter (GAL1) that could be induced adding galactose in the culture broth. The promoter is strongly repressed by the presence of glucose.

Copper incorporation into BALL

The changing in the aeration conditions from shaking to static conditions results in an increase in the specific activity of recombinantly expressed BALL laccase in *E. coli*: this behaviour was reported previously by Durao [18]. Cu metabolism in *E. coli* is dependent on oxygen availability and an increased intracellular accumulation of Cu can be observed under anaerobic growth conditions. Therefore, *E. coli* cells were grown in microaerobic conditions to promote higher intracellular Cu content and therefore a better Cu incorporation into BALL enzyme.

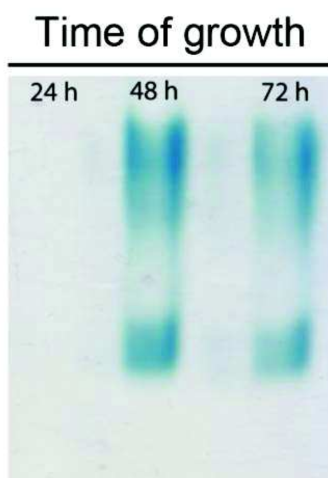

Figure S1 Native-PAGE of the culture broth of *S. cerevisiae* cells expressing OB1 laccase collected at 24, 48, and 72 hours after growth in the expression medium. Proteins in the broth were precipitated at 75% (w/vol) ammonium sulfate saturation. In each lane ≈ 0.5 mL of culture broth was loaded. The gel was developed by incubation with the laccase activity staining solution. The presence of two bands in native-PAGE is probably due to the presence of two different glycosylated forms of OB-1 expressed in *S. cerevisiae*. For our purpose, the two forms were co-purified and characterized together.

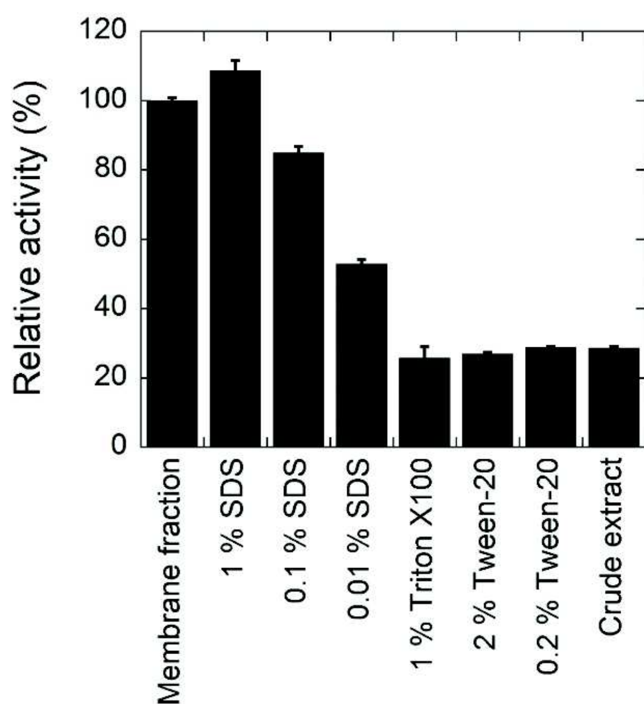

Figure S2 Detection of enzymatic activity of MmPPO in the membrane fraction (obtained by centrifugation at 10000xg) and in the crude extract (obtained by centrifugation at 40000xg) in the absence of a detergent as well as in the soluble fraction after solubilization in the presence of different detergents. The enzymatic activity in the membrane fraction is taken as 100%.

Table S1 Laccases from different sources under investigation in this work. Specific activity was determined based on the activity on ABTS at pH 5.0, 25 °C, and the protein concentration assayed with the Biuret method (for BALL laccase estimated by using the molar extinction coefficient)

Enzyme	Source	Specific activity[a] (U/mg prot)	Purity (%)	Producer
Tv - Sigma	*T. versicolor*	13.1	≥ 90	Sigma-Aldrich – cat. S38429
Tv – LAC C	*T. versicolor*	5.0 (200)	≥ 90	ASA Spezialenzyme GmbH – LAC C
Tv – LAC 4	*Trametes sp.*	0.1 (8.3)	≥ 90	ASA Spezialenzyme GmbH – LAC 4
Mt – LAC 3	*Myceliopthora thermophila*, expressed in *A. niger*	14.0 (130)[b]	≥ 70	ASA Spezialenzyme GmbH – LAC 3
Po	*P. ostreatus*	7.5	≥ 90	Sigma-Aldrich – cat. S75117
Ab	*A. bisporus*	10.5 (53.2)[b]	≥ 80	ASA Spezialenzyme GmbH – LAC A
OB1	Basidiomycete PM-1	3.2	≥ 90	This work
Th – LAC 5	*Thielavia sp.*	8.0 (59.1)	≥ 90	ASA Spezialenzyme GmbH – LAC 5
BALL	*Bacillus licheniformis*	20.0	≥ 90	This work
MmPPO	*Marinomonas mediterranea*	4.0[b]	≈ 5	This work
R. vernicifera	*Rhus vernicifera*	1.4	≥ 90	Sigma-Aldrich – cat. L2157

[a]The specific activity value determined at pH 4.5 and 37 °C is reported in parentheses.

[b]Specific activity was corrected for the purity degree determined by SDS-PAGE analysis when purity was < 90%.

Table S2 Volumetric yield of OB1 laccase activity (on ABTS as substrate) obtained in *S. cerevisiae* cultures grown under different conditions. 3% DMSO was added directly to the *S. cerevisiae* colture broth as inducer. The presence of DMSO could enhance the protein expression level in response to cellular stress conditions. Actually, it was demonstrated that DMSO can modify membrane physical properties and alter the regulation of intracellular biochemical pathways, inducing oxidative stress in the yeast *Saccharomyces cerevisiae* [a,b].

[a] Routledge S. J., Mikaliunaite L., Patel A., Clare M., Cartwright S. P., Bawa Z. & Bill R. M. *Methods* (2016) **95**, 26-37.

[b] Sadowska-Bartosz I., Pączka A., Mołoń M. & Bartosz G. *FEMS yeast research* (2013) **13**(8), 820-830.

	Temperature of growth (°C)			
	20		**30**	
	2 mM Cu^{++} (U/L)	**4 mM Cu^{++} (U/L)**	**2 mM Cu^{++} (U/L)**	**2 mM Cu^{++}+ 3% DMSO (U/L)**
pEMBL-OB1	0.3	0.1	0.3	1.4
pVTU-OB1	1.1	1.6	19.0	0.7