

## **Additional File 1**

### **AMB Express**

#### **H<sub>2</sub>O<sub>2</sub> as a candidate bottleneck for MnP activity during cultivation of *Agaricus bisporus* in compost**

Aurin M. Vos<sup>1</sup>, Edita Jurak<sup>2</sup>, Jordi F. Pelkmans<sup>1</sup>, Koen Herman<sup>1</sup>, Gill Pels<sup>2</sup>, Johan J. Baars<sup>3</sup>, Ed Hendrix<sup>3</sup>, Mirjam A. Kabel<sup>2</sup>, Luis G. Lugones<sup>1</sup>, Han A. B. Wösten<sup>1\*</sup>

<sup>1</sup>Microbiology, Department of Biology, Utrecht University, Padualaan 8, 3584 CH Utrecht, The Netherlands;

<sup>2</sup>Wageningen University, Laboratory of Food Chemistry, Bornse Weilanden 9, 6708 WG, Wageningen, The

Netherlands; <sup>3</sup>Plant Breeding, Wageningen University and Research Centre, 6700 AJ Wageningen, The Netherlands

\*Corresponding author

Prof. dr. Han A.B. Wösten

Department of Microbiology, Utrecht University

Padualaan 8, 3584 CH Utrecht

The Netherlands

Telephone: 31 30 2533448

Fax: 31 30 2513655

E-mail: [h.a.b.wosten@uu.nl](mailto:h.a.b.wosten@uu.nl)

**Table S1:** Primers used in this study.

<b>Primer</b>	<b>Primer name</b>	<b>Sequence</b>
<b>1</b>	Actin prmtr F	AAGCTTAGCCGAGAGAAGATGCCCC
<b>2</b>	Actin prmtr R	CCATGGTTTGTATTTCGTGTGTTTCG
<b>3</b>	Actin trmnr F	GGATCCGCTGATGGTGCTTTATGATAAATAAAGTCCTTGGG
<b>4</b>	Actin trmnr R	GAATTCTACTACTACCCCCAAAACCGACATCATCC
<b>5</b>	Act-Pr_F_infu	CCAGGGGGATCGTTAAAGCTTAGCCGAGAGAAG
<b>6</b>	Act-Ter_R_infu	CGCCGAATTGGCGCGGAATTCTACTACTACCCCC

**Table S2:** Statistical differences over time in MnP and Lcc activity and chitin content in compost as presented in Figure 2. Time points sharing a letter are not significantly different (ANOVA with Bonferroni post hoc test,  $p < 0.05$ ).

	A15			MnP1-1		
	MnP	Lcc	Chitin	MnP	Lcc	Chitin
<b>PII</b>	-	-	A	-	-	A
<b>Casing</b>	A	A	AB	A	A	AB
<b>Venting</b>	B	B	BC	B	B	BC
<b>After 2nd flush</b>	C	C	C	C	C	C

**Table S3:** Relative abundance of lignin residues as percentage of total molar area as presented in Figure 3. Standard deviations are indicated between brackets.

		<b>G-residues</b>							
		<b>Guaiacol</b>	<b>4-methylguaiacol</b>	<b>Ethyl-guaiacol</b>	<b>4-vinylguaiacol</b>	<b>Vanillin</b>	<b>Trans-isoeugenol</b>	<b>Acetovanillone</b>	<b>Guaiacylacetone</b>
	<b>WS</b>	22 (4)	2.8 (0.5)	1.9 (0.5)	37.4 (6.4)	3.5 (0.7)	2 (0.5)	1.2 (0.3)	2.8 (0.6)
	<b>PII</b>	24.5 (1.4)	3.3 (0.3)	2.1 (0.4)	32.9 (2.9)	2.3 (0.2)	3.4 (0.2)	1.7 (0.2)	2.3 (0.2)
<b>Casing</b>	<b>A15</b>	26.6 (5.4)	2.9 (0.8)	1.9 (0.6)	23.2 (6.2)	4.6 (1)	2.9 (1)	4.2 (0.7)	3.1 (0.8)
	<b>MnP1-1</b>	26.7 (5.6)	2.8 (0.7)	1.9 (0.4)	22.4 (5)	4.6 (0.9)	2.8 (0.6)	4.3 (0.9)	3.1 (0.6)
<b>Venting</b>	<b>A15</b>	31.7 (3.6)	2.5 (0.4)	1.7 (0.3)	15.9 (1.9)	4.9 (0.6)	2.2 (0.3)	5.4 (0.5)	3.3 (0.4)
	<b>MnP1-1</b>	31.9 (1.6)	2.5 (0.2)	1.8 (0.2)	15.5 (1)	5 (0.4)	2.2 (0.2)	5.3 (0.3)	3.3 (0.2)
<b>After 2nd</b>	<b>A15</b>	35.3 (2.9)	2 (0.2)	1.7 (0.2)	13.6 (1.1)	6 (0.4)	1.7 (0.1)	6.2 (0.3)	3.1 (0.2)
	<b>MnP1-1</b>	35.3 (2.8)	2 (0.2)	1.6 (0.2)	13.4 (1.5)	6 (0.8)	1.8 (0.3)	6.4 (0.8)	3.3 (0.4)

  

		<b>S-residues</b>						
		<b>Syringol</b>	<b>4-methylsyringol</b>	<b>4-vinylsyringol</b>	<b>Syringaldehyde</b>	<b>4-Allylsyringol</b>	<b>Acetosyringone</b>	<b>Syringylacetone</b>
	<b>WS</b>	10 (1.8)	1.3 (0.3)	8.1 (1.5)	1.4 (0.2)	2.1 (0.4)	1.8 (0.4)	1.8 (0.3)
	<b>PII</b>	10.2 (1.5)	1.3 (0.1)	8.5 (1.5)	1.2 (0.2)	3 (0.3)	2 (0.1)	1.5 (0.2)
<b>Casing</b>	<b>A15</b>	12.8 (2.6)	1.2 (0.3)	6.6 (1.3)	1.6 (0.3)	2.6 (0.5)	3.7 (0.7)	2.1 (0.4)
	<b>MnP1-1</b>	13.2 (2.8)	1.3 (0.3)	6.5 (1.3)	1.7 (0.3)	2.6 (0.5)	3.8 (0.7)	2.3 (0.4)
<b>Venting</b>	<b>A15</b>	15.4 (1.9)	1.1 (0.2)	5.2 (0.6)	1.7 (0.2)	2 (0.2)	4.3 (0.4)	2.6 (0.3)
	<b>MnP1-1</b>	15.6 (1.1)	1.2 (0.1)	5.1 (0.5)	1.6 (0.1)	2 (0.1)	4.4 (0.3)	2.6 (0.2)
<b>After 2nd</b>	<b>A15</b>	14.8 (1.4)	0.8 (0.1)	4.4 (0.2)	2 (0.2)	1.7 (0.1)	4.2 (0.3)	2.6 (0.2)
	<b>MnP1-1</b>	14.3 (1.7)	0.8 (0.1)	4.2 (0.6)	2.1 (0.2)	1.7 (0.2)	4.5 (0.5)	2.6 (0.3)

**Text 1:** Nucleotide sequence of the adapted *mnp1* over-expressed in *A. bisporus*.

CCATGGCTTTCAA AATTCTTCTCAGCCTTATTTTGGCTTTGAACGCCGTCCAATTTATTGCTGGTAC  
GCACTTTACTCATATCCTCATATTATTTGCCGACAAGTCAACGATGTAGCTGTTCCCTACCAGGAGG  
GCGCAATGTGCCGATGGCACCACTGTTTTCTAATGAGGCCTGTTGCGTTCCTGCCATTATAGCA  
GATATCCAGCCGAACTTGTTTCGAAAACGAGTGCGGTGAAGAGGTAATGATTTAATTTTTTGATAGA  
GTTACGATATTCAACAGCATATTTATACTTTAGGTCCATGAAACCTTGCGGGCATTATTCATGATG  
CTATCGGATTCTCCAGAGCAGCTGGGTAGGTTCTGACTGTCTGTGGCGGCAGCTAGTATCGCTAAA  
ATGATTTCCAGTGGTGGTGGTGTCTGATGGTTCTCTCGTCACTTTTCGGCGATGTGGAGACGACATTT  
GCCGCTAACGCAGGAATCGACGAGATTGTAGAGACATTACGACCTTTTATAAACAGCCACAACAT  
CAGTGCCGGCGATTTGTGAGCCTGGACTCATAATCTGTGTTGCATCCTGTTAACGCTCTTCCTAGCA  
TCCAGTTTGCAACCGTTGTTGGATTGACCAACTGCCCCGGCGCACCTCGCATACCCTTTTTCTTAGG  
CCGCCAGACGCTACCGCCGCTTCCCCCGATGGTCTTGTCCCTGAGCCCTTCGGTCAGTCTCTCATC  
AGCCTTTCCGGTATAATCTACAACCCAAGTTGATAATCAACAGATTCCGTCACGAAAATTCTTGAA  
CGCTTCGATGATGCTGGTTTTACCCCCACTGAAGTTGTTGCATTGCTTGCATCGTGAGTCTCTAGAA  
CAAGATCGAGAAATATGTGTCCAATCCCCCGTAGTCATACTGTTCGAGCTTCGGACACTATTGAG  
CCCGGTGTAAGTTTATTTCTTCGTCAGTATGCATTCTCATCTCATGAGCACCTTATTCCAGCTCGAA  
GGTAAGACATTCCTATCAATAAGAAACGTCTCAGTATCCAACGCAACCTCTAGTGTCCCCTTCG  
ACTCGACTCCCGGAGAATTTGACAGGCAGTTCTTTATCGAGACTATGCTTAAAGGCACTTCCTTCC  
CAGGGTAAGTAGTTCGTTGACATTCGTTACGCATCCTAATTTTGGCAAAGCACTGGGGGGAACCAA  
GGTGAAGCCTTATCTCCCTTGCCGGGAGAACTTCGTCTCGAATCCGATGGACTTGTACGTATACAG  
TCTTTTGTGTCTGAATTTGTGATTGTTAACATTATTTATCATTAGTTGGCTCGAGGTCTGTCTTGCT  
AACAAAAGGCATCTGATAACATTGAAGGACTAACTTCATCATAGATGAACGAACCGCATGTGACT  
GGCAACTCTTCGCTAGTAAGAATGTTTGCCAACCAAGAAGAATAGCTCACCTAAAATTCCACGATA  
GCAGATCAACAAAAAATGGCATCGGCATTTAGCGACGCGATGGTCAAACCTGTCTCTCGTCGGGCA  
AGATAAGAGCCAGTTGATTGATTGTTTCAGACGTCATTCCGCGAACGATCCCTTTGACAAATGAACC  
ATACTTCCCTGCCGACTTGACCAAAGATGATCTTGAGCAGACCGTGAGTCCAATAGACATTCACAA  
TACGATTTCCACTCTGAGCCTGCTTGTTTTTGATACAGTGCCCGGATGAATTCCTGATTACCCTTC  
CAACCCCAGTGTCACTTCGGTCGCGCCAGTGTAAGTTTATGAACAGCTCCAGACGTCTGCTCTTAT  
TCACTCTGCGCAAATATCTAGTCCCACCTCGTAAGGATCC

**Text 2:** Amino acid sequence of adapted *mnp1* over-expressed in *A. bisporus*.

MAFKILLSLILALNAVQFIAAVPTRRAQCADGTTVSNEACCVLLPIIADIQ  
PNLFENECGEEVHETLRASFHDAIGFSRAAGGGGADGSLVTFGDVETTF  
ANAGIDEIVETLRPFINSHNISAGDFIQFATVVGLTNCPGAPRIPFFLGRPD  
ATAASPDGLVPEPFDSVTKILERFDDAGFTPTEVVALLASHTVAASDTIEP  
GLEGVPFDPSTPGEFDRQFFIETMLKGTSPGTGGNQGEALSPLPGELRLES  
DGLLARDERTACDWQLFATDQQKMASAFSDAMVKLSLVGQDKSQLIDC  
SDVIPRTIPLTNEPYFPADLTKDDLEQTCPDEFDPDYPSNPSVTSVAPVPTS