

Valorisation of wine wastes by *de novo* biosynthesis of resveratrol using a recombinant xylose-consuming industrial *Saccharomyces cerevisiae* strain

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Table S1. List of plasmids used in the study.

Name	Parent plasmid	Cloned biobricks	Description	Reference
pCfB2312			Episomal plasmid for Cas9 expression under <i>TEF1</i> promoter, <i>KanMX</i> selection marker	1
pCfB3042			gRNA helper vector targeting X-4 insertion site, <i>NatMX</i> selection marker	2
pCfB3049			gRNA helper vector targeting XII-4 insertion site, <i>NatMX</i> selection marker	2
pCfB3040			EasyClone-MarkerFree Integrative Vector for XII-4 insertion site (Chr XII:830227..831248)	2
pCfB2767			Multi-integrative plasmid, Ty4, P_{TDH3} - <i>AtPAL2</i> , P_{FBA1} - <i>AtC4H</i> , P_{PGK1} - <i>At4CL2</i> , P_{TEF1} - <i>VvVST1</i> , <i>KIURA3</i>	3
B430	pCfB3040	BB_CEC02	MarkerFree plasmid, X-4, P_{TDH3} - <i>AtPAL2</i> , P_{FBA1} - <i>AtC4H</i> , P_{PGK1} - <i>At4CL2</i> , P_{TEF1} - <i>VvVST1</i>	This study

Table S2. List of primers used in the study.

Name	Sequence (5' to 3')
PR-1496 (CYB5/ATR2_fw)	<u>CGTGCGA</u> UTCATTTCGTTCAACAAATAATAAGC
PR-1555 (CYB5/ATR2_rv)	<u>CACGCGA</u> UTCACCAGACATCTCTCAA
PR-2221 (universal_up_conf_rv)	GTTGACACTTCTAAATAAGCGAATTTC
PR-2220 (universal_down_conf_fw)	CCTGCAGGACTAGTGCTGAG
PR-905 (X-4_up_conf_fw)	CTCACAAAGGGACGAATCCT
PR-906 (X-4_down_conf_rv)	GACGGTACGTTGACCAGAG
PR-897 (XII-4_up_conf_fw)	GAACTGACGTCGAAGGCTCT
PR-898 (XII-4_down_conf_fw)	CGTGAAATCTCTTTGCGGTAG
ID224 (ADH1_test_fw)	GAAATTCGCTTATTTAGAAAGTGTC
PR-22847 (ADH1_C4L_seq_fw)	CGACCTCATGCTATACCT
PR-22848 (4CL_seq_int_rv)	TGTGTTCTTTGAGAGTTGGT
PR-22849 (4CL_seq_int_fw)	TGGTTTGGGATGTAGATATCTG
PR-22850 (4CL_seq_vst1_rv)	AAGTAGTAGTCGGCGTAATC
ID340 (PGK1_test_fw)	TACAGATCATCAAGGAAGTAATTATC
ID339 (TEF1_test_rv)	GCTCATTAGAAAGAAAGCATAGC
PR-22788 (VST1_seq_rv)	GCTCCTATTCTATCTGCTG
PR-22851 (PAL2_int2_fw)	GCAAATACCGACCAAGAA
PR-22852 (PAL2_int3_fw)	TCACCAGAAGCAGTAATAG
PR-22790 (PAL2_int_seq_fw)	GGGTTAGCCAAGTATTGC
PR-22853 (FBA1_PAL2_seq_rv)	GAAGAGAACAGACAAATGG
PR-22789 (NAT5_PAL2_seq_fw)	GACGAAACCGAAAGTGATGATG
ID504 (tdh3UPfwd1)	TCTGCTGTAACCCGTACATG
PR-22854 (C4H_seq_fw)	ACGGTGAACATTGGAGAA
PR-22791 (FBA1_C4H_seq_fw)	CTCTTTCGGGCTCAATTG
PR-22792 (CYC1_C4H_seq_rv)	CCTGTTACTTCTTGCAGACATC
ID18 (CYC1_rv)	CACGCGTCTGTACAGAAA
PR-22857 (ATR2_seq_int_fw)	GTTACACCGTTTTTTGATGCT

* Overhangs used for USER cloning are underlined.

Table S3. List of biobricks used in the study.

Biobrick	Description	Template	Forward primer	Reverse primer
BB_CEC02	<i>ScCyb5←PGK1p - TEF1p→AtATR2</i>	pCfB2767	PR-1496	PR-1555

Table S4. List of yeast strains used in the study.

Yeast strain	Parent strain	Relevant genotype	Reference
L323 (reported also as Ethanol Red RBP)	Ethanol Red®	<i>X-4: TDH3p→AtPAL2, FBA1p→AtC4H, PGK1p→At4CL2, TEF1p→VvVST1</i>	4
XylC2 V1 (ST5415)	Ethanol Red®	<i>X-3: TEF1p→RKI1, TDH3p→RPE1</i> <i>XI-3: TEF1p→TKL1, TDH3p→PsTAL1</i> <i>XII-2: TEF1p→CpXylA, TDH3p→PsSUT1</i> <i>XII-5: TEF1p→CpXylA, TDH3p→PsXYL3</i> <i>ΔGRE3::TEF1p→CpXylA/</i> <i>ΔGRE3::TEF1p→CpXylA</i>	5
L326	XylC2 V1 (ST5415)	<i>X-4: TDH3p→AtPAL2, FBA1p→AtC4H, PGK1p→At4CL2, TEF1p→VvVST1</i> <i>X-3: TEF1p→RKI1, TDH3p→RPE1</i> <i>XI-3: TEF1p→TKL1, TDH3p→PsTAL1</i> <i>XII-2: TEF1p→CpXylA, TDH3p→PsSUT1</i> <i>XII-5: TEF1p→CpXylA, TDH3p→PsXYL3</i> <i>ΔGRE3::TEF1p→CpXylA/</i> <i>ΔGRE3::TEF1p→CpXylA</i>	This study
L543	L326	<i>XII-4: ScCyb5←PGK1p - TEF1p→AtATR2</i> <i>X-4: TDH3p→AtPAL2, FBA1p→AtC4H, PGK1p→At4CL2, TEF1p→VvVST1</i> <i>X-3: TEF1p→RKI1, TDH3p→RPE1</i> <i>XI-3: TEF1p→TKL1, TDH3p→PsTAL1</i> <i>XII-2: TEF1p→CpXylA, TDH3p→PsSUT1</i> <i>XII-5: TEF1p→CpXylA, TDH3p→PsXYL3</i> <i>ΔGRE3::TEF1p→CpXylA/</i> <i>ΔGRE3::TEF1p→CpXylA</i>	This study

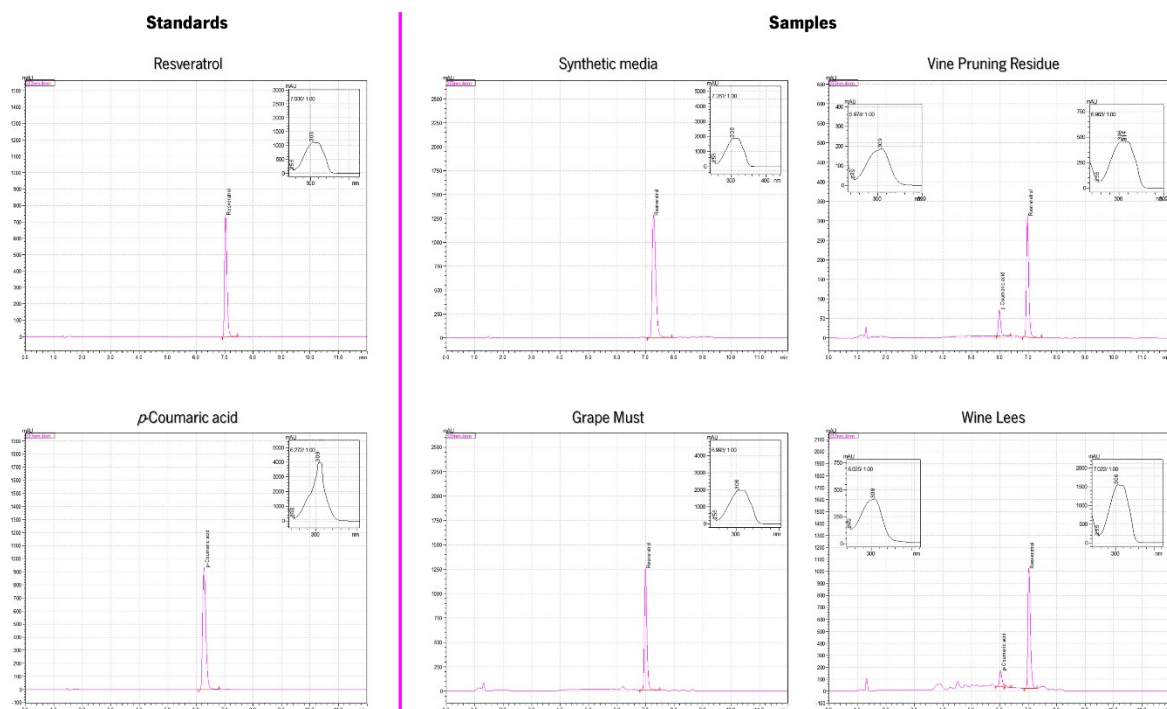


Fig. S1. Chromatograms from the analysis of standards and samples for resveratrol and *p*-coumaric acid detection and quantification.

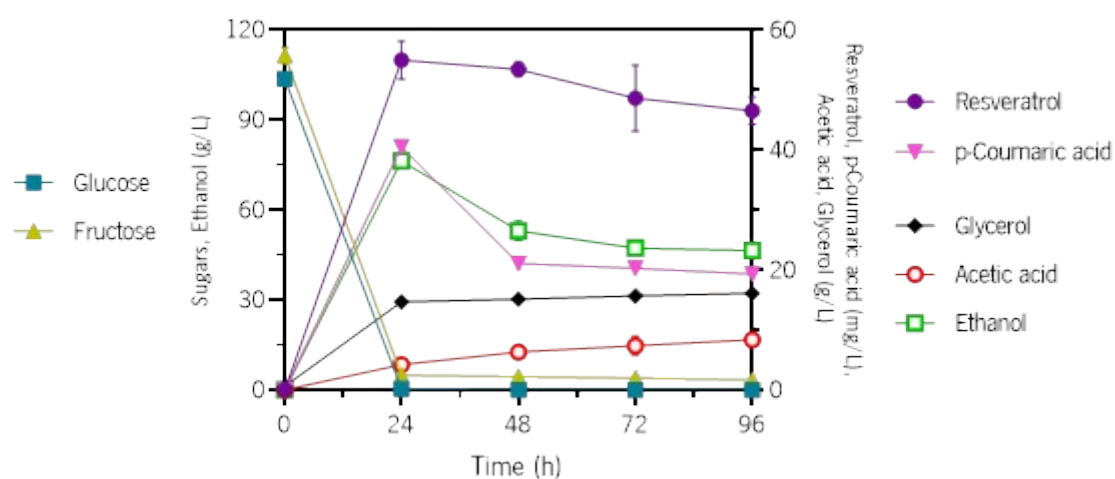


Fig. S2. Fermentation profile of media with 95 % of grape must dissolved supplemented with 7.5 g/L of yeast extract.

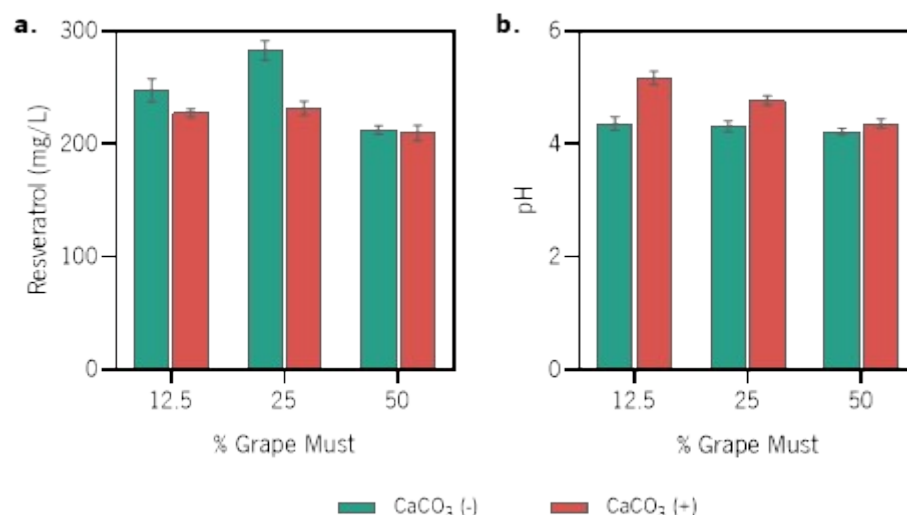
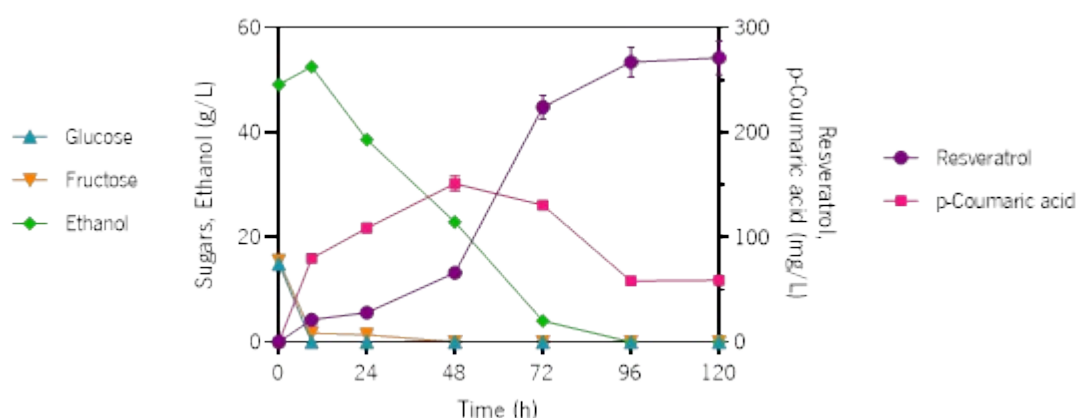


Fig. S3. Difference between fermentation without (CaCO₃ (-)) and with (CaCO₃ (+)) of different concentrations of grape must dissolved in the fermentation media, from 12.5 to 50 %, supplemented with 7.5 g/L of yeast extract.



References

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**DNA sequence of the Resveratrol Biosynthetic Pathway (*TDH3p*→*AtPAL2*,
FBA1p→*AtC4H*, *PGK1p*→*At4CL2*, *TEF1p*→*VvVST1*) used in this work (9955 bp).**

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DNA sequence of *ScCYB5/AtATR2* used in this work (3933 bp).

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