

Supplementary Tables

Table 1. Yeast strains used in this study

Strain	Description	Reference
BY4741	<i>MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0</i>	¹
BY4742	<i>MATa his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0</i>	¹
S288c (<i>MATa</i>)	<i>MATa</i> prototroph	¹
S288c (<i>MATa</i>)	<i>MATa</i> prototroph	¹
VR1	Natural isolate and former production strain in Brazilian bio-ethanol production with sugar cane	Mario Lucio Lopes (Fermentec, Piracicaba, Brazil)
VR1-5B	Haploid (<i>MATa</i>) segregant from VR1 with similarly high ethanol tolerance	This study

Table 2. Plasmids used in this study

Plasmid	Gene	Reference
YCplac111		²
YCplac111(<i>MKT1-BY</i>)	<i>MKT1</i> from BY4741	This study
YCplac111(<i>MKT1-VR</i>)	<i>MKT1</i> from VR1-5B	This study
pFA6-kanMX4		³

Table 3. Primers used for SNP detection

Marker	Position	Nucleotide		Primer coordinates	Primer combination		Annealing temperature
		BY 4741	VR1-5B		BY4741	VR1-5B	
S58	41,900	C	T	chr XIV 42,986 - 42,967	TGATGTTGAGTAC CTCCCAG	TGATGTTGAGTAC CTCCCAG	64
				chr XIV 41,883 - 41,900	CGCCACCACCAG CTACAC	CGCCACCACCAGC TACAT	
S59	81,570	C	G	chr XIV 80,641 - 80,660	TGCAGAACGATC AACAGAGG	TGCAGAACGATCA ACAGAGG	60
				chr XIV 81,590 - 81,570	ATTGTAGAGAGA GAACGCAAC	ATTGTAGAGAGAG AACGCAAG	
S60	118,249	C	G	chr XIV 117,380 - 117,399	AAGAGCTGGACA TTGAGGAG	AAGAGCTGGACAT TGAGGAG	62
				chr XIV 118,267 -	TGGACACCACGC	TGGACACCACGCT	

				118,249	TTTGACC	TTGACG	
S61	163,817	C	T	chr XIV 162,880 - 162,899	CAGAGATTGAGC AGTTCAGC	CAGAGATTGAGCA GTTTCAGC	60
				chr XIV 163,839 - 163,817	AGGCTATAAGAA TTTCAAAAACC	AGGCTATAAGAAT TTCAAAAAC T	
S62	206,786	C	T	chr XIV 205,964 - 205,983	TGAAGGATGGAG TGTTTCACG	TGAAGGATGGAGT GTTTCACG	64
				chr XIV 206,806 - 206,786	CGAGATTTATCAT CATGAGCC	CGAGATTTATCAT CATGAGC T	
S63	254,474	G	A	chr XIV 253,639 - 253,658	AGAGTTCAAGGC TGCTGATG	AGAGTTCAAGGCT GCTGATG	66
				chr XIV 254,494 - 254,474	GCACCAGTTCTTT CTTG GT TG	GCACCAGTTCTTT CTTG GT TA	
S64	295,690	G	T	chr XIV 295,093 - 295,112	ACCATGTGAGGA AAGTCCTG	ACCATGTGAGGAA AGTCCTG	60
				chr XIV 295,710 - 295,690	CGGCAGTGACTA AAAATC TT TG	CGGCAGTGACTAA AAATC TT T	
S65	334,339	G	A	chr XIV 334,782 - 334,763	CCACCTTGCTTTG TCATCTG	CCACCTTGCTTTGT CATCTG	62
				chr XIV 334,319 - 334,339	CCAGATTATATTG CTCCTG A G	CCAGATTATATTG CTCCTG A A	
S66	383,709	T	A	chr XIV 382,989 - 383,008	TACTTGCAGTGTC CTCTTG G	TACTTGCAGTGTC CTCTTG G	60
				chr XIV 383,733 - 383,709	CCAATTCACAATA AATATAACACT T	CCAATTCACAATA AATATAACACT A	
S67	417,421	C	T	chr XIV 418,015 - 417,996	GGGTCATCTACTA CAAGTGC	GGGTCATCTACTA CAAGTGC	60
				chr XIV 417,398 - 417,421	AATTTTTGAGAAA GTTTCAAAAGC	AATTTTTGAGAAA GTTTCAAAAG T	
S67-1	445,671	G	A	chr XIV 445,651 - 445,671	TGCACATCATTCA TATCAGC G	TGCACATCATTCA TATCAGC A	62
				chr XIV 445,852 - 445,832	CACTCCTAGAAG AGA ACT GT T	CACTCCTAGAAGA GA ACT GT C	
S67-2	454,997	C	T	chr XIV 454,977 - 454,997	CTATGGAACAAT CACACTT G C	CTATGGAACAATC ACACTT G T	62
				chr XIV 455,285 - 455,264	TAGATTTATGGGC TTGTGATT C	TAGATTTATGGGC TTGTGATT T	
S68	468,912	C	T	chr XIV 468,229 - 468,248	TCATCTAGTGCTT CTCCTG C	TCATCTAGTGCTT CTCCTG C	62
				chr XIV 468,931 - 468,912	AGAATACCTCAC CCCATC T C	ATAATACCTCACC CCATC T T	
S68-1	480,343	C	T	chr XIV 480,324 - 480,343	GCAAGAAGGAGC TGACAT C C	GCAAGAAGGAGCT GACAT C T	66
				chr XIV 480,506 - 480,487	ACGGTGGACGAC TACTGG T T	ACGGTGGACGACT ACTGG T G	

S68-2	484,452	C	T	chr XIV 484,432 - 484,452	GATGAAGATTCC GATTGGGAC	GATGAAGATTCCG ATTGGGAT	64
				chr XIV 484,869 - 484,851	TAGAGCTGTCAC GTTGCTT	CAGAGCTGTCACG TTGCTC	
S68-3	489,321	G	C	chr XIV 489,302 - 489,321	ACGAAGGCATTG ATTCCACG	ACGAAGGCATTGA TTCCACC	60
				chr XIV 489,489 - 489,468	TGCCAGATGTAAT TCACTAAAC	TGCCAGATGTAAT TCACTAAAG	
S69	504,614	A	G	chr XIV 504,593 - 504,614	GCCTAAAAGATT CATCAACAGA	GCCTAAAAGATTT ATCAACAGG	64
				chr XIV 504,851 - 504,830	GACCAAATGATA GTTGTATGGC	GACCAAATGATAG TTGTATGGT	
S70	546,739	**	**	chr XIV 546,351 - 546,370	GGTCAACGTAGA TGAGGTAG	GGTCAACGTAGAT GAGGTAG	54
				chr XIV 547,273 - 547,254	AGCTTGGAGAAG CATGAGAG	AGCTTGGAGAAGC ATGAGAG	
S71	586,448	A	T	chr XIV 586,112 - 586,131	CAACAGAGTCAA GCTCTGAG	CAACAGAGTCAAG CTCTGAG	66
				chr XIV 586,468 - 586,448	AAGTAAGGGTGC TATGAGCTA	AAGTAAGGGTGCT ATGAGCTT	
S72	635,537	A	G	chr XIV 634,366 - 634,385	TAGACCACTTCCT TGTCGTG	TAGACCACTTCCT TGTCGTG	62
				chr XIV 635,558 - 635,537	CCATACCCACAGT AAATATGTA	CCATACCCACAGT AAATATGTG	
S73	705,570	C	A	chr XIV 706,456 - 706,437	CATCTGCTCTTCG AATGCTC	CATCTGCTCTTCG AATGCTC	64
				chr XIV 705,550 - 705,570	GCTATTGCTACAA TATCTGCC	GCTATGGCTACAA TATCTGCA	

** The region from 546,739 till 547,083 is deleted in VR1-5B. Two primers were chosen that flank this region. Amplification of the region by PCR results in fragments with different lengths, by which the parent strains could be distinguished.

References

1. C. B. Brachmann, A. Davies, G. J. Cost et al., *Yeast* **14** (2), 115 (1998).
2. R. D. Gietz and A. Sugino, *Gene* **74** (2), 527 (1988).
3. A. Wach, A. Brachat, R. Pohlmann et al., *Yeast* **10** (13), 1793 (1994).

Supplementary Figure legends

Supplementary Figure 1. SNP nucleotide frequency versus SNP chromosomal position for comparison of strain VR1-5B and BY4741.

As an example, the nucleotide frequency of each SNP on chromosome XIV of strain VR1-5B compared with BY4741 is plotted against the SNP chromosomal position. Similar plots were obtained for all other chromosomes. These two parent strains have been crossed to obtain the segregants that were phenotyped for ethanol tolerance.

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