

Identification of the α -galactosidase *MEL* genes in some populations of *Saccharomyces cerevisiae*: a new gene *MEL11*

GENNADI I. NAUMOV^{1,2}, ELENA S. NAUMOVA^{1,2}, HILKKA TURAKAINEN²
AND MATTI KORHOLA^{3*}

¹ State Institute for Genetics and Selection of Industrial Microorganisms, I Dorozhnyi 1, Moscow 113545, Russia

² Department of Biosciences, Division of Genetics, P.O. Box 56 (Viikinkaari 5), FIN-00014 University of Helsinki, Finland

³ Primalco Ltd, P.O. Box 350 FIN-00101 Helsinki, Finland

(Received 6 September 1995)

Summary

In this report we mapped a new *MEL11* gene and summarize our population studies of the α -galactosidase *MEL* genes of *S. cerevisiae*. The unique family of structural *MEL* genes has undergone rapid translocations to the telomeres of most chromosomes in some specific *Saccharomyces cerevisiae* populations inhabiting olive oil processing waste (alpechin) and animal intestines. A comparative study of *MEL* genes in wine, pathogenic and alpechin populations of *S. cerevisiae* was conducted using genetic hybridization analysis, molecular karyotyping and Southern hybridization with the *MEL1* probe. Five polymeric genes for the fermentation of melibiose, *MEL3*, *MEL4*, *MEL6*, *MEL7*, *MEL11*, were identified in an alpechin strain CBS 3081. The new *MEL11* gene was mapped by tetrad analysis to the left telomeric region of chromosome I. In contrast, in wine and pathogenic populations of *S. cerevisiae*, *MEL* genes have been apparently eliminated. Their rare Mel⁺ strains carry only one of the *MEL1*, *MEL2*, or *MEL8* genes. One clinical strain YJM273 was heterozygotic on the *MEL1* gene; its *mel1*⁰ allele did not have a sequence of the gene.

1. Introduction

The polymeric α -galactosidase *MEL* genes controlling melibiose fermentation were briefly described several years ago by Roberts *et al.* (1959) and Santa Maria & Vidal (1970) in some *S. cerevisiae* strains isolated from animal intestines and olive oil processing waste (alpechin), but during following decades they had remained forgotten. A family of movable genes *MEL1–MEL10* of *S. cerevisiae* was later identified and is of interest for evolutionary and ecological yeast genetics (Naumov, 1989; Naumov *et al.* 1990, 1991, 1995a; Turakainen *et al.* 1993a, 1994). The *MEL* genes are dispersed throughout the genome in some strains, and they have been mapped to the telomeric regions of different chromosomes (Naumov *et al.* 1995b; Turakainen *et al.* 1993b; Vollrath *et al.* 1988).

Screening of the *MEL* genes in natural yeast strains allows the differentiation of some populations and the saturation of the *S. cerevisiae* genetic map with new functional telomeric markers. By genetic and mol-

ecular analysis of natural *S. cerevisiae* strains, we have now extended the family of *MEL* genes to include a new member, the *MEL11* gene.

2. Materials and methods

(i) Strains and culture conditions

The strains of *S. cerevisiae* used and their genotypes are listed in Table 1. The methods and media for cultivation and hybridization of yeasts have been described earlier (Naumov *et al.* 1986). Hybrids of heterothallic yeasts were obtained by mass mating of cells and by isolation of zygotes with a micromanipulator. Crosses between heterothallic and homothallic strains were performed by the 'haploid cell-to-spore' mating method using the micromanipulator. Melibiose fermentation was first determined on a pH-indicator agar medium with eosin–methylene blue (EMB) after 1 d of incubation (Scheda & Yarrow, 1968). The yeasts that did not ferment melibiose on EMB agar medium were then tested in Durham fermentation tubes. The composition of the liquid fermentation medium was (g/l): yeast extract, 10; peptone, 20; sugar, 20. Mel⁺ strains usually began

* Correspondence to Matti Korhola, Department of Biosciences, Division of General Microbiology, P.O. Box 56 (Viikinkaari 9), FIN-00014 University of Helsinki, Finland. Tel: (358 0) 70859212, Fax: (358 0) 70859262.

Table 1. Strains of *Saccharomyces cerevisiae* from which monosporic cultures were obtained

Strain ^a	Genotype ^b	Reference or source
X2180-1A	<i>a SUC2 mal mel gal2</i>	R. K. Mortimer
S288C	α <i>SUC2 mal mel gal2</i>	R. K. Mortimer
YNN 295	α <i>ura3 lys2 ade1 ade2 his7 trp1-Δ1</i>	R. W. Davis
YP1	<i>a mel ura3 ade2 lys2</i>	E. J. Louis
N2	α <i>SUC MAL MEL1 GAL</i>	Naumov <i>et al.</i> (1990)
C.B.11	<i>a SUC MAL6 MEL1 GAL lys2</i>	Naumov <i>et al.</i> (1990)
CBS 4411	<i>HO suc mal MEL3 MEL4 MEL5 MEL6 MEL7 GAL</i>	Naumov <i>et al.</i> (1990)
CBS 5378	<i>HO suc mal MEL3 MEL4 MEL6 MEL7 MEL8 MEL9 MEL10 gal4</i>	Naumov <i>et al.</i> (1991)
CBS 3081	<i>HO suc MAL MEL GAL</i>	Naumov <i>et al.</i> (1983, 1995a)
VKM Y-1830	<i>ho SUC MAL MEL2 GAL</i>	Naumov <i>et al.</i> (1990)
VKM Y-1703	<i>ho SUC MAL MEL GAL</i>	Naumov (1988)
61-190	<i>HO SUC MEL GAL</i>	H. J. Phaff
L579	<i>HO SUC MAL MEL GAL</i>	Naumov <i>et al.</i> (1993)
L1425	<i>HO SUC MAL MEL GAL</i>	Naumov <i>et al.</i> (1993)
YJM273	<i>HO SUC MAL MEL GAL</i>	McCusker <i>et al.</i> (1994)
YJM455	<i>HO SUC MAL MEL GAL</i>	J. H. McCusker
ML2-3D	α <i>mal MEL2 gal2</i>	Naumov <i>et al.</i> (1991)
OL2-5B	α <i>SUC2 MEL3 gal2</i>	Naumov <i>et al.</i> (1990)
OL2-4B	<i>a SUC2 MEL3 gal2</i>	Naumov <i>et al.</i> (1990)
OL0-11C	α <i>SUC2 MEL4 GAL</i>	Naumov <i>et al.</i> (1990)
OL11-11B	α <i>SUC2 MEL6 gal2</i>	Naumov <i>et al.</i> (1990)
OL13-7A	<i>a SUC MEL6 gal2</i>	Naumov <i>et al.</i> (1990)
OL8-9B	<i>a SUC2 MEL7 GAL</i>	Naumov <i>et al.</i> (1990)
OL33-2C	<i>a SUC2 MEL7 gal2</i>	Naumov <i>et al.</i> (1990)
NR2-3D	<i>a suc MEL8 gal</i>	Naumov <i>et al.</i> (1991)
S56	α <i>mel ura3 I R-TG_{1,3}::URA3</i>	Louis & Borts (1995)
S194	α <i>mel ura3 I L-TG_{1,3}::URA3</i>	Louis & Borts (1995)
TM11a	<i>a MEL11 ura3 ade2 lys2</i>	Present study

^a CBS = Centraalbureau voor Schimmelcultures, Delft, Holland; NCYC = National Collection of Yeast Cultures, Nutfield, England; VKM = All-Russian Collection of Microorganisms, Moscow, Russia; YJM = Department of Biochemistry, Stanford University Medical Centre, USA; ATCC = American Type Culture collection, Rockville, USA; VKPM = All-Russian Collection of Industrial Microorganisms, Moscow, Russia. C.B.11 is an inbred line of NCYC 74. CBS 4411 = VKPM Y-61, CBS 5378 = VKM Y-1232, CBS 3081 = VKM Y-1234, YJM273 = CBS 7835, S56 equivalent to ATCC 96004, S194 = ATCC 96003, YP1 = ATCC 90839.

^b Genotypes of haploid cells or spores are given. Generally accepted genetic nomenclature is used.

fermenting melibiose (Serva or Aldrich Chemicals) in 1 d, but some strains took 2–8 d (see footnotes for Table 2). Mel⁻ strains did not ferment melibiose during a test period of 10 d. The genes for sucrose, galactose and maltose fermentation were used as control markers.

To localize the new *MEL11* gene, we used two telomeric testers, S56 and S194, each marked by the *URA3* gene integrated in the right and the left end of chromosome I, respectively. The tester strains were derived from S288C marked by the *ura3*⁻ mutation by integrating a *URA3*-containing vector having telomeric TG_{1,3} sequences (Louis & Borts, 1995). Segregation of uracil auxotrophy was determined on the following medium (g/l): dextrose, 20; Difco yeast nitrogen base, without amino acids, 6.7; adenine, 0.3;

lysine, 0.3; agar, 20. Control segregation for the red *ade2* marker was screened on YPD medium.

(ii) Isolation of genomic DNA

Total DNA from the yeast strains was prepared by the method described by Sherman *et al.* (1981), digested with restriction endonucleases (Boehringer Mannheim or New England Biolabs), and electrophoresed in agarose gels.

(iii) CHEF gel electrophoresis

The preparation of chromosomal DNA has been described elsewhere (Naumov *et al.* 1992). A CHEF-DR[®] II apparatus (Bio-Rad, Richmond, CA, USA) was used to separate chromosomal DNAs. The electro-

phoresis buffer was 0.5 × TBE. The buffer was circulated around the gel and cooled to 14 °C. Electrophoresis was conducted at 200 V for 15 h with a switching time of 60 s and then for 8 h with a switching time of 90 s. *S. cerevisiae* YNN 295 chromosomes (Bio-Rad) were used as a standard.

(iv) Southern blot analysis

Southern blot analysis of DNA restriction fragments was done essentially according to Maniatis *et al.* (1982). The chromosomal DNA separated by CHEF was treated with depurination solution (0.55 M HCl), denatured, neutralized, and transferred to nitrocellulose filters which were then baked at 80 °C for 2 h. The *MEL1* probe was a 2.8 kb *Bam*H I-*Sal* I fragment (Ruohola *et al.* 1986) inserted in pGEM3 (Promega). The *TRP5* probe was a 3.3 kb *Bam*H I-*Bam*H I fragment isolated from pA-B3 (Balzi *et al.* 1987). The *ADC1* probe was a 1.5 kb *Bam*H I-*Hind* III fragment isolated from pAAH5 (Ammerer, 1983). The probes were labelled with digoxigenin-11-dUTP using the Nonradioactive DNA Labeling Kit (Boehringer Mannheim, Mannheim, Germany). Hybridization and colorimetric detection of hybridization were performed as recommended by the supplier (Boehringer Mannheim).

3. Results

(i) Monogenic determination

We first compared three wine *Mel*⁺ strains, one strain isolated from *Drosophila*, and two clinical isolates with the reference testers (C.B.11 and VKM Y-1830)

carrying the *MEL1* and *MEL2* genes, respectively. Both crossing with the *Mel*⁻ tester X2180-1A and Southern hybridization of chromosomal DNA with the cloned *MEL1* gene showed that all six strains contained only one *MEL* gene each (Table 2, monogenic segregation of hybrids CW1–CW5; Fig. 1*b*, lanes 4–9). In three strains, L1425, L579 and YJM455 (Fig. 1*a*, *b*, lanes 4, 5 and 8, respectively) the *MEL1* probe hybridized to the chromosomal doublet containing chromosomes VII/XV while in VKM Y-1703 these two chromosomes were separated (Fig. 1*a*, lane 6). It is known that the *MEL2* and *MEL8* genes are located on chr. VII and chr. XV, respectively (Naumov *et al.* 1990, 1991). The *TRP5* probe (chr. VII) hybridized to the same band as the *MEL1* probe (not shown), indicating that VKM Y-1703 apparently possesses the *MEL2* gene. According to the sizes of hybridizing bands, strains 61-190 and YJM273 may have the *MEL1* gene. Tetrad recombination analysis showed the fine *MEL* genotypes of five strains to be as follows: L579 and L1425 – *MEL8*; VKM Y-1703 – *MEL2*; 61-190 and YJM273 – *MEL1* (Table 2). In some cases (hybrids CW6, CW8, CW11, and CW12), genetically appropriate substitutions of homothallic *Mel*⁺ parent strains by their *Mel*⁺ heterothallic segregants from crosses with *Mel*⁻ tester X2180-1A were made (see footnotes to Table 2).

The original strain YJM273 was heterozygotic on the *MEL1* gene, as shown by the monogenic segregation 48 *Mel*⁺:51 *Mel*⁻ found among its meiotic products. Moreover, the *mel1*⁰ allele does not have a silent *MEL* sequence (Fig. 1*b*, lane 10). Strain YJM273 is also heterozygotic on genes *GAL* and *MAL* (McCusker *et al.* 1994). Another clinical strain,

Table 2. Identification of genotypes of *Saccharomyces cerevisiae* strains L579, L1425, VKM Y-1703, 61-190, and YJM273

Hybrid	Origin of <i>Mel</i> ⁺ hybrids ^a	No. of tetrads segregating as <i>Mel</i> ⁺ : <i>Mel</i> ⁻			Genotypes
		4:0	2:2	3:1	
CW1	L579 × X2180-1A	0	8	0	<i>MEL8/mel</i>
CW2	L1425 × X2180-1A	0	21	0	<i>MEL8/mel</i>
CW3	1703 × X2180-1A	0	14	0	<i>MEL2/mel</i>
CW4	61-190 × X2180-1A	0	12	0	<i>MEL1/mel</i>
CW5	273-10B × X2180-1A	0	21	0	<i>MEL1/mel</i>
CW6	CW1-6D × NR2-3D	18	0	0	<i>MEL8/MEL8</i>
CW7	L579 × ML2-3D	2	0	8	<i>MEL8/MEL2</i>
CW8	CW2-8B × NR2-3D	33	0	0	<i>MEL8/MEL8</i>
CW9	L1425 × ML2-3D	8	6	18	<i>MEL8/MEL2</i>
CW10 ^b	1703 × ML2-3D	23	0	0	<i>MEL2/MEL2</i>
CW11	CW4-2D × N2	22	0	0	<i>MEL1/MEL1</i>
CW12 ^c	CW5-5B × C.B.11	91	<i>Mel</i> ⁺ :0 <i>Mel</i> ⁻		<i>MEL1/MEL1</i>

^a Equivalent strains: CW1-6D (α *GAL mal*) = L579, CW2-8B (α *GAL SUC*) = L1425, CW4-2D (α *gal2 SUC mal*) = 61-190, CW5-5B (α *GAL SUC mal*) = 273-10B (*HO GAL SUC mal*).

^b A large number of its segregants showed delayed melibiose fermentation.

^c Data on random spore analysis are given.

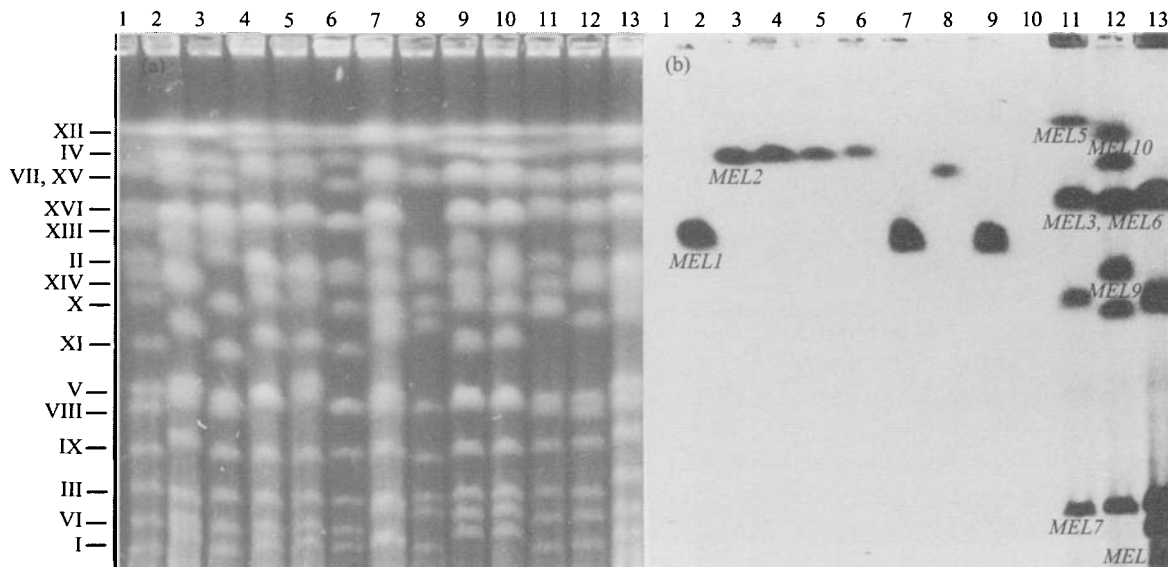


Fig. 1. Molecular karyotyping and Southern blot analysis of the chromosomal DNAs of *Mel*⁺ strains of *S. cerevisiae* isolated from different populations: wine (lanes 3–6, VKM Y-1830, L1425, L579 and VKM Y-1703, respectively), *Drosophila* (lane 7, 61-190), pathogenic-source (lanes 2, 8, 9 and 10, C.B.11, YJM455, YJM273-10B and YJM273-2A, respectively), pig faeces (lane 11, CBS 4411), alpechin (lanes 12 and 13, CBS 5378 and CBS 3081, respectively). Ethidium bromide-stained gel (a) *MEL1* probe (b). The linkage group numbering refers to the chromosomes of the strain YNN 295 (lane 1). All cultures studied are monosporic. Strain YJM73-2A is *Mel*⁻.

Table 3. Genetic identification of the genes *MEL3*, *MEL4*, *MEL4*, *MEL7*, and *MEL11* in *Saccharomyces cerevisiae* CBS 3081

Hybrid	Origin of <i>Mel</i> ⁺ hybrids	No. of tetrads segregating as <i>Mel</i> ⁺ : <i>Mel</i> ⁻			Genotypes ^a
		4:0	2:2	3:1	
OG0	3081 × X2180-1A	57	0	5	(<i>MEL</i>) ₅ / <i>mel</i>
OG1	OG0-3A × X2180-1A	5	5	17	(<i>MEL</i>) ₂ / <i>mel</i>
OG2	OG0-3C × X2180-1A	4	3	21	(<i>MEL</i>) ₂ / <i>mel</i>
OG3	OG0-3D × X2180-1A	4	2	23	(<i>MEL</i>) ₂ / <i>mel</i>
OG4	OG1-3B × X2180-1A	0	22	0	<i>MEL11</i> / <i>mel</i>
OG5	OG1-3C × S288C	0	23	0	<i>MEL6</i> / <i>mel</i>
OG6	OG1-3D × X2180-1A	0	22	0	<i>MEL11</i> / <i>mel</i>
OG7	OG2-1A × X2180-1A	0	22	0	<i>MEL7</i> / <i>mel</i>
OG8	OG2-1B × X2180-1A	0	23	0	<i>MEL4</i> / <i>mel</i>
OG9	OG2-1D × S288C	0	23	0	<i>MEL4</i> / <i>mel</i>
OG10	OG3-2B × S288C	0	22	0	<i>MEL3</i> / <i>mel</i>
OG11	OG3-2C × X2180-1A	0	22	0	<i>MEL3</i> / <i>mel</i>
OG12	OG3-2D × X2180-1A	0	22	0	<i>MEL7</i> / <i>mel</i>
OG13	OG1-3C × OG3-2C	7	7	13	<i>MEL6</i> / <i>MEL3</i>
OG14 ^b	OG1-3C × OL2-5B	65 <i>Mel</i> ⁺ :25 <i>Mel</i> ⁻			<i>MEL6</i> / <i>MEL3</i>
OG15 ^b	OG1-3C × OL11-11B	110 <i>Mel</i> ⁺ :0 <i>Mel</i> ⁻			<i>MEL6</i> / <i>MEL6</i>
OG16	OG2-1A × OL8-9B	27	0	0	<i>MEL7</i> / <i>MEL7</i>
OG17	OG2-1D × OL0-11C	24	0	0	<i>MEL4</i> / <i>MEL4</i>
OG18	OG3-2C × OL2-4B	28	0	0	<i>MEL3</i> / <i>MEL3</i>
OG19	OG3-2C × OL13-7A	4	3	20	<i>MEL3</i> / <i>MEL6</i>
OG20	OG3-2D × OL33-2C	22	0	0	<i>MEL7</i> / <i>MEL7</i>
OG21	OG1-3D × YP1	0	27	0	<i>MEL11</i> / <i>mel</i>

^a Additional genotypes of segregants studied: OG0-3A, α *GAL SUC2 MAL*; OG0-3C, *HO GAL SUC2 mal*; OG0-3D, α *gal2 suc2 mal*; OG1-3B, α *SUC2 GAL mal*; OG1-3C, α *SUC2 GAL MAL*; OG1-3D, α *SUC2 gal2 MAL*; OG2-1A, α *SUC2 gal2 mal*; OG2-1B, *HO GAL SUC2 mal*; OG2-1D, α *SUC2 gal2 mal*; OG3-2B, α *gal2 SUC2 mal*; OG3-2C, α *gal2 suc2 mal*; OG3-2D, α *gal2 suc2 mal*.

^b Data on random spore analysis are given.

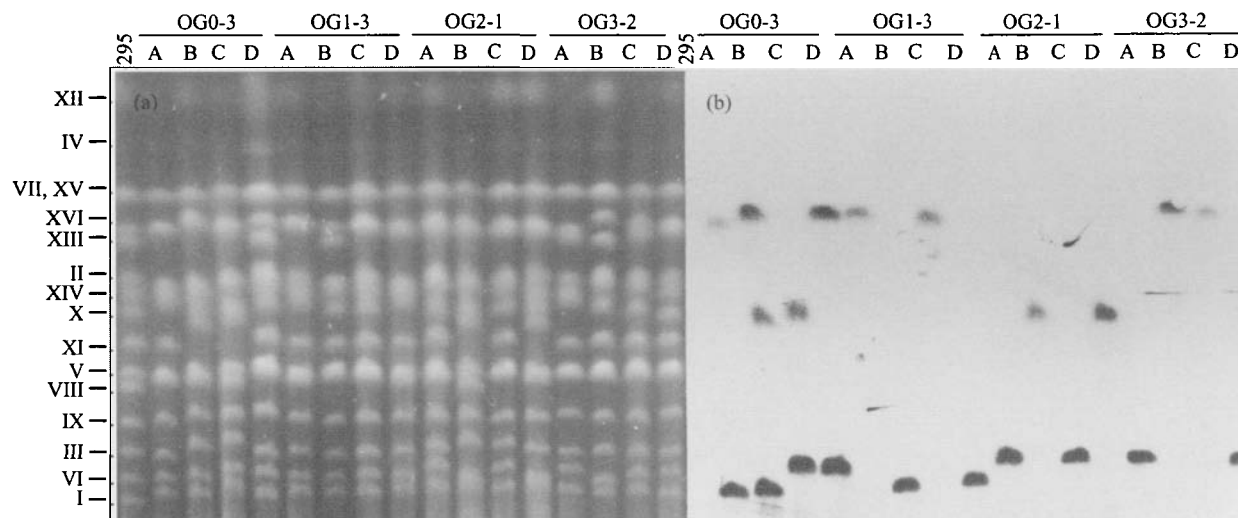


Fig. 2. Molecular karyotyping and Southern blot analysis of chromosomal DNAs from the first (OG0-3) and second (OG1-3, OG2-1 and OG3-2) generations of segregants descended from the strain CBS 3081. Ethidium bromide-stained gel (a) corresponding to hybridization of chromosomal DNAs with the *MEL1* probe (b). The linkage group numbering refers to the chromosomes of the strain YNN 295.

YJM455, showed the segregation 4 Mel^+ :8 Mel^- . However, in this strain a cosegregation of ' Mel^- ', ' Gal^- ' and ' ρ^- ' properties was observed, suggesting that the Mel^- phenotype was probably caused by mitochondrial mutations in these segregants. All monosporic clones analysed of strain YJM455 were Mal^+ Suc^+ . The *MEL* gene of strain YJM455 was not determined precisely. This strain showed a unique karyotype pattern (Fig. 1a, lane 8) due to the absence of the doublet containing chromosomes XIII and XVI seen in the standard strain YNN 295 (Fig. 1a, lane 1). The chromosomal band comigrating with chromosomes VII and XV hybridized to the *MEL1* probe as well as to the *TRP5* (chr. VII) and the *ADC1* promoter (chr. XV) probes (not shown) suggesting that YJM455 may possess the *MEL8* or *MEL2* gene.

(ii) Polygenic determination

Molecular karyotyping and Southern hybridization revealed that strain CBS 3081 isolated from olive oil processing waste (alpechin), had four chromosomal bands hybridizing with the *MEL1* probe, one of which had a new location, chromosome I (Fig. 1b, lane 13). According to the intensity of the hybridization signal, the uppermost band probably contained two hybridizing chromosomes (XIII and XVI). By comparison to the standard strain YNN 295 (Fig. 1a, lane 1), having a known order and sizes of chromosomes, and by comparison to strains with known *MEL* genes as reference testers (Fig. 1b, lanes 2, 3, 11 and 12), it was possible to conclude that strain CBS 3081 might have the *MEL3*, *MEL4*, *MEL6*, *MEL7* and *MEL11* genes, two of which, *MEL3* and *MEL6*, are located in a doublet containing chromosomes XVI/XIII (Naumov *et al.* 1990). The complete genotype of strain CBS 3081 was identified by genetic hybridization analysis (Table 3, Fig. 3). A polygenic segregation for melibiose

fermentation with an absolute predominance of tetrads segregating as 4 Mel^+ :0 Mel^- was observed in a cross between a monosporic highly fertile homothallic culture of CBS 3081 and a haploid Mel^- tester X2180-1A (Table 3, hybrid OG0). The ratio of the different types of tetrads confirmed that strain CBS 3081 contained five *MEL* genes. One complete tetrad of the hybrid OG0: OG0-3A, OG0-3B, OG0-3C, OG0-3D containing Mel^+ segregants only was isolated and all of its *MEL* genes were identified. Southern blot analysis of this tetrad suggested the following genotypes for the segregants: OG0-3A – *MEL3/6*, *MEL11*; OG0-3B – *MEL3*, *MEL4*, *MEL6*, *MEL11*; OG0-3C – *MEL4*, *MEL7*; and OG0-3D – *MEL6/3*, *MEL7* (Fig. 2a and b). Identification of all *MEL* genes of the heterozygotic hybrid OG0 necessitated determining by genetic analysis the genotypes of at least three segregants from the complete tetrad OG0-3 and then cloning by recombination all of the five *MEL* genes. Three backcrosses of segregants OG0-3A, OG0-3C, and OG0-3D with their Mel^- parents (hybrids OG1, OG2 and OG3, respectively) were studied (Figs. 2, 3). Each of the hybrids gave digenic segregation (Table 3). One complete tetrad, 4⁺:0⁻, from each of the three hybrids was analysed further. The backcrosses of the segregants from the tetrads OG1-3, OG2-1, and OG3-2 with the Mel^- testers (Table 3, hybrids OG4-OG12) and Southern blot analysis (Fig. 2b) showed that the segregants in the new generation contained all the *MEL* genes isolated.

Mel^- segregants were observed in hybrid OG1-3C × OG3-2C, proving that strain CBS 3081 carried two *MEL* genes localized on chromosomes of similar sizes (Table 3, hybrid OG13; Fig. 2b).

Taking into account the chromosomal bands hybridizing to the *MEL1* probe (Fig. 2b), a limited number of the *MEL* testers (*MEL3*, *MEL4*, *MEL6* and *MEL7*) were used in order to identify the isolated

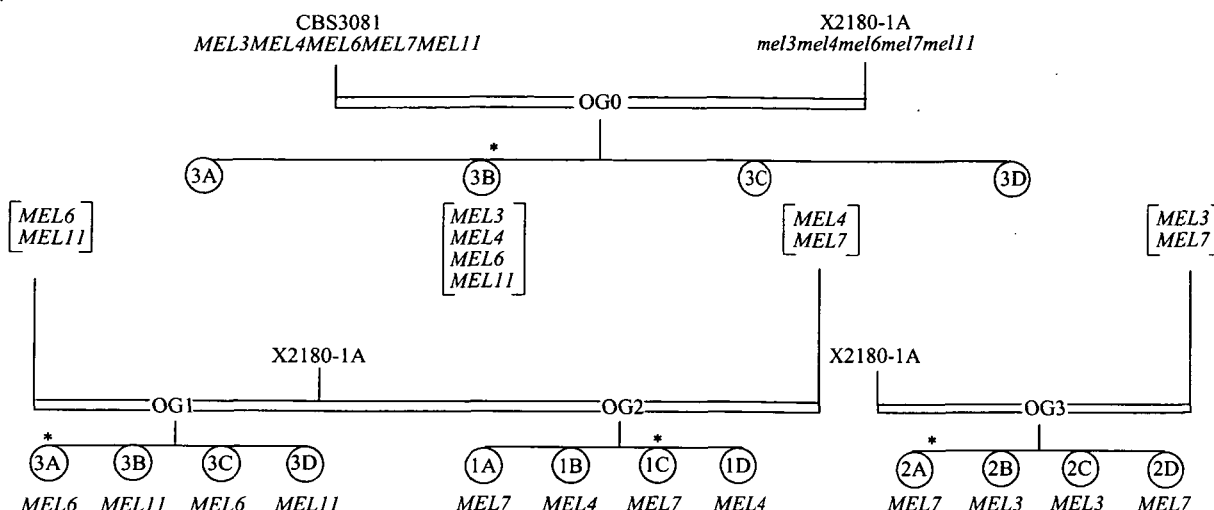


Fig. 3. Pedigree of the segregants studied from the cross CBS 3081 \times X2180-1A and genetic isolation of *MEL3*, *MEL4*, *MEL6*, *MEL7*, and *MEL11* genes. Asterisks indicate strains in which the genotype was determined by molecular methods only.

Table 4. Meiotic segregation of the *URA2* gene integrated into the right and left telomeres (*TG₁₋₃::URA3*) of chromosome I and the new *MEL11* gene

Hybrid	Marker pairs ^a	P ^b	N	T
OG22	IR:: <i>URA3</i> / <i>MEL11</i>	5	2	24
OG23	IL:: <i>URA3</i> / <i>MEL11</i>	306	0	0

^a Monogenic segregation of the control marker *ade2* was also observed in both hybrids.

^b Types of tetrads: P = parental ditype, N = non-parental ditype, T = tetratype.

genes. The recombination analysis data indicated that the segregants OG1-3C, OG2-1A, OG2-1D, OG3-2C, and OG3-2D contained *MEL6*, *MEL7*, *MEL4*, *MEL3*, and *MEL7*, respectively (Table 3, hybrids OG14–OG20; Fig. 3) while the segregants OG1-3B and OG1-3D carried the new *MEL11* gene (Figs. 2b, 3). Knowing the genotypes of these segregants, we could determine the genotypes of the hybrids OG1, OG2, and OG3 as *MEL6 MEL11/mel6 mel11*, *MEL4 MEL7/mel4 mel7*, and *MEL3 MEL7/mel3 mel7*, respectively. The genotype of the initial tetrad OG0-3 was established as: 3A, *MEL6 MEL11*; 3C, *MEL4 MEL7*; 3D, *MEL3 MEL7*. Therefore, the hybrid OG0 is heterozygotic on these five *MEL* genes; and its *Mel*⁺ parent, the monosporic culture of strain CBS 3081, has the haploid genotype *MEL3 MEL4 MEL6 MEL7 MEL11*.

The precise location of the *MEL11* gene was established on the basis of recombination with the *URA3* marker integrated in the telomeres of chromosome I. First, we constructed strain TM11a (OG21-37A) having the genotype: *a MEL11 ura3 ade2 lys2* by crossing with strain YP1 (Table 3, hybrid OG21). The constructed strain was crossed with two telomeric testers, S56 and S194 (Table 4, hybrids OG22 and

OG23). The *URA3* integrated in the right telomere of chromosome I, and the *MEL11* gene was inherited independently (Table 4, hybrid OG22), while all 306 tetrads analysed of the hybrid OG23 were non-recombinant parent ditypes. Thus, the *MEL11* gene was genetically mapped in the telomeric region of the left arm of chromosome I.

(iii) Restriction analysis

The total DNA from the segregant OG1-3D carrying the *MEL11* gene was digested with restriction endonucleases, separated in agarose gel, transferred to nitrocellulose filter, and hybridized to the *MEL1* probe. The restriction map of the *MEL11* locus (not shown) was identical with that of the *MEL5*, *MEL7* and *MEL9* loci (Turakainen *et al.* 1993a).

4. Discussion

Hybrid brewing lager yeasts *S. pastorianus* (syn *S. carlsbergensis*) and wine strains of *S. bayanus* (syn. *S. uvarum*) are usually *Mel*⁺ (Naumov *et al.* 1993; Vaughan Martini & Martini, 1987) while cultivated and wild strains of *S. cerevisiae* generally are *Mel*⁻. However, there are some very specific *S. cerevisiae* populations, inhabiting alpechin and animal intestines, in which an accumulation of polymeric *MEL* genes has taken place (Naumov *et al.* 1995a). In the present study, we identified five *MEL* genes in the alpechin strain CBS 3081: *MEL3*, *MEL4*, *MEL6*, *MEL7*, *MEL11*. The *MEL11* gene has not previously been reported in the literature. Earlier, the complete genotypes of two other strains have been established. Strain CBS 4411 isolated from pig faeces harbored the *MEL3*, *MEL4*, *MEL5*, *MEL6*, and *MEL7* genes (Naumov *et al.* 1990) and the alpechin strain CBS 5378 had seven genes: *MEL3*, *MEL4*, *MEL6*, *MEL7*, *MEL8*, *MEL9*, and *MEL10* (Naumov *et al.* 1991).

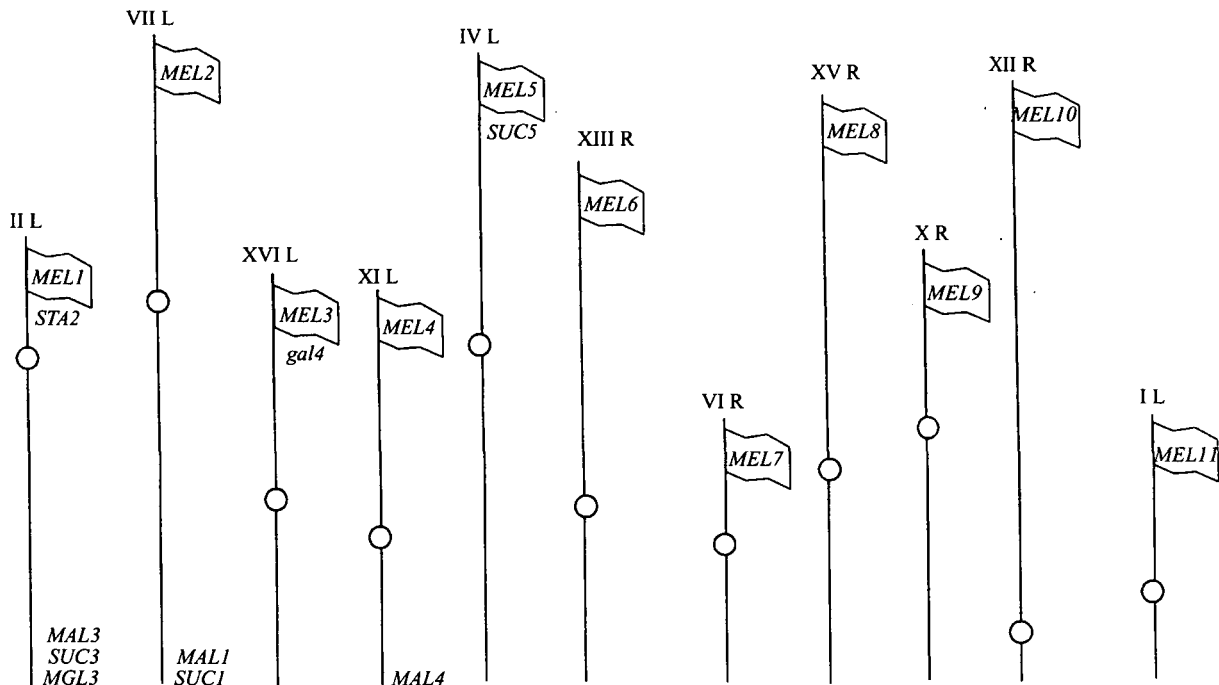


Fig. 4. Evolutionary dynamics of the chromosome location of fermentation *MEL*, *MAL*, *SUC*, *STA*, and *MGL* genes in some populations of *Saccharomyces cerevisiae* (Lyness *et al.* 1992; Mortimer *et al.* 1992; Naumov *et al.* 1990, 1991, 1995b, present study).

In contrast, in wine and pathogenic populations of *S. cerevisiae* *Mel*⁺ strains are very rare. Among 46 different wine strains (listed elsewhere, Naumova *et al.* 1993), only four strains were *Mel*⁺ and only two out of 15 clinical strains could ferment melibiose (McCusker *et al.* 1994; J. H. McCusker, pers. comm.). In the present study and in a previous one (Naumov *et al.* 1990), we found altogether eight strains having a monogenic determination for melibiose fermentation. Two wine strains, VKM Y-1830 and VKM Y-1703 carried the *MEL2* gene, while the other two, L579 and L1425, had *MEL8*. Strain 61-190 isolated from *Drosophila* possessed the *MEL1* gene and clinical isolates, NCYC 74 (C.B.11), YJM273, and YJM455, had the *MEL1*, *MEL1*, and *MEL2/8* genes, respectively. The rarity of *Mel*⁺ strains in pathogenic and wine populations of *S. cerevisiae*, the different monogenic determination of the *Mel*⁺ character and the possible heterozygosity *MEL/mel* suggest that *Mel*⁺ strains appear randomly and that an elimination of *MEL* genes then takes place in these populations.

Thus, *MEL* genes are accumulated in some populations of *S. cerevisiae* while *MEL* genes have been lost in others. It is important to emphasize that the genetic drift of *MEL* genes involves the plastic part of yeast genome – telomeric regions (Fig. 4) (Louis & Haber, 1990a,b; Louis *et al.* 1994). In addition, a Ty delta sequence has been found in the *MEL* downstream region (Turakainen *et al.* 1994). The abrupt end in the homology between *MEL5/MEL7/MEL9* and *MEL4/MEL6/MEL8* loci in the middle of this sequence suggests that some of these *MEL* gene duplication/translocation events might be Ty delta-

mediated. The *MEL* genes are not, however, the only movable gene family (Fig. 4). The telomeric *SUC*, *MAL*, *MGL*, and, probably, *STA* genes controlling fermentation of sucrose, maltose, α -methylglucoside and soluble starch (dextrins), respectively, behave similarly. In some populations of *S. cerevisiae* and wild species of *S. paradoxus*, an elimination of active *MAL* alleles has been found (Naumov, 1977; Naumov *et al.* 1994). An accumulation of *gal4* mutations, along with mutations in other *GAL* genes, takes place in some wine *S. cerevisiae* strains (Naumov & Gudkova, 1979a,b). It is interesting to note that the *GAL4* gene is also located at the end of a chromosome (Fig. 4).

The polymorphism of the telomeric genes for fermentation of various sugars, and in particular the *MEL* genes, represents a unique genetic system which allows us to study the rapid microevolution of yeast genome at the level of chromosomes and genes.

We would like to thank Professors L. Halkka and D. Bamford for the possibility of doing the main part of this research at the Department of Genetics, University of Helsinki, and Drs E. J. Louis and J. H. McCusker for kindly providing yeast strains and their descriptions. This research was supported by a grant from the Foundation for Biotechnical and Industrial Fermentation Research (Finland) and partly by the grant 'Frontiers in Genetics' from the Russian Academy of Sciences to G.I.N. and E.S.N.

References

- Ammerer, G. (1983). Expression of genes in yeast using the *ADC1* promoter. *Methods in Enzymology* **101**, 192–201.
- Balzi, E., Chen, W., Ulaszewski, S., Capioux, E. & Goffeau,

- A. (1987). The multidrug resistance gene *PDR1* from *Saccharomyces cerevisiae*. *Journal of Biological Chemistry* **262**, 16871–16879.
- Louis, E. J. & Borts, R. H. (1995). A complete set of marked telomeres in *Saccharomyces cerevisiae* for physical mapping and cloning. *Genetics* **139**, 125–136.
- Louis, E. J. & Haber, J. E. (1990a). The subtelomeric Y' repeat family in *Saccharomyces cerevisiae*. An experimental system for repeated sequence evolution. *Genetics* **124**, 533–545.
- Louis, E. J. & Haber, J. E. (1990b). Mitotic recombination among subtelomeric Y' repeats in *Saccharomyces cerevisiae*. *Genetics* **124**, 547–559.
- Louis, E. J., Naumova, E. S., Lee, A., Naumov, G. & Haber, J. E. (1994). The chromosome end in yeast: its mosaic nature and influence on recombinational dynamics. *Genetics* **136**, 789–802.
- Lyness, C. A., Jones, C. R. & Meaden, P. G. (1993). The *STA2* and *MEL1* genes of *Saccharomyces cerevisiae* are idiomorphic. *Current Genetics* **23**, 92–94.
- Maniatis, T., Fritsch, E. F. & Sambrook, J. (1982). *Molecular Cloning. A Laboratory Manual*. Cold Spring Harbor, New York: Cold Spring Harbor Laboratory Press.
- McCusker, J. H., Clemons, K. V., Stevens, D. A. & Davis, R. W. (1994). Genetic characterization of pathogenic *Saccharomyces cerevisiae* isolates. *Genetics* **136**, 1261–1269.
- Mortimer, R. K., Contopoulou, C. R. & King, J. S. (1992). Genetic and physical maps of *Saccharomyces cerevisiae*, edition 11. *Yeast* **8**, 817–902.
- Naumov, G. I. (1977). Comparative genetics of yeast. XVI. Genes for maltose fermentation in *Saccharomyces carlsbergensis* N.C.Y.C. 74. *Soviet Genetics* **12**, 1374–1386.
- Naumov, G. I. (1988). A hybridological study of the yeast *Saccharomyces* from the expedition collection of V. I. Kudrjavev (during 1934 and 1936). *Mikologiya i Fitopatologiya* **22**, 295–301 (in Russian).
- Naumov, G. I. (1989). Identification of melibiose fermentation polymeric genes in *Saccharomyces cerevisiae*. *Doklady Biological Sciences* **304**, 53–55.
- Naumov, G. I. & Gudkova, N. K. (1979a). Comparative genetics of yeast. XVIII. Microevolution of *Saccharomyces bayanus*. *Soviet Genetics* **15**, 380–387.
- Naumov, G. I. & Gudkova, N. K. (1979b). Regressive evolution of *Saccharomyces*. *Doklady Biological Sciences* **245**, 791–793.
- Naumov, G. I., Kondratieva, V. I. & Naumova, E. S. (1986). Methods for hybridization of homothallic yeast diploids and haploids. *Soviet Biotechnology* **6**, 29–32.
- Naumov, G. I., Kondratieva, V. I., Naumova, T. I. & Gudkova, N. K. (1983). Genetic bases for classification of *Saccharomyces cerevisiae*. A study of survival of hybrid ascospores. *Zhurnal Obshchei Biologii* **44**, 648–660 (in Russian).
- Naumov, G. I., Naumova, E. S. & Gaillardin, C. (1993). Genetic and karyotypic identification of wine *Saccharomyces bayanus* yeasts isolated in France and Italy. *System. Applied Microbiology* **16**, 274–279.
- Naumov, G. I., Naumova, E. S. & Korhola, M. (1992). Genetic identification of natural *Saccharomyces sensu stricto* yeasts from Finland, Holland and Slovakia. *Antonie van Leeuwenhoek* **61**, 237–243.
- Naumov, G. I., Naumova, E. S. & Korhola, M. (1995a). Chromosomal polymorphism of *MEL* genes in some populations of *Saccharomyces cerevisiae*. *FEMS Microbiology Letters* **127**, 41–45.
- Naumov, G. I., Naumova, E. S. & Louis, E. J. (1995b). Genetic mapping of the α -galactosidase *MEL* gene family on right and left telomeres of *Saccharomyces cerevisiae*. *Yeast* **11**, 481–483.
- Naumov, G. I., Naumova, E. S. & Michels, C. A. (1994). Genetic variation of the repeated *MAL* loci in natural populations of *Saccharomyces cerevisiae* and *Saccharomyces paradoxus*. *Genetics* **136**, 803–812.
- Naumov, G. I., Naumova, E. S., Turakainen, H., Suominen, P. & Korhola, M. (1991). Polymeric genes *MEL8*, *MEL9* and *MEL10* – new members of α -galactosidase gene family in *Saccharomyces cerevisiae*. *Current Genetics* **20**, 269–276.
- Naumov, G. I., Turakainen, H., Naumova, E. S., Aho, S. & Korhola, M. (1990). A new family of polymorphic genes in *Saccharomyces cerevisiae*: α -galactosidase genes *MEL1*–*MEL7*. *Molecular and General Genetics* **224**, 119–128.
- Naumova, E. S., Naumov, G. I. & Korhola, M. (1993). Molecular karyotypes of various genetic lines of the yeast *Saccharomyces cerevisiae*. *Russian Biotechnology* **4**, 1–4.
- Roberts, C., Ganesan, A. T. & Haupt, W. (1959). Genetics of melibiose fermentation in *Saccharomyces italicus* var. *melibiosi*. *Heredity* **13**, 499–517.
- Ruohola, H., Liljeström, P. L., Torkkeli, T., Kopu, H., Lehtinen, P., Kalkkinen, N. & Korhola, M. (1986). Expression and regulation of the yeast *MEL1* gene. *FEMS Microbiology Letters* **34**, 179–185.
- Santa Maria, J. & Vidal, D. (1970). Segregación anormal del 'mating type' en *Saccharomyces*. *Inst. Nac. Invest. Agron.* **30**, 1–21.
- Scheda, R. & Yarrow, D. (1968). Variation in the fermentative pattern of some *Saccharomyces* species. *Archiv für Mikrobiologie* **61**, 310–316.
- Sherman, F., Fink, G. R. & Lawrence, C. W. (1981). *Methods in Yeast Genetics*. Cold Spring Harbor, New York: Cold Spring Harbor Laboratory Press.
- Turakainen, H., Aho, S. & Korhola, M. (1993a). *MEL* gene polymorphism in the genus *Saccharomyces*. *Applied and Environmental Microbiology* **59**, 2622–2630.
- Turakainen, H., Naumov, G., Naumova, E. & Korhola, M. (1993b). Physical mapping of the *MEL* gene family in *Saccharomyces cerevisiae*. *Current Genetics* **24**, 461–464.
- Turakainen, H., Kristo, P. & Korhola, M. (1994). Consideration of the evolution of the *Saccharomyces cerevisiae* *MEL* gene family on the basis of the nucleotide sequences of the genes and their flanking regions. *Yeast* **10**, 1559–1568.
- Vaughan Martini, A. & Martini, A. (1987). Three newly delimited species of *Saccharomyces sensu stricto*. *Antonie van Leeuwenhoek* **53**, 77–84.
- Vollrath, D., Davis, R. W., Connelly, C. & Hieter, P. (1988). Physical mapping of large DNA by chromosome fragmentation. *Proceedings of the National Academy of Sciences, USA* **85**, 6027–6031.