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

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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, 1995*a*; Turakainen *et al.* 1993*a*, 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.* 1995*b*; Turakainen *et al.* 1993*b*; 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 molecular analysis of natural *S. cerevisiae* strains, we have now extended the family of *MEL* genes to include a new member, the *MEL11* gene.

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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

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

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

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

[a] Equivalent strains: CW1-6D (*α GAL mal*) = L579, CW2-8B (*αGAL SUC*) = L1425, CW4-2D (*a 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.

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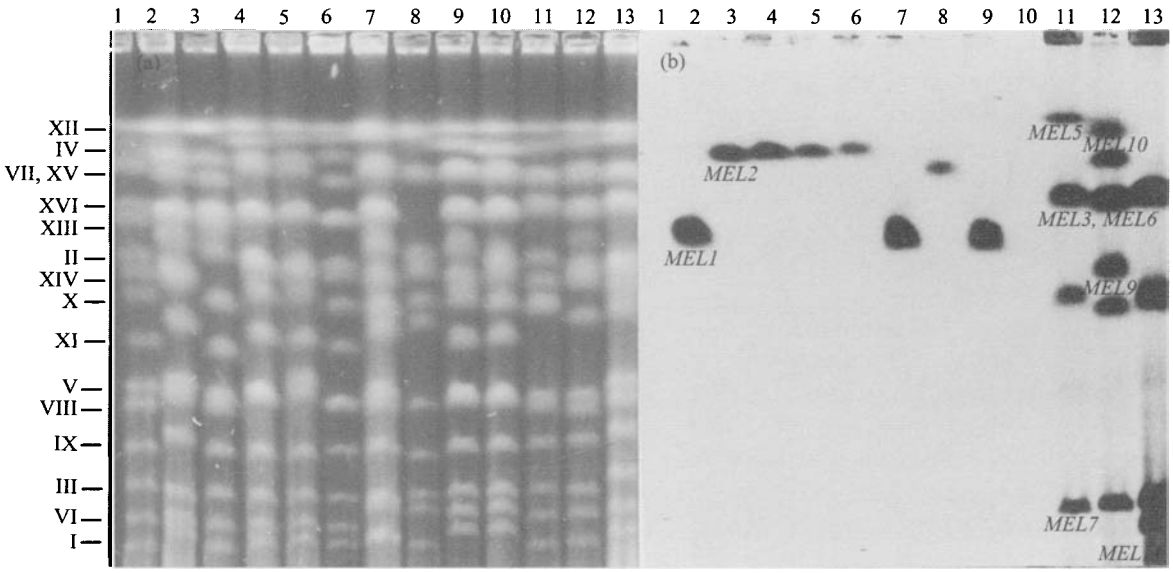

Fig. 1. Molecular karyotyping and Southern blot analysis of the chromosomal DNAs of Mel$^+$ strains of *S. cerevisiae* isolated from different populations: wine (lanes 3–6, VKM Y-1830, L1425, L579 and VKM Y-1703, respectively), *Drosophila* (lane 7, 61-190), pathogenic-source (lanes 2, 8, 9 and 10, C.B.11, 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 Mel$^+$ hybrids	No. of tetrads segregating as Mel$^+$:Mel$^-$ 4:0	2:2	3:1	Genotypes[a]
OG0	3081 × X2180-1A	57	0	5	$(MEL)_5/mel$
OG1	OG0-3A × X2180-1A	5	5	17	$(MEL)_2/mel$
OG2	OG0-3C × X2180-1A	4	3	21	$(MEL)_2/mel$
OG3	OG0-3D × X2180-1A	4	2	23	$(MEL)_2/mel$
OG4	OG1-3B × X2180-1A	0	22	0	*MEL11/mel*
OG5	OG1-3C × S288C	0	23	0	*MEL6/mel*
OG6	OG1-3D × X2180-1A	0	22	0	*MEL11/mel*
OG7	OG2-1A × X2180-1A	0	22	0	*MEL7/mel*
OG8	OG2-1B × X2180-1A	0	23	0	*MEL4/mel*
OG9	OG2-1D × S288C	0	23	0	*MEL4/mel*
OG10	OG3-2B × S288C	0	22	0	*MEL3/mel*
OG11	OG3-2C × X2180-1A	0	22	0	*MEL3/mel*
OG12	OG3-2D × X2180-1A	0	22	0	*MEL7/mel*
OG13	OG1-3C × OG3-2C	7	7	13	*MEL6/MEL3*
OG14[b]	OG1-3C × OL2-5B	65 Mel$^+$:25 Mel$^-$			*MEL6/MEL3*
OG15[b]	OG1-3C × OL11-11B	110 Mel$^+$:0 Mel$^-$			*MEL6/MEL6*
OG16	OG2-1A × OL8-9B	27	0	0	*MEL7/MEL7*
OG17	OG2-1D × OL0-11C	24	0	0	*MEL4/MEL4*
OG18	OG3-2C × OL2-4B	28	0	0	*MEL3/MEL3*
OG19	OG3-2C × OL13-7A	4	3	20	*MEL3/MEL6*
OG20	OG3-2D × OL33-2C	22	0	0	*MEL7/MEL7*
OG21	OG1-3D × YP1	0	27	0	*MEL11/mel*

[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, *a SUC2 GAL MAL*; OG1-3D, *αSUC2 gal2 MAL*; OG2-1A, *α SUC2 gal2 mal*; OG-1B, *HO GAL SUC2 mal*; OG2-1D, *a SUC2 gal2 mal*; OG3-2B, *a gal2 SUC2 mal*; OG3-2C, *α gal2 suc2 mal*; OG3-2D, *α gal2 suc2 mal.*
[b] Data on random spore analysis are given.

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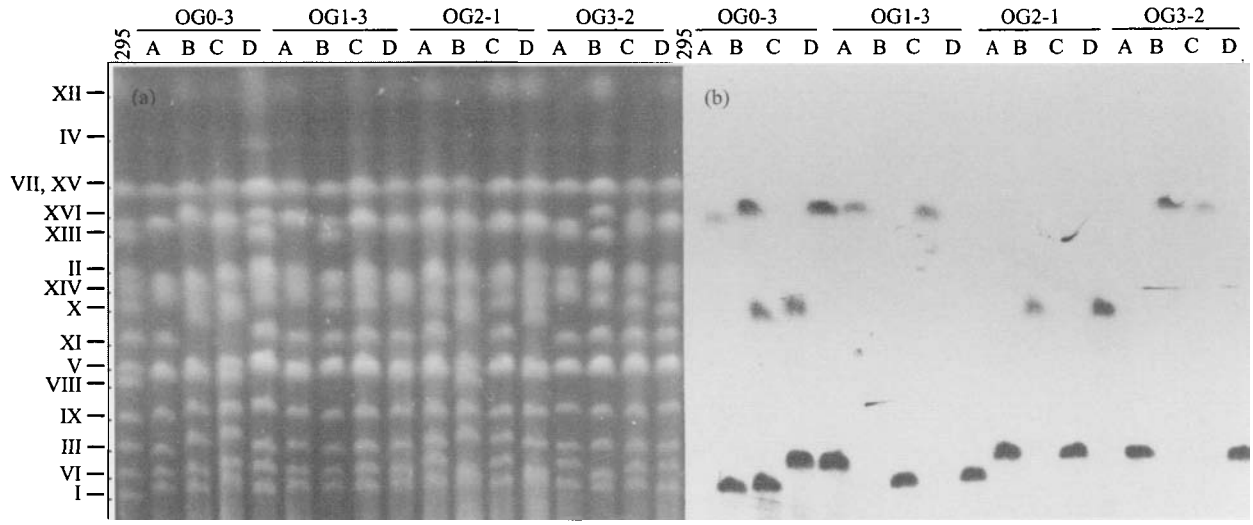

Fig. 2. Molecular karyotyping and Southern blot analysis of chromosomal DNAs from the first (OG0-3) and second (OG1-3, OG2-1 and OG3-2) generations of segregants descended from the strain CBS 3081. Ethidium bromide-stained gel (*a*) corresponding to hybridization of chromosomal DNAs with the *MEL1* probe (*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. 1 *a*, lane 8) due to the absence of the doublet containing chromosomes XIII and XVI seen in the standard strain YNN 295 (Fig. 1 *a*, 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. 1 *b*, 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. 1 *a*, lane 1), having a known order and sizes of chromosomes, and by comparison to strains with known *MEL* genes as reference testers (Fig. 1 *b*, 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. 2 *a* 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. 2*b*) 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. 2*b*).

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

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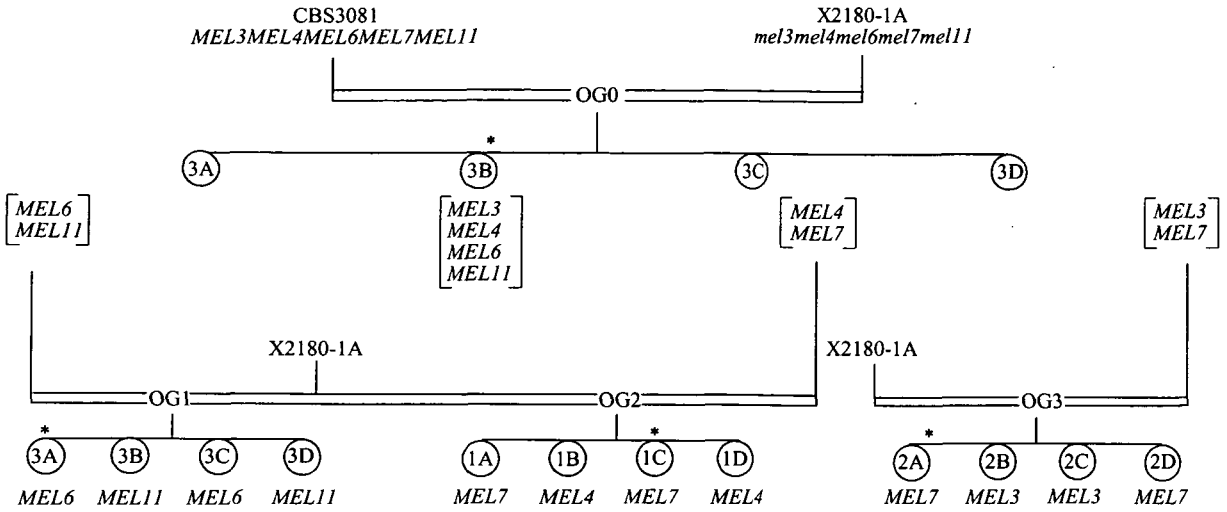

Fig. 3. Pedigree of the segregants studied from the cross CBS 3081 × X2180-1A and genetic isolation of *MEL3*, *MEL4*, *MEL6*, *MEL7*, and *MEL11* genes. Asterisks indicate strains in which the genotype was determined by molecular methods only.

Table 4. *Meiotic segregation of the URA2 gene integrated into the right and left telomeres ($TG_{1\text{-}3}$::URA3) of chromosome I and the new MEL11 gene*

Hybrid	Marker pairs[a]	P[b]	N	T
OG22	IR::*URA3*/*MEL11*	5	2	24
OG23	IL::*URA3*/*MEL11*	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. 2*b*, 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 TM11*a* (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.* 1993*a*).

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.* 1995*a*). 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).

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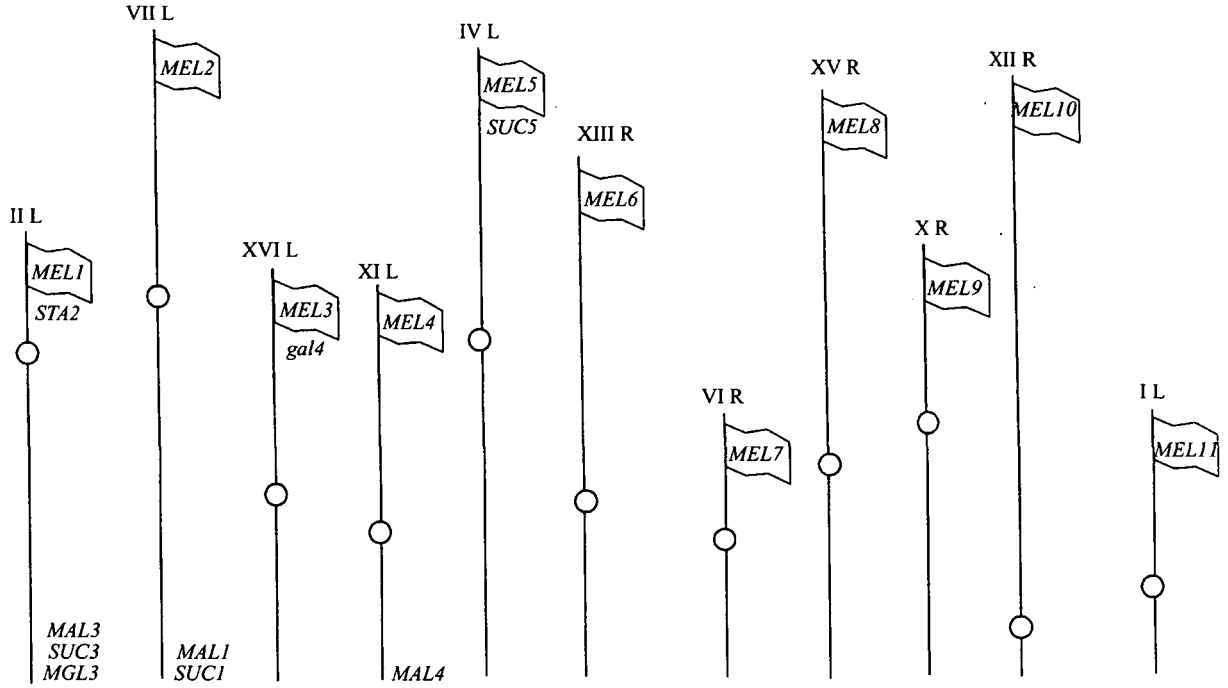

Fig. 4. Evolutionary dynamics of the chromosome location of fermentation *MEL*, *MAL*, *SUC*, *STA*, and *MGL* genes in some populations of *Saccharomyces cerevisiae* (Lyness *et al.* 1992; Mortimer *et al.* 1992; Naumov *et al.* 1990, 1991, 1995*b*, present study).

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, 1990*a*, *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, 1979*a*, *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.

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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., Capiaux, 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. (1990*a*). 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. (1990*b*). 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. Kudrjavtzev (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. (1979*a*). Comparative genetics of yeast. XVIII. Microevolution of *Saccharomyces bayanus*. *Soviet Genetics* **15**, 380–387.

Naumov, G. I. & Gudkova, N. K. (1979*b*). 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 diplonts and haplonts. *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. (1995*a*). 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. (1995*b*). 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. (1993*a*). *MEL* gene polymorphism in the genus *Saccharomyces*. *Applied and Environmental Microbiology* **59**, 2622–2630.

Turakainen, H., Naumov, G., Naumova, E. & Korhola, M. (1993*b*). 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.