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Chromosomal reorganization during meiosis of *Saccharomyces cerevisiae* baker's yeasts

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Abstract The genomic constitution of two *S. cerevisiae* baker's yeasts and their meiotic products have been analyzed by pulsed-field gel-electrophoresis, hybridization with specific gene probes, marker segregation, and flow cytometry. The parental strains have chromosomal patterns substantially different from those of laboratory strains used as controls. This pattern is partly the result of there being more than one copy of homologous chromosomes of different size, as judged by Southern-blot hybridization carried out with specific gene probes. Flow cytometry indicated that the strains have a 2.7 C DNA content. Tetrad analysis showed disomy for some chromosomes and tetrasomy for others. When two complete tetrads were subjected to molecular analysis the results confirmed instances of segregation of homologous chromosomes of different size. However, the presence of chromosomal bands absent in the parentals and the disappearance of chromosomal bands present in the parental strains were frequently seen. This result was attributed to two different phenomena: (1) the presence of multiple Ty1 and Ty2 transposable elements which seem to undergo interchromosomal translocation together with amplification, giving rise to differences in chromosomal size; (2) the presence of multiple Y′ subtelomeric regions, giving rise to asymmetrical homologous recombination and, as a consequence, differences betwen the size of the recombinant chromosomes and the non-recombinant parental chromosomes. Chromosomal reorganization occurs with a very high frequency during meiosis. By contrast, mitosis is very stable, as judged by the reproducible electrophoretic karyotype shown by the parental strains in successive generations.

Key words Baker's yeast · Chromosomal polymorphism · Meiotic instability · Chromosomal reorganization

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Introduction

Analysis of the electrophoretic karyotypes of laboratory and industrial yeasts has revealed the presence of chromosomal-length polymorphism between strains of *S. cerevisiae*. This polymorphism is much more extensive in industrial yeasts (Bidenne et al. 1992). When electrophoretic karyotype analysis is complemented with genetic methods, an extraordinary complexity of the chromosomal constitution of industrial yeasts is evident. Many of them are aneuploid and possess a variable number of chromosomal copies depending on the specific chromosome involved (Bakalinsky and Snow 1990; Vezinhet et al. 1990; Adams et al. 1992; Ibeas and Jiménez 1996). Furthermore, hybridization of chromosomes from industrial yeasts with specific probes indicates that individual strains contain different-sized homologous chromosomes as well as hybrid chromosomes resulting from interchromosomal translocation (Bidenne et al. 1992).

Other investigators have also found evidence that industrial yeast populations undergo radical changes in their genomic structure, including changes in ploidy level and chromosomal loss (Adams et al. 1992). This phenomenon has been attributed to adaptation to specific environments which is frequently accompanied by large-scale duplication of the genome, large deletions, and polymorphisms for chromosomal length (Adams et al. 1992; Yoda et al. 1993).

The systematic segregation during meiosis of more than one band corresponding to a particular chromosome has confirmed that the bands represent homologous chromosomes of different length (Bakalinski and Snow 1990; Bi-

denne et al. 1992; Wicksteed et al. 1994). Homologous recombination within subtelomeric Y′ repeat sequences is thought to contribute to chromosomal-size differences and, therefore, chromosomal-length polymorphism (Vezinhet et al. 1990; Jäger et al. 1992). However, differences in size have also been detected in homologous chromosomes lacking Y′ regions (Bidenne et al. 1992). Consequently, in addition to homologous recombination within Y′ regions, intrachromosomal (deletion/duplication) or interchromosomal (translocation) mechanisms have been suggested to be responsible for this polymorphism (Aguilera and Klein 1993). Examples of spontaneous rearrangements include the non-reciprocal translocation of part of the left arm of chromosome III onto chromosome I observed in some strains of *S. cerevisiae* and the deletions that occur in mutator *DEL1* strains and which have breakpoints at two Ty transposable elements (Aguilera and Klein 1993). There are also spontaneous reciprocal translocations that result from interactions between Ty elements located on chromosomes VII and XIII (Aguilera and Klein 1993).

When analyzing this phenomenon Yoda et al. (1993) found that DNA length polymorphism is generated during meiosis and sporulation. These authors noticed that the length of chromosome-III-DNA of a parent strain was remarkably different from reported values, and that the length of this chromosome in some sporulation-deficient mutants varied from that of the parent strain. This polymorphism was generated by the loss or addition of a specific unit of approximately 100 kb. Furthermore, Jäger et al. (1992) attributed the strong aneuploidy and chromosomal-length polymorphism generated in the meiosis of a *S. cerevisiae* strain to different numbers of Ty elements and Y′ repeats, as well as other subtelomeric elements which would lead to impaired chiasma formation.

We report here the meiotic analysis carried out with two baker's strains and their meiotic products. Our results support a previous suggestion that Ty elements and Y′ repeat sequences are responsible for both inter- and intra-chromosomal reorganization occurring during meiosis. However, in the baker's strains analyzed these reorganizations occur with an extremely high frequency.

Materials and methods

Strains. The *S. cerevisiae* strains used in this work are listed in Table 1. The baker's yeasts V1 and V2 were chosen for their high frequency of sporulation and tetrad formation (over 50%), as well as spore viability (about 60%), as compared to other industrial strains (Codón and Benítez 1995; Codón et al. 1995).

Enzymes and chemicals. Proteinase K was obtained from Merck A. G. (Darmstadt Germany); Zymolyase 20 000 from Seikagako (Seikagako kogyo Co. Ltd. Tokyo Japan); N-lauroylsarcosine, as well as all other chemicals used, were purchased from the Sigma Chemical Co. (St. Louis, Missouri, USA).

Tetrad analysis. Tetrad analysis was performed as described by Sherman et al. (1986) after providing the cells with optimal sporulation conditions (Codón et al. 1995). Tetrads were printed via a velvet replicator system onto various diagnostic media to determine their phenotypes: minimal medium SD to determine auxotrophies; YPG medium for petite phenotype; PRE1 and SPO1 to establish the ability to sporulate. Finally, the meiotic products were mixed with either MMY1 and MMY2 as testers to determine their capacity to conjugate.

Electrophoretic karyotype. The basis procedure followed for chromosomal DNA preparation was that of Naumov et al. (1992). The gel was prepared with 0.5×TBE buffer and 0.9% agarose. The system used was a CHEF-DRII gel-electrophoresis apparatus from Bio-Rad Laboratories (Richmond, California, USA). Electrophoresis was carried out at 14 °C and 200 V for 15 h with a switching time of 60 s, and then for 8 h with a switching time of 90 s. A standard set of *S. cerevisiae* YNN295 chromosomes was obtained commercially (Bio-Rad). Once chromosome separation was completed, the gel was stained with ethidium bromide, washed and photographed with a Polaroid MP4 camera, using 667 film, a Kodak Wratten 22 A filter and U. V. illumination of 300 nm, from a Fotodyne 3-3002 (New Berlin Wyoming USA) transilluminator.

Southern-blot analysis. The chromosomal DNA separated by CHEF was treated with de-purination solution (0.25 N HCl) for 4 min, denatured for 3 min, neutralized for another 3 min and transferred to nitrocellulose filters. Each filter was used once (that is, there was

Table 1 *S. cerevisiae* strains used in this work

Strain	Genotype	Source
V1	Unknown	CGL [a]
V2	Unknown	CGL
V1–17	Unknown	This work. A complete tetrad from strain V1
V1–29	Unknown	This work. A complete tetrad from strain V1
V1–30	Unknown	This work. A complete tetrad from strain V1
V1–38	Unknown	This work. A complete tetrad from strain V1
V2–4	Unknown	This work. A complete tetrad from strain V2
V2–9	Unknown	This work. A complete tetrad from strain V2
V2–13	Unknown	This work. A complete tetrad from strain V2
V2–27	Unknown	This work. A complete tetrad from strain V2
YNN295	*MATα ura3 lys2 ade1 ade2 his trp1 Δ1*	Bio-Rad [b]
MMY1	*MATα ura3 Δ52 Cyh*^{r}	SERI [c]
MMY2	*MAT***a** *ura3 Δ52 Cyh*^{r}	SERI [c]
DS81	*MATα/MAT***a** *mal/mal gal2/gal2 SUC2/SUC2 CUP1/CUP1*	Codón et al. 1995

[a] CGL, Compañía General de Levaduras, Valladolid, Spain
[b] Bio-Rad, Richmond, California, USA
[c] SERI, Solar Energy Research Institute, Golden, Colorado, USA

one gel for each probe). The basic procedure followed was that of Naumov et al. (1992). All the experiments were carried out at least twice.

Flow cytometry. Yeast cells were grown with vigorous agitation at 30 °C in tubes with 10 ml of YPD. When the culture reached stationary phase (approximately 5×10^8 cells/ml) the cells were harvested by centrifugation, washed and re-suspended in 70% ethanol and maintained at –20 °C for 30 min. About 5×10^5 cells were then re-suspended into 50 mM sodium citrate, centrifuged and newly re-suspended into 0.5 ml of 50 mM sodium citrate containing 50 µg of RNAase. The preparation was incubated for 2 h at 37 °C and after this time 0.5 ml of 50 mM sodium citrate containing 2 µg of propidium iodine was added. The samples were incubated for 30 min at room temperature, sonicated for 30 s and the DNA content measured in a flow cytometer (Becton-Dickinson FACScan analyzer) following published procedures (Hutter and Eipel 1979).

Results

Meiotic analysis of the baker's yeasts V1 and V2

To carry out the genetic analysis, complete tetrads were micromanipulated after the baker's yeasts V1 and V2 were grown in sporulation conditions. Only 12 out of 127 micromanipulated tetrads from strain V1 and 7 of 78 from strain V2 were recovered (the percent survival of ascospores following ascus dissection of strains V1 and V2 was 58 and 64% respectively). Of these, four complete tetrads of each of the two strains were analyzed with regards to several features: the presence of auxotrophic markers, the petite phenotype, and especially the capacity to sporulate and/or conjugate (Tables 2 and 3). No meiotic products of strain V1 were able to both sporulate and conjugate; some conjugated or sporulated and some did neither (Table 2). In two tetrads, two spores were of **a** mating type whereas two were unable to either conjugate or sporulate; one tetrad had two spores able to sporulate and two that were of **a** mating type; the fourth tetrad had two spores unable to sporulate or conjugate, one which sporulated and one of **a** mating type. The absence of spores of the α mating type in the other tetrads analyzed is compatible with tetrasomy of chromosome III so that the strain is **a**/**a**/α/α; the **a** alleles are functional for both sporulation and conjugation whereas the α alleles only function for sporulation but not in conjugation. However, other possibilities cannot be discounted.

Similarly, when analysing tetrads of the V2 strain, some meiotic products either conjugated or sporulated and some did neither (Table 3). Segregation shown by the V2 strain was similar to that of V1: two tetrads in which two spores are α and **a** respectively and two that neither conjugate nor sporulate; one tetrad in which two meiotic products sporulate and two that do neither; a fourth tetrad had two α and one **a** meiotic products and one that did neither. Results similar to these have been subsequently observed in further tetrads analyses.

The results are compatible with tetrasomy for chromosome III of the type **a**/**a**/α/α. Of the two α and two **a** alleles one of each is apparently functional for both conjugation and sporulation while the other is not.

Table 2 DNA content, ability to sporulate and/or conjugate, auxotrophies and petite phenotype of baker's yeast V1 and four complete tetrads

Strain		DNA content (C) [a]	Sporulation	Mating type	Auxotrophy	Petite
V1		2.7	+	–	–	–
Ascosporal clones						
17	A	1.7	–	–	–	–
	B	2.1	+	–	–	–
	C	1.0	–	**a**	–	–
	D	1.3	–	–	–	–
29	A	1.3	–	**a**	+	–
	B	1.3	+	–	–	–
	C	1.2	+	–	–	–
	D	1.3	–	**a**	+	–
30	A	1.7	–	**a**	–	–
	B	1.3	–	**a**	–	–
	C	1.2	–	–	–	–
	D	1.2	–	–	–	–
38	A	1.3	–	–	–	–
	B	1.4	–	**a**	–	–
	C	1.0	–	–	–	+
	D	1.6	–	**a**	–	–

[a] 1 C and 2 C is the DNA content in the G_1 phase of a haploid and a diploid cell respectively; in this study the laboratory strains YNN295 (15 ng DNA/10^6 cells) and DS81 (29 ng DNA/10^6 cells) were used as haploid and diploid controls respectively (their DNA content obtained by flow cytometry was 7 and 13 arbitrary units per 10^4 cells)

Table 3 DNA content, ability to sporulate and/or conjugate, auxotrophies and the petite phenotype of the baker's yeast strain V2 and four complete tetrads

Strain		DNA content (C) [a]	Sporulation	Mating type	Auxotrophy	Petite phenotype
V2		2.7	+	–	–	–
Ascosporal clones						
4	A	1.2	–	–	+	+
	B	1.1	–	–	+	+
	C	1.3	–	**a**	–	–
	D	1.3	–	α	–	–
9	A	1.5	–	**a**	–	–
	B	1.5	–	α	+	–
	C	1.4	–	–	–	–
	D	1.6	–	–	+	+
13	A	1.4	+	–	–	–
	B	1.1	–	–	+	+
	C	1.1	–	–	+	+
	D	1.6	+	–	–	–
27	A	1.2	–	α	–	+
	B	1.5	–	–	+	–
	C	1.6	–	**a**	+	–
	D	1.3	–	α	–	+

With regards to the segregation of the auxotrophies and the petite phenotype, strain V2 showed a 2 : 2 segregation for both features, indicating heterozygosis for a gene located in a chromosome present in a disomic condition (Table 3). By contrast, the low frequency of both auxotrophies (2 out of 16 spores) and the petite phenotype (1 out of 16 spores) in the V1 strain, indicated heterozygosis of a gene located in a chromosome present in a trisomic or, more probably, tetrasomic condition (Table 2).

DNA content of the baker's yeasts V1 and V2 and four complete tetrads of the V1 and of the V2 strains

The DNA content of strains V1 and V2 and their meiotic products was determined using the laboratory yeasts YNN295 and DS81 as haploid and diploid controls, respectively. The results obtained are shown in Tables 2 and 3. When compared with YNN295 and DS81, strains V1 and V2 seemed to be 2.7 C (where 1 C is the DNA content of a haploid cell). Four complete tetrads of the V1 strain and four of V2 were also analyzed. These meiotic products had a DNA content which varied between 1 and 2.1 C. This variation was observed even within the same tetrad (i. e. tetrad 17 from the V1 strain). However, in general, the meiotic products of these complete tetrads seemed to have received an almost identical DNA content (Tables 2 and 3). Considering that the viable meiotic products of a complete tetrad could have received anywhere from n to 2n DNA, this evenness in DNA distribution is worth nothing. The fact that similar results had been observed when analyzing the meiotic products of complete tetrads in another bakers' strain with a DNA content of 3 C (Gasent-Ramírez et al. 1995) suggests that perhaps the only way to obtain four viable spores is to evenly distribute the DNA. Also, the aneuploidy of V1 and V2 (2.7 C) would explain the lack of viability of the complete tetrads observed in these strains. In addition, the aneuploidy of some of the meiotic products which were able to sporulate (1.2, 1.3, 1.4, 1.6 and 2.1 C respectively) would explain the lack of viability of their spores when 5–10 tetrads from each sporulated meiotic product were micromanipulated. The meiotic product 17 B (Table 2) was 2.1 C but the complete lack of viability of its spores indicates the strain to be genomically unbalanced. In addition, the bakers' strains may be highly heterozygous, so that the lack of enough homology prevents recombination. When meiotic recombination is abolished this results in a lack of spore viability, presumably through non-disjunction of the non-recombined homologues with ensuing aneuploidy (Hugerat and Simchen 1993; Aguilera and Klein 1995).

Our results also indicate that strains V1 and V2 are heterothallic. Fully homothallic yeasts yield ascosporal colonies containing cells with DNA contents almost equal to that of their respective parentals. However, heterozygosity for a gene conferring homothallism cannot be presently ruled out. When α or **a** spores were mated to tester laboratory strains, the viability of the meiotic products after sporulating the hybrids was also very low. Most hybrids gave asci with 2–3 spores; after micromanipulating 12 tetrads of the only hybrid which produces some 4-spore asci, a single viable meiotic product was recovered that was not investigated further.

Electrophoretic karyotype

It is generally agreed that chromosomal polymorphism is more extensive in industrial yeasts than in laboratory strains (Bidenne et al. 1992). In many industrial yeasts it has been possible to demonstrate the presence of a variable number of chromosomal bands which seem to be due to chromosomal-length polymorphism. Furthermore, industrial populations of yeasts have been shown to undergo changes which include chromosomal loss, variations in ploidy levels, and both inter- and intra-chromosomal rearrangements (Adams et al. 1992).

To establish the possible presence of these changes in strains V1 and V2, these yeasts and two complete tetrads of each strain were subjected to karyotype analysis by the pulse-field technique, using contour-clamped homogeneous electric-field electrophoresis (Figs. 1 and 2).

The chromosomal banding patterns of both baker's strains and their meiotic products were very different from each other and from that of the laboratory strain YNN295 used as a control. The karyotype patterns were readily distinguishable from each strain and their meiotic products. Variations in both the number and length of bands were detected. The number of bands varied from 15 in the laboratory strain YNN295 to more than 20 in some of the meiotic segregants of the industrial strains. Furthermore the chromosomal-length polymorphism is clearly evident because each profile can be defined not only by the number of bands and their positions but also by their relative intensities.

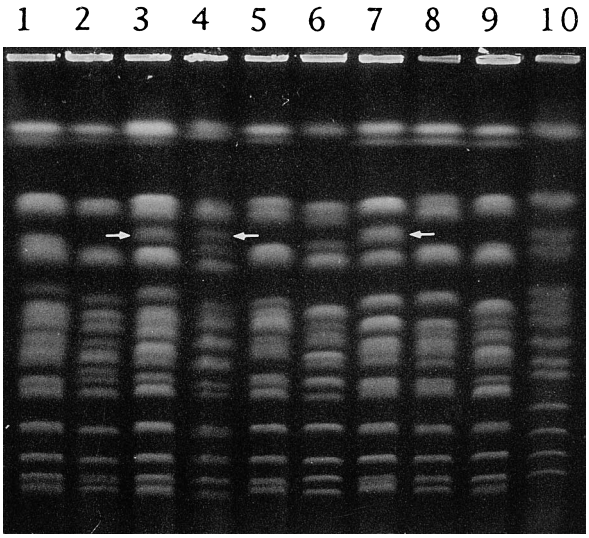


Fig. 1 Electrophoretic karyotype of baker's yeast strain V1 and two complete tetrads. *Lane 1*, strain V1; *lanes 2–5*, tetrad 17, spores A, B, C, D; *lanes 6–9*, tetrad 29, spores A, B, C, D; *lane 10* control strain YNN295. New chromosomal bands in the meiotic products, absent in the parental strain, (*lane 1*) are shown by *arrows*

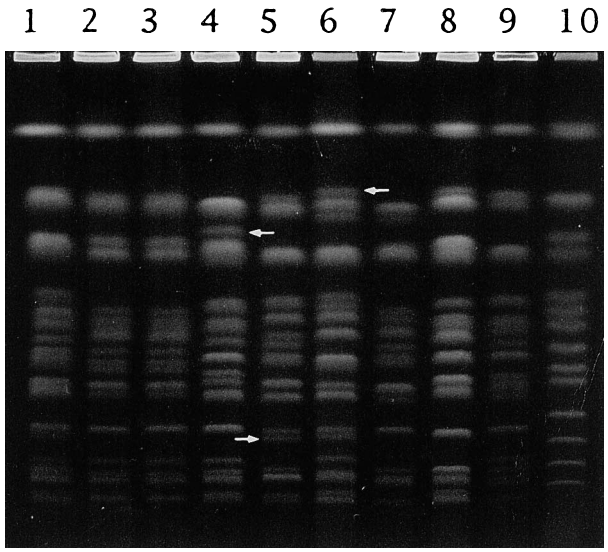


Fig. 2 Electrophoretic karyotype of baker's yeast strain V2 and two complete tetrads. *Lane 1*, strain V2; *lanes 2–5*, tetrad 4, spores A, B, C, D; *lanes 6–9*, tetrad 9, spores A, B, C, D; *lane 10* control strain YNN295. New chromosomal bands in the meiotic products, absent in the parental strain, (*lane 1*) are shown by *arrows*

Our results also demonstrate the presence of homologous chromosomes of different size in both parental strains, which segregated in their meiotic products. Moreover, new chromosomal bands, not observed in the parental strains, were present in their meiotic segregants (indicated by arrows in Figs. 1 and 2).

Chromosomal identification by Southern-blot hybridization analysis

To assign the different bands observed in the electrophoretic karyotypes to specific chromosomes in both V1 and V2 and their complete tetrads, as well as the laboratory control yeast YNN295, Southern blots of electrophoretic CHEF gels were hybridized with reference probes (Naumov et al. 1992). Chromosomal identification in the strains was examined using cloned genes of *S. cerevisiae*, some of which have a single location on the *S. cerevisiae* genome, i.e. *LEU2* in chromosome III, *TRP1* in chromosome IV, *URA3* in chromosome V, *CUP1* in chromosome VIII, *SUC2* in chromosome IX, *ADC1* in chromosome XV, and *GAL4* in chromosome XVI. After electrophoretic karyotyping the chromosomes were transferred to nitrocellulose filters and hybridized with the cloned gene-probes of *S. cerevisiae*.

As Figs. 3 and 4 show, the DNA of both V1 and V2 (and their meiotic products) has a high homology to that of the laboratory strain since the conditions used were highly stringent. In fact hybridization was only possible for DNA-DNA homologies of over 90%. Also, the probes used indicated that the genes *LEU2, TRP1, URA3, CUP1, ADC1* and *GAL4* were located in the same chromosomes (III, IV, V, VIII, XV and XVI respectively) as in the laboratory strains of *S. cerevisiae*.

However, some unexpected results were observed Figure 3 A indicates a single band corresponding to chromosome III in baker's strain V1 that was smaller than that of the laboratory strain YNN295. The two complete tetrads have a similar band, although of different intensities, which might be explained by differences in the DNA content of the wells during the electrophoresis or to the presence of more than one copy of *LEU2* in the same chromosomal band. Although the strain is 2.7 C, the copy number of each chromosome is unknown. However segregation of some markers indicated disomy of some chromosomes and trior tetra-somy for some others. Thus, segregation of the mating-type located on chromosome III pointed to the presence of four copies of chromosome III. Therefore differences in intensities of the chromosomal band could in fact be due to the presence of more than one copy of chromosome III in some meiotic products. Alternatively, in spite of strain V1 being *S. cerevisiae*, this yeast may be highly heterozygous so that some segregants show lower homologies than others.

With regards to strain V2, chromosome III also seems to be smaller than that of laboratory strain YNN295 (Fig. 4 A). The parental strain yielded a single chromosome-III band. However, some of the meiotic products of V2 had bands corresponding to chromosome III but of different size. These new bands are not seen in the parental V2 strain (Fig. 4 A). The hybridization does not seem to be due to partial homology with other genes present in different chromosomes but rather to true *LEU2* genes. Furthermore, differences in intensities were so strong that some of the Southern blots had to be exposed for longer periods of time and involved the use of larger amounts of DNA, otherwise in some of the meiotic products no *LEU2* band seemed to be present. As before, this could be attributed to the high heterozygosity of strain V2. In fact, lack of homology has been shown to prevent recombinational repair and results in chromosomal-loss aneuploidy (Aguilera and Klein 1993).

When chromosome IV was identified using a *TRP1* probe (Figs. 3 B and 4 B) the result was a strong hybridization in the expected position according to the control YNN295. Again there were differences in intensity which were probably due to several copies of this chromosome in the parentals V1 and V2 which then segregated unevenly in the meiotic products. Some chromosomal bands seemed to be smaller than that of the control.

When the *URA3* probe was used, chromosomal bands were observed in the same position as that of chromosome V of the control YNN295 (Figs. 3 C and 4 C). However, in both V1 and V2 at least two bands were seen which segregated unevenly in the meiotic products with regards to both the number of bands as well as their position and intensities (Figs. 3 C and 4 C). Again, some meiotic products had chromosomal bands which did not appear in the parentals. In addition, both the parental strains V1 and V2 and their meiotic products exhibit a chromosomal band of high molecular weight bearing a copy of the *URA3* gene present

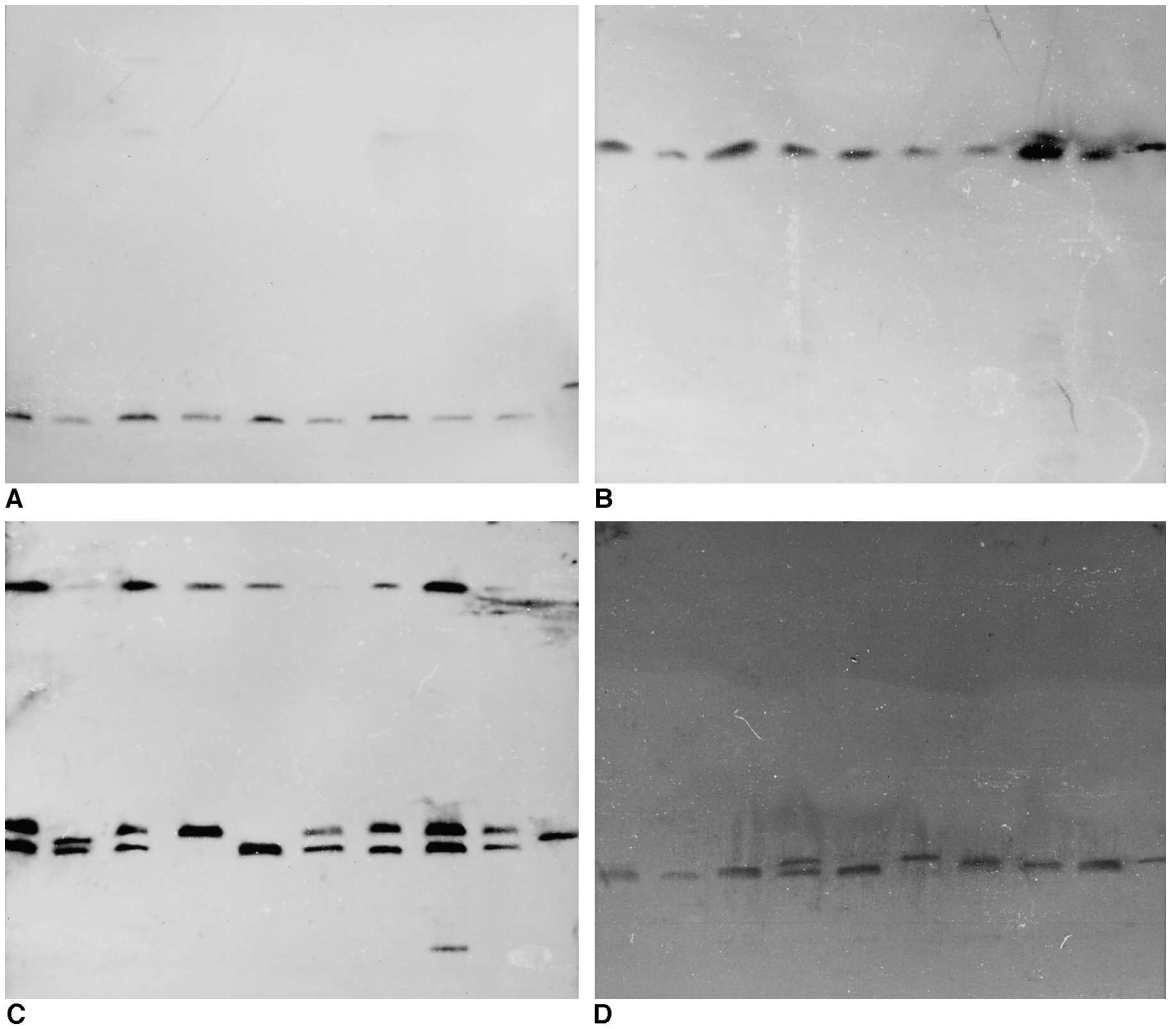


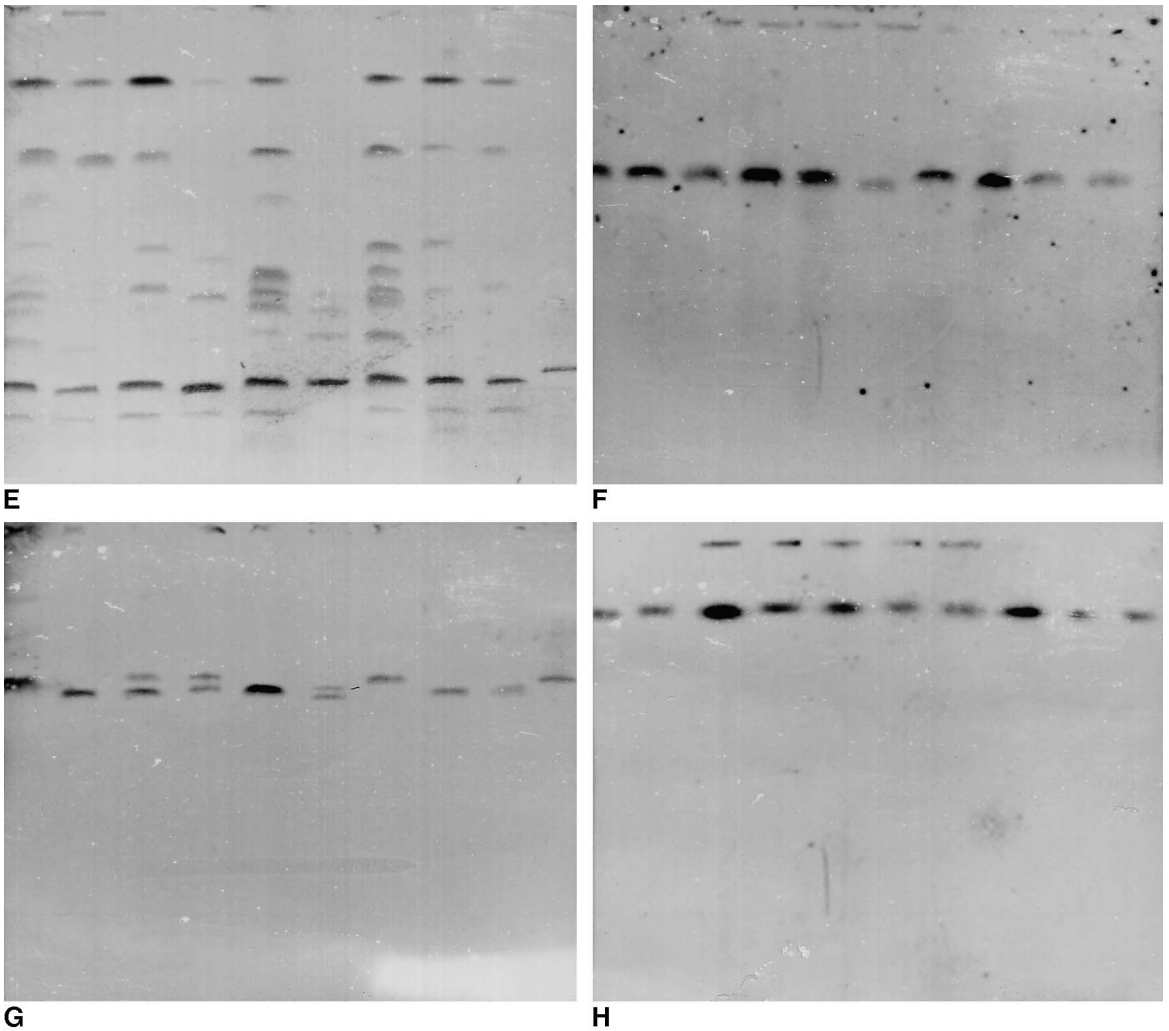
Fig. 3A–H Southern-blot hybridization analysis of chromosomal DNAs of the strains described in Fig. 1, corresponding to the hybridization of chromosomal DNAs with cloned genes *LEU2* (**A**), *TRP1* (**B**), *URA3* (**C**), *CUP1* (**D**), *SUC2* (**E**), *ADC1* (**F**), *GAL4* (**G**) and *rDNA* (**H**)

on a chromosome much longer than the standard chromosome V of the control strain YNN295.

Results of the *GAL4* (Figs. 3G and 4G) and *CUP1* (Fig. 3D and 4D) probes, located on chromosome XVI and VIII respectively, were similar to the previous ones: namely, the presence of chromosomal bands in the position expected according to the control YNN295; the appearance of new bands in the meiotic products of slightly different sizes from that of the parental; and different intensities indicating the inheritance of different numbers of chromosomes XVI and/or VIII in the different meiotic products.

The baker's strains V1 and V2 produced multiple bands hybridizing with a *SUC2* probe from chromosome IX (Figs. 3E and 4E). These bands can be regarded as indicating the presence of many *SUC* copies on different chromosomes. All strains of *S. cerevisiae* carry a *SUC2* gene but some of them carry additional *SUC* genes, highly homologous to *SUC2*, in different chromosomes that vary among strains (Aguilera and Klein 1993; Ness and Aigle 1995). Because the intensity of the bands was variable within each strain, there must have been differences in DNA homologies. The genetic strain YNN295 has a single band. Baker's strains and their meiotic products had the most intense band in the expected position (chromosome IX) with a variable degree of homology of the *SUC* gene in the other chromosomes. Segregation of the *SUC* gene in the meiotic products was very heterogeneous but, as before, new bands of slightly different size seem to appear in the meiotic products which are absent in the parentals. The results therefore indicate the presence of several *SUC* genes in different chromosomes which segregate in the meiotic products in V1 and V2 but not in the laboratory strain YNN295.

The *ADC1* promoter, a probe corresponding to chromosome XV, was located in the expected position with respect to the laboratory strain YNN295. Because chromosome XV is very large (1140 kb, Grivell and Planta 1990) differences in chromosome length were not so clearly seen as



with smaller chromosomes, such as chromosome III. This was especially true for strain V1 and its meiotic products (Fig. 3F). V2, however, showed a single chromosome XV band, though some of the meiotic products had at least two bands or else a single band but in a slightly higher or lower position than that of the parental (Fig. 4F). The fact that both V1 and V2 strains and all the four meiotic products show positive hybridization with the *ADC1* probe indicates that these bakers' yeasts are *S. cerevisiae*, and are neither its sibling species, *S. paradoxus*, *S. bayanus* or *S. pastorianus*, nor a hybrid between any of these and *S. cerevisiae*.

Only *S. cerevisiae*, shows hybridization with the *ADC1* promoter probe (Naumov et al. 1992). The other *Saccharomyces* species do not contain DNA homologous to the *ADC1* probe.

Both strains and their meiotic products, together with the laboratory strain, displayed a single band of lower intensity corresponding to partial homology of the *ADC1* promoter with a different gene located an another chromosome (Figs. 3F and 4F).

The oligo of the rDNA probe was tested to check chromosome XII, which is the largest (2600 kb, Grivell and Planta 1990). The rDNA seems to be present at the same position in all the laboratory and the baker's strains as well as in the meiotic products. As before, the small length differences detected in the smaller chromosomes were not evident in chromosome XII due to the size of this chromosome (Figs. 3H and 4H).

The presence of the mobile elements Ty1 and Ty2 in the baker's yeasts and their meiotic products

The results of hybridization of the probes for the mobile elements Ty1 and Ty2, which assume multiple chromosomal locations in *S. cerevisiae*, are presented in Figs. 5 and 6. Both Ty1 and Ty2 transposable elements were found on many chromosomes in the strains tested and the complete tetrads analyzed. However, both Ty1 and Ty2 sequences seem to be more frequent in baker's yeasts than in the laboratory strain. The Ty2 sequence has been found in strains of *S. cerevisiae* but not in *S. paradoxus* or *S. bayanus* (Naumov et al. 1992), thus confirming strains V1 and V2 to be *S. cerevisiae*.

Also, differences in intensity within the same strain are attributable to amplification in the same chromosome

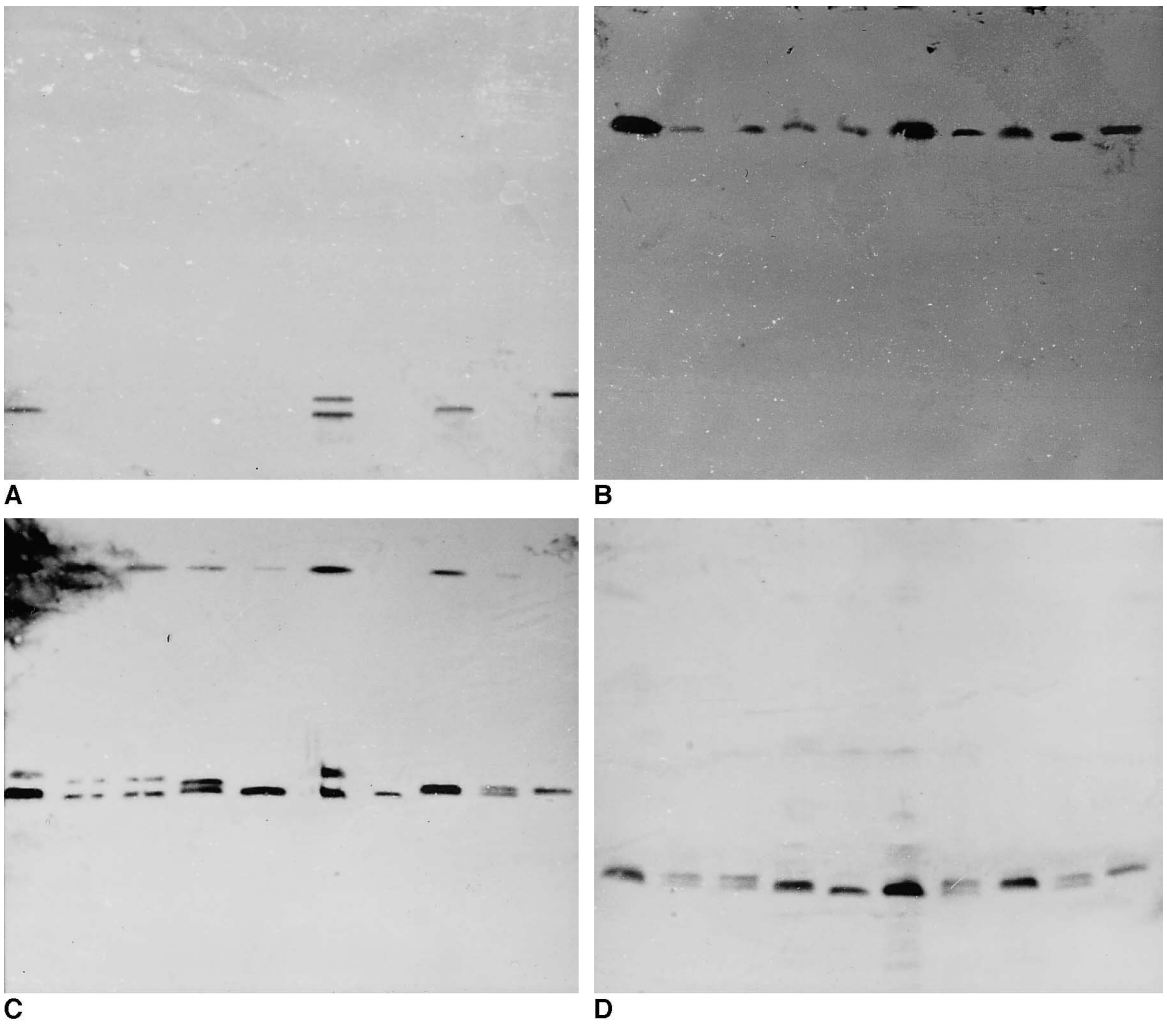


Fig. 4A–H Southern-blot hybridization analysis of chromosomal DNAs of the strains described in Fig. 2, corresponding to the hybridization of chromosomal DNAs with cloned genes *LEU2* (**A**), *TRP1* (**B**), *URA3* (**C**), *CUP1* (**D**), *SUC2* (**E**), *ADC1* (**F**), *GAL4* (**G**) and *rDNA* (**H**)

rather than to differences in DNA homologies. Figures 5 and 6 also show an unequal distribution of the Ty1 and Ty2 elements among the meiotic products and among the different chromosomes. The presence of so many copies of Ty1 and Ty2 transposons in the baker's yeasts as compared to the laboratory control is in line with the great polymorphism found in these strains (Figs. 1 and 2).

Together with the unequal distribution of bands and intensities among the meiotic products, Ty1 and Ty2 segregation also indicated the presence of new bands of hybridization apparently absent in either of the parentals V1 and V2 (shown by arrows in Figs. 5 and 6). The intensity of some individual bands in some of the meiotic products seems to be due to amplification of the Ty1 and Ty2 probes in just that particular meiotic product rather than to differences in the overall DNA content with regards to the parentals or to other meiotic products.

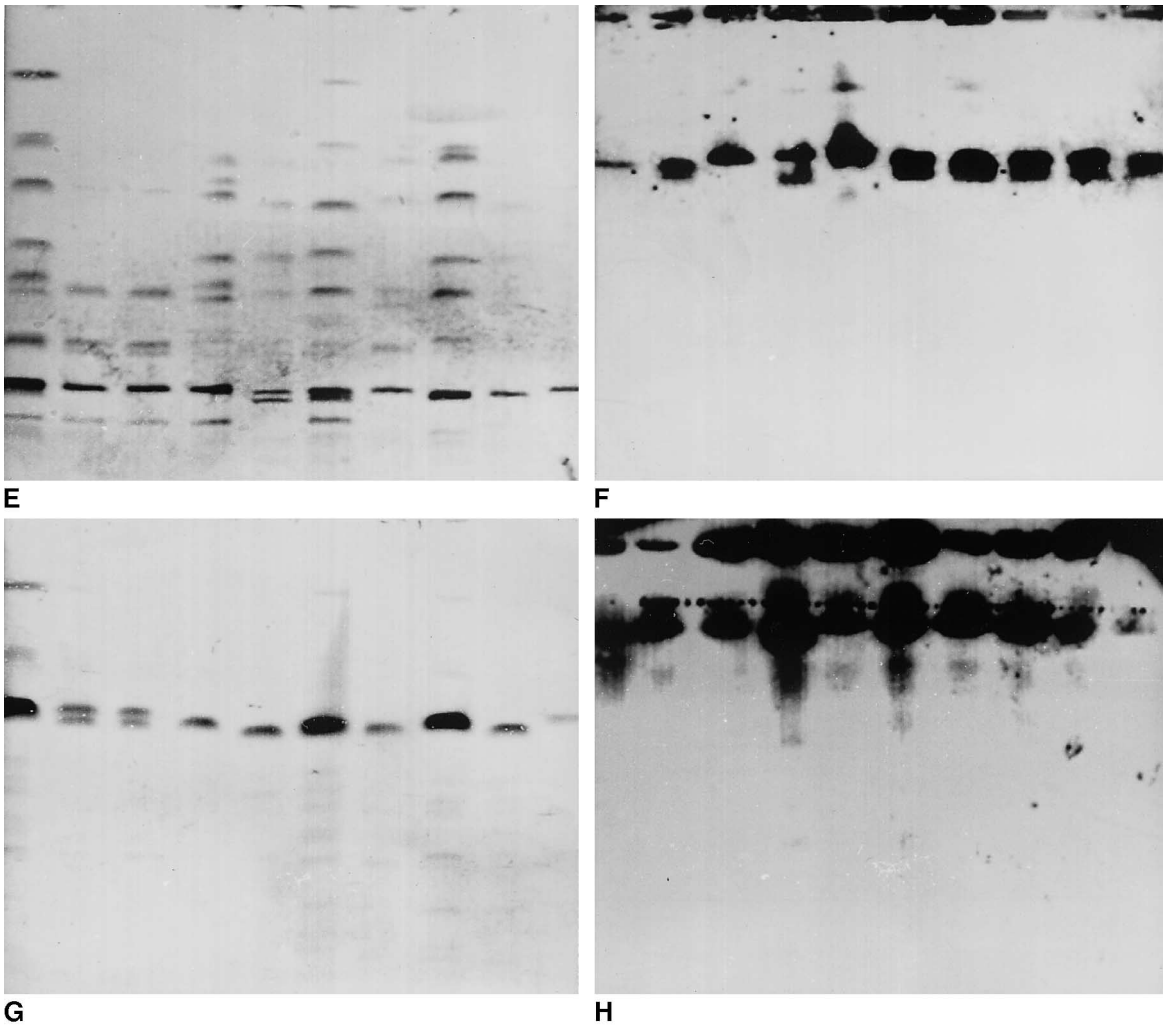
It seems, therefore, that both Ty1 and Ty2 elements undergo extensive translocation to other chromosomes, as well as amplification, when the parental strains undergo meiosis.

The presence of telomeric Y′ sequences in V1 and V2 and their meiotic products

The telomere-associated Y′ sequences, which are assumed to have multiple chromosomal locations in *S. cerevisiae*, were also used as probes for the baker's strains V1 and V2 and their meiotic products.

When the telomeric probes Y′16 and Y′29 (Fig. 7) were tested, large differences in both the number of hybridization signals and their intensity among the laboratory and the baker's strains were observed (Figs. 8 and 9). Both Y′16 and Y′29 sequences seem to be present on almost all the chromosomes in all three strains (baker's and laboratory) and in the meiotic products of the baker's yeasts.

The near absence of segregation (absence/presence) of the Y′16 and Y′29 sequences among the meiotic products (in nearly all cases the four meiotic products hybridize) as



compared to other probes with multiple locations, such as SUC (Figs. 3E and 4E) and especially Ty1 and Ty2 (Figs. 5 and 6), indicated that nearly all homologous chromosomes possess at least one copy of the Y′ sequence.

Telomeric sequences have also been implicated in generating polymorphism in some organisms, as happens with the Ty1 and Ty2 transposable elements. Homologous recombination within the multiple copies of the subtelomeric repeat sequences contributes to chromosomal size differences.

Therefore, data on Y′29 and Y′16 distribution (Figs. 8 and 9), together with those of the Ty1 and Ty2 elements (Figs. 5 and 6), support the high degree of polymorphism found for chromosomal patterns in these baker's yeasts.

Discussion

It has been well established that most baker's and other industrial yeasts are polyploid or aneuploid. The unbalanced chromosome complements of industrial yeasts have been suggested to be advantageous since aneuploids are so widespread (Bakalinski and Snow 1990; Adams et al. 1992). It has also been suggested that extra sets of chromosomes might give rise to more-vigorous strains (Adams et al. 1992) and that a certain chromosomal constitution might reflect optimal fitness in each industrial environment (Yamamoto et al. 1991).

In the present study we have found that the DNA content of the baker's strains V1 and V2 was 2.7 C while the DNA content of their meiotic products varies from 1 to 2.1 C (Tables 2 and 3).

The aneuploidy (greater than 2 C and less than 3 C) shown by V1 and V2 could explain the lack of viability of their meiotic products (58% for strain V1 and 64% for V2) (Codón et al. 1995). This lack of ascosporal viability indicated that the strains are genetically unbalanced, having several copies of some homologous chromosomes and probably a single copy of some others. The segregation observed in the meiotic products for characteristics such as the petite phenotype, auxotrophies or the capacity to sporulate and/or conjugate supports this view (Tables 2 and 3).

The aneuploidy of the parental V1 and V2 strains was revealed when gene probes for the identification of the different chromosomes were employed: some meiotic prod-

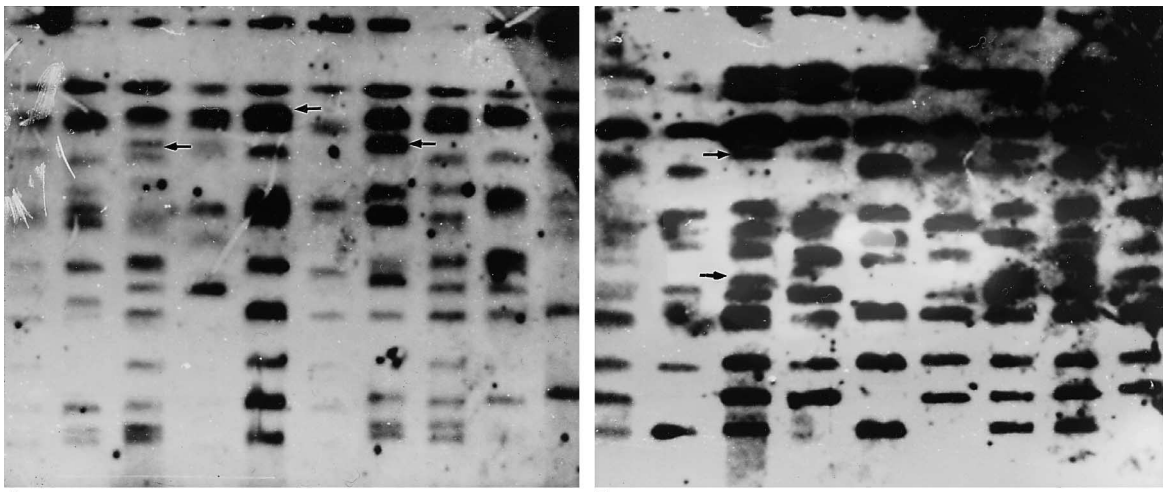
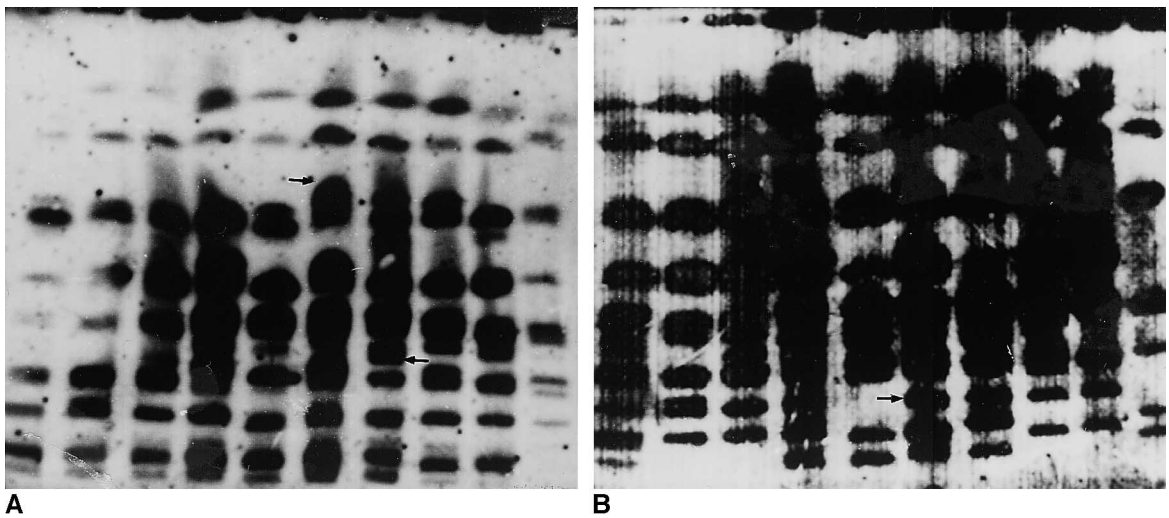


Fig. 5A, B Southern-blot hybridization analysis of chromosomal DNAs of the strains described in Fig. 1, corresponding to the hybridization of chromosomal DNAs with the probes Ty1 (**A**) and Ty2 (**B**). [In **B**, strain YNN295 (*lane 10* in **A**) is missing]. New chromosomal bands in the meiotic products, absent in the parental strain, (*lane 1*) are shown by *arrows*

ucts of V1 and V2 had bands of a stronger intensity, indicating the presence of more than one copy of the same chromosome (Figs. 3 and 4). Furthermore, due to chromosomal-length polymorphism for homologous chromosomes, segregation in the meiotic products was frequently seen so that sometimes a meiotic product inherited the longest homologous chromosome and another meiotic product the shortest. There were also meiotic products which inherited both bands (Figs. 3 and 4). In wine yeasts the observation of two bands, indicating normal and enlarged variants, is also frequent (Bidenne et al. 1992). The segregation during meiosis of these bands confirms that they represent homologous chromosomes that differ in length.

Other authors found spore viability of only about 20% after analyzing the progeny arising from a certain diploid (Jäger et al. 1992). Further analysis showed aneuploidies in all spore clones from n+1 to n+4 or even higher. Low homology between the genomes of the parental, together with chromosome-length polymorphism, was suggested as the explanation for both lack of viability of the meiotic products and the aneuploidy generated. However, in wine yeasts Bidenne et al. (1992) demonstrated that the difference in the length of homologous chromosomes did not impair meiotic recombination. In accordance with Jäger et al. (1992) the genomes of the V1 and V2 seem to be too heterogeneous for the formation of efficient chiasma between their chromosomes. Consequently, together with the low viability of the spores, tetrad analysis by electrophoretic karyotyping indicated such strong polymorphism in chro-

Fig. 6A, B Southern-blot hybridization analysis of chromosomal DNAs of the strains described in Fig. 2, corresponding to hybridization of chromosomal DNAs with the probes Ty1 (**A**), and Ty2 (**B**). New chromosomal bands in the meiotic products, absent in the parental strain, (*lane 1*) are shown by *arrows*



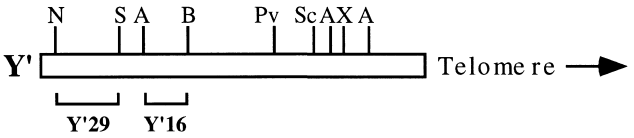


Fig. 7 Fragments of the Y′ sequence used for hybridization. Y′sequences according to Louis and Haber (1990a, b). *S* = *Sal*I; *A* = *Asp*718; *B* = *Bam*HI; *Pv* = *Pvu*I; *Sc* = *Sac*I; *X* = *Xho*I

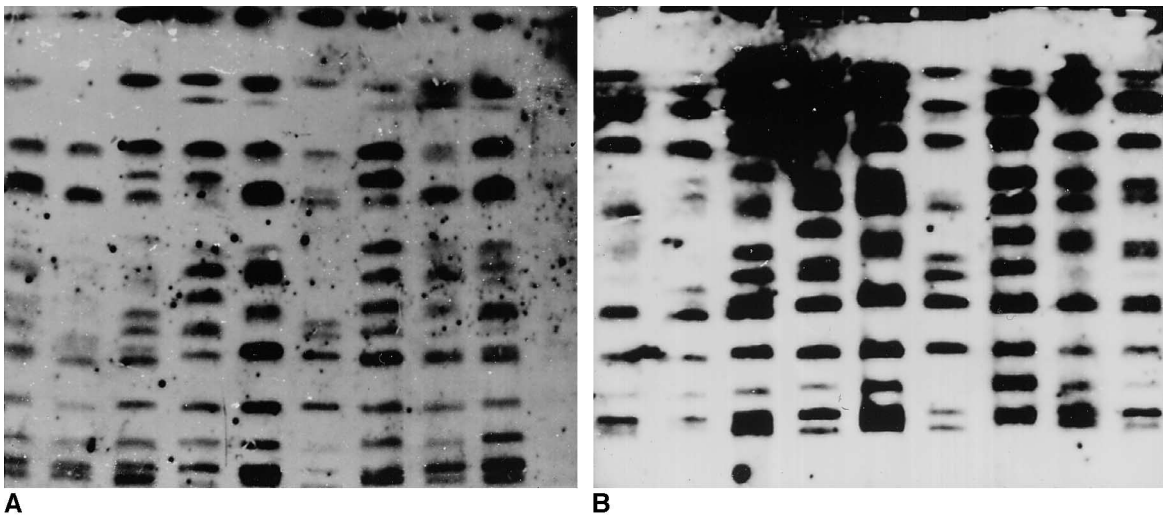
mosome length that new bands, absent in the parental, were very often found in the meiotic products (Figs. 1–6). Furthermore, due to the lack of homology between the chromosomes of the baker's strain and laboratory test strains, when those meiotic products able to mate were crossed with a laboratory haploid strain almost no viable spores were obtained from the hybrids after micromanipulation.

Most of the probes used for the Southern-blot hybridization (*URA3, CUP1, LEU2, TRP1, GAL4, ADC1* and *rDNA*) gave either a single main chromosomal band or two very close ones which segregated in the meiotic products (Figs. 3 and 4). This indicates polymorphism for homologous chromosomes but with the location of the probe on a single chromosome. However, in both V1 and V2, multiple bands hybridized with the *SUC2* probe from chromosome IX (Figs. 3E and 4E). These bands indicated the presence of many *SUC* copies on different chromosomes, which segregated further in the tetrads. The intensity of the bands varied with the chromosome, the highest intensity being in chromosome IX corresponding to the laboratory strain containing the *SUC2* gene. In some strains of wine yeasts, *SUC* genes are present not only on the expected chromosome but also on other bands, and this has been explained by the telomeric location of the *SUC* genes (Bidenne et al. 1992). In other wine strains there is single band for the *SUC2* gene (Codón et al., unpublished). This difference in the *SUC2* gene contribution of wine yeasts is understandable since many of them are totally unable to metabolize sucrose (Benítez et al. 1983). The presence of multiple copies of the *SUC* gene in V1 and V2 suggests adaptation to the industrial environment since baker's strains are grown in molasses with sucrose as the main carbon source (Trivedi et al. 1986). Ness and Aigle (1995) have also found that industrial strains grown on molasses show multiple *SUC* genes throughout the genome.

The nature of the length polymorphisms found in V1 and V2 and their meiotic products has been accounted for by the different number of Ty elements and Y′ repeats as well as other subtelomeric elements which would lead to impaired chiasma formation. When Southern-blot analyses were carried out using Ty1 and Ty2 transposable-element probes, as well as Y′16 and Y′29 subtelomeric probes, the results indicated the presence of these elements on almost all the chromosomes of V1 and V2 (Figs. 5, 6, 8 and 9). Furthermore, the meiotic products of both strains displayed amplification to a different degree in the different chromosomes, as judged by the intensity of hybridization, together with the translocation of these elements to new chromosomal bands (since they appeared only in the meiotic products and not in the parentals).

The Ty1 and Ty2 elements are unequally distributed both among the industrial groups and among the different chromosomes of yeasts (Naumov et al. 1992; Ibeas and Jiménez 1996). Both sequences seem to be more frequent in baker's yeasts than in other groups such as wine, brewer's or distiller's strains (Codón et al., unpublished). Ty elements transpose at low frequencies but can engage in homologous recombination with non-allelic Ty elements (ectopic recombination) giving rise to deletions, inversions and translocations in yeast (Grivell and Planta 1990; Aguilera and Klein 1993). In fact, in *S. cerevisiae* laboratory

Fig. 8A, B Southern-blot hybridization analysis of chromosomal DNAs of the strains described in Fig. 1, corresponding to the hybridization of chromosomal DNAs with the probes Y′16 (**A**), and Y′29 (**B**) respectively (Naumov et al., 1992). [In **B**, strain YNN295 (*lane 10* in **A**) is missing]



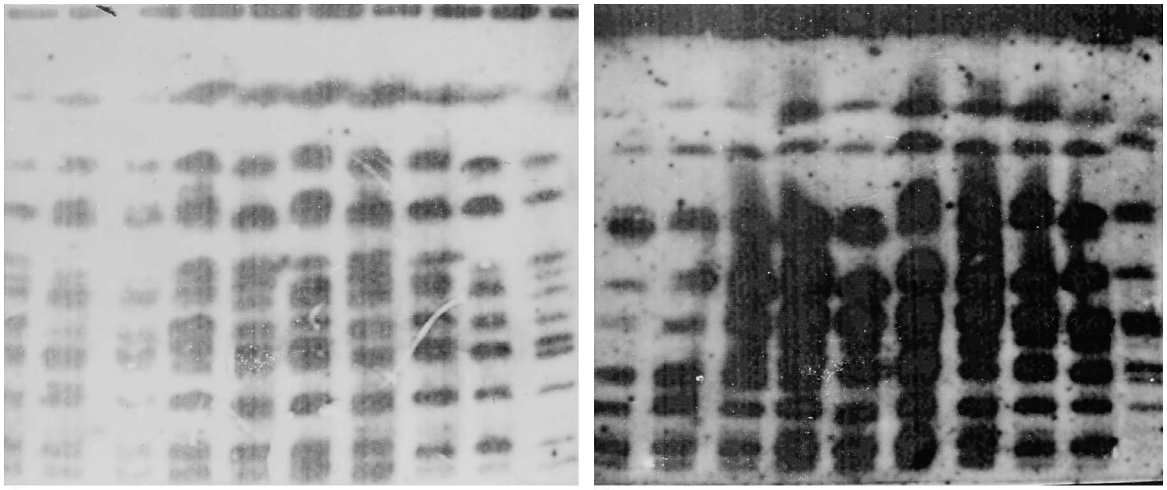


Fig. 9A, B Southern-blot hybridization analysis of chromosomal DNAs of the strains described in Fig. 2, corresponding to the hybridization of chromosomal DNAs with the probes Y′16 (**A**) and Y′29 (**B**) respectively (Naumov et al. 1992)

strains both reciprocal and non-reciprocal recombination events have been detected, the latter representing a gene-conversion event (Kupiec and Petes 1988 a). However, the frequency of such events is low so that a Ty-specific system that represses ectopic exchange has been suggested (Kupiec and Petes 1988 a). Furthermore, Kupiec and Petes (1988 b) found a large variety of different types of ectopic recombination mediated by Ty elements, and which was greater in mitosis than in meiosis. The presence of so many copies of Ty1 and Ty2 elements in V1 and V2 and their tetrads (Figs. 5 and 6) is in line with the high polymorphism found in these strains as compared to other industrial groups. However, rearrangements of large chromosome fragments in bakers' strains, as a result of the movement of Ty elements in the DNA, seem to be occurring at an extremely high frequency and only in meiosis since mitosis appears to be very stable. Almost no changes in the karyotype have been detected in these strains after about 200 generations in continuous culture (J. M. Gasent-Ramírez, unpublished). Yoda et al. (1993) described DNA length polymorphism generated by the loss or addition of 100 kb for DNA only during meiosis or sporulation and suggested the presence of Ty elements as the explanation for such a process. The appearance of new bands in the meiotic products of strains V1 and V2 (Figs. 1 and 2), together with the observation of translocation and amplification of Ty1 and Ty2 elements after meiosis (Figs. 5 and 6), supports this suggestion.

Since Y′ telomeric sequences of *S. cerevisiae* have also been shown to generate polymorphism in yeasts (Louis et al. 1994) we examined their number and distribution in V1 and V2 and their meiotic products. Y′s share the dispersed nature of Tys and recombinational interactions among Y′s, as happens with Tys, may also explain the homogenization of Y′s within a strain, even at different chromosomal locations, as well as the copy number and location differences between strains (Louis and Haber 1990 b). In the present work, data on Y′ distribution, together with that of the Ty1 and Ty2 elements, support the high degree of chromosome pattern polymorphism found in baker's yeasts (Rank et al. 1991). When the telomeric probes Y′29 and Y′16 were tested in the present study, large differences in both the number of hybridization signals and their intensity among the chromosomes were observed. Both Y′29 and Y′16 seemed to be present in almost all the chromosomes and with a high frequency on each of them (Figs. 8 and 9). The appearance of new bands in the meiotic products and the disappearance of chromosomal bands present in the parental were also seen (Figs. 8 and 9), indicating changes in chromosome size. Louis and Haber (1990 a) found duplications that occurred by ectopic recombinational interactions between Y′s at different chromosome ends, as well as by unequal sister chromatid exchange and losses which resulted in the chromosome end becoming Y′-less. Several of the ectopic duplications also resulted in an originally Y′-less chromosome end acquiring a Y′ region (Louis et al. 1994). These phenomena could explain the changes in chromosomal bands observed in Figs. 8 and 9 and in Figs. 3–6.

The lack of segregation (presence/absence) of most Y′ subtelomeric regions in the meiotic products of V1 and V2 indicates the presence of these regions in all homologous chromosomes. Their presence enhances the possibilities for unequal recombination, and resulting polymorphism, in successive generations. While subtelomeric repeat-sequence recombination contributes to chromosome-size differences in some species (Bidenne et al. 1992), in wine yeasts there are strains with different-sized homologous chromosomes which lack Y′ sequences (Bidenne et al. 1992).

The most striking result of the present work is the enormous difference which exists between V1 and V2 and their meiotic products with regards to chromosomal bands, their number, position and intensities.

These genomic rearrangements appear to be caused by the presence of a high frequency of Ty1 and Ty2 elements, as well as Y′ subtelomeric regions, all of which have been presumably implicated in DNA reorganization.

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References

Adams J, Puskas-Rozsa S, Simlar J, Wilke CM (1992) Adaptation and major chromosomal changes in populations of *Saccharomyces cerevisiae.* Curr Genet 22: 13 – 19

Aguilera A, Klein HL (1993) Chromosome aberrations in simpler eukaryotes. In: Kirsch IR (ed) The causes and consequences of chromosomal aberrations. CRC Press, Boca Raton, Florida, USA, pp 51 – 90

Bakalinsky AT, Snow R (1990) The chromosomal constitution of wine strains of *Saccharomyces cerevisiae.* Yeast 6: 367 – 382

Benítez T, del Castillo L, Aguilera A, Conde J, Cerdá-Olmedo E (1983) Selection of wine yeasts for growth and fermentation in the presence of ethanol and sucrose. Appl Environ Microbiol 45: 1429 – 1436

Bidenne C, Blondin B, Dequin S, Vezinhet F (1992) Analysis of the chromosomal DNA polymorphism of wine strains of *Saccharomyces cerevisiae.* Curr Genet 22: 1 – 7

Codón AC, Benítez T (1995) Variability of the physiological features and of the nuclear and mitochondrial genomes of bakers' yeasts: System Appl Microbiol 18: 343 – 352

Codón AC, Gasent-Ramírez JM, Benítez T (1995) Factors which affect the frequency of sporulation and tetrad formation in *Saccharomyces cerevisiae* baker's yeasts. Appl Environ Microbiol 61: 630 – 638

Gasent-Ramírez JM, Codón AC, Benítez T (1995) Characterization of genetically transformed *Saccharomyces cerevisiae* bakers' yeasts able to metabolize melibiose. Appl Environ Microbiol 61: 2113 – 2121

Grivell LA, Planta RJ (1990) Yeast: the model "eurokaryote"? Trends Biotechnol 8: 241 – 243

Hugerat Y, Simchen G (1993) Mixed segregation and recombination of chromosomes and YACs during single-division meiosis in spo13 strains of *Saccharomyces cerevisiae.* Genetics 135: 297 – 308

Hutter KS, Eipel HE (1979) DNA determination of yeast by flow cytometry. J Gen Microbiol 113: 369 – 375

Ibeas JI, Jimenéz J (1996) Genomic complexity and chromosomal rearrangements in wine-laboratory yeast hybrids. Curr Genet 30: 410 – 416

Jäger D, Gysler C, Niederberger P (1992) Aneuploidy events in meiotic progeny of *Saccharomyces cerevisiae.* 16th Int Conf Yeast Genet Mol Biol, S640

Kupiec M, Petes TD (1998 a) Meiotic recombination between repeated transposable elements in *Saccharomyces cerevisiae.* Mol Cell Biol 8: 2942 – 2954

Kupiec M, Petes TD (1988 b) Allelic and ectopic recombination between Ty elements in yeast. Genetics 119: 549 – 559

Louis EJ, Haber JE (1990 a) The subtelomeric Y′ repeat family in *Saccharomyces cerevisiae:* an experimental system for repeated-sequence evolution. Genetics 127: 533 – 545

Louis EJ, Haber JE (1990 b) Mitotic recombination among subtelomeric Y′ repeats in *Sacharomyces cerevisiae.* Genetics 124: 547 – 559

Louis EJ, Naumova ES, Lee A, Naumov G, Haber JE (1994) The chromosome end in yeast: its mosaic nature and influence on recombinational dynamics. Genetics 136: 789 – 802

Naumov GI, Naumova ES, Lantto RA, Louis EJ, Korhola M (1992) Genetic homology between *Saccharomyces cerevisiae* and its sibling species *S. paradoxus* and *S. bayanus:* electrophoretic karyotypes. Yeast 8: 599 – 612

Ness F, Aigle M (1995) RTM1: a member of a new family of telomeric repeated genes in yeast. Genetics 140: 945 – 956

Rank GH, Casey GP, Xiao W, Pringle AT (1991) Polymorphism within the nuclear and 2-μm genomes of *Saccharomyces cerevisiae.* Curr Genet 20: 189 – 194

Sherman F, Fink GR, Hicks JB (1986) Methods in yeast genetics. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York

Trivedi NB, Jacobson GK, Tesch W (1986) Baker's yeast. CRC Crit Rev Biotechnol 24: 75 – 109

Vezinhet F, Blondin B, Hallet JN (1990) Chromosomal DNA patterns and mitochondrial DNA polymorphism as tools for identification of enological strains of *Saccharomyces cerevisiae.* Appl Microbiol Biotechol 32: 568 – 571

Wicksteed BL, Collins I, Dershowitz A, Stateva LI, Green RP, Oliver SG, Brown AJP, Newlon CS (1994) A physical comparison of chromosome III in six strains of *Saccharomyces cerevisiae.* Yeast 10: 39 – 57

Yamamoto N, Yamamoto N, Amemiya H, Yokomori Y, Shimizu K, Totosuka A (1991) Electrophoretic karyotypes of wine yeasts. Am J Enol Vitic 42: 358 – 363

Yoda A, Kontani T, Ohshiro S, Yagishita N, Hisatomi T, Tsuboi M (1993) DNA-length polymorphisms of chromosome III in the yeast *Saccharomyces cerevisiae.* J Ferment Bioeng 75: 395 – 398