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Chromosomal polymorphism and adaptation to specific industrial environments of *Saccharomyces* strains

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Abstract Several industrial *Saccharomyces* strains, including bakers', wine, brewers' and distillers' yeasts, have been characterized with regards to their DNA content, chromosomal polymorphism and homologies with the DNA of laboratory strains. Measurement of the DNA contents of cells suggested that most of the industrial yeasts were aneuploids. Polymorphisms in the electrophoretic chromosomal pattern were so large that each strain could be individually identified. However, no specific changes relating to a particular group were observed. Hybridization using different probes from laboratory strains was very strong in all cases, indicating that all industrial strains possess a high degree of DNA homology with laboratory yeasts. Probes *URA3*, *CUP1*, *LEU2*, *TRP1*, *GAL4* or *ADC1* demonstrated the presence of one or two bands, two especially in bakers' strains. Also, results indicate that all hybridized genes are located on the same chromosomes both in laboratory and industrial strains. Translocation from chromosome VIII to XVI seems to have occurred in a distillers' strain, judging by the location of the *CUP1* probe. Finally, when the *SUC2* probe is used, results indicate a very widespread presence of the *SUC* genes in only bakers' and molasses alcohol distillers' strains. This clearly suggests that amplification of *SUC* genes is an adaptive mechanism conferring better fitness upon the strains in their specific industrial conditions. The widespread presence of *Ty1* and *Ty2* elements as well as *Y′* subtelomeric sequences could account for the inter- and intrachromosomal changes detected.

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Introduction

Industrial *Saccharomyces cerevisiae* yeasts have long been considered as microorganisms that possess an unknown chromosomal constitution and a great deal of genetic heterogeneity (Bakalinski and Snow 1990). Aneuploidy and/or polyploidy among industrial yeasts is so widespread that the maintenance of an unbalanced chromosome set has been suggested to be advantageous (Sancho et al. 1986; Adams et al. 1992). Increasing the number of favourable genes might give rise to more vigorous strains (Adams et al. 1992). For instance, the characteristics required of a good baking yeast include high potential glycolytic activity, ability to adapt rapidly to changing substrates, high invertase activity, a high potential maltose fermentation rate in dough fermentation, storage stability and osmotic resistance to salts and sugars (Reed and Nagodawithana 1991). Since bakers' yeasts are grown in molasses where the main carbon source is sucrose, it is conceivable that any chromosomal change resulting in increasing growth rate, the duplication of invertase genes, for instance, will be favoured (Kamel and Stauffer 1993; Trivedi et al. 1986). In fact, bakers' strains have undergone a selective presssure because man has favoured those genotypes with the highest growth rates and productivities in molasses. The growth rate of other industrial yeasts in molasses was reduced by a half (Jiménez and Benítez 1986) or one third (Martínez-Force and Benítez 1993) as compared to that in laboratory media.

Traditionally industrial yeasts have been characterized according to their physiological properties, such as fermentation and assimilation of certain carbon and nitrogen sources (Barnett 1992). However, the high variability found in these parameters made it necessary to develop new techniques for strain identification such as detecting polymorphisms in the length of the restriction fragments of mitochodrial DNA (Querol et al. 1992a, b; Kitamoto et al. 1991) or chromosome electrophoresis (Naumov et al. 1992a; Rank et al. 1991). The

latter has shown that almost every industrial yeast displays a characteristic pattern (Naumov et al. 1992a) due to the extensive polymorphisms existing within and between different industrial yeasts (Yamamoto et al. 1991). In fact, some authors (Bidenne et al. 1992; Vezinhet et al. 1990) have detected interchromosomal changes (translocations) in addition to intrachromosomal ones (deletions and duplications) as well as the presence of a variable number of chromosomes with high or low homology (Rank et al. 1991) when compared to those of haploid reference laboratory strains. This homology is such that, although chromosomes from brewer's yeasts have been shown to complement laboratory auxotrophic strains, sequence divergence of homologous DNA was sufficiently high to prevent genetic recombination (Pedersen 1988; Russell et al. 1987). In addition, wine and laboratory strains seem to be genetically quite distinct from bakers' yeast, since there is an apparent incompatibility between nuclear and mitochondrial genomes from different sources (Codón et al. 1995).

Nuclear genes code for those functions required for optimal fitness in each industrial environment (Yamamoto et al. 1991). Therefore, much of the nuclear genomic polymorphism is under strong selection pressure (Bakalinski and Snow 1990). DNA-length polymorphisms of sporulation-deficient mutants were described as being generated by the loss or addition of 100 kb DNA during meiosis or sporulation (Yoda et al. 1993). Other authors (Grivell and Planta 1990) suggested that most changes detected concern intergenic regions but that large-scale rearrangements are invariably the result of the movement of *Ty* elements in or out the DNA. This study aims to determine polymorphisms of chromosomes of industrial yeasts.

Materials and methods

Strains

The strains used in this work are listed in Table 1.

Enzymes and chemicals

Proteinase K was obtained from Merck A.G. (Darmstadt, Germany), Zymolyase 20000 from Seikagako (Seikagako kogyo Co. Ltd, Tokyo, Japan) and *N*-lauroylsarcosine, as well as all other chemicals used, were purchased from Sigma Chemical Co. (St. Louis, Mo., USA).

Electrophoretic karyotype

The basic procedure followed for chromosomal DNA preparation was that of Naumov et al. (1992a). The gel was prepared with 0.5 × TRIS borate/EDTA buffer (0.045 M TRIS borate; 0.001 M EDTA, pH 8) and 0.9% agarose. The system used was a CHEF-DRII gel electrophoresis apparatus from Bio-Rad Laboratories (Richmond, Calif., 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 the chromosome separation was finished, 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 UV illumination of 300 nm, from a Fotodyne 3-3002 (New Berlin, Wyo., USA) transilluminator.

Southern blot analysis

The chromosomal DNA separated by contour-clamped homogeneous-electric-field electrophoresis (CHEF) was treated with depurination solution (0.25 M HCl) for 4 min, denatured for 3 min, neutralized for another 3 min and transferred to nylon filters. Each filter was used only once. The basic procedure followed was that of Naumov et al. (1992a), and experiments were carried out at least twice.

Flow cytometry

Yeast cells were grown with vigorous stirring at 30 °C in tubes with 10 ml yeast/peptone/dextrose medium (1% Bacto-yeast extract, 2% Bacto-peptone, 2% glucose). When the culture reached the stationary phase the cells were harvested by centrifugation, washed and resuspended in 70% ethanol and maintained at −20 °C for 30 min. About 5×10^5 cells were then resuspended in 50 mM sodium citrate pH 7.5, centrifuged and again resuspended in 0.5 ml 50 mM sodium citrate containing 50 mg RNase. The preparation was incubated for 2 h at 37 °C and then 0.5 ml 50 mM sodium citrate containing 2 mg propidium iodide was added. The samples were incubated for 30 min at room temperature and sonicated for 30 s. The DNA content was measured in a flow cytometer (Becton-Dickinson FACScan analyzer) following procedures described (Hutter and Eipel 1979).

Results

DNA content and electrophoretic karyotype of the industrial and laboratory strains

DNA content was determined in the industrial strains used in this research (Table 1). The laboratory yeasts YNN295 and DS81 were used as haploid and diploid controls respectively. Results obtained are summarized in Table 2, and those of some strains, taken as examples, expressed in Fig. 1. When compared with YNN295 and DS81 laboratory yeasts, the DNA content of the industrial yeasts varied from 1.3 *n* in some bakers' strains to 3 *n*. There was no uniformity within an industrial group, so that the DNA content of the bakers' strains varied from 1.3 *n* to 3 *n*, as indicated, and the same was observed with distillers' (1.3–1.6 *n*) and brewers' (1.6–2.2 *n*) yeasts. Only 2 out of 16 industrial strains, the bakers' strain ALKO743 and ALKO1611, were 3 *n*.

Industrial and laboratory yeasts were subjected to karyotype analysis by pulsed-field technique, using CHEF (Fig. 2). The karyotype patterns were readily distinguishable for each strain. The number of chromosomal bands varied from 14 in the laboratory strain YNN295 to more than 20, according to the strain. Furthermore, these variations were detected in the number, intensity and length of the bands, even when two strains, for example SB2 and SB11, came from the same source (Table 1). It was not possible to assign a

Table 1 Strains used in this work. *G* genetic line, *B* bakers', *W wine, Br* brewers', *D* distillers'

Strain		Genotype	Source
DADI	B	Unknown	La Cruz del Campo, Sevilla, Spain
VS	B	Unknown	La Cruz del Campo, Sevilla, Spain
CT	B	Unknown	La Cruz del Campo, Sevilla, Spain
SB2	B	Unknown	Horno San Buenaventura, Sevilla, Spain
SB11	B	Unknown	Horno San Buenaventura, Sevilla, Spain
V1	B	Unknown	Compañía General de Levadura, Valladolid, Spain
V2	B	Unknown	Compañía General de Levadura, Valladolid, Spain
DS81	G	*MATα/MATa SUC2/SUC2 mal/mal gal2/gal2 CUP1/CUP1*	This work
ACA21	W	Unknown	A. Casas, Departamento de Microbiología, Sevilla, Spain
ACA22	W	Unknown	A. Casas, Departamento de Microbiología, Sevilla, Spain
ATCC9080	Br	Unknown	American Type Culture Collection
NCYC396	Br	Unknown	National Collection of Yeast Cultures, UK
ALKO743	B	Unknown	Alko Ltd., Helsinki, Finland
ALKO554	B	Unknown	Alko Ltd., Helsinki, Finland
TS146[a]	B	Unknown	Alko Ltd., Helsinki, Finland
ALKO1245	D	Unknown	Alko Ltd., Helsinki, Finland
ALKO1523	D	Unknown	Alko Ltd., Helsinki, Finland
ALKO1611	B	Unknown	Alko Ltd., Helsinki, Finland
YNN295	G	*MATa ura3 lys2 ade1 ade2 his trp1Δ*1	Bio-Rad, Richmond, Calif., USA

[a] TS146 has been isolated from rye bread sour dough. This yeast is obviously naturally selected and propagated in sour dough (thus, not grown industrially on molasses)

Table 2 DNA content (arbitrary units), and ploidy (*n*) of the strains in this work. *G* genetic line, *B* bakers', *W* wine, *Br* brewers', *D* distillers'

Strain		DNA content	Ploidy (*n*)
YNN295	G	3.5	1
DS81	G	7	2
DADI	B	5.5	1.5
VS	B	9.5	2.5
CT	B	10	2.7
SB2	B	9.8	2.6
SB11	B	9.7	1.3
V1	B	10	2.7
V2	B	10	2.7
ACA21	W	7	1.9
ACA22	W	7	1.9
ALKO1245	D	6	1.6
ALKO1523	D	5	1.3
ALKO743	B	11	3
ALKO554	B	5	1.3
ALKO1611	B	11	3
TS146	B	6	1.6
ATCC9080	Br	6	1.6
NCYC396	Br	8.5	2.2

specific pattern to any industrial group (e.g. baker's yeasts).

Chromosome identification by Southern blot hybridization analysis

DNA homologies between the different industrial yeasts and the laboratory strains, as well as chromosome identification in the industrial yeasts, were examined by Southern blot analysis. Cloned genes of *S. cerevisiae* were used, some of them having a single location on the *S. cerevisisae* genome (Naumov et al. 1992a). After electrophoretic karyotyping, the chromosomes were transferred to nylon filters and hybridized with the cloned gene probes of *S. cerevisiae*. Southern blot hybridization patterns of lane 16 in Fig. 3 seem to be slightly different from those of lane 1, while the strain tested was the same (YNN295); because of variable amounts of DNA of this strain in the gel used, only a faint signal was obtained with some probes in these lanes. The conditions used were very stringent, and hybridization was hence only possible for DNA · DNA homologies of more than 90%. Therefore, as Fig. 3 shows, the DNA of all the industrial yeasts (bakers', wine, brewers' and distillers') was highly homologous to that of the laboratory strains. Also, the probes used indicated the genes *LEU2*, *TRP1*, *URA3*, *CUP1*, *ADC1* and *GAL4* to be located on the same chromosomes (III, IV, V, VIII, XV and XVI respectively) as in the laboratory strains of *S. cerevisiae*. However, Fig. 3A seems to indicate that more than one copy of chromosome III of different lengths were present in some bakers' strains. Figure 3B, however, shows strong hybridization in chromosome IV and a weaker one in a different position, even though *TRP1* genes have been described to be present in a single copy (Naumov et al. 1992a). This second band appears to be present in most bakers' strains (DADI, VS, CT, SB2, SB11 and TS146) but never in any other strain whatsoever. It is still to be determined what this

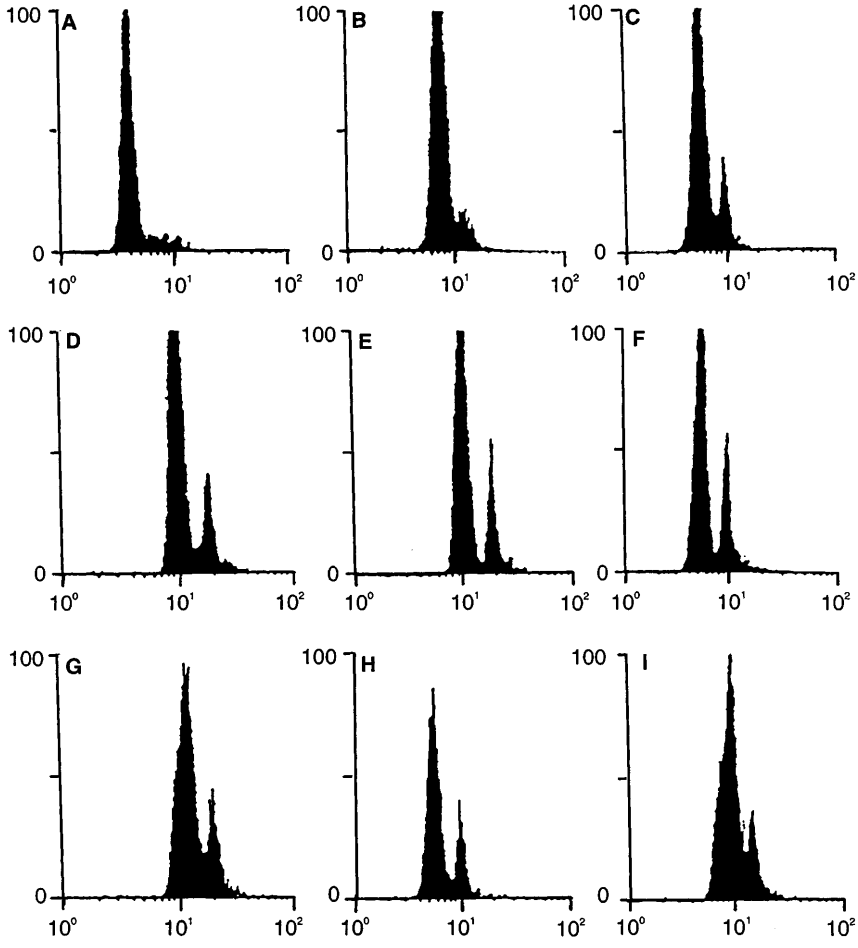

Fig. 1A–I DNA content (arbitrary units) measured by flow-cytometric analysis of the laboratory yeast YNN295 (haploid) (**A**) and DS81 (diploid) (**B**), and the strains TS146 (**C**), SB2 (**D**), V1 (**E**), DADI (**F**), ALKO1611 (**G**), ALKO1245 (**H**) and VS (**I**). The figure represents the numbers of cells (*ordinate*) that had an absorbance (arbitrary units) indicative of the DNA content (abscisa)

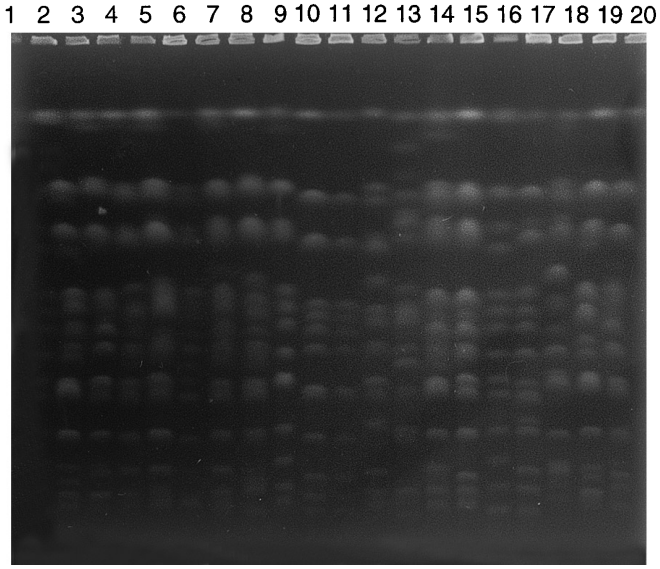

Fig. 2 Chromosomal patterns of industrial and laboratory yeasts. Lanes *1* and *16* laboratory strain YNN295; lanes 2–8 bakers' yeasts DADI (*2*), VS (*3*), CT (*4*), SB2 (*5*), SB11 (*6*), V1 (*7*) and V2 (*8*); lane *9* laboratory strain DS81; lanes *10* and *11* wine yeasts ACA21 (*10*) and ACA22 (*11*); lanes *12* and *13* brewers' yeasts ATCC9080 (*12*) and NCYC396 (*13*); lanes *14* and *15* bakers' yeasts ALKO743 (*14*) and ALKO554 (*15*); lanes *17–19* distillers' strains TS146 (*17*), ALKO1245 (*18*) and ALKO1523 (*19*); lane *20* bakers' yeast ALKO1611

hybridising sequence codes for. The fact that chromosome IV has over 1600 kb (Grivell and Planta 1990) makes it impossible to differentiate more than one copy of chromosome IV, with different lengths, in any of the strains.

When the *URA3* probe was used (Fig. 3C) the presence of more than one chromosome V, of variable size, in some bakers' yeasts was observed. With regards to the *CUP1* probe (Fig. 3D) the very intense band in the laboratory strain DS81, already described as copper-resistant, indicates gene amplification of *CUP1*. Some of industrial strains seem to have undergone *CUP1* gene amplification in chromosome VIII (Fig. 3D). However, the possibility exists of sequence heterology of functionally homologous genes resulting in the differential hybridization observed. This has already happened for probes derived from laboratory strains used in hybridization with distilling (Keiding 1985) and brewers' strains (Petersen et al. 1987). Distillers' strain ALKO1245 displayed, together with *CUP1* gene amplification, translocation of *CUP1* from chromosome VIII to a different chromosome, probably chromosome XVI as earlier found for some other strains by Naumov et al. (1992b). Results also indicate the presence of more than one chromosome VIII in some bakers' yeasts of different size.

When *ADC1* (from chromosome XV, Fig. 3F) and *GAL4* (from chromosome XI, Fig. 3G) were used as probes, results were comparable to that of *LEU2* (Fig. 3A). In addition to chromosome XVI, the *GAL4* probe hybidizes with at least two extra bands in laboratory strains. However, in industrial yeasts, it seems to show hybridization with other chromosomes. As before, the reason could be partial homology with other genes.

The presence of multiple bands hybridizing with a *SUC2* probe, from chromosome IX, was observed in bakers' yeasts and in one cane molasses distillers' strain (Fig. 3E). These bands can be regarded as indicating the presence of many *SUC* genes located on different chromosomes. The intensity of the bands was variable within a strain so that the reason has to be differences in DNA homologies. When an oligomer of the rDNA probe (Naumov et al. 1992a) was tested to check chromosome XII (the largest, 2600 kb) (Grivell and Planta 1990), results show that this rDNA occupies the same position in all the strains except TS146, ALKO1523 and ALKO1611 (not shown). Heterology with the rDNA probe could account for the observed result.

Presence of the mobile elements *Ty1* and *Ty2* as well as telomeric *Y′* sequences in the industrial yeasts

The results of hybridization of the probes for the mobile elements *Ty1* and *Ty2* as well as the telomere-associated *Y′* sequences (Fig. 4), which assume multiple chromosomal locations in *S. cerevisiae*, are presented in Fig. 5. Both *Ty1* and *Ty2* transposable elements were found on many chromosomes in all strains tested (Fig. 5A, B). However, both sequences seemed to be more frequent in bakers' and, to a lesser extent, in laboratory yeasts than in wine and brewers' yeasts and some of the distillers' strains. Besides, differences of intensity within the same strain have to be attributed to amplification rather than DNA homologies. Figure 5A, B also shows unequal distribution among industrial groups and among different chromosomes of the *Ty1* and *Ty2* elements.

When the telomeric probes *Y′29* and *Y′16* were tested in this study, differences in both the number of hybridization signals and their intensity among the strains were observed (Fig. 5C, D). The *Y′16* sequence seems to be present in nearly every chromosome and, moreover,

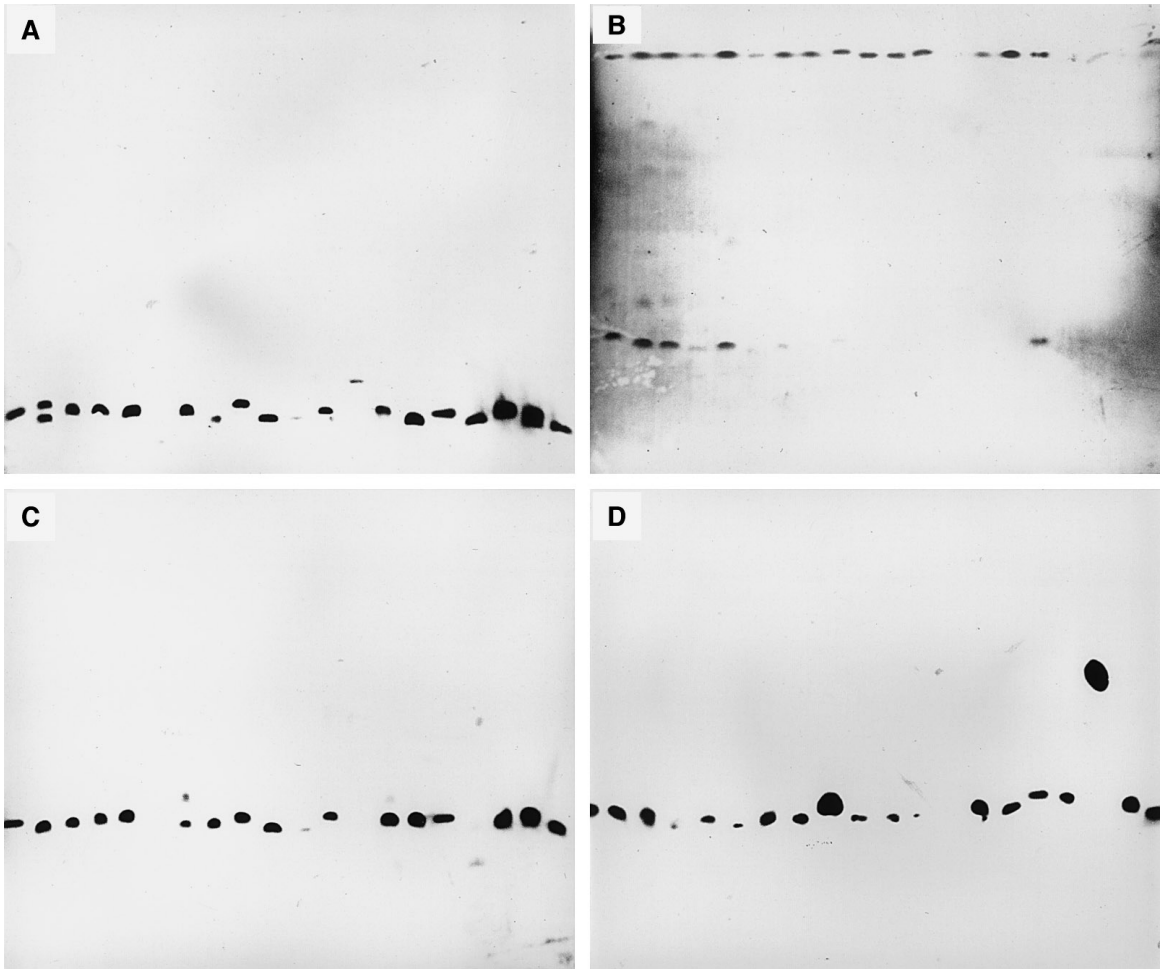

Fig. 3A–D

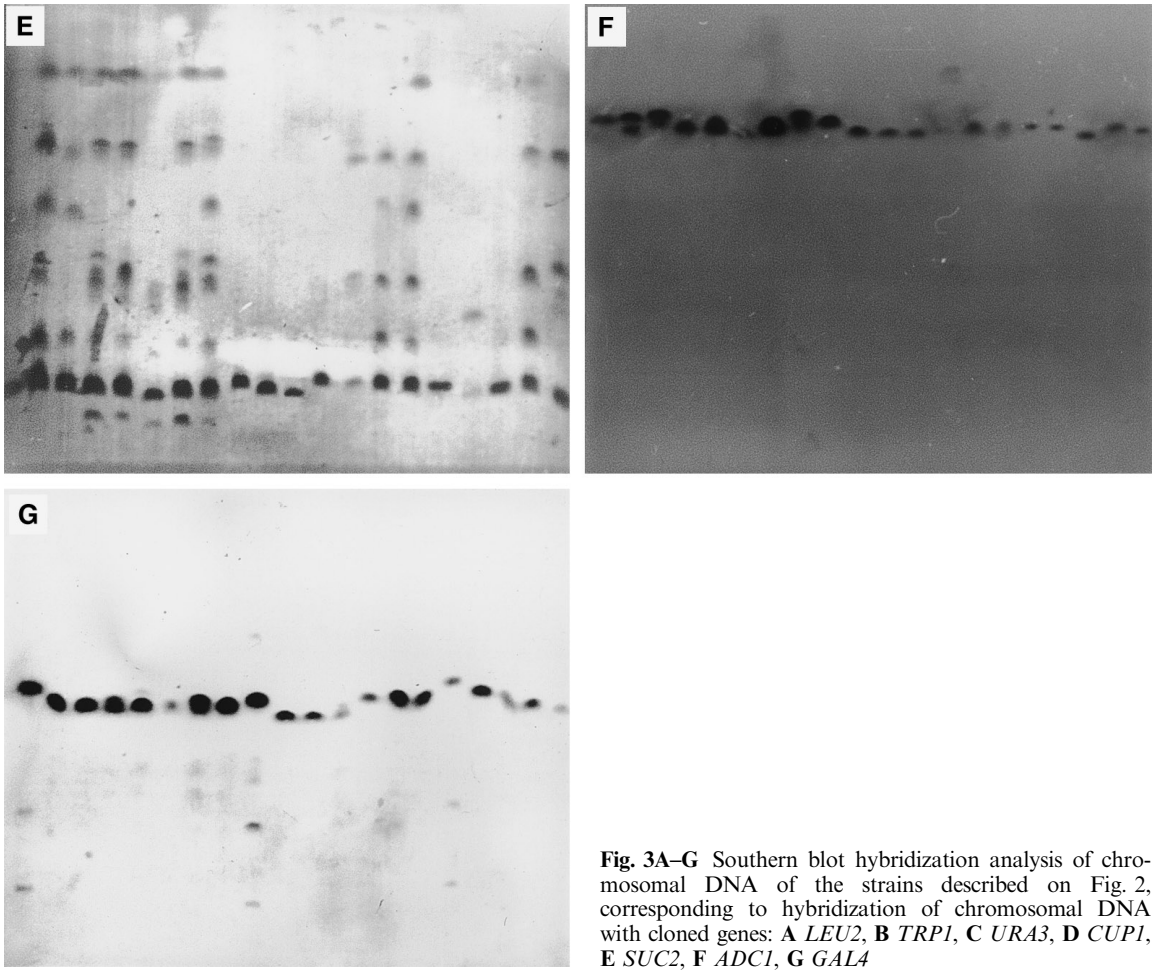

Fig. 3A–G Southern blot hybridization analysis of chromosomal DNA of the strains described on Fig. 2, corresponding to hybridization of chromosomal DNA with cloned genes: **A** *LEU2*, **B** *TRP1*, **C** *URA3*, **D** *CUP1*, **E** *SUC2*, **F** *ADC1*, **G** *GAL4*

with a high frequency in each of them in most strains. The presence of *Y′16* was scarce in wine and laboratory yeasts. By comparison, *Y′29* was less represented in the yeast populations.

Discussion

Most bakers' and other industrial yeasts (wine, brewers' and distillers') are polyploid or aneuploid. Unbalanced chromosome complements of industrial yeasts have been suggested to be advantageous since aneuploidies are so extended (Bakalinski and Snow 1990; Adams et al. 1992). However, the suggestion that extra sets of chromosomes might give rise to more vigorous strains (Adams et al. 1992) and those of a chromosomal constitution reflecting optimal fitness in each industrial environment (Yamamoto et al. 1991) does not seem to be supported by the data in Table 2. The selective pressure exerted on bakers' strains is totally different from that on wine, brewers' or distillers' yeast; nevertheless, the DNA content within bakers' strains varied from 1.3 *n* to 3 *n*, with similar variations in the other yeasts. Supporting these data, Bakalinski and Snow (1990), Guijo et al. (1997) and Ibeas and Jiménez (1996) demonstrated the complexity of the chromosomal constitution of wine yeasts, concluding that the strains were aneuploid. On the other hand, the aneuploidy shown by some of the bakers' strains such as DADI (1.5 *n*) or VS (2.5 *n*) might explain the lack of viability of their meiotic products (0% for DADI and 10% for VS strains; Gasent-Ramírez et al. 1995). This lack of viability had already indicated that the strains were strongly unbalanced. Strains ACA21 and ACA22 were 1.9 *n*, and the fairly high viability of their meiotic products (over 60%; Jiménez and Benítez 1987) seems to support the idea of chromosomal balance.

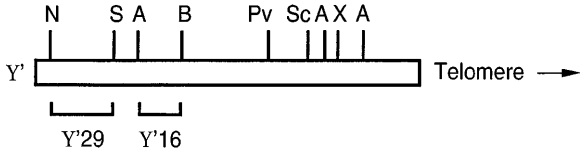

Fig. 4 Fragments of the *Y′* sequence used for hybridization. *Y′* sequences according to Louis and Haber (1990). *S Sal*I, *A* Asp-718, *B Bam*HI, *Pv Pvu*I, *Sc Sac*I, *X Xho*I

The identification of industrial yeasts has not been easy; most of them belong to the same species, *S. cere-*

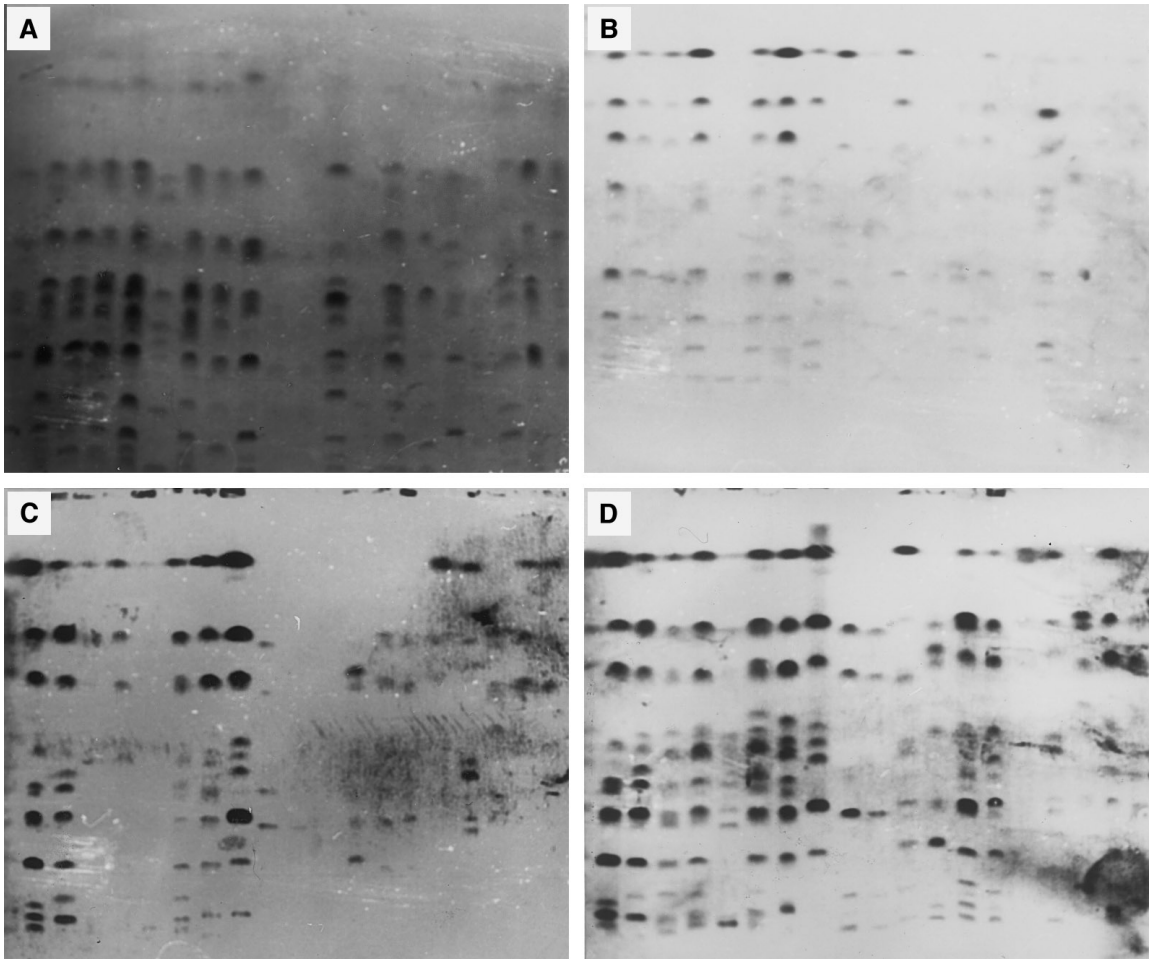

Fig. 5A–D Southern blot hybridization analysis of chromosomal DNA of the strains described in Fig. 2, corresponding to hybridization of chromosomal DNA with the probes: **A** *Ty1*, **B** *Ty2*, **C** *Y′16*, **D** *Y′29* (Naumov et al. 1992)

visiae, and they seldom have suitable genetic markers. Hence chromosomal patterns have come to be used as the only reliable technique in yeast characterization (Yamamoto et al. 1991; Bakalinski and Snow 1990; Naumov et al. 1992a; Querol et al. 1992a). The industrial strains of *S. cerevisiae* show similar patterns, but individual bands have different mobilities due to chromosome-length polymorphism (Yamamoto et al. 1991). Almost invariably, these changes allow each single industrial strain to be perfectly distinguishable from the others. Polymorphism is much more extensive in industrial yeasts than in laboratory strains (Bidenne et al. 1992). Bakalinski and Snow (1990) said that the range of variation they observed between the karyotypes of the laboratory strains and those of the wine strains did appear to exceed that commonly encountered between laboratory strains. In this work the karyotype pattern was specific for each strain (Fig. 2). In accordance with these results, Vezinhet et al. (1990) and Yamamoto et al. (1991) described a marked polymorphism in enological yeast strains. Bidenne et al. (1992) found differences in the size of specific chromosomes reaching 45% on wine yeasts.

Although it has been possible to demonstrate the presence of a variable number and size of chromosomes in the strains of this study (Fig. 2) as well as in other industrial strains (Naumov et al. 1992a; Rank et al. 1991) the underlying molecular basis for chromosomal-length polymorphism remains unknown. The possibility that strong selection pressure accounts for this polymorphism has been suggested by some authors (Yamamoto et al. 1991; Rank et al. 1991; Bakalinski and Snow 1990). Furthermore, industrial populations of yeasts have been shown to undergo changes, including chromosome loss and variations in the ploidy level (Adams et al. 1992); rearrangements have also been detected in strong selective continuous culture where the isolated variants were selectively advantageous (Adams et al. 1992). It is interesting to note that this selection pressure acting on industrial yeasts and accounting for chromosomal polymorphism seems to exist to a much lesser extent in wine yeasts (Fig. 2; Vezinhet et al. 1990; Martínez et al. 1995). It has been postulated that, in the absence of recombination, the composition of a population will remain monomorphic, except for those periods when the population undergoes adaptative shifts (Rosenzweig et al. 1994). Sancho et al. (1986), following

the distribution of characteristics of wine yeasts, found a very marked sexual isolation. They suggested that this sexual isolation in the yeast populations prevented the random distribution of taxonomic characters. Other explanations however, cannot be ignored.

Southern blot analysis using gene probes of *S. cerevisiae* with a single location on the genome (Naumov et al. 1992a) indicates these genes to be located on the same chromosome and to be highly homologous in the laboratory and the industrial yeasts (Fig. 3), and that more than one homologous chromosome, of different lengths, are present mostly in bakers' yeasts. The *S. cerevisiae* complex is made up of four species presently known: *S. cerevisia*e, *S. bayanus*, *S. pastorianus* and *S. paradoxus*. Naumov et al. (1992a) found that from eight *S. cerevisiae* gene probes used (those employed in this study) only four of them showed reliable homology with the genes of *S. bayanus;* signals of very low intensity were obtained with probes such as *CUP1* and *TRP1* and no hybridization with *ADC1*. In general terms, these results can be interpreted in the sense that different intensities of hybridization for the different strains provide a rough estimate of the differing rates of divergence among the genes that were probed (Naumov et al. 1992a). It is also interesting to note that, according to Naumov et al. (1992a), the variation shown here is intraspecific rather than interspecific, since all isolates examined are members of the same species. The reason is that neither *S. paradoxus* nor *S. bayanus* strains showed any hybridization with the *ADC1* promoter probe (Naumov et al. 1992a). In fact, these authors suggested the possibility of using the *ADC1* promoter sequence as a specific probe to distinguish *S. cerevisiae* from *S. paradoxus* and *S. bayanus*.

On the other hand, the *SUC* gene family includes the six loci *SUC1*, *2*, *3*, *4*, *5* and *7*. All of these genes except *SUC2* are located near chromosome ends, have highly homologous sequences and are dispersed on different chromosomes (Carlson et al. 1985). As the different *SUC* genes share a high homology, the *SUC2* probes hybridized with all of the *SUC* genes present in the bakers' yeasts. The results of Fig. 3E show a high diversity of *SUC* genes (number and location) in the genome of these different industrial yeasts. Ness and Aigle (1995) also found multiple *SUC* genes in the genome of the yeasts used on molasses, whereas laboratory strains carried only the *SUC2* gene. *SUC* genes encode the invertase enzyme and the major carbon source in molasses is sucrose. This can therefore be explained by the adaptation of these yeasts to growth on molasses. Thus, the *SUC* genes show genetic diversity in this population of yeasts and seem to be a good example of genomic rearrangement playing a major role in evolution and environmental adaptation in a yeast population using molasses. These suggestions are supported by the following facts. (1) The specific activity of the invertase enzyme varies enormously from one strain to another; however, on average, bakers' and distiller strains have between 10 and 15 times more activity than laboratory yeasts, whereas wine yeasts have about twice as much activity (Jiménez and Benítez 1986; Codón and Benítez 1995). (2) Laboratory yeasts reduce their growth rate in molasses to about 80% of that in laboratory medium; industrial yeasts such as wine strains reduce their growth rate in molasses to 35%–45% (Martínez-Force and Benítez 1993, 1995); however, all the bakers' strains tested grow at the same rate or even faster in molasses than in laboratory medium (Codón and Benítez 1995). (3) In contrast to distillers' and bakers' yeasts, those strains originally not growing on molasses, like the majority of wine strains, show only the *SUC2* gene and no *SUC* telomeric genes (Fig. 3E; Bidenne et al. 1992; Ness and Aigle 1995). These wine yeasts ferment grape juice containing glucose and fructose instead of sucrose. (4) During fermentation in molasses, biomass production increased considerably after addition of fungal invertase (Park and Sato 1982), which indicated that the sucrose-hydrolysing ability could be a limiting step in productivity. Moreover Gozalbo (1992) found that a high copy number of *SUC4* promoter regions caused increased expression of the invertase genes, thus resulting in the synthesis of external proteins even under repressing conditions. A distillers' strain, originating from a cane molasses distillery (ALKO1523), also had multiple copies of the *SUC* gene. Distilled alcoholic drinks are produced from many alcoholic materials, such as fermented fruit juices or cereal grains (Reed and Nagodawithana 1991). Distillers' yeasts carry out the alcoholic fermentation under conditions that vary greatly because of the many diverse substrates and processes, a fact that might explain the heterogeneity of this industrial group.

Comparative genetic studies of yeast strains of different origins have revealed many gene families such as polymeric *MEL* genes (Naumov et al. 1990, 1991; Turakainen et al. 1991), and *MAL* loci (Naumov et al. 1994). Some natural strains contain different combinations of these genes. The telomeric location of these genes may be an explanation (Bidenne et al. 1992). Subtelomeric regions have been found to harbour multicopy genes involved in carbon-source metabolism. These include the *MAL*, *SUC* and *MEL* genes (Louis and Haber 1990; Louis et al. 1994). Furthermore, it seems that these regions might contain a transposable sequence such as *Ty5* (Louis et al. 1994).

Grivell and Planta (1990) stated that rearrangements of large chromosome fragments are always the result of the movement of *Ty* elements in the DNA. The presence of so many copies of *Ty1* and *Ty2* elements in most bakers' yeasts (Fig. 5) is in line with the great polymorphism found in these strains as compared to other industrial groups. Furthermore, Yoda el al. (1993) described DNA-length polymorphism as generated by the loss or addition of 100 kb DNA during meiosis or sporulation and suggested the presence of *Ty* elements as the reason for such a process. In addition, Naumov et al. (1992a) reported on the *Ty2* sequence being only found in some strains of *S. cerevisiae* but not in *S. paradoxus* or *S. bayanus*. These results confirm that the industrial

strains discussed here are *S. cerevisiae* (Fig. 5B). Differences in distribution of *Ty2* appear to be due to industrial rather than taxonomic features (Ibeas and Jiménez 1996).

Telomeric sequences have been involved in generating polymorphism in some organisms. Furthermore, evidence for a human factor in the evolution of *S. cerevisiae* derives from the presence of a large number of chromosomes of small size and the clustering of fermentation markers at chromosome ends. This reflects selection pressure for high fermentation rates and hence for duplication and crossing-over events (Spencer and Spencer 1990). In this work, data on *Y′29* and *Y′16* distribution together with those on the *Ty1* and *Ty2* elements support, on the one hand, the high degree of polymorphism found for chromosome patterns in bakers' yeasts (Fig. 3) and the high copy numbers of polymeric gene families such as those found for the *SUC* gene (Fig. 3E). On the other hand, the different patterns shown by *Y′29* and *Y′16* telomeric sequences indicate that different chromosome regions undergo changes in intra- or intergenic rearrangements at different rates.

The selective pressure exerted on the nuclear genome seems to be supported by data on mitochondrial DNA. Whereas mitochondrial DNA polymorphism has been described as sufficiently extensive to be useful in endonuclease fingerprinting of both enological and brewers' strains (Vezinhet et al. 1990); bakers' strains had exactly the same pattern (Codón and Benítez 1995). So, although the association of mitochondrial (mt) DNA with nuclear gene products would suggest that both genomes are subjected to similar evolutionary mechanisms, the comparison of nuclear and mtDNA polymorphisms in bakers' strains indicates that mtDNA is relatively stable when compared to the nuclear genome. On the other hand, ethanol seems to be a strong mutagenic factor in yeast mitochondria, even though it has no genetic effects on the nuclei of the yeast cells (Jiménez and Benítez 1988). These different selective pressures could explain differences in polymorphisms observed in nuclear and mitochondrial genomes in bakers', wine and distillers' yeasts (Ibeas and Jiménez 1996).

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