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Orthogonal-field-alternation Gel Electrophoresis Banding Patterns of DNA from Yeasts

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Chromosomal DNAs from various yeast species were separated by orthogonal-field-alternation gel electrophoresis (OFAGE). To this end we developed a spheroplasting and lysis method to obtain intact DNA from both ascomycetous and basidiomycetous yeasts. The OFAGE banding patterns of 22 ascomycetous and four basidiomycetous yeast strains were compared. The strains represented species from the genera: *Brettanomyces, Candida, Cryptococcus, Filobasidiella, Geotrichum, Hansenula, Kluyveromyces, Pachysolen, Pichia, Rhodosporidium, Rhodotorula, Saccharomyces, Saccharomycodes, Saccharomycopsis, Schizosaccharomyces* and *Zygosaccharomyces*. Variations occurred in the number of bands and their positions in the gel, not only among strains of different genera but also among species from the same genus and even between varieties of the same species. The ascomycetous yeasts, with the exception of *Saccharomyces cerevisiae*, only showed one to five bands of DNA larger than 1000 kilobase pairs (kb) in general none smaller. The patterns of the four basidiomycetous yeasts revealed also a few large DNA bands but in addition one to six bands ranging in size from 500 to 1000 kb, with the exception of a single smaller chromosome in *Rhodotorula mucilaginosa*. From the OFAGE banding patterns of strains studied here it appears that in *Sacch. cerevisiae* the partitioning of DNA over chromosomes is unique. But rather than the large number of chromosomes, the presence of four chromosomes with less than 500 kb of DNA is characteristic for *Sacch. cerevisiae*.

KEY WORDS — Orthogonal-field-alternation gel electrophoresis; karyotyping; ascomycetous yeasts; basidiomycetous yeasts.

INTRODUCTION

The development of electrophoretic techniques for separating DNA molecules with sizes from 30 to 2000 kilobase pairs (kb) (Schwartz and Cantor, 1984; Carle and Olson, 1984) has provided a tool for the direct demonstration that genetic linkage groups in the yeast *Saccharomyces cerevisiae* correlate to physically distinct chromosomal DNA molecules (Schwartz and Cantor, 1984; Carle and Olson, 1984, 1985). These DNAs may be separated into 12 well-resolved bands ranging in size from 260 kb up to about 2000 kb by pulsed-field gel electrophoresis (Schwartz and Cantor, 1984) or its improved version: orthogonal-field-alternation gel electrophoresis (OFAGE) (Carle and Olson, 1984). The banding pattern obtained after ethidium bromide staining has been correlated to 15 out of the 17 genetically defined chromosomes (I–XI and XIII–XVI) by hybridization to DNA probes derived from cloned, chromosome-specific genes (Schwartz and Cantor, 1984; Carle and Olson, 1984, 1985; Mortimer and Schild, 1985). Chromosome XII, containing more than 100 copies of rRNA genes, did not reproducibly enter the gels, and chromosome XVII could not be detected due to the lack of cloned marker genes. Carle and Olson (1985) demonstrated the existence of chromosome length polymorphisms among strains of *Sacch. cerevisiae* which might be caused by extensive rearrangements near the telomeres (Horowitz *et al.*, 1984) or by yeast transposons (Ty elements). They also proposed this banding pattern obtained by OFAGE as an electrophoretic karyotype.

The question arose whether this technique could also be applied to other yeasts from various taxonomic entities. For instance, only three chromosomes have been found in *Schizosaccharomyces pombe* (Gygax and Thuriaux, 1984; Erard and

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Barker, 1985) and *Candida albicans* (Hilton *et al.*, 1985), whereas for many other species insufficient data are available. As a first approach to answer this question we studied OFAGE patterns of 26 different species belonging to various genera of ascomycetous and basidiomycetous yeasts. These species were chosen because they represent yeasts that are important in laboratory, industry or medicine.

MATERIALS AND METHODS

Strains

The taxonomic classification and sources of yeast strains used in this study are compiled in Table 1.

Sample preparation with Zymolyase

Yeasts were grown to late logarithmic phase at 30°C in 2-litre Erlenmeyer flasks each containing 250 ml YEPD medium (YEPD: 10 g yeast extract, 20 g Bacto-peptone, 20 g glucose per litre distilled water). We modified the procedure of Schwartz and Cantor (1984) by using 150 μg Zymolyase 60·000 per 10^{10} cells. Subsequently the blocks were rinsed with 10 ml NDS (NDS: 0·5 M-EDTA, pH 7·5, 10 mM–Tris–HCl, pH 7·5, 1% (v/v) sodium *N*-lauroylsarcosinate) and lysed in 20 ml NDS containing 4 mg proteinase K (Boehringer, Mannheim) for 16 h at 50°C. Blocks could be stored for at least 3 weeks at 4°C in fresh NDS without degradation of DNA.

Sample preparation with Novozym

Approximately 10^{10} late-logarithmic-phase cells were harvested and subsequently washed in 20 ml 0·05 M–EDTA, pH 7·5, at room temperature. The final cell pellet was resuspended in 20 ml buffer (pH 7·5) containing 0·05 M–EDTA, 10 mM–Tris–HCl, and 10 mM–dithiothreitol, and incubated for 15 min at 30°C with gentle shaking. Cells were spun down, washed with 20 ml CPE buffer (CPE: mix 100 ml 40 mM–citric acid, 120 mM–Na_2HPO_4, pH 6·0 with 4 ml 0·5 M–EDTA, pH 7·5) and resuspended in 3 ml CPES buffer (CPES: CPE buffer containing 1·2 M–sorbitol and 5 mM–dithiothreitol). Cells were acclimatized at 38°C for 5 min. To prepare spheroplasts, 20 mg Novozym 234 (Novo Biolabs, Denmark) was dissolved in 5 ml of 1% (w/v) low-gelling agarose, prepared in CPE buffer and cooled down to 38°C, and immediately mixed with the cell suspension. The mixture was pipetted into a precooled matrix and allowed to gel at 0°C. The agarose blocks were removed from the matrix and incubated for 1 h at 30°C in 20 ml CPE buffer. Lysis with NDS was performed as described above.

Electrophoresis

The agarose blocks were inserted into preformed slots of a 10·3 × 10·3 × 0·5 cm 1·5% (w/v) agarose gel in electrophoresis buffer and subjected to OFAGE in an apparatus built according to Carle and Olson (1984). Electrophoresis buffer was 0·45 × TBE (1 × TBE: 90 mM–Tris base, 90 mM–boric acid, 2·5 mM–EDTA, disodium salt, pH 8·2). During the run usually 24 h, the current was maintained at 135 mA and the temperature in the electrophoresis tank was kept between 16 and 20°C. During this period, the voltage decreased from 300 to 250 V. Switching intervals were either 15, 55 or 65 s, as indicated. The gels were stained with 0·5 μg/ml ethidium bromide for 1 to 2 h and destained overnight in 10 mM–EDTA, pH 7·5. Gels were photographed on a transilluminator at 300 nm.

RNAse treatment

When a smear of RNA obscured the DNA banding pattern (which frequently was observed after sample preparation with Zymolyase), the gel was incubated for 2 h at 37°C with gentle shaking in a sealed bag containing 30 ml electrophoresis buffer and 1·5 ml R Nase solution (R Nase solution: 500 μg/ml pancreatic RNase and 100 U/ml T_1 RNase—both preparations from Boehringer, Mannheim—in 10 mM – Tris–HCl, pH 7·5, 15 mM–NaCl; any DNase contamination was inactivated by heating for 10 min at 100°C, followed by a 4–h period of cooling to room temperature.) The RNase was removed from the gel by rinsing twice with electrophoresis buffer during 1 h. Alternatively, the sample blocks could be pretreated with RNase. To this end the blocks were rinsed three times with 10 ml ET (ET: 0·5 M – EDTA, pH 7·4, 10 mM – Tris–HCl, pH 7·5) at 4°C for 30 min per cycle and incubated in 18 ml ET to which 2 ml RNase solution had been added. Blocks were then rinsed twice with 10 ml NDS and incubated in 20 ml NDS containing 4 mg proteinase K for 2 h at 50°C. Blocks could be stored in fresh NDS at 4°C for at least 3 weeks without any remarkable degradation of DNA.

Table 1. Taxonomic classification and sources of yeast strains studied.

Classification[a]	Genus and species name	Strain	References and notes
Ascomycetes			
IA	*Schizosaccharomyces pombe*	CBS[b] 356	
IB	*Saccharomycodes ludwigii*	CBS 821	
IC	*Hansenula anomala*	CBS 5759	
	Hansenula nonfermentans	CBS 5764	
	Hansenula polymorpha	CBS 4732	
	Kluyveromyces marxianus var. *lactis*	CBS 683	
	Kluyveromyces marxianus var. *marxianus*	CBS 712	
	Pachysolen tannophilus	CBS 4044	
	Pichia stipitis	CBS 5773	
	Saccharomyces cerevisiae	CBS 1513	*S. carlsbergenis*[f]
		AB972	Sandmeyer and Olson (1982)
		X3402-15C	Mortimer and Hawthorne (1973)
		CBS 8066	JJ101
		CBS 395	*S. uvarum*[f]
		CBS 1171	Type strain
	Saccharomycopsis lipolytica	CBS 6124	*Yarrowia lipolytica*[f]
	Zygosaccharomyces bailii	CBS 680	
	Zygosaccharomyces rouxii	CBS 732	
II	*Geotrichum candidum*	CBS 772.71[c]	
Basidiomycetes			
III	*Filobasidiella neoformans*	CBS 132	
	Filobasidium uniguttulatum	CBS 1730	
IV	*Leucosporidium antarcticum*	CBS 5942	
	Rhodosporidium toruloides	CBS 14	
Imperfect yeasts			
V	*Brettanomyces custersii*[d]	CBS 5512	
	Brettanomyces intermedius[d]	CBS 73	
	Candida albicans[d]	CBS 562	
	Candida shehatae[d]	CBS 5813	
	Candida utilis[d]	CBS 621	
	Cryptococcus laurentii[e]	CBS 139	
	Phaffia rhodozyma[e]	CBS 5905	
	Rhodotorula mucilaginosa[e]	CBS 17	
	Trichosporon cutaneum[e]	CBS 2466	

[a]Classification according to Kreger-van Rij (1984) (with exception of *Geotrichum candidum* which was included in the yeasts according to Barnett *et al.* (1983): I, family of Saccharomycetaceae; IA, subfamily of Schizosaccharomycetoideae; IB, subfamily of Nadsonioideae; IC, subfamily of Saccharomycetoideae; II, family of Endomycetaceae; III, family of Filobasidiaceae; IV, family of teliospore-forming yeasts; V, family of Cryptococcaceae.
[b]Centraal Bureau voor Schimmelcultures. Yeast Division, Julianalaan 67, 2628 BC Delft, The Netherlands.
[c]This strain was obtained from CBS-Baarn, Oosterstraat 1, 3742 SK Baarn, The Netherlands.
[d]Ascomycetous yeasts.
[e]Basidiomycetous yeasts.
[f]Previous name.

RESULTS

Sample preparation

For *Sacch. cerevisiae* we modified the method described by Schwartz and Cantor (1984). To eliminate background as much as possible we investigated parameters like age and concentration of cells, Zymolyase concentration and the times required for spheroplasting and subsequent lysis. The results are compiled in Table 2. Although the concentration of cells did not seem to influence the background, it appeared that at lower cell concentrations bands migrated faster than at higher concentrations.

Table 2. Parameters in sample preparation with Zymolyase.

Parameter	Optimum	Range tested
NDS incubation time	16 h	0·5–16 h
Zymolyase concentration	80 U/ml[a]	40–900 U/ml[a]
Zymolyase incubation time	4 h	2–16 h
Dithiothreitol concentration in spheroplasting buffer	10 mM	6–60 mM
Concentration of cells in the agarose blocks	1×10^9/ml	$0{\cdot}2–3 \times 10^9$/ml
Age of cells	20 h	3–30 h

The parameters were varied one by one in the order indicated in the table. It was found that proteinase K or pronase could be omitted from the NDS buffer. The range tested indicates limits within which the parameters may be varied without affecting the quality of the banding pattern. The values in the table were estimated for *Sacch. cerevisiae* X3402-15C; for other strains we routinely used 900 U Zymolyase/ml and incubated for 4 h.
[a]Determined for a 16-h incubation.

The cell walls of basidiomycetous yeasts were not degraded by Zymolyase. Therefore, Novozym was used instead. This enzyme has maximum activity at pH 5 to 6, but even at pH 6·0 the high concentration of EDTA used to prevent DNAse activity in the Zymolyase method could not be obtained and consequently the EDTA concentration was reduced to 20 mM. Although this might have caused the DNA degradation observed with some strains, the method with Novozym worked well for most strains, not only for the basidiomycetous yeasts but also for the ascomycetous yeasts. No difference was observed between the patterns from X3402–15C spheroplasts prepared by either Zymolyase or Novozym.

Saccharomyces cerevisiae *X3402–15C*

Sacch. cerevisiae X3402–15C (Mortimer and Hawthorne, 1973) was used for a co-migrating reference in all gels. Apart from the intensity of chromosome I (X3402–15C is disomic for this chromosome) the pattern is similar to that of strain AB972 (Sandmeyer and Olson, 1982) from which all bands were correlated to genetically defined chromosomes by DNA–DNA hybridizations using cloned genes (Carle and Olson, 1985). Without further checking we have assumed that this correlation is similar for X3402–15C and used an identical band-numbering system. In our gels, band 10 was resolved into two bands, which we called 10 A and 10 B. Band 12 consistently migrated approximately 9 mm into the gels and ran rather straight (Figure 1A, lanes 1 and 6) instead of following bent lanes as did the DNA molecules in bands 1–11. The origin of this band is unclear. The DNA from chromosome XII, containing over 100 copies of rDNA (Petes, 1979), is unable to enter the gel over more than a few mm (Carle and Olson, 1985), but a Southern blot of an OFAGE hybridized to an rDNA probe showed a straight-running smear migrating outside the bent lanes and terminating at the position of band 12 (P. de Jonge *et al.*, unpublished results). A similar smear may be seen in Figure 1C, lane 2, and Figure 1E, lane 2. It started just below the wells where a band probably representing large chromosmal DNA was found. Moreover, Carle and Olson (1985) correlated band 12 to chromosome IV by hybridization with the *SUP2* gene as specific probe. Since we have observed band 12 in the pattern of almost all yeast strains studied when switching times of at least 50 s were used (Figure 1A and C–G), and since it is unlikely that all strains have a chromosome of the same size, we think that band 12 is an artefact of the OFAGE. We used this band as an indicator for cell lysis. When it was absent we concluded that cells had not lysed sufficiently during preparation of DNA, or that the DNA had been degraded extensively. In the latter case an intense smear was usually seen at the bottom of the gels. Since band 12 was present in all gels and appeared to be independent of

the species used, it was not included in our comparison of the number of bands from various yeasts.

Four other Saccharomyces cerevisiae strains

To get an idea of the variations in the banding patterns of different strains of the same species we compared four *Sacch. cerevisiae* strains to X3402–15C. Figure 1A shows the banding patterns of the chromosomal DNAs from these yeast strains obtained by OFAGE after staining the gel with ethidium bromide. They all reveal a more or less similar pattern in having at least 12 bands ranging in size from about 250 kb up to approximately 2000 kb. The existence of chromosome length polymorphisms among the strains is apparent from Figure 1A, B and the diagrammatic representation in Figure 3. The most striking differences were found between strain CBS 395 (previous name *Sacch. uvarum*) and the reference strain.

Another strain, previously classified as *Sacch. carlsbergensis*, also revealed remarkable chromosome length polymorphism. Like many industrial strains this brewery yeast is known to be aneuploid. We observed at least 17 bands including very weak bands adjacent to more intensive bands. There are at least three bands at the position of band 1 of the reference (hardly visible in Figure 1A, but well resolved in the 15 s-pulsed gel, as shown in Figure 1B). Therefore, it seemed unlikely that each of these bands represented one of the 17 genetically defined chromosomes of haploid *Sacch. cerevisiae*. It might rather be ascribed to polymorphisms in length of the chromosomes and the aneuploidy of the strain.

In the taxonomic type strain of *Sacch. cerevisiae* (CBS 1171) the doublet in band 5 was resolved into two bands. Just above band 2 a weak band was barely visible. It was better resolved on a 15 s-pulsed gel (Figure 1B, lane 4). The intensity was approximately half of that of band 2 and this suggested aneuploidy rather than heterozygosity.

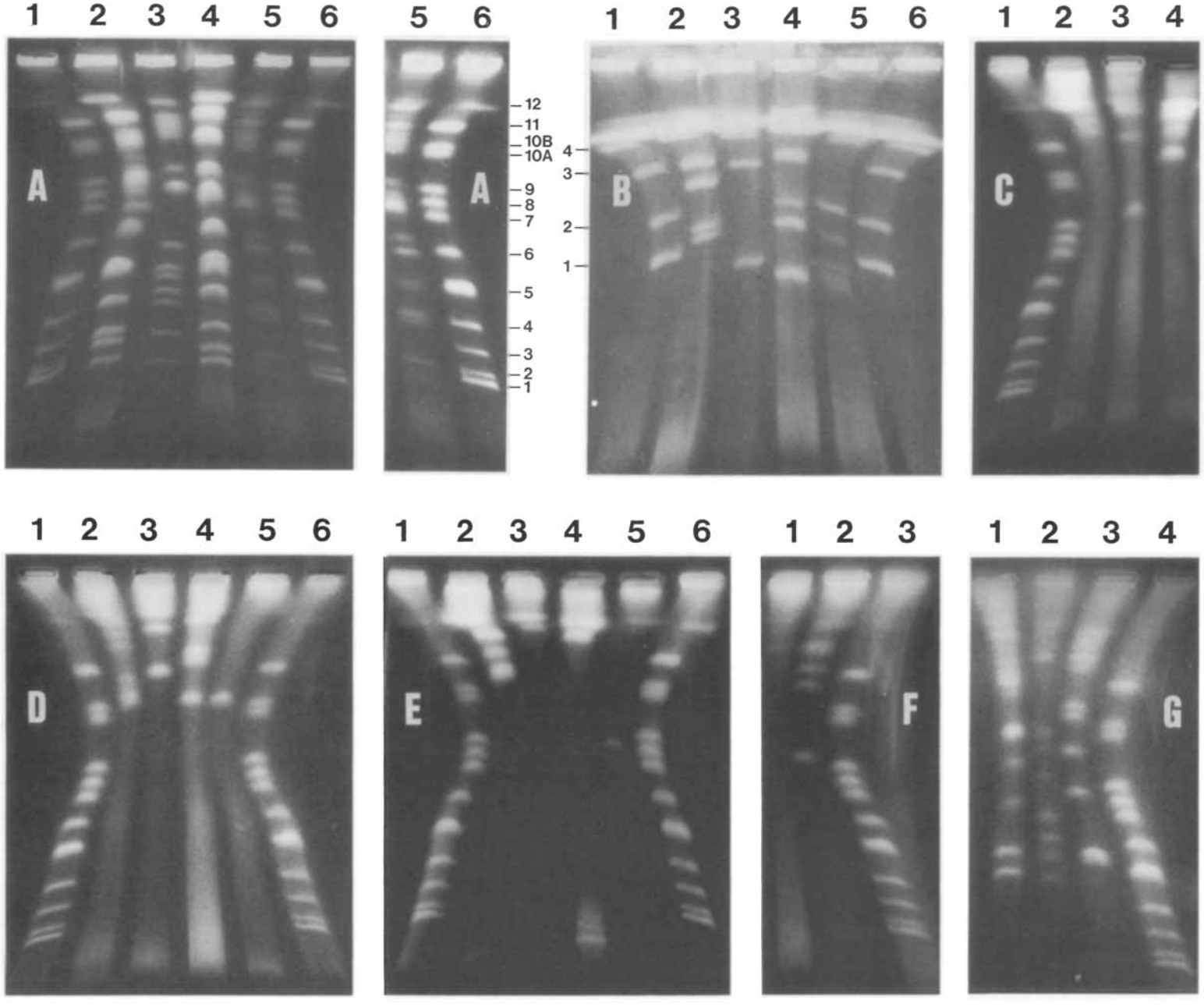
The pattern of strain CBS 8066–a rapidly growing homothallic laboratory strain, also known as JJ101–resembled that of the reference strain. When a 15 s-switching time was used, band 1 resolved into two bands of equal intensity (Figure 1B, lane 2). Dissection of asci from this strain showed a 2:2 segregation of the long and short chromosomes (data not shown). This result suggested heterozygosity in this homothallic strain.

With the exception of chromosome length polymorphisms within individual strains, the general pattern of *Sacch. cerevisiae* revealed the presence of at least 12 bands, ranging in sizes from approximately 250 kb up to about 2000 kb. To investigate how far this pattern is common among the yeasts we compared yeasts from various genera and families by OFAGE.

Other yeast species and genera

In order to get an impression of the variation in number of physically distinct chromosomes in yeasts from various genera and families we selected 26 strains from 25 species of interest in the laboratory, the food industry or the clinic and not belonging to the genus *Saccharomyces* (Table 1). They represented five families: Saccharomycetaceae (including three out of the four subfamilies; 11 species), Endomycetaceae (one species), Filobasidiaceae (two species), teliospore-forming yeasts (two species) and Cryptococcaceae (nine species). Five of the selected strains, including four basidiomycetes, did not give satisfactory results due to insufficient growth, spheroplasting or lysis, or due to DNA degradation. These were *Brettanomyces intermedius, Filobasidium uniguttulatum, Leucosporidium antarcticum, Phaffia rhodozyma* and *Trichosporon cutaneum*. The banding patterns of 17 of the remaining 21 strains are shown in Figure 1C–G and Figure 2. Figure 3 is a schematical representation of all 21 strains. For reasons of simplicity we distinguished three size classes: small (< 500 kb), medium (500–1000 kb) and large (> 1000 kb). Bands in the large class were often obscured by a smear, probably originating from degradation products (cf. Figure 1F and G). Especially large chromosomal DNAs migrating between the well and band 12 might remain undetected. Therefore, unless indicated otherwise, band 12 and higher bands are not included in our comparative analysis, but the possible presence of such large chromosomes in the yeasts studied here should not be ignored.

The patterns of three *Hansenula* species differed in all bands (Figure 1C). *H. anomala* had only two bands below band 12 in addition to one faint band just below the well, while *H. nonfermentans* and *H. polymorpha* each showed three bands below band 12. The latter two species may be more related to each other than to *H. anomala* as could be inferred from a phylogenetic diagram proposed by Wickerham (1970). With the exception of one band from *H. nonfermentans*, just falling into the medium-size class, the other bands from the


1 2 3 4 5 6
A
5 6
A
12
11
10B
10A
9
8
7
6
5
4
3
2
1
1 2 3 4 5 6
B
4
3
2
1
1 2 3 4
C
1 2 3 4 5 6
D
1 2 3 4 5 6
E
1 2 3
F
1 2 3 4
G

Hansenula species corresponded to chromosomes larger than 1000 kb.

Another example of difference in chromosome sizes between two species of the same genus was found in *Zygosaccharomyces bailii* and *Z. rouxii*. While *Z. bailii* showed one band, *Z. rouxii* revealed five bands from chromosomes larger than band 11 of the reference, thus exceeding 1300 kb (Mortimer and Schild, 1985).

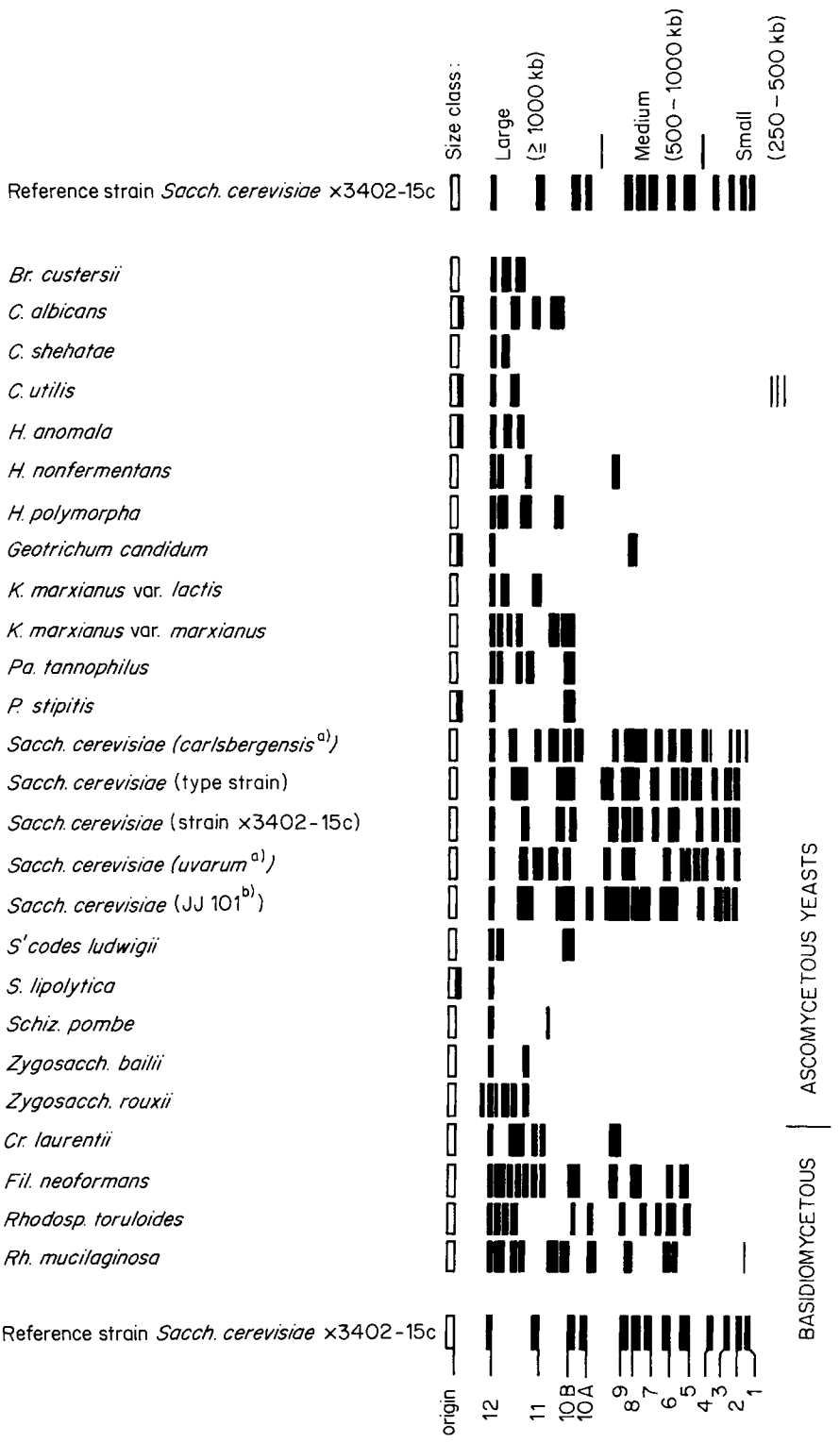
Even in two varieties of a species a different number of bands was seen (Figure 1E, lanes 2 and 3). Whereas the variety *marxianus* of *Kluyveromyces marxianus* displayed five bands, the variety *lactis* only had two. Other representatives of the subfamily of Saccharomycetoideae like *Pachysolen tannophilus, Pichia stipitis* (Figure 1D, lanes 4 and 5) and *Saccharomycopsis lipolytica* all gave different patterns with, respectively, four, one and no bands in the large-size class. The latter two species also had a faint band just migrating out of the well. *Saccharomycodes ludwigii* had two bands and *Schizosaccharomyces pombe* (Figure 2) one weak band in the large-size class only. In the case of *Schiz. pombe*, however, the absence of band 12 and a smear extending from the front of the gel to approximately 700 kb indicated poor lysis or degradation.

Species from ascomycetous genera of imperfect yeasts like *Brettanomyces* (one species), and the less well-defined genus *Candida* (three species), as well as *Geotrichum candidum* (Figure 1F, lane 1 and Figure 1E, lanes 2–5), were similar with respect to the restricted number of large chromosomal DNA bands and the absence of small and medium-sized chromosomes, with two exceptions. *Geotrichum candidum* showed, besides one large DNA band just entering the gel, one band of medium-sized DNA and *C. utilis* revealed three bands of DNA smaller than 260 kb. We have not investigated a possible extrachromosomal origin, i.e. mitochondrial or plasmid DNA, and therefore cannot exclude the presence of small chromosomes in this strain. Apart from large DNA molecules banding just below the wells (Figure 1E, lanes 2–4), *C. utilis* and *C. shehatae* only showed one band each, slightly downstream from band 12, whereas *C. albicans* produced two more bands in the large-size class. This again confirmed that different patterns occurred among species within the same genus. Although *Brettanomyces custersii* only showed two bands superimposed on a background of degraded DNA, it is not very likely that small chromosomes (down to 250 kb) were obscured from detection.

The general feature for ascomycetous yeasts, with the exception of *Saccharomyces cerevisiae*, was the presence of one to five bands of chromosomal DNA

Figure 2. OFAGE banding pattern of *Schizosaccharomyces pombe*. Switching time 65 s. (1). *Sacch. cerevisiae* strain X3402 15C; (2) and (3) *Schiz. pombe*.

Figure 1. Ethidium bromide—stained agarose gels after OFAGE of chromosomal DNA from various yeasts. Switching times 55 s (A). 15 s (B) and 65 s (C–G). *Sacch. cerevisiae* strain X3402-15C was used as reference and co-electrophoresed in the outer lanes of each gel. (A) and (B) represent strains of *Sacch. cerevisiae*. A1, B1 and A6, B6, X3042-15C; A2 and B2, CBS 8066; A3 and B3, CBS 395 (previous name *S. uvarum*); A4 and B4, CBS 1171 (type strain); A5 and B5, CBS 1513 (previous name *Sacch. carlsbergensis*). Due to their low intensity some of the bands in lane 5 may be lost by photographic reproduction. Therefore, a longer exposed photograph of lanes A5 and A6 is shown besides. C1. X3402-15C: C2. *Hansenula anomala;* C3, *Hansenula non-fermentans*, C4, *Hansenula polymorpha;* D1 and D6, X3402-15C; D2, *Kluyveromyces marxianus* var. *marxianus*; D3, *Kluyveromyces marxianus* var. *lactis*; D4, *Pachysolen tannophilus*; D5, *Pichia stipitis*, E1 and E6, X3402-15C; E2, *Candida albicans; E3, Candida shehatae*; E4, *Candida utilis*; E5, *Geotrichum candidum*; F1. *Brettanomyces custersii*; F2, *Cryptococcus laurentii*; F3, X3402-15C; G1, *Filobasidiella neoformans*; G2, *Rhodosporidium toruloides*; G3, *Rhodotorula mucilaginosa*; G4, X3402-15C.


Size class :
Large (≧ 1000 kb)
Medium (500 – 1000 kb)
Small (250 – 500 kb)
Reference strain *Sacch. cerevisiae* x3402-15c
Br. custersii
C. albicans
C. shehatae
C. utilis
H. anomala
H. nonfermentans
H. polymorpha
Geotrichum candidum
K. marxianus var. *lactis*
K. marxianus var. *marxianus*
Pa. tannophilus
P. stipitis
Sacch. cerevisiae (carlsbergensis[a])
Sacch. cerevisiae (type strain)
Sacch. cerevisiae (strain x3402-15c)
Sacch. cerevisiae (uvarum[a])
Sacch. cerevisiae (JJ 101[b])
S. codes ludwigii
S. lipolytica
Schiz. pombe
Zygosacch. bailii
Zygosacch. rouxii
Cr. laurentii
Fil. neoformans
Rhodosp. toruloides
Rh. mucilaginosa
ASCOMYCETOUS YEASTS
BASIDIOMYCETOUS
Reference strain *Sacch. cerevisiae* x3402-15c
origin
12
11
10B
10A
9
8
7
6
5
4
3
2
1

larger than 1000 kb, whereas small and medium-sized chromosomes were found only occasionally.

In contrast to the ascomycetous yeasts, medium-sized chromosomes appeared much more frequently in the OFAGE banding patterns of four basidiomycetous yeast species. These belong to three different families (Figure 1G). *Filobasidiella neoformans, Rhodosporidium toruloides* and *Rhodotorula mucilaginosa* revealed rather similar patterns as far as the partitioning of their chromosomal DNA molecules over large and medium-sized molecules was concerned. These strains showed four, six and four bands in the medium class, and seven, four and five bands in the large class, respectively. In addition, a weak band corresponding to DNA of 260 to 290 kb was obtained from *Rhodotorula mucilaginosa*. Its relatively low intensity might be caused by aneuploidy or by the presence of extrachromosomal DNA. *Cryptococcus laurentii* (Figure 1F, lane 2) gave fewer bands than the other three basidiomycetous yeasts. Only one band was found in the medium class and three or four bands in the large class; the thick upper band perhaps was composed of two co-migrating DNA molecules. Unfortunately, *F. uniguttulatum* resisted our standard spheroplasting method. The low intensities of the bands were possibly caused by insufficient lysis. We saw one or two bands in the large-size class and four bands in the medium class, but likely missed some smaller ones. Thus, in three out of the four basidiomycetous yeasts studied here, both large and medium-sized chromosomes were present, whereas small chromosomes were rare. The total number of bands varied from nine to 11, and thus exceeded the value found for most ascomycetous yeasts. *Cr. laurentii*, with only five bands, from which only one was medium-sized, resembled the ascomycetous yeasts.

Taking the results together it appears that *Sacch. cerevisiae* is exceptional under the ascomycetous yeasts in having at least ten chromosomes smaller than 1000 kb. Even if basidiomycetes are included, the presence of four chromosomes smaller than 500 kb still makes *Sacch. cerevisiae* a unique yeast.

DISCUSSION

The results obtained show a great variation in length and number of chromosomes among various yeasts, not only among species of the same genus but even between varieties of one species. However, the exact number of chromosomes in the various yeasts cannot simply be inferred from the number of bands in the OFAGE. It should be emphasized that apart from co-migrating chromosomal DNA molecules, also very large or unusually folded molecules that do not enter the gel might escape detection. For example, chromosome XII (about 1700 kb) from *Sacch. cerevisiae* failed to enter the gel in a reproducible way (Carle and Olson, 1985). This might be due to its having more than 100 repeats of rDNA (Petes, 1979), or to the fact that it originates from the nucleolus. The observation of only a single band in *Schiz. pombe* is contradictory to the presence of three complementation groups in this species (Gygax and Thuriaux, 1984). From the approximate ratio of linear lengths of chromosomes I, II and III (i.e. 2:1·5:1·0) (Erard and Barker, 1985) and the haploid genome size (i.e. $1{\cdot}5 \times 10^4$ kb (Bostock, 1970), it can be calculated that the lengths of these chromosomes are about $6{\cdot}7 \times 10^3$, $5{\cdot}0 \times 10^3$ and $3{\cdot}3 \times 10^3$ kb, respectively. Such large chromosomal DNAs might not have entered the gel. Alternatively, poor lysis or degradation might have occurred, as suggested by the absence of band 12. It would be interesting to investigate the relation, if any, between the three bands observed in *C. albicans* and the three genetic linkage groups which separately can be lost from heat-shocked diploid cells and therefore have been suggested to be physically linked on individual chromosomes (Hilton *et al.*, 1985). We do not think that these three bands together represent the whole genome, since we also found a faint band just below the well, which might correspond to one or more large chromosomes.

Since approximately the same haploid genome size, i.e. $1{\cdot}4 \times 10^4$ kb, has been reported for *Sacch. cerevisiae* (Lauer *et al.*, 1977), *C. albicans* (Hilton *et al.*, 1985) and the remotely related fission yeast

Figure 3. Schematic representation of OFAGE banding patterns. The bands of the reference strain, *Sacch. cerevisiae* X3402 15C, are numbered according to Carle and Olson (1985). The lengths of chromosomal DNA molecules in the marker bands were taken from Carle and Olson (1985) and Mortimer and Schild (1985). The positions of other bands were related to those of the bands of the reference. For reasons of simplicity the bands of X3402–15C in the outer lanes were connected by straight lines. This gives deviations of the positions of bands in the intermediate lanes, especially for the small DNA molecules, as can be seen by comparing the relevant patterns to those in Figure 1A. [a]Previous name; [b]CBS 8066.

Table 3. Mean sizes of chromosomes in various yeasts and some other organisms.

Organism	Haploid genome size (Mb)[a]	Haploid no. of chromosomes	No. of bands in OFAGE	Mean size of chromosomes (Mb)	References
Sacch. cerevisiae	14	17	—	0·8	Mortimer and Schild (1985), Lauer *et al.* (1977)
		16	—	0·9	Carle and Olson (1985), Kuroiwa *et al.* (1984)
		—	12	1·2	Carle and Olson (1984, 1985), this study
C. albicans	14[b]	3	—	4·7	Hilton *et al.* (1985)
		—	4	3·5	This study
H. polymorpha	—[c]	—	3	4·7	This study
K. lactis	20	9±1	—	2·3	Galeotti and Williams (1978), Whittacker and Leach (1978)
K. marxianus var. *lactis*	—[c]		2	7·0	This study
K. marxianus var. *marxianus*	—[c]	—	5	2·8	This study
Schiz. pombe	15	3	—	4·7	Gygax and Thuriaux (1984), Erard and Barker (1985), Bostock (1970)
		—	⩾1	⩽14	This study
Fil. neoformans	—[c]	—	11	1·3	This study
E. coli K-12	3·9	1	—	3·9	Bachmann and Brooks Low (1980)
Neurospora crassa	45	7	—	6·4	Perkins and Barry (1977)
Man	3000	23	—	130	DuPraw (1970)

[a]Mb = 10^6 base pairs.
[b]Calculated from the amount of DNA per haploid cell.
[c]Data not available. For the calculation we used a value of 14 Mb.

Schiz. pombe (Bostock, 1970), we assume that this value will not differ considerably for the other yeasts. From this haploid genome size and the fact that for euploid yeasts the number of OFAGE bands represents a minimum figure for the number of different chromosomes, an impression of the mean size of the chromosomal DNA molecules may be obtained (Table 3). It appears that *Sacch. cerevisiae* and basidiomycetous yeasts like *Fil. neoformans* are exceptional among the yeasts in this respect. These values might even be rare in nature, as comparison with such unrelated organisms as *E. coli, Neurospora crassa* and man suggests.

The high number of chromosomes in *Sacch. cerevisiae* as compared with other yeasts might be caused by the selection of this yeast for fermentation processes by man. The location of *SUC* and *MAL* genes at the ends of chromosomes (Mortimer and Schild, 1985) would be in favour of this hypothesis. It was recently shown that the *SUC* genes are flanked by homologous sequences embedded in telomere-adjacent regions, the X and Y elements (Carlson *et al.*, 1985). Thus man-induced amplification of *SUC* genes could have been coupled with the generation of extra telomeres and consequently of new, smaller chromosomes. We find this explanation unlikely for two reasons. First, if small chromosomes have arisen from larger ancestor chromosomes, *SUC* genes would be preferably found on the cleavage products. However, only the presumed ancestor gene *SUC2* (Carlson *et al.*, 1985) has been found on a small chromosome (IX) whereas three out of the six *SUC* genes are located on chromosomes larger than 1000 kb (chromosomes VII, XIII and IV) and two *SUC* genes are mapped on medium-sized chromosomes (VIII and II) (Mortimer and Schild, 1985). Second, the same holds for the highly conserved telomere-adjacent Y sequences. If these were involved in generating new chromosomes (Dunn *et al.*, 1984), one might expect at least some residual Y sequences in the telomeres of small chromosomes. Using a Y-specific probe (clone 131A from Chan and Tye (1983)), we did not find hybridization with bands 1, 2, 3 and 6 on OFAGE blots of *Sacch. cerevisiae* X3402–15C (H. Y. Steensma and M. Linnekamp, unpublished results). Thus the three smallest chromosomes and one representative of the medium-size class are deprived of this element. Therefore, it seems unlikely that amplication of *SUC* genes caused the high number of chromosomes in *Sacch. cerevisiae*.

Chan and Tye (1983) found that a probe specific for the repetitive, telomere-adjacent Y element hybridized to multiple bands on genomic blots of *C. utilis, P. rhodanensis* and *Saccharomycopsis lipolytica*. Since the intensities were much less than on genomic blots of *Sacch. cerevisiae*, the authors concluded that telomeres in those yeasts contained Y-like repeats which differed substantially from those in *Sacch. cerevisiae*. Our results indicate that the lower intensities might also be explained by the much smaller number of chromosomes in these strains.

Whether OFAGE can be used for taxonomic purposes will depend on improvement of the separation of bands in the large-size class, as most yeasts have chromosomes larger than 1000 kb. While this manuscript was refereed, Carle *et al.* (1986) reported a new technique, called field-inversion gel electrophoresis (abbreviated FIGE) in which DNA molecules migrate in straight lanes. This method will be more appropriate for comparison of strains than OFAGE, although better separation of DNA molecules larger than 1000 kb has not been achieved.

REFERENCES

Bachmann, B. J. and Brooks Low, K. (1980). Linkage map of *Escherichia coli K–12*, Edition 6. *Microbiol. Rev.* **44,** 1–56.

Barnett, J. A., Payne, R. W. and Yarrow, D. (Eds) (1983). *Yeasts, characteristics and identification.* Cambridge University Press.

Bostock, C. J. (1970). DNA synthesis in the fission yeast *Schizosaccharomyces pombe*. *Exp. Cell Res.* **60,** 16–26.

Carle, G. F., Frank, M. and Olson, M. V. (1986). Electrophoretic separations of large DNA molecules by periodic inversion of the electric field. *Science* **232,** 65–68.

Carle, G. F. and Olson, M. V. (1984). Separation of chromosomal DNA molecules from yeast by orthogonal-field-alternation gel electrophoresis. *Nucleic Acids Res.* **12,** 5647–5664.

Carle, G. F. and Olson, M. V. (1985). An electrophoretic karyotype for yeast. *Proc. Natl. Acad. Sci. U.S.A.* **82,** 3756–3760.

Carlson, M., Celenza, J. L. and Eng, F. J. (1985). Evolution of the dispersed *SUC* gene family of *Saccharomyces* by rearrangements of chromosome telomeres. *Mol. Cell. Biol.* **5,** 2894–2902.

Chan, C. S. M. and Tye, B. K. (1983). Organization of DNA sequences and replication origins at yeast telomeres. *Cell* **33,** 563–573.

Dunn, B., Szauter, P., Pardue, M. L. and Szostak, J. W. (1984). Transfer of yeast telomeres to linear plasmids by recombination. *Cell* **39,** 191–201.

DuPraw, E. J. (1970). DNA and chromosomes. In Koffler, H. (Ed.), *Molecular and Cellular Biology Series*. Holt, Rinehart and Winston Inc.

Erard, M. and Barker, D. G. (1985). Electron microscopic studies of condensed mitotic chromosomes in the fission yeast *Schizosaccharomyces pombe*. *Biol. Cell* **55,** 27–34.

Galeotti, C. L. and Williams, K. L. (1978). Giemsa staining of mitotic chromosomes in *Kluyveromyces lactis* and *Saccharomyces cerevisiae*. *J. Gen. Microbiol.* **104,** 337–341.

Gygax. A. and Thuriaux, P. (1984). A revised chromosome map of the fission yeast *Schizosaccharomyces pombe*. *Current Genet.* **8,** 85–92.

Hilton, C., Markie, D., Corner, B., Rikkerink, E. and Poulter, R. (1985). Heat shock induces chromosome loss in the yeast *Candida albicans*. *Mol. Gen. Genet.* **200,** 162–168.

Horowitz, H., Thorburn, P. and Haber, J. E. (1984). Rearrangements of highly polymorphic regions near telomeres of *Saccharomyces cerevisiae*. *Mol. Cell. Biol.* **4,** 2509–2517.

Kreger-van Rij, N. J. W. (Ed.) (1984). *The Yeasts: a Taxonomic Study*, 3rd revised and enlarged edition. Elsevier Science Publishers B.V.

Kuroiwa, T., Kojima, H., Miyakawa, I. and Sando, N. (1984). Meiotic karyotype of the yeast *Saccharomyces cerevisiae*. *Exp. Cell Res.* **153,** 259–265.

Lauer, G. O., Roberts, T. M. and Klotz, L. C. (1977). Determination of the nuclear DNA content of *Saccharomyces cerevisiae* and implications for the organization of DNA in yeast chromosomes. *J. Mol. Biol.* **114,** 507–526.

Mortimer, R. K. and Hawthorne, D. C. (1973). Genetic mapping in *Saccharomyces* IV. Mapping of temperature-sensitive genes and use of disomic strains in localizing genes. *Genetics* **74,** 33–54.

Mortimer, R. K. and Schild, D. (1985). Genetic map of *Saccharomyces cerevisiae*, Edition 9. *Microbiol. Rev.* **49,** 181–212.

Perkins, D. D. and Barry, E. G. (1977). The cytogenetics of *Neurospora*. *Advances Genetics* **19,** 133–285.

Petes, T. D. (1979). Yeast ribosomal DNA genes are located on chromosome XII. *Proc. Natl. Acad. Sci. U.S.A.* **76,** 410–414.

Sandmeyer, S. B. and Olson, M. V. (1982). Insertion of a repetitive element at the same position in the 5′-flanking regions of two dissimilar yeast tRNA genes. *Proc. Natl. Acad. Sci. U.S.A.* **79,** 7674–7678.

Schwartz, D. C. and Cantor, C. R. (1984). Separation of yeast chromosome-sized DNAs by pulsed field gradient gel electrophoresis. *Cell* **37,** 67–75.

Whittacker, P. A. and Leach, S. M. (1978). Interspecific hybrid production between the yeasts *Kluyveromyces lactis* and *Kluyveromyces fragilis* by protoplast fusion. *FEMS Microbiol. Letts* **4,** 31–34.

Wickerham, L. J. (1970). Hansenula, H. et P. Sydow. In Lodder. J. (Ed.), *The Yeasts, a Taxonomic Study*. North-Holland Publishing Company, pp. 226–315.