Estimation of chromosome number and size by pulsed-field gel electrophoresis (PFGE) in medically important *Candida* species

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The chromosomal DNAs of eight medically important *Candida* species, *C. albicans*, *C. stellatoidea*, *C. tropicalis*, *C. parapsilosis*, *C. krusei*, *C. guilliermondii*, *C. kefyr* and *C. glabrata*, were analysed by pulsed-field gel electrophoresis under various conditions. The corresponding bands in the gels were assigned by three kinds of DNA probe which hybridized to DNA of all the species: rDNA, TUB2 and PEP4. The best conditions for separating the chromosomal DNAs were investigated and the numbers and molecular sizes of the chromosome bands were determined for each species. The chromosomal DNAs of the species were separated into 5–14 bands ranging in size from 0.5 to 4.5 Mb. Based on the quantification of the chromosome band intensities using a laser fluorescent gel scanner, the chromosome numbers were estimated. The apparent average total number of chromosomes per cell was 16 for *C. albicans*, 16 for *C. stellatoidea*, 12 for *C. tropicalis*, 14 for *C. parapsilosis*, 8 for *C. krusei*, 8 for *C. guilliermondii*, 18 for *C. kefyr*, and 14 for *C. glabrata*; the total chromosomal DNA size of each species per cell was calculated at about 31 Mb, 33 Mb, 31 Mb, 26 Mb, 20 Mb, 12 Mb, 29 Mb and 14 Mb, respectively.

Introduction

The technique of electrophoretic karyotyping by pulsed-field gel electrophoresis (PFGE), which makes it possible to separate yeast chromosomal DNAs according to their size in agarose gels, is useful for genetic and molecular investigations. There are several different kinds of PFGE techniques: OFAGE (orthogonal field alternation gel electrophoresis), FIGE (field inversion gel electrophoresis), TAFE (transverse alternating field electrophoresis), and CHEF (contour-clamped homogeneous electric field gel electrophoresis). CHEF is one of the most effective techniques for separating large chromosomal DNAs (Chu *et al.*, 1986; Birren *et al.*, 1989; Gunderson & Chu, 1991). The migration of the chromosomal DNA molecules has been followed using a fluorescent microscope during PFGE (Schwartz & Koval, 1989; Smith *et al.*, 1989). Theoretical studies of PFGE have determined the likely relationship between chain length and the mobility of chromosomes in various conditions (Deutsch & Madden, 1989; Viovy, 1989). However, optimal conditions for chromosome migration

for any organism are still most efficiently determined experimentally by running gels under various PFGE conditions, because the theory of chromosome movement is not yet completely understood.

The PFGE technique has been applied to the epidemiological study of pathogenic yeasts (for a review see Merz, 1990). The medically important *Candida* species *C. albicans*, *C. stellatoidea*, *C. tropicalis*, *C. parapsilosis*, *C. krusei*, *C. guilliermondii*, *C. kefyr* and *C. glabrata* have been analysed by PFGE and some species-specific karyotypes have been recognized (Suzuki *et al.*, 1988; Magee & Magee, 1987; Monod *et al.*, 1990; Iwaguchi *et al.*, 1990). In these studies, with the exception of *C. albicans*, the chromosomes have not always been resolved unequivocally by PFGE and the chromosome number and molecular size remain to be precisely determined. In this study, we tried to optimize conditions to separate all the chromosomes of several *Candida* species and estimated their total numbers and sizes by PFGE.

Methods

Strains and plasmids. DNA chromosome size markers were prepared from *Saccharomyces cerevisiae* (X2180-1A: Mortimer & Schild, 1985) and *Schizosaccharomyces pombe* (HM422-h⁻: Fan *et al.*, 1988). The 24

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Abbreviation: PFGE, pulsed-field gel electrophoresis.

Table 1. *Candida* strains used in this study

Species	Strains		
	1	2	3
<i>C. albicans</i>	FC18	NUM1000	NUM812
<i>C. stellatoidea</i>	IFO 1397	IFO 0692	IFO 1398
<i>C. tropicalis</i>	NUM37	NUM267	NUM400
<i>C. parapsilosis</i>	NUM303	NUM301	NUM304
<i>C. krusei</i>	IFO 0013	IFO 0841	IFO 1162
<i>C. guilliermondii</i>	NUM4	NUM679	NUM892
<i>C. kefyr</i>	IFO 0586	IFO 882	IFO 1065
<i>C. glabrata</i>	TIMM1063	TIMM1062	TIMM1064

strains, from the eight medically important *Candida* species, used in this study are listed in Table 1.

The plasmids used as hybridization probes were pHS100 for TUB2 (Smith *et al.*, 1988), pAT68 for rDNA (Sugihara *et al.*, 1986) and pPRA1-2 for PEP4 (Lott *et al.*, 1989). The precise fragments used for TUB2 and rDNA probes were described previously (Iwaguchi *et al.*, 1990; Asakura *et al.*, 1991). The 1.5 kb *Eco*RI-*Xho*I fragment of pPRA1-2 was used as the PEP4 probe.

Preparation of yeast chromosomal DNAs. Yeast chromosomal DNAs were prepared for PFGE as described previously (Iwaguchi *et al.*, 1990).

PFGE. Yeast chromosomal DNAs were separated by PFGE using the Pulsaphor system with a hexagonal electrode array (Pharmacia-LKB). The sample plugs containing DNAs were applied to a 0.8% agarose gel which was prepared from 130 ml molten agarose (Agarose HGS; Nakarai tesque Co., Japan) and run in a running buffer (1 × TBE) at 10 °C under the various conditions described in Results. Gels were stained with ethidium bromide for 30 min, destained overnight in distilled water and photographed under UV light (302 nm).

Southern hybridization. This was performed as described previously (Iwaguchi *et al.*, 1990).

Fluorescent quantification. Ethidium bromide intercalates into DNA and can be excited by light with a wavelength of about 530 nm. The emitted fluorescence was detected by a fluorescent-bioimage analyser (FMBIO system, Takara Co., Japan) and the fluorescent images were stored on an optical disk. The emitted intensities of the images were quantified using the program for FMBIO, with an Apple Macintosh IIcx computer (Ishino *et al.*, 1992).

Results and Discussion

PFGE conditions for separating chromosomal DNAs of the *Candida* species

Many factors, such as voltage, switch interval, running time, agarose concentration of the gel, running temperature, running buffer, and angle of the alternating electric field, are known to affect DNA migration in PFGE gels (Birren *et al.*, 1989). Among these factors, the switch interval, voltage and running time significantly affect DNA migration and are easily controlled. A low voltage and long switch interval are required to separate large

DNA molecules. The voltage and switch interval have a roughly inverse relationship for effective separation of a certain size of chromosomes (Smith *et al.*, 1987; Vollrath & Davis, 1987; Gunderson, 1991). Previously, we systematically changed the switch interval and voltage in order to determine the optimal conditions for separating *C. albicans* chromosomes (Iwaguchi *et al.*, 1990). Here we investigated the optimum conditions for separating chromosomes of other *Candida* species.

The effects of the voltage and the switch interval upon the migration of different-sized chromosomes were examined using *Sacch. cerevisiae* and *Sch. pombe* chromosomes, whose sizes are already known (Fig. 1). For a given switch interval, the higher the voltage, the greater the migration of chromosomes in the gel. However, high voltages did not always give better separation of the chromosomes. Some of the chromosome separation profiles of *Candida* species are shown in Fig. 2, with those of *Sacch. cerevisiae* and *Sch. pombe* as controls. At a switch interval of 100 s, virtually none of the chromosomes migrated at 180 V, whilst at 200 V significant migration was observed. At 220 V the separation profiles were improved further but the bands were fuzzy. Similar effects were observed with a 1000 s switch interval. In general, at a lower voltage the chromosomes were separated sharply but with low mobility, whilst at higher voltages the chromosomes were more mobile but were not separated sharply. Thus, small differences in voltage at a given switch interval could result in significant improvements in chromosome separation. We therefore determined the optimum voltage for each switch interval, and from chromosome band profiles under various conditions, we were able to determine the most satisfactory combinations for separation (Fig. 3).

The resolution range is too narrow to separate chromosomes of the *Candida* species using one set of conditions. Based on the above results, we altered parameters in succession. We first used a short switch interval to separate smaller chromosomes, followed by a longer switch interval to separate larger ones. We were then able to determine the optimum conditions, which included a ramping switch interval (in which the switch interval is gradually changed) (Fig. 4). Under condition (a), which had been used previously (Asakura *et al.*, 1991), chromosomal DNAs smaller than 1.6 Mb were well resolved (Fig. 4a). Condition (b) could resolve chromosomes smaller than 2.2 Mb, but not those larger than 2.2 Mb (Fig. 4b). Under condition (c), chromosome bands of *Candida* species were separated over the entire size range from 0.25 to 3.5 Mb (Fig. 4c). The resolution of DNA molecules larger than 3.5 Mb was achieved under condition (d) (Fig. 4d). Our four conditions are able to effectively resolve chromosomes in all eight *Candida* species studied. Furthermore, chromosomal DNAs in

Fig. 1. Chromosome mobility with various switch interval and voltage combinations. Chromosomes of *Sacch. cerevisiae* and *Sch. pombe* were separated in 0.8% agarose gels for running times ranging from 24 to 48 h. The mobilities of some of the chromosomes are plotted. The chromosome sizes are indicated on the right of the figure.

Fig. 2. Separation profiles of chromosomal DNAs. (a) The 100 s switch interval combined with different voltages. Chromosomes from the medically important *Candida* species were separated in a 0.8% agarose gel for 24 h, with 100 s switch interval, and voltages of 180, 200 or 220 V. Lanes: 1, *Sacch. cerevisiae*; 2, *C. albicans* strain FC18; 3, *C. stellatoidea* strain IFO 1397; 4, *C. tropicalis* strain NUM37; 5, *C. parapsilosis* strain NUM303; 6, *C. krusei* strain IFO 13; 7, *C. guilliermondii* strain NUM4; 8, *C. kefyr* strain IFO 586; 9, *C. glabrata* strain TIMM1063; 10, *Sch. pombe*. (b) The 1000 s switch interval with different voltages. Chromosomes were separated with a 1000 s switch interval and voltages of 50, 80 or 110 V, the remaining conditions being the same as above.

Fig. 3. Relationship between switch interval and voltage for the best chromosome resolution. The points were determined from the gel profiles run under various conditions as described for Fig. 2.

two additional strains of each species (described in Table 1) were separated under these conditions.

Estimation of the molecular size of the *Candida* chromosomes

Estimation of chromosome size is often difficult because chromosome mobility does not always correlate with chromosome size in PFGE. However, at least within a certain range of size, a linear relationship of chromosome mobility and size has been reported (Vollrath & Davis, 1987; Birren *et al.*, 1989). We determined the chromosome size of the *Candida* species from standard curves of various conditions using *Sacch. cerevisiae* and *Sch. pombe* chromosomes as size markers (Fig. 5). Chromosome size in the ranges of less than 1.6 Mb, 1.6–2.2 Mb, 2.2–

Fig. 4. The best separating conditions for the *Candida* chromosomal DNAs. Conditions (a), (b) and (c) are a combination of the first and second conditions. The first and the second conditions were used to separate relatively short and long chromosomes, respectively. Condition (d) separates a wide range of longer chromosomes by a ramping switch interval. Condition (a), a 100 s switch interval at 180 V for 15 h followed by a 300 s switch interval at 140 V for 20 h; (b), a 200 s switch interval at 150 V for 24 h followed by a 700 s switch interval at 100 V for 48 h; (c), a 300 s switch interval at 130 V for 24 h followed by a 1000 s switch interval at 90 V for 48 h; (d), a linear ramping switch interval from 1000 s to 3000 s at 50 V for 144 h in a 0.7% gel (Chromosomal-grade agarose; Bio-Rad). Samples were the same as in Fig. 2. Pointers on the left of the photographs indicate the migration of 2.2, 1.6 and 1.2 Mb *Sacch. cerevisiae* chromosomes; pointers on the right of panel (d) indicate the migration of 5.7, 4.6 and 3.5 Mb *Sch. pombe* chromosomes.

Fig. 5. Standard curve made by the marker chromosomes of *Sacch. cerevisiae* and *Sch. pombe*. The chromosomes were run under the same conditions as described in Fig. 4: conditions (a, Δ), (b, $\blacktriangle$), (c, $\circ$) and (d, $\bullet$).

Fig. 6. Scheme of chromosome band profiles of the representative strains used. The chromosome bands stained with ethidium bromide are represented as bars positioned according to their molecular size on a log scale that is indicated on the left. The numbers under each species correspond to the columns of strains in Table 1. The probes (r, rDNA; t, TUB2; p, PEP4) hybridized to the chromosomes as shown in the scheme. Some unseparated bands which hybridized to the rDNA probe are indicated with a bracket (]). Uncertain hybridization is indicated by parentheses.

3.5 Mb, and more than 3.5 Mb, was determined mainly from the standard curves described by conditions (a), (b), (c) and (d), respectively. To assign the same chromosome between the chromosome bands separated by the different conditions, their band profiles were carefully compared. Furthermore, some chromosome bands were

specifically identified by hybridization using rDNA, TUB2 and PEP4 probes which hybridized to DNAs of all species (Fig. 6). The chromosomal DNAs of the *Candida* species were separated into 5–14 bands ranging in size from 0.5 to 4.5 Mb. The resultant molecular sizes of chromosome DNAs, for one representative strain of

each species, are shown in Table 2, and a schematic illustration of the electrophoretic patterns, i.e. karyotypes, of all *Candida* strains examined is shown in Fig. 6.

Estimation of the total chromosome number of the Candida cell

Following separation of chromosomes by PFGE under optimum conditions, the relative DNA content of each chromosome band could be estimated from the fluorescence of bound ethidium bromide. The fluorescent intensities of the chromosome bands were measured by a laser-excited fluorescence image analyser and divided by the corresponding chromosome sizes. The resultant value was called specific intensity. If chromosome DNAs are completely separated, the band number is equal to the total chromosome number of the cell. However, some bands contain multiple chromosomes. From the specific intensity, which reflects the number of chromosome molecules in a band, the relative chromosome number in bands can be estimated if ethidium bromide binds uniformly to all parts of the chromosomes and whole molecules of each chromosome in a sample plug migrate to each band. So, the total chromosome number of the cell should be the sum of the relative chromosome number if the chromosomes are not a perfect diploid or polyploid. It has been shown that a perfect diploid is rare in *C. albicans* (Iwaguchi *et al.*, 1990).

The chromosomes of a *Sacch. cerevisiae* haploid strain and a *C. albicans* strain, whose chromosome number was already known, were analysed and total chromosome number per cell was estimated (Table 3). Bands representing two chromosomes gave roughly twice the specific intensity value of bands known to be single chromosomes in *Sacch. cerevisiae*. When we calculated the total chromosome size (about 14 Mb) from the total chromosome number and each chromosome size, it was consistent with the haploid chromosome size. The *C. albicans* band representing the 3500 kb and 3140 kb chromosomes gave similar specific intensity to the 2200 kb band. However, we assumed that the two bands contain three and two chromosomes, respectively, because the specific intensity per chromosome gradually decreased with increasing chromosome size, suggesting that longer chromosomes may become more entangled in the gel matrix, and the ratio of the specific intensities of the 3500 kb to the 3140 kb bands separated in a different gel was roughly 2:1, suggesting that the band representing the 3500 kb and 3140 kb chromosomes contains at least three chromosomes. The total chromosome size of *C. albicans* FC18 was calculated as about 33 Mb, suggesting diploid size. We thus showed that it is possible to estimate total chromosome number by this method.

Similarly, taking careful account of the specific intensity of each band separated in various PFGE conditions and other information such as the ploidy of the species and the DNA content of yeast cells (see below), the total chromosome numbers of each *Candida* species were estimated (Table 4). However, it was very difficult to determine how many chromosomes were present in some bands because the specific intensity decreased with increasing chromosomal size. We therefore cannot exclude the possibility that this approach to karyotypic analysis may contain some inaccuracies. Whilst it is generally believed that the chromosome number of a given species is constant, we found variability for *C. krusei*, *C. kefyr* and *C. glabrata*. It has been shown that heat shock or treatment with antimicrotubule agents can induce chromosome loss and lead to aneuploidy (Hilton *et al.*, 1985; Barton & Gull, 1992).

From the estimated chromosome numbers and sizes, the total chromosome size per cell was calculated. As shown in Table 4, the total size ranges from about 12 Mb to about 33 Mb. *C. albicans*, *C. stellatoidea*, *C. tropicalis* and *C. kefyr* have a similar total chromosome size, of about 30 Mb. *C. guilliermondii* had the smallest size, 12 Mb, of the species tested.

Ploidy of the Candida species

Of the eight *Candida* species studied here, it has been reported that the diploid species are *C. albicans* (Riggsby *et al.*, 1982), *C. stellatoidea* (Kwon-Chung *et al.*, 1987), *C. tropicalis* (Kamiryo *et al.*, 1991), *C. parapsilosis* (Whelan & Kwon-Chung, 1988) and *C. krusei* (Whelan & Kwon-Chung, 1988). The haploid species are *C. guilliermondii* (Suzuki *et al.*, 1986) and *C. glabrata* (Whelan *et al.*, 1984). Ploidies have been determined on the basis of DNA content, UV killing rate, heterozygosity of a gene, and the respective mutation rate. However, it is impossible to define aneuploidy by these methods. So, the assignments of haploid and diploid are not strictly correct; rather, aneuploid near to haploid and diploid, respectively, is more accurate. We have evaluated ploidy on the basis of total chromosome size. The total chromosome sizes of *C. albicans*, *C. stellatoidea*, *C. tropicalis* and *C. kefyr* were about 30 Mb and that of *C. parapsilosis* was about 26 Mb. The haploid sizes of both *Sacch. cerevisiae* and *Sch. pombe* have been estimated at about 14 Mb (Fan *et al.*, 1988; Mortimer & Schild, 1985). Assuming a common genome content in all yeast species, *C. albicans*, *C. stellatoidea*, *C. tropicalis*, *C. kefyr* and *C. parapsilosis* are likely to be diploid species. This is consistent with the fact that probes hybridized to two chromosome bands in some strains of each species. We cannot exclude the possibility that the probe DNA region has been duplicated in a different

Table 2. Molecular size of *Candida* chromosomes separated by PFGE

Species	Molecular size (kb)	No. of bands
<i>C. albicans</i>	3500 3140 2200 1800 1630 1400 1130 1020	8
<i>C. stellatoidea</i>	3850 3500 3400 2640 2580 1700 1330 1270 1100 900 880 600	12
<i>C. tropicalis</i>	3500 3100 2800 2600 2300 2100 1380 1020	8
<i>C. parapsilosis</i>	3300 2200 2000 1700 1550 1450 1360 1310 1030 980	10
<i>C. krusei</i>	3700 3100 2800 1450 1400	5
<i>C. guilliermondii</i>	2130 2000 1600 1450 1350 980 520	7
<i>C. kefyr</i>	3200 2300 2100 1980 1800 1680 1630 1530 1480 1420 1350 1250 1100 1050	14
<i>C. glabrata</i>	2100 1720 1420 1380 1150 1050 990 760 720 640 520 510	12

Table 3. Calculation of chromosome number and total chromosome size

Size (kb)	Intensity	Specific intensity*	Ratio†	Chr. no.	Chr. no. × size
(a) <i>Sacch. cerevisiae</i> X-2180A					
2200	26523	12.06	0.73	1	2200
1560	27124	17.39	1.05	1	1560
1200	34088	28.41	1.71	1	1200
1200	—	—	—	1	1200
1020	30806	30.20	1.82	1	1020
945	—	—	—	1	945
850	12012	14.13	0.85	1	850
800	12102	15.13	0.91	1	800
770	10886	14.14	0.85	1	770
700	10615	15.16	0.91	1	700
630	19045	30.23	1.82	1	630
580	—	—	—	1	580
460	7742	16.83	1.01	1	460
370	5551	15.00	0.90	1	370
290	4316	14.88	0.90	1	290
245	4065	16.59	1.00	1	245
Total				16	13820
(b) <i>C. albicans</i> FC18					
4050‡	15215	3.75	0.20	1	4050
3500	54937	15.69	0.85	2	3500
3140	—	—	—	1	3140
2200	34108	15.50	0.84	2	4400
1800	26331	14.62	0.79	2	3600
1630	26758	16.41	0.89	2	3260
1400	22683	16.20	0.88	2	2800
1130	19795	17.51	0.95	2	2260
1020	18691	18.32	1.00	2	2040
Total				16	32550

* Specific intensity represents the intensity divided by the chromosome size.

† Specific intensity relative to the value of the smallest chromosome.

‡ This band was not detected in a different sample prepared. This chromosome contains rDNA and frequently changes in size (Asakura *et al.*, 1991).

chromosome, but such duplication is likely to be a rare event (Iwaguchi *et al.*, 1990). The total chromosome size of *C. krusei* was about 20 Mb, which may be too small to represent a diploid complement but too large to be haploid. However, it has been shown previously that

Table 4. Estimated chromosome number and total chromosome size in *Candida* species

Species		Strain*			Mean
		1	2	3	
<i>C. albicans</i>	Number	16	16	16	16
	Size (Mb)	32.6	30.3	29.3	30.7
<i>C. stellatoidea</i>	Number	16	16	16	16
	Size (Mb)	33.9	32.3	32.3	32.8
<i>C. tropicalis</i>	Number	12	12	12	12
	Size (Mb)	29.5	30.8	31.4	30.6
<i>C. parapsilosis</i>	Number	14	14	14	14
	Size (Mb)	25.8	26.8	26.4	26.3
<i>C. krusei</i>	Number	7	8	9	8
	Size (Mb)	19.3	20.0	20.2	19.8
<i>C. guilliermondii</i>	Number	8	8	8	8
	Size (Mb)	11.6	11.5	11.6	11.6
<i>C. kefyr</i>	Number	19	19	17	18
	Size (Mb)	31.1	29.2	27.4	29.2
<i>C. glabrata</i>	Number	13	14	14	14
	Size (Mb)	13.7	14.2	14.4	14.1

* The numbers correspond to the columns of strains in Table 1.

C. krusei is heterozygous at a *URA* gene (Whelan & Kwon-Chung, 1988), and rDNA and TUB2 probes hybridized to two chromosome bands in the *C. krusei* strains used here. *C. krusei* is therefore probably diploid. *C. krusei* may contain a very different-sized genome to those of other yeasts. This is consistent with evolutionary data suggesting that *C. krusei* is only distantly related to other *Candida* species (Barns *et al.*, 1991; Hendriks *et al.*, 1991). *C. guilliermondii* (Suzuki *et al.*, 1986) and *C. glabrata* (Whelan *et al.*, 1984) appear to be haploid species; the total chromosome sizes of *C. guilliermondii* and *C. glabrata* were calculated as about 12 Mb and 14 Mb, respectively. On the other hand, the rDNA probe hybridized to two bands in all three *C. glabrata* strains and one strain of *C. guilliermondii* whilst TUB2 and PEP4 hybridized to only one band. We have already observed the hybridization of the rDNA probe to two chromosomes in many *C. glabrata* strains (Asakura *et al.*, 1991). In this case, the chromosome containing the

rDNA gene may have a homologue, or the rDNA region may be duplicated on a different chromosome. Further detailed studies using the PFGE and hybridization techniques are needed to clarify the organization of the chromosomes in these *Candida* species.

Karyotypes of the *Candida* species

Karyotypes have been analysed in *C. albicans* (Merz *et al.*, 1988; Lott *et al.*, 1987; Mahrous *et al.*, 1990; Lasker *et al.*, 1989; Snell *et al.*, 1987; Magee *et al.*, 1988; Iwaguchi *et al.*, 1990), *C. stellatoidea* (Kwon-Chung *et al.*, 1989; Rikkerink *et al.*, 1990), *C. tropicalis* (Hawley & Marcus, 1989; Kamiryo *et al.*, 1991), *C. parapsilosis* (Carruba *et al.*, 1991), *C. kefyr* (Sor & Fukuhara, 1989) and *C. glabrata* (Kaufmann & Merz, 1989; Asakura *et al.*, 1991). Only *C. albicans* has been studied extensively. Chromosome bands, separated by PFGE, were assigned by many DNA probes, and the haploid number has been estimated as eight chromosomes in the diploid species of *C. albicans*.

Species-specific *Candida* karyotypes have been reported previously (Monod *et al.*, 1990; Magee & Magee, 1987; Suzuki *et al.*, 1988). We have shown previously that the intraspecies variation of *C. albicans* is not so great as to be confused with the interspecies variation in the eight medically important *Candida* species (Iwaguchi *et al.*, 1990). This observation was confirmed by the present study, in which chromosomes were much better separated than before. Furthermore, the probes which hybridized to all the *Candida* species were useful in species differentiation. For example, whilst the chromosome distribution between *C. albicans* and *C. parapsilosis* looked similar, the rDNA probe hybridized to chromosomes of very different sizes. We have observed that the size of the one chromosome homologue is highly conserved in *C. albicans* whilst chromosome 2, containing rDNA, is variable (Iwaguchi *et al.*, 1990; Asakura *et al.*, 1991). *C. albicans* chromosome 2 is too variable to distinguish between strains. The variation has been shown to be derived from a change in length of the rDNA repeat region (Wickes *et al.*, 1991; Iwaguchi *et al.*, 1992). A similarly highly variable chromosome containing rDNA has also been observed in *C. glabrata* (Asakura *et al.*, 1991).

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