International Journal of Systematic Bacteriology (1999), **49**, 1925–1931 Printed in Great Britain

Karyotypes of *Saccharomyces sensu lato* species

Randi Føns Petersen,[1,2] Torsten Nilsson-Tillgren[2] and Jure Piškur[1]

Author for correspondence: Jure Piškur. Tel: +45 45 25 25 18. Fax: +45 45 93 28 09.
e-mail: imjp@pop.dtu.dk

[1] Department of Microbiology, Technical University of Denmark, DTU-301, DK-2800 Lyngby, Denmark

[2] Department of Genetics, Institute of Molecular Biology, University of Copenhagen, Denmark

An improved pulsed-field electrophoresis program was developed to study differently sized chromosomes within the genus *Saccharomyces*. The number of chromosomes in the type strains was shown to be nine in *Saccharomyces castellii* and *Saccharomyces dairenensis*, 12 in *Saccharomyces servazzii* and *Saccharomyces unisporus*, 16 in *Saccharomyces exiguus* and seven in *Saccharomyces kluyveri*. The sizes of individual chromosomes were resolved and the approximate genome sizes were determined by the addition of individual chromosomes of the karyotypes. Apparently, the genome of *S. exiguus*, which is the only *Saccharomyces sensu lato* yeast to contain small chromosomes, is larger than that of *Saccharomyces cerevisiae*. On the other hand, other species exhibited genome sizes that were 10–25% smaller than that of *S. cerevisiae*. Well-defined karyotypes represent the basis for future genome mapping and sequencing projects, as well as studies of the origin of the modern genomes.

Keywords: *Saccharomyces*, karyotypes, genome structure, pulsed-field electrophoresis, genome duplication

INTRODUCTION

Pulsed-field electrophoresis has made possible the separation of large yeast DNA molecules, thus allowing determination of the number and size of nuclear chromosomes, denoting the nuclear karyotype (Carle & Olson, 1985; Johnston & Mortimer, 1986). In addition, pulsed-field electrophoresis studies can serve as a rapid and relatively easy approach to the characterization and identification of yeast species. Yeasts belonging to the genus *Saccharomyces* can be divided into the *sensu stricto* and *sensu lato* groups. The sibling species of the *Saccharomyces cerevisiae* complex have been shown to exhibit uniform karyotypes (Cardinali & Martini, 1994; Carle & Olson, 1985; Naumov *et al*., 1992, 1995; Vaughan-Martini *et al*., 1993). Their chromosomes have been divided into three different classes on the basis of their size: light (<500 kb), medium (500–1000 kb) and heavy (>1000 kb) (Vaughan-Martini *et al*., 1993). Karyotype patterns of the type strains of *S. cerevisiae* and *Saccharomyces paradoxus* are very similar, while *Saccharomyces bayanus* has a species-specific karyotype (Naumov *et al*., 1992). *Saccharomyces pastorianus* (syn. *Saccharomyces carlsbergensis*) has been reported to be a hybrid and has been shown to possess one chromosome set originating from *S. cerevisiae* and another from a non-*S. cerevisiae* parent (Nilsson-Tillgren *et al*., 1983; Pedersen, 1986a, b; Vaughan Martini & Kurtzman, 1985; Vaughan-Martini & Martini, 1987).

The karyotypes of the *Saccharomyces sensu lato* yeasts exhibit much heterogeneity. When these species were analysed in different laboratories, the number of bands ranged from five to 14. *Saccharomyces kluyveri* chromosomes may be resolved into five bands, according to Jäger & Philippsen (1989); however, in another study, it was found to contain seven chromosomes of the heavy class that were all well above 1000 kb (Vaughan-Martini *et al*., 1993). In two different studies, the *Saccharomyces exiguus* chromosomes were separated into 11 bands (Jäger & Philippsen, 1989) and 14 bands, of which some were double (Naumova *et al*., 1996). The remaining four *Saccharomyces sensu lato* species, *Saccharomyces castellii*, *Saccharomyces dairenensis*, *Saccharomyces servazzii* and *Saccharomyces unisporus*, were shown to contain numbers of chromosomes within these two extremes. *S. unisporus* and *S. servazzii* strains yielded chromosome banding patterns very similar to each other, exhibiting between nine and 13 bands from 580 to 2200 kb (Jäger & Philippsen, 1989; Naumov *et al*., 1995). *S. dairenensis* and *S. castellii* chromosomes were resolved into eight to 11 bands with sizes from 460 to 2200 kb (Jäger &

Philippsen, 1989; Naumov *et al*., 1995). The results obtained in the two main studies (Vaughan-Martini *et al*., 1993; Naumov *et al*., 1995) were similar in most instances. However, until this point, chromosome identification within the *Saccharomyces sensu lato* yeasts was not adequate because several bands in the previous studies apparently contained overlapping chromosomes. Therefore, we focused on the development of a better separation technique, allowing the number and size of chromosomes to be determined more precisely.

METHODS

Yeast strains. The yeast species investigated in the present study were mostly type strains obtained from the Agricultural Research Service (NRRL), National Center for Agricultural Utilization Research, US Department of Agriculture, Peoria, IL, USA: *S. cerevisiae* NRRL Y-12632^T, *S. castellii* NRRL Y-12630^T, *S. dairenensis* NRRL Y-12639^T, *S. exiguus* NRRL Y-12640^T, *S. servazzii* NRRL Y-12661^T, *S. unisporus* NRRL Y-1556^T and *S. kluyveri* NRRL Y-12651^T. Non-type strains included in the study were *S. cerevisiae* YPH755, which is an *ade*$^-$ derivative of YPH149 (Gerring *et al*., 1991), *S. unisporus* CBS 2422 from the culture collection of the Centraalbureau voor Schimmelcultures (CBS), Delft, The Netherlands, and *S. kluyveri* XM 8-5 from L. Marsh, Albert Einstein College of Medicine, Bronx, NY, USA.

Preparation of chromosome-sized DNA. Chromosome plugs were prepared following a modified protocol of Gerring *et al*. (1991). Yeast cells were grown to stationary phase (OD_{600} 10–14) in rich medium [YPD; 10 g yeast extract (1% w/v), 20 g peptone (2% w/v) and 40 ml 50% glucose (2% w/v) l^{-1}]. Samples (1 ml) were spun down and washed twice in 1 ml EDTA/Tris solution (50 mM EDTA, 10 mM Tris, pH 7·5). Protoplasts were prepared with zymolyase [0·15 ml EDTA/Tris solution plus 1 µl zymolyase (20 mg 100 T ml^{-1} 10 mM sodium phosphate buffer, pH 7·5)] and placed at 42 °C for 30 s. Low-melting-point agarose (0·25 ml of a 1%, w/v, solution in 125 mM EDTA, pH 7·5) was added at 42 °C and mixed with cells. The cell suspension was gently pipetted into the plug holders and placed on ice. After the plugs had hardened they were transferred to a 12 × 75 mm Falcon (glass) tube and washed as described previously (Gerring *et al*., 1991). Plugs were stored at 4 °C in EDTA/Tris solution. Plugs stored in this fashion are stable for several years.

Pulsed-field gel electrophoresis. Yeast chromosomes were separated by pulsed-field gel electrophoresis using either Bio-Rad CHEF-DRII or Bio-Rad Chef-Mapper. Gels were prepared as 1% ultra-pure agarose (KILOrose; Clontech) (1·1 g agarose in 0·5 × TBE). Plugs were inserted in the gel and fixed with agarose. Gels were run in 0·5 × TBE at 14 °C. After electrophoresis, the gels were stained in electrophoresis buffer containing 0·5 µg ethidium bromide ml^{-1} for 4–24 h.

Separation program for *Saccharomyces sensu lato* chromosomes on Bio-Rad CHEF-DRII. The CHEF-DRII apparatus (Bio-Rad) allows programming of two different blocks. In order to get optimal separation of all *Saccharomyces sensu lato* chromosomes, a gel must be subjected to two CHEF-DRII runs. Long pulse-times allow separation of large chromosomes while shorter pulse-times give better separation of smaller chromosomes. The best separation of all chromosomes was obtained when the large chromosomes were separated first followed by separation of smaller chromosomes. The first run (Run 1) lasted for 18 h. Run 1 was divided into block A1, pulse-time 240 s for 8 h, and block B1, pulse-time 160 s for 10 h. The second run (Run 2) lasted for 22 h. Run 2 was divided into block A2, pulse-time 90 s for 14 h, and block B2, pulse-time 60 s for 8 h. Run 2 followed Run 1 immediately and was performed in the same buffer. It was essential to restart the CHEF-DRII between Run 1 and Run 2. The power supply was set to 150 V.

Separation program for *Saccharomyces sensu lato* chromosomes on Bio-Rad Chef Mapper-multistate. The Chef Mapper-multistate program (Bio-Rad) allows for programming of several blocks with different parameters. The program used for separation of the *Saccharomyces sensu lato* chromosomes resembled the program designed for the CHEF-DRII. Again it was found that separation of the large chromosomes resulted in better total separation and the program was designed starting out with long pulse-times. The Chef Mapper program was divided into four blocks (A + B + C + D) and the total run time was 52 h. Block A was pulse-time 240 s for 12 h, block B was pulse-time 160 s for 16 h, block C was pulse-time 90 s for 16 h and block D was pulse-time 60 s for 8 h.

Determination of chromosome and genome sizes. The sizes of single chromosomes were calibrated against the sizes of the marker strain, *S. cerevisiae* YPH755. It was assumed that logarithm of the molecular mass is a linear function of the gel mobility. The total genome size was calculated by adding the sizes of single chromosomes.

RESULTS

Molecular karyotypes of *Saccharomyces sensu lato* species

Several different separation programs were tested on chromosomes of *Saccharomyces sensu lato* type strains. The results of the optimized four-step pulsed-field electrophoresis separation are shown in Fig. 1. The number and sizes of chromosomal bands together with the total sizes of the genomes were calculated on the basis of the migration of the YPH755 chromosomes. The sizes of individual chromosomes and genome sizes are depicted in Table 1.

The separation of chromosomes was not perfectly linear. Lack of linearity was particularly true for chromosomes larger than 1000 kb. The chromosome sizes reported here are still therefore to be regarded as approximate. In addition, the intensity of single chromosomes within the same preparation varied (Fig. 1). Even though the chromosomes were separated using different programs, it is not possible to exclude completely the possibility that some single bands were actually composed of two chromosomes. This should be taken into account when the genome sizes are compared.

The four-step program generally allowed separation of all *Saccharomyces sensu lato* chromosomal bands. However, the resolution of bands varied slightly among different runs and different plugs and occasionally particular bands could not be distinguished. Chromosomal bands were numbered from the bottom of the gel.

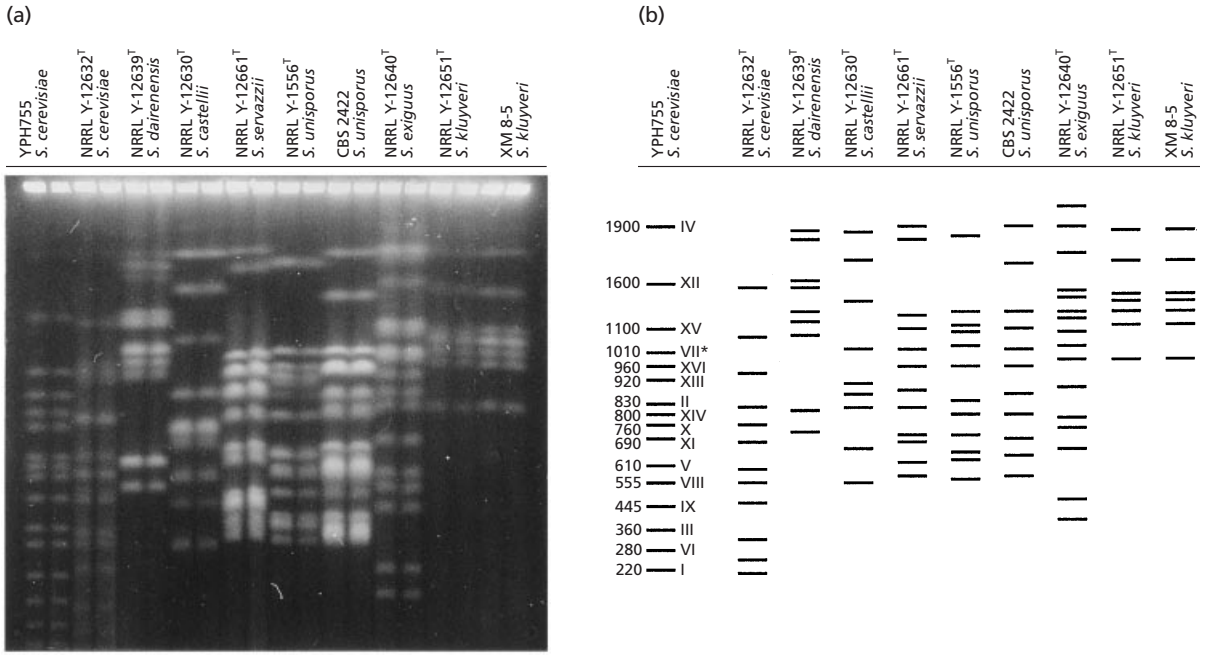

Fig. 1. Karyotypes of *Saccharomyces sensu lato* species. (a) Chromosome gel. (b) Schematic illustration of the separated chromosomes based on many independent experiments. Chromosome-sized DNA from *S. cerevisiae* YPH755, *S. cerevisiae* Y-12632^T, *S. dairenensis* Y-12639^T, *S. castellii* Y-12630^T, *S. servazzii* Y-12661^T, *S. unisporus* Y-1556^T, *S. unisporus* CBS 2422, *S. exiguus* Y-12640^T, *S. kluyveri* Y-12651^T and *S. kluyveri* XM 8-5 was separated by pulsed-field electrophoresis. The chromosomes were separated using a four-step program: step 1, pulse 240 s, 8 h; step 2, pulse 160 s, 10 h; step 3, pulse 90 s, 14 h; step 4, pulse 60 s, 8 h. *, A YPH755 chromosome has been fragmented at *RAD*2 resulting in separation of chromosome VII and XV. Note also that the two *S. unisporus* strains exhibited slightly different patterns.

Table 1. Numbers of chromosomes and chromosome and genome sizes of *Saccharomyces sensu lato* yeasts

Chromosomes are numbered by size from the bottom of the gel (see Fig. 1). The numbers presented are in kb and are means, obtained by compiling the results from various pulsed-field gels. All strains are type strains except *S. cerevisiae*, which is represented by YPH149/YPH755 (Gerring *et al.*, 1991). Note that the size of the sequenced strains of *S. cerevisiae* is approximately 13400 kb (Goffeau *et al.*, 1996).

	S. dairenensis	*S. castellii*	*S. servazzii*	*S. unisporus*	*S. exiguus*	*S. kluyveri*	*S. cerevisiae*
No. of chromosomes	9	9	12	12	16	7	16
Sizes of individual chromosomes:							
1	730	550	570	560	395	970	220
2	800	660	620	630	470	1110	280
3	1060	820	690	660	660	1300	360
4	1200	860	720	720	740	1410	445
5	1300	890	740	800	780	1510	555
6	1510	1000	870	840	880	1750	610
7	1600	1320	960	960	970	1950	690
8	1800	1720	1010	1020	1020	–	760
9	1920	1900	1100	1080	1080	–	800
10	–	–	1250	1110	1250	–	830
11	–	–	1850	1300	1300	–	920
12	–	–	1950	1880	1470	–	960
13	–	–	–	–	1510	–	1010
14	–	–	–	–	1780	–	1100
15	–	–	–	–	1950	–	1600
16	–	–	–	–	2100	–	1900
Genome size	11920	9720	12330	11560	18355	10000	13040

Table 2. Chromosome numbers and estimated genome sizes compared with previously published results

Data were obtained from Naumov *et al.* (1995), Naumova *et al.* (1996), Jäger & Philippsen (1989) and Vaughan-Martini *et al.* (1993). The sequenced strain of *S. cerevisiae* contains 16 chromosomes and its genome size, including repetitive sequences, is approximately 13400 kb (Goffeau *et al.*, 1996).

Species	Chromosome number				Estimated genome size (kb)	
	Present study	Vaughan-Martini	Naumov/ Naumova	Jäger & Philippsen*	Present study	Vaughan-Martini
S. dairenensis	9	6	7	8	11920	7920
S. castellii	9	8	–	–	9720	9415
S. servazzii	12	9	9–13†	10†	12330	10430
S. unisporus	12	11	9–13†	10	11560	11375
S. exiguus	16	11	14–16‡	11†	18355	10800
S. kluyveri	7	7	–	5	10000	9550

* Only a few species were represented by type strains in this study.

† The chromosome number for the type strain was not stated specifically.

‡ Data from Naumova *et al.* (1996); other data in this column are from Naumov *et al.* (1995).

The *S. cerevisiae* type strain was included in the analysis. Only 12 bands were resolved using the four-step program, demonstrating that a number of chromosomes ran as non-distinguishable double bands. Thus, the karyotypes obtained using pulsed-field electrophoresis may not always express the true number of chromosomes in a species. In the following analysis, our results are compared with the previously published data on *Saccharomyces sensu lato* type strains.

The present results show that the *S. dairenensis* type strain (Fig. 1) contains nine chromosomes with sizes ranging from 730 to 1920 kb. The *S. dairenensis* chromosomal bands were generally well resolved and could be distinguished using the four-step program. However, bands 5 and 6, as well as 7 and 8, occasionally ran close to each other. The total genome size was calculated to be 11920 kb (Table 2), which is larger than the size reported by Vaughan-Martini *et al.* (1993) (7929 kb). Note that the number of bands observed by us in this strain was higher than that reported by Vaughan-Martini *et al.* (1993), Jäger & Philippsen (1989) and Naumov *et al.* (1995).

S. castellii (Fig. 1) contains nine chromosomes with a size range similar to that of *S. dairenensis*. The chromosomes ranged from 550 to 1900 kb. The *S. castellii* chromosomal bands were generally well resolved. Band 4 appeared as one band with a high intensity and may be a double band. Occasionally, band 9 was not easily detectable. It either appeared as a very weak band or it ran as a double band with band 8. The total genome size was calculated to be 9720 kb, which is similar to the size reported by Vaughan-Martini *et al.* (1993) (9415 kb).

Separation of *S. servazzii* chromosomes (Fig. 1) identified 12 chromosomal bands. Chromosome sizes ranged from 570 to 1950 kb. With only two chromosomes belonging to the large size class, most chromosomes were medium-sized. Bands 1–4 ran in one group; sizes ranged from 570 to 720 kb. Bands 3 and 4 were sometimes difficult to distinguish. Band 3 was very thick. Bands 8 and 9 appeared with high intensity on the gel and may each contain two chromosomes. The genome size was calculated as 12330 kb. Vaughan-Martini *et al.* (1993) reported the presence of 11 bands and a genome size of 10430 kb for the *S. servazzii* type strain.

As with *S. servazzii*, 12 bands were visible upon separation of chromosomes from the *S. unisporus* type strain (Fig. 1). Furthermore, the chromosomes were very much within the same size range, 560–1880 kb. Bands 2 and 3 were sometimes difficult to distinguish because they often ran as a double band. Similarly, bands 9 and 10 were sometimes difficult to distinguish. The genome size was calculated to be 11560 kb. Vaughan-Martini *et al.* (1993) reported 11 bands and a genome size of 11375 kb. In the case of *S. unisporus*, two strains were analysed. They exhibited a slightly different separation pattern of nuclear chromosomes. A large chromosome of 1400 kb was present in CBS 2422, but it was not present in the type strain. Instead, the type strain contained two bands of 1110 and 720 kb. Note that the karyotypes of *S. servazzii* and *S. unisporus* exhibit a similar pattern and many chromosomes may be homologous. This is in agreement with the sequencing data on the rDNA genes which have been taken to show that these two species are closely related (James *et al.*, 1997; Kurtzman & Robnett, 1991; Oda *et al.*, 1997).

Sixteen chromosomal bands were identified in *S. exiguus* (Fig. 1). The chromosome sizes ranged from 395 to 2100 kb, thus belonging to all three size classes. Two chromosomes were smaller than 500 kb, five

chromosomes were between 500 and 1000 kb and nine chromosomes were larger than 1000 kb. Chromosomal bands 10 and 11, 12 and 13 and 15 and 16 often ran as double bands. The large number of heavy chromosomes results in the highest genome size obtained within the *Saccharomyces sensu lato* species, 18 355 kb. According to the present results, this species has the largest genome among *Saccharomyces* yeasts. Vaughan-Martini *et al.* (1993) reported 11 bands and a total genome size of 10 800 kb. Note that this is the only species among the *Saccharomyces sensu lato* species that contains small chromosomes (< 500 kb).

S. kluyveri contained seven large chromosomes (Fig. 1). Their sizes ranged from 970 to 1950 kb and the genome size was 10 000 kb. All chromosomes were resolved as single bands by the four-step program. The number of chromosomes and the genome size agree with the results obtained by Vaughan-Martini *et al.* (1993).

The chromosome number and genome sizes obtained in the present study were compared with previously published results (Table 2). A large variation between the results from the different studies is evident. We suggest that this is due to poorer resolution obtained by the pulsed-field programs utilized in the previous studies. It may be that some chromosomes are more easily recovered upon extraction than others and that the extraction procedure is important. The discrepancy may therefore also be a result of the experimental extraction procedure (different lysing enzymes, buffers, etc.) as well as variations in the recovery of specific chromosomes.

Further discrepancies were observed in the estimated genome sizes, particularly in the case of *S. dairenensis* and *S. exiguus*. These differences are correlated with the number of chromosomes observed. In the present study, we documented a larger number of chromosomes and therefore also a larger estimated genome size for the two species. In general, *S. kluyveri* is reported to have the fewest chromosomes and to posses a genome size at the lower end of the range shown by the *Saccharomyces sensu lato* group. It is also interesting to point out that all species except *S. exiguus* have a smaller genome than that of the sequenced strain of *S. cerevisiae*, which is 13 400 kb (Goffeau *et al.*, 1996).

DISCUSSION

The genus *Saccharomyces* consists of several species that exhibit a lot of variation within the organization of their nuclear (Jäger & Philippsen, 1989; Naumov *et al.*, 1992, 1995; Naumova *et al.*, 1996; Petersen, 1998; Vaughan-Martini *et al.*, 1993) and mitochondrial (Groth, 1998; Petersen, 1998; Piškur *et al.*, 1998) genomes. One possible approach to understanding the origin of the present polymorphism is to characterize and compare the organization of the modern yeast genomes. Therefore, karyotypes of type strains of some *Saccharomyces sensu lato* yeasts were studied in more detail. The number of chromosomes for some species belonging to the *Saccharomyces sensu lato* group described in this report was higher than generally reported previously (Jäger & Philippsen, 1989; Naumov *et al.*, 1995; Naumova *et al.*, 1996; Vaughan-Martini *et al.*, 1993; Table 2). In addition, the genome size estimates varied from the previous estimates (Table 2). In the case of *S. exiguus*, this difference was almost 8 Mb, and for *S. dairenensis* the difference was 3 Mb. These discrepancies are possibly due to the fact that the separation of chromosomes in our electrophoresis experiments was more efficient and thus several 'novel' distinctive chromosome bands could be detected. However, the genome sizes shown in Table 2 should still be considered as approximate for the following reasons. The lack of perfect linearity in the separation profile for chromosomes larger that 1000 kb may contribute to errors in the size estimates of individual chromosomes. The ploidy of several of the yeast species examined, *S. dairenensis*, *S. servazzii*, *S. unisporus* and *S. exiguus*, is not known and these yeasts are not available as monospore strains. Thus, these strains, which are likely to be diploid, may contain homologous chromosome pairs that are polymorphic and thereby produce 'extra' chromosome bands on the gel. In short, a single chromosome could be represented by two differently sized copies. On the other hand, the patterns of *S. kluyveri* and *S. castellii* type strains are the same as in the case of monospore or haploid isolates (data not shown). In addition, some chromosomes could exhibit similar electrophoretic mobility and consequently not separate into distinguishable bands. Despite these uncertainties, several conclusions can be reached from our results.

On the basis of sequence analysis of nuclear and mitochondrial genes (James *et al.*, 1997; Kurtzman, 1993; Kurtzman & Robnett, 1991; Oda *et al.*, 1997; Petersen, 1998; Peterson & Kurtzman, 1991), the *Saccharomyces sensu lato* yeasts can be separated into several sub-groups consisting of phylogenetically closely related species: (i) *S. servazzii* and *S. unisporus*, (ii) *S. castellii* and *S. dairenensis*, (iii) a group containing *S. exiguus* and (iv) a group containing *S. kluyveri*. It is apparent from our results (Fig. 1, Table 1) that *S. servazzii* and *S. unisporus*, two closely related yeasts, contain the same number of chromosomes and have very similar karyotypes. *S. castellii* and *S. dairenensis*, which are also relatively closely related, contain the same number of chromosomes, but only a minority of them seem to be similar in size. The numbers of chromosomes and karyotypes of *S. exiguus* and *S. kluyveri* differ substantially from any other *Saccharomyces sensu lato* yeasts.

The numbers and sizes of chromosomes in the *Saccharomyces sensu lato* group show significant heterogeneity. The *Saccharomyces sensu lato* chromosomes are either large or medium in size. Small chromosomes (< 500 kb) have been detected only in *S. exiguus*. In other genera closely related to *Saccharo-*

myces, such as *Kluyveromyces*, *Torulaspora* and *Zygosaccharomyces*, the chromosome number is in general lower than in *S. cerevisiae* and small chromosomes have not been detected (de Jonge *et al*., 1986; Oda & Tonomura, 1995; Sor & Fukuhara, 1989). Thus, it is likely that the progenitor of *Saccharomyces* and other related genera had a small number of chromosomes. However, during evolution of the genus *Saccharomyces*, the number of chromosomes has increased. Two different models could explain the corresponding evolutionary mechanism. One model, the 'chromosome breakage' model, suggests that some of the progenitor's large chromosomes broke and generated several smaller chromosomes. According to this model, extensive synteny should be found in the modern species. Alternatively, the 'mosaic' model proposes that the structure of the modern chromosomes is of a more recent origin and is the result of extensive gene jumping between and along chromosomes, as well as the frequent generation of novel chromosomes. According to this model, the progenitor chromosome structure cannot be deduced from the structure of the modern chromosomes. The genomes of several *Saccharomyces sensu lato* yeasts are currently being analysed in our laboratory to test the above models.

Another aspect of analysis of these genomes is to find out when the apparent duplication in the *S. cerevisiae* genome (Keogh *et al*., 1998; Wolfe & Shields, 1997) took place and whether it is also present in other yeasts. Duplicated sequences in *S. cerevisiae* cover approximately 2 Mb of the 12 Mb sequenced genome (Wolfe & Shields, 1997). In addition, the total genome, which is 13·4 Mb, includes approximately 1·3 Mb of repeated sequences, such as the rDNA genes (Goffeau *et al*., 1996). The total genome size prior to the duplication event was suggested to be approximately 11–12 Mb (Keogh *et al*., 1998). It is interesting to point out that this pre-duplication size generally matches the size of a majority of the *Saccharomyces sensu lato* yeasts. *S. cerevisiae* and *S. exiguus* exhibit genome sizes that are larger than those of other yeasts and both contain small chromosomes (Table 2). Whether the size and number of chromosomes in *S. exiguus* are correlated with duplication of some part(s) of the genome is still not known. So far it is also unclear whether the origin of the *Saccharomyces* small chromosomes is mono- or polyphyletic.

ACKNOWLEDGEMENTS

We thank Thomas H. Andersen and Jeanne Hvidtfeldt for technical help and assistance and Cletus Kurtzman and Michael L. Nielsen for their comments. This work was supported by the Danish Research Council, the Carlsberg Foundation and the Novo Nordisk Foundation.

REFERENCES

Cardinali, G. & Martini, A. (1994). Electrophoretic karyotypes of authentic strains of the sensu stricto group of the genus *Saccharomyces*. *Int J Syst Bacteriol* **44**, 791–797.

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

Gerring, S. L., Connelly, C. & Hieter, P. (1991). Positional mapping of genes by chromosome blotting and chromosome fragmentation. *Methods Enzymol* **194**, 57–77.

Goffeau, A., Barrell, B. G., Bussey, H. and 13 other authors (1996). Life with 6000 genes. *Science* **274**, 546–567.

Groth, C. (1998). *Saccharomyces sensu stricto yeasts*: *characterization of mitochondrial DNA*. MSc thesis, University of Copenhagen, Copenhagen, Denmark.

Jäger, D. & Philippsen, P. (1989). Many yeast chromosomes lack the telomere-specific Y′ sequence. *Mol Cell Biol* **9**, 5754–5757.

James, S. A., Cai, J., Roberts, I. N. & Collins, M. D. (1997). A phylogenetic analysis of the genus *Saccharomyces* based on 18S rRNA gene sequences: description of *Saccharomyces kunashirensis* sp. nov. and *Saccharomyces martiniae* sp. nov. *Int J Syst Bacteriol* **47**, 453–460.

Johnston, J. R. & Mortimer, R. K. (1986). Electrophoretic karyotyping of laboratory and commercial strains of *Saccharomyces* and other yeasts. *Int J Syst Bacteriol* **36**, 569–572.

de Jonge, P., de Jongh, F. C. M., Meijers, R., Steensma, H. Y. & Scheffers, W. A. (1986). Orthogonal-field-alternation gel electrophoresis banding patterns of DNA from yeasts. *Yeast* **2**, 193–204.

Keogh, R. S., Seoighe, C. & Wolfe, K. H. (1998). Evolution of gene order and chromosome number in *Saccharomyces*, *Kluyveromyces* and related fungi. *Yeast* **14**, 443–457.

Kurtzman, C. P. (1993). Systematics of the ascomycetous yeasts assessed from ribosomal RNA sequence divergence. *Antonie Leeuwenhoek* **63**, 165–174.

Kurtzman, C. P. & Robnett, C. J. (1991). Phylogenetic relationships among species of *Saccharomyces*, *Schizosaccharomyces*, *Debaryomyces* and *Schwanniomyces* determined from partial ribosomal RNA sequences. *Yeast* **7**, 61–72.

Naumov, G. I., Naumova, E. S., Lantto, R. A., Louis, E. J. & Korhola, M. (1992). Genetic homology between *Saccharomyces cerevisiae* and its sibling species *S. paradoxus* and *S. bayanus*: electrophoretic karyotypes. *Yeast* **8**, 599–612.

Naumov, G. I., Naumova, E. S. & Korhola, M. (1995). Karyotypic relationships among species of *Saccharomyces* sensu lato: *S. castellii*, *S. dairensis*, *S. unisporus* and *S. servazzii*. *Syst Appl Microbiol* **18**, 103–108.

Naumova, E. S., Naumov, G. I. & Korhola, M. (1996). Use of molecular karyotyping for differentiation of species in the heterogeneous taxon *Saccharomyces exiguus*. *J Gen Appl Microbiol* **42**, 307–314.

Nilsson-Tillgren, T., Kielland-Brandt, M. C., Holmberg, S., Petersen, J. G. L. & Gjermansen, C. (1983). Is lager yeast a species hybrid? Utilization of intrinsic genetic variation in breeding. In *Genetics of Industrial Microorganisms*, pp. 143–147. Edited by Y. Ikeda & T. Beppu. Tokyo: Kodanska.

Oda, Y. & Tonomura, K. (1995). Electrophoretic karyotyping of the yeast genus *Torulaspora*. *Lett Appl Microbiol* **21**, 190–193.

Oda, Y., Yabuki, M., Tonomura, K. & Fukunaga, M. (1997). A phylogenetic analysis of *Saccharomyces* species by the sequence of 18S-28S rRNA spacer regions. *Yeast* **13**, 1243–1250.

Pedersen, M. B. (1986a). DNA sequence polymorphisms in the genus *Saccharomyces*. III. Restriction endonuclease fragment patterns of chromosomal regions in brewing and other yeast strains. *Carlsberg Res Commun* **51**, 163–183.

Pedersen, M. B. (1986b). DNA sequence polymorphisms in the

genus *Saccharomyces*. IV. Homoeologous chromosomes III of *Saccharomyces bayanus*, *S. carlsbergensis*, and *S. uvarum*. *Carlsberg Res Commun* **51**, 185–202.

Petersen, R. F. (1998). *Genome dynamics and evolution of the mitochondrial and nuclear genomes in Saccharomyces sensu lato species*. PhD thesis, University of Copenhagen, Copenhagen, Denmark.

Peterson, S. W. & Kurtzman, C. P. (1991). Ribosomal RNA sequence divergence among sibling species of yeasts. *Syst Appl Microbiol* **14**, 124–129.

Piškur, J., Smole, S., Groth, C., Petersen, R. F. & Pedersen, M. B. (1998). Structure and genetic stability of mitochondrial genomes vary among yeasts of the genus *Saccharomyces*. *Int J Syst Bacteriol* **48**, 1015–1024.

Sor, F. & Fukuhara, H. (1989). Analysis of chromosomal DNA patterns of the genus *Kluyveromyces*. *Yeast* **5**, 1–10.

Vaughan Martini, A. & Kurtzman, C. P. (1985). Deoxyribonucleic acid relatedness among species of the genus *Saccharomyces* sensu stricto. *Int J Syst Bacteriol* **35**, 508–511.

Vaughan-Martini, A. & Martini, A. (1987). Three newly delimited species of *Saccharomyces* sensu stricto. *Antonie Leeuwenhoek* **53**, 77–84.

Vaughan-Martini, A., Martini, A. & Cardinali, G. (1993). Electrophoretic karyotyping as a taxonomic tool in the genus *Saccharomyces*. *Antonie Leeuwenhoek* **63**, 145–156.

Wolfe, K. H. & Shields, D. C. (1997). Molecular evidence for an ancient duplication of the entire yeast genome. *Nature* **387**, 708–713.