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Loss and fragmentation of chromosome 5 are major events linked to the adaptation of *rad52-ΔΔ* strains of *Candida albicans* to sorbose

Encarnación Andaluz¹, Jonathan Gómez-Raja¹, Belén Hermosa¹, Toni Ciudad¹, E. Rustchenko², Richard Calderone³, and Germán Larriba^{1,*}

¹Departamento de Microbiología, Facultad de Ciencias, Universidad de Extremadura, 06071 Badajoz, Spain

²Department of Biochemistry and Biophysics, University of Rochester Medical Center, Rochester, NY 14642, USA

³Microbiology and Immunology, Georgetown University School of Medicine, Washington DC 20007, USA

Abstract

C. albicans can adapt and grow on sorbose plates by losing one copy of Chr5. Since *rad52* mutants of *S. cerevisiae* lose chromosomes at a high rate, we have investigated the ability of *C. albicans rad52* to adapt to sorbose. *Carad52-ΔΔ* mutants generate Sou⁺ strains earlier than wild type but the final yield is lower, probably because they die at a higher rate in sorbose. As other strains of *C. albicans*, CAF2 and *rad52-ΔΔ* derivatives generate Sou⁺ strains by a loss of one copy of Chr5 about 75% of the time. In addition, *rad52* strains were able to produce Sou⁺ strains by a fragmentation/deletion event in one copy of Chr5, consisting of loss of a region adjacent to the right telomere. Finally, both CAF2 and *rad52-ΔΔ* produced Sou⁺ strains with two apparent full copies of Chr5, suggesting that additional genomic changes may also regulate adaptation to sorbose.

Index descriptors

Sorbose adaptation; *Candida albicans*; Rad52; chromosome loss; chromosome fragmentation

Introduction

Candida albicans, as well as other *Candida* species, have become prevalent opportunistic human pathogens. *Candida albicans* is a diploid organism (Olaiya and Sogin, 1979) with a high degree of heterozygosity that lacks a complete sexual cycle. Still, genome sequence analysis has identified a locus (*MTL*, for mating type-like locus) similar to the *MAT* locus of *S. cerevisiae* (Hull and Johnson, 1999). Furthermore, strains of opposite mating type can mate whether they are genetically engineered (Hull *et al.*, 2000) or *in vitro* selected (Magee and Magee, 2000). However, although there is no doubt that karyogamy follows conjugation (Bennet *et al.*, 2005), meiosis has not been demonstrated so far and tetraploids derived from these crosses likely become diploid by a loss of chromosomes thus completing a parasexual cycle (Bennet and Johnson, 2003).

*Corresponding author: E-MAIL glarriba@unex.es

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Importantly, progeny of a particular strain undergo changes in the karyotype *in vitro* as well as *in vivo* (see below). These genotypic changes are frequently accompanied by alterations in a number of phenotypic traits, an observation that has led to the widely accepted hypothesis that *C. albicans* can achieve phenotypic variability by genomic arrangements (Rustchenko-Bulgac *et al.*, 1990; Rustchenko and Sherman, 2003; Magee and Chibana, 2002; Larriba, 2004). For instance, gain- and loss-of-assimilation function *in vitro* is associated with gross rearrangements involving chromosomes 2, 5 and 6 (Rustchenko *et al.*, 1994). Gross chromosomal rearrangements in chromosomes 5, 6 and 7 also occur during the course of infection (Legrand *et al.*, 2004). The systematic analysis by Rustchenko and colleagues demonstrated that in the environments that kill cells or prevent their propagation, *C. albicans* survives due to different specific chromosome alterations (reviewed by Rustchenko and Sherman 2003; Rustchenko, 2006). In the most detailed study of the adaptation to sorbose, it was suggested that the loss of an entire homologue of Chr5 is required because this chromosome carries multiple genes for negative regulators, which are functionally redundant. Especially relevant for some of our current results was the finding of a critical portion of 209 kb located on the right arm of Chr 5 (Kabir *et al.*, 2005). The loss of this portion allows adaptation to sorbose, thus, mimicking the loss of an entire homologue. More recently, it has been reported that aneuploidy in general, and more specifically formation of an isochromosome composed of the two left arms of chromosome 5 were associated with azole resistance. Interestingly, this arm has *ERG11* required for ergosterol biosynthesis and *TAC1*, which is a positive regulator of the drug efflux pump (Selmecki *et al.*, 2006). This fact may also explain why the gain of one copy of this chromosome occurs in some *C. albicans* strains resistant to fluconazole (Perepnikhatka *et al.*, 1999).

In addition to changes in karyotype, derivatives of wild type strains of *C. albicans* frequently show loss of heterozygosity (LOH) in several markers (Forche *et al.*, 2004; Tavanti *et al.*, 2004). Furthermore, it has been reported that loss of heterozygosity (LOH), caused by mutation or mitotic recombination, occurs independently at different loci across the whole genome during *in vivo* passage and may be accompanied or not by changes in karyotypes as well as alteration in phenotypes (Forche *et al.*, 2005).

Studies in *S. cerevisiae* have indicated that loss of chromosomes is prevented by both homologous recombination (HR) and checkpoint functions. It is well known that *S. cerevisiae* cells lose chromosomes at a high rate in *rad52* strains (Mortimer *et al.*, 1981; Yoshida *et al.*, 2003), as well as in *rad50*, *rad51* and *rad54* mutants (Hiraoka *et al.*, 2000; Klein, 2001; Yoshida *et al.*, 2003). HR acts synergistically with DNA damage checkpoints to assure the correct repair and segregation of chromosomes between the mother and daughter cells (Klein, 2001).

We have recently characterized the *RAD52* from *C. albicans*. As expected, it is required for several cellular events that require homologous recombination, including integration of linear DNA with long flanking homology and repair of DNA damage caused by UV or the radiomimetic MMS. In the present study, we have investigated the appearance of Sou⁺ mutants in sorbose plates in *rad52-ΔΔ* strains. We have found that the absence of Rad52 leads to the earlier appearance of Sou⁺ mutants, but the final yield of Sou⁺ mutants is lower. As described for several Rad52⁺ strains (Janbon *et al.*, 1998; Andaluz *et al.*, 2002), adaptation of both CAF2 and *rad52-ΔΔ* derivatives to sorbose mostly occurred through loss of one full copy of Chr5, but also through other genetic alterations that do not imply obvious alterations of this chromosome. Furthermore, a particular mechanism of adaptation to sorbose consisting of a long deletion of Chr5 was restricted to *rad52* strains.

2. Materials and methods

2.1. Strains and media

The *C. albicans* strains used in this study are indicated in Table 1. They are derived from the prototrophic strain SC5314 (Gillum *et al.*, 1984), used in the sequencing of the genome. *C. albicans* cells were grown routinely at 28°C in YEPD (2% glucose, 1% yeast extract, 2% Bacto Peptone).

2.2. Analysis of Sou⁺ mutants

The formation of Sou⁺ mutants was analyzed as described before (Andaluz *et al.*, 2002), except that for the time course experiments, sorbose plates were inoculated with cells from a YEPD liquid culture (instead of a colony) at a cell density of about 3000-5000 cfu/plate. The viability experiment has been described before (Janbon *et al.*, 1999; Andaluz *et al.*, 2002). Briefly, a cell suspension from a YEPD liquid culture was appropriately diluted and about 800 cells were plated in duplicate on sorbose plates. A YEPD plate was also inoculated to provide a direct estimation of the total CFU plated. To determine the number of viable cells, at the indicated times the entire agar disc from one series of sorbose plates was transferred onto the surface of YEPD plates. The second set of plates was monitored daily to determine the number of Sou⁺ mutants. In order to correlate the appearance of Sou⁺ mutants and viability, we considered that the Sou⁺ mutant had been formed four (Rad52⁺) or five (Rad52⁻) days before the first visual observation of the colony (see reconstruction experiment). All Sou⁺ colonies were verified by growing them on new sorbose plates before being analyzed for the *MTL* locus, karyotype or the indicated SNP marker(s). Finally, a reconstruction experiment was performed to determine the lag time between the mutational event and the appearance of the microscopic colony. For this purpose, Sou⁺ cells from a colony were plated on a new sorbose plate and the time of the appearance of the colony recorded (Janbon *et al.*, 1999; Wang *et al.*, 2004).

2.3. Determination of heterozygosity/homozygosity of selected regions of chromosomes 2, 4 and 5

Loss of chromosome 5 was first investigated by analyzing LOH at the *MTL* locus. For PCR analysis of the *MTL* loci, we used the protocol and oligonucleotides reported (Rustad *et al.*, 2002), except that the annealing temperature was 57°C instead 55°C. The presence of one or both homologues of Chr5 was further investigated by analyzing previously characterized single nucleotide polymorphisms (SNP) in *Sfi*I fragments, SNP 1445/2395 in 5M and SNP 1341/2493 in 5I, from that chromosome. These SNPs correspond to SNP110 and SNP118 respectively in the SNP map (<http://albicansmap.ahc.umn.edu/html/snp.html>). Similarly, for chromosome 2, we amplified and sequenced the region around the SNP marker 1314/2038 (SNP68) in *Sfi*I fragment 2U. For these experiments, the regions containing the polymorphisms were amplified using the appropriate oligonucleotides and the PCR product sequenced. Primer sequences can be obtained upon request from Anja Forche (<http://albicansmap.ahc.umn.edu/html/snp.html>). A region of 1009 bp of chromosome 4, including *RBT7*, was investigated using oligonucleotides indicated in Table 2. The *RBT7* ORF exhibits restriction fragment length polymorphism (RFLP) for several restriction enzymes (Anja Forche, personal communication). Following purification, this band was treated with *Alu*I and the fragments analyzed in an agarose gel. A strain homozygous for *RBT7* obtained in our laboratory was used as a control.

2.4. DNA extraction and analysis

Standard techniques were routinely used for DNA manipulations (Sherman *et al.*, 1982). Genomic DNA was prepared from protoplasts obtained by the incubation of cells with

Zymolyase stabilized with 1M sorbitol and lysed in 50 mM Tris, 50 mM EDTA, 0.6% sodium dodecyl sulfate (SDS).

2.5. Preparation of native chromosomes

A 0.1-ml sample of an exponentially growing culture of *C. albicans* was used to inoculate 10-ml of YEPD. The culture was maintained in a rotatory shaker at 30°C for 48 h. Cells were harvested by centrifugation, washed twice with 50 mM EDTA, pH 8.0, and suspended in 1-ml of CPES (40 mM citric acid, 120 mM sodium phosphate, 20 mM EDTA, pH 8, 1.2 M sorbitol and 5 mM dithiothreitol) supplemented with 0.2 mg of Zymolyase 20,000A. A 1-ml aliquot of CPE (same as CPES but lacking sorbitol and DTT) containing 1% low-melting-point agarose at 50°C was then added and gently mixed. Aliquots of 200 µl were then transferred into a sample mold and kept at -20°C. Upon solidification, plugs were transferred to test tubes, supplemented with 6 ml of CPE and incubated at 30°C for 4 h. CPE buffer was replaced with 5 ml of TESP buffer (1M Tris-HCl, 0.5 M EDTA, 2% SDS, 1 mg/ml of proteinase K) and incubated overnight at 50°C. Samples were then washed 3x with TE (10 mM Tris-HCl, 1 mM EDTA) at 50°C and 6x at room temperature. The plugs were stored at 4°C in 50 mM EDTA, pH 8.

2.6. Electrophoretic chromosome separation

Two different protocols were then used to separate chromosomes. In the first protocol, all chromosomes were separated but homologues of chromosomes 6 and 7 can not be distinguished. The gel samples were electrophoresed in 0.6% agarose for 24 h at 80 V with a 120-300 sec linear ramp and then for 48 h at 80 V with a 420-900 sec linear ramp in a rotating-gel electrophoresis apparatus (Rotaphor, Biometra) using TBE buffer (0.5x, pH 8). A second protocol was also used to separate both homologues of the smaller chromosomes, 6 and 7 (Legrand *et al.*, 2004; Lephard *et al.*, 2005). In this case, the gel samples were run in 1% agarose at 100V with a linear ramp of 60-120 sec, 120° included angle for 48 h and then at 130V with a 300-420 sec linear ramp, 120° included angle for 48 h in the same apparatus. The intensity of the ethidium bromide fluorescence was quantified with the Corel Photo-Paint program.

2.7. Southern blot analysis

For Southern blotting, electrophoresed samples were transferred to TM-nitrocellulose and hybridized with the probes (below) at high stringency (65°C in 6× SSC, 5×Denhardt's solution, 0.5% SDS and then washed with 0.1x SSC, 0.1% SDS). The probes consisted of: 1) markers of *Sfi*I fragments of Chr5 (*CDC1* for 5I and *GCD11* for 5M (kindly provided by BB Magee); 2) a 521 bp probe specific of *RAD17* located close to the left telomere in *Sfi*I fragment 4BB; 3) a 456 bp probe of *ILV3*, an ORF located in *Sfi*I fragment 5I at the telomere-proximal side of *HIS1* (Wu *et al.*, 2005); and 4) a 453 bp probe of the *HTS1* ORF located at the telomere-proximal side of *ILV3*. These probes were amplified by PCR from genomic DNA, using the oligonucleotides indicated in Table 2. These oligonucleotides were designed according to the Candida Genome Database (<http://www.candidagenome.org/>).

3. Results

3.1. Kinetics of appearance of Sou⁺ colonies from CAF2 and rad52 strains

As shown in Table 3 (Expt. 1), when inoculated at a concentration of about 3000 cells per plate, CAF2 generated Sou⁺ colonies most of them developing in a period of three to four days following a 12 day incubation period, with a final yield of 15-25%. A similar pattern and yield was produced by the heterozygote (*CaRAD52/Carad52Δ*) and a reconstituted strain (TCR3.2.1)(Ciudad *et al.*, 2004). On the other hand, for two *rad52-ΔΔ* strains plated at the same cell density (only TCR2.2 is shown) a significantly reduced yield (2.5 -5%) of Sou⁺

colonies was observed. In spite of its lower final yield, the time of appearance of these *rad52-ΔΔ* Sou⁺ colonies was earlier on average, and in fact between days 5 and 9, the Rad52⁺ strain produced a larger number of colonies than any of the Rad52⁺ counterparts (Table 3). Three additional experiments, one of which is shown Table 3 (Expt.2), indicated that the bias towards the earlier generation and the lower final yield of sorbose-resistant strains in *rad52-ΔΔ* cells was highly reproducible. We found, however, some variability with regard to the exact period of maximum production of Sou⁺ (for instance, for CAF2, days 13-15th in expt. 1 and 10-12th day in expt. 2).

Viability of the four strains in the presence of sorbose was extremely low, but *rad52-ΔΔ* cells were particularly sensitive to the sugar (Fig. 1A). The fact that null cells die more quickly explains the lower final colony yield of these mutants. Once again, the relative timing of the appearance and final yield of the Sou⁺ colonies in this experiment followed essentially the pattern described in Table 3 (not shown).

Colonies formed from *rad52-ΔΔ* cells were bigger on average (Fig. 1B), probably as a consequence of their earlier appearance. However, regardless of the nature of the parental Sou⁺ strain (i.e., Rad52⁺ or Rad52⁻ cells), almost all Sou⁺ colonies contained cells that were truly adapted to sorbose; not only did colonies increase in size with time, but more than 90% of cells readily formed colonies when transferred to new sorbose agar plates. Most Rad52⁺ cells formed visible colonies in four days, whereas a similar percentage of *rad52-ΔΔ* did so in five days. This observation is probably derived from the lower growth rate of *rad52-ΔΔ* cells as compared with the Rad52⁺ counterparts (Chauhan *et al.*, 2005; Andaluz *et al.*, 2006).

Since sorbose adaptation of *C. albicans* cells is mediated by genetic events, it is not surprising to find differences between Rad52⁺ and Rad52⁻ strains. In *rad52* mutants of *S. cerevisiae*, chromosome loss is increased as compared to wild type cells (Mortimer *et al.*, 1981; Hiraoka *et al.*, 2000; Yoshida *et al.*, 2003). Since Rad52p plays a similar role in both organisms, one could expect an increase in the loss of chromosomes in *rad52-ΔΔ* strains of *C. albicans*, which would explain the earlier generation of Sou⁺ mutants. Furthermore, it is likely that loss of chromosomes in the presence of sorbose is not restricted to Chr5 but affects other chromosomes leading frequently to cell death [as described for the conversion of tetraploids into diploids (Bennet and Johnson, 2003)], which, in turn, would be responsible for the lower yield of Sou⁺. Indeed, *rad52-ΔΔ* strains of *C. albicans* exhibit a much higher frequency of spontaneous chromosome loss than the parental Rad52⁺ strain from which they were derived (manuscript in preparation). This propensity to lose chromosomes seems to be significantly increased in the presence of sorbose.

3.2. Analysis of the MTL locus in Sou⁺ strains

As mentioned above, it is well documented that the loss of one copy of Chr5 is a major mechanism of adaptation to sorbose in *C. albicans* strains 3153A and CAF2 (Janbon *et al.*, 1998; 1999; Andaluz *et al.*, 2002). In order to investigate whether the Sou⁺ cells from our experiments shared this feature, we analyzed the loss of heterozygosity at the *MTL* locus. As shown in Fig. 2A, parental CAF2 and *rad52-ΔΔ* (lanes 1 and 5) were heterozygous for *MTL* since they carry both *MTLa1* and *MTLa2* genes. On the other hand, although most of the Sou⁺ derivatives were homozygous for *MTL* as shown for two of them derived from CAF2 (C-Sou⁺1, lane 2, and C-Sou⁺2, lane 3) and four derived from *rad52-ΔΔ* (*r*-Sou⁺1 to *r*-Sou⁺4, lanes 6-9 respectively), there was no absolute correlation between the Sou⁺ phenotype and homozygosity at the *MTL* locus. Three examples of this, one for CAF2 (C-Sou⁺3, lane 4) and two for *rad52-ΔΔ* *r*-Sou⁺5 and *r*-Sou⁺6, lanes 10 and 11 respectively) are shown in Fig. 2A. A screen of 43 Rad52⁺ Sou⁺ strains indicated that most of them (approximately 70%) were homozygous (2 α and 28 α), but a significant percentage (30%) were heterozygous for *MTL* (13 α). A similar screen of 97 Rad52⁻ Sou⁺ strains yielded values of 83% (50% α and 33%

α) and 17% respectively (Table 4). As expected, strains carrying *MTLa1* also carried *MTLa2* and cells lacking *MTLa1* also lacked *MTLa2* (not shown). In summary, most *Sou*⁺ cells are homozygous for the *MTL* locus but a significant percentage (30% for CAF2 and 17% for *rad52-ΔΔ*) are still heterozygous. Curiously, our results indicate a bias towards the generation of *MTL* α strains (28 out of 30) versus *MTL* *a* (2 out of 30) in the CAF2 *MTL*-homozygous derivatives. Recently, it has been reported that the size of the MRS influences the frequency of chromosome loss. For instance, with regard to Chr5, the copy with the largest MRS was preferentially lost (Lephart *et al.*, 2005). We do not know whether this MRS-based bias operates in our case, since the size of MRS alleles of chromosome 5 from strain CAF2 has not been reported. A similar preference for the loss of the *MTLa* allele recently has been observed by Wu *et al.* (2005) during the spontaneous generation of sixteen *MTL*-homozygous offspring from four natural *a/a* strains. This may indicate that there are deleterious, not necessarily lethal, alleles in the *a* copy of Chr5 from CAF2. However, the distribution between *a* and α strains was 50% and 33% in the *Sou*⁺ derivatives of the *rad52-ΔΔ* strain. The same was true for CAI4, where *MTL* *a*- and *MTLa*-*Sou*⁺ strains were recovered at a similar frequency (Lephart *et al.*, 2005). Therefore, it is likely that during the generation of strain CAI4 from CAF2, putative deleterious alleles have become homozygous and have conserved the homozygosity in the CA4-derived *rad52-ΔΔ* mutants.

3.3. Karyotypes and SNP analysis of *Sou*⁺ strains homozygous for the *MTL* locus

The lack of either *MTL* locus could result not only from the loss of the entire Chr5, but also from any deletion that includes that region. Conversely, the presence of both *MTL* loci does not guarantee the presence of two full copies of Chr5. A visual inspection of the karyotypes (Fig. 2Ba) of parental CAF2 (*Sou*⁻) (lane 1) and two *Sou*⁺ derived strains homozygous for the *MTL* locus (lanes 2-*MTL* *a*- and 3-*MTLa*-) suggests that whereas the former carries two copies of Chr5, most of the latter seemed to have lost one copy of that chromosome. The same was true for parental *rad52-ΔΔ* (*a/a*) (lane 5) and four *rad52-ΔΔ* *Sou*⁺ strains homozygous for the *MTL* locus (two *MTLa* and two *MTLa*) (lanes 6-9). Quantification of the ethidium bromide signal using chromosome 2 as an internal standard confirmed these suspicions. The Chr5/Chr2 ratio was about 1 in both parental CAF2 and *rad52-ΔΔ* *Sou*⁻ strains but dropped to one half in all the *MTL* homozygous derivatives, with the exception of the strain *r-Sou*⁺2 (lane 7). An analysis of a SNP marker of Chr2 (SNP68) indicated that all the strains were heterozygous for that marker and yielded the same amount of each base at each polymorphic site (data not shown). Since the relative ethidium bromide signal of Chr2 looked normal, these results suggest that all the strains were in fact disomic for Chr2 and support our approach in determining the copy number of Chr5. In two *rad52-ΔΔ* *Sou*⁺ strains, an additional extrachromosomal band was also observed (lanes 6 and 9 of Fig. 2Ba, and 2Ca, black arrows). Southern analysis of the electrophoretic karyotypes indicated that a probe of the *Sfi*I 5I fragment (*CDC1*) only labelled Chr5 (Fig. 2Bc). Similarly, a probe of the *Sfi*I 5M fragment (*GCD11*) only labelled Chr5 in all the *MTL* homozygous strains (Fig. 2Bb and 2Cb). None of these probes labelled any of the new chromosomal bands (Fig 2B and 2C, lanes 6 and 9), suggesting that they originated by a break of chromosomes other than Chr5. On the other hand, the relative intensity of the labelled band corresponding to Chr5 was consistent with the presence of two copies of this chromosome in both parental strains (Fig. 2Bb and 2Bc; lanes 1 and 5) and one copy in each of the *MTL* homozygous strains, again with the exception of strain *r-Sou*⁺2 (lane 7), suggesting that this strain likely had duplicated Chr5. Finally, in order to be sure that the homozygosity of the *MTL* locus extends to other regions of Chr5, we analyzed two intergenic regions of this chromosome located at both sides of the centromere known to be polymorphic in strain SC5314 because of the presence of several SNPs (Forche *et al.*, 2004; A. Forche, personal communication; Sanyal *et al.*, 2004): one in the left arm, *Sfi*I fragment 5M, (SNP110 in contig 19-10198), and the other in right arm, *Sfi*I fragment 5I (SNP118 in contig 19-10194) (Fig. 2, bottom). Both regions conserved the heterozygosities in parental *Sou*⁻ CAF2 and *rad52-ΔΔ*

strains but became homozygous in the Sou⁺ derivatives that were homozygous for the *MTL* locus. Moreover, *a*-strains carried one allele and *α* strains the other allele (Table 5). Accordingly, in strains homozygous for the *MTL* locus, whose proportions were similar for CAF2 (70%) and *rad52*-ΔΔ (83%), most if not all Sou⁺ strains arise by chromosome loss.

3.4. Karyotypes and SNP marker analysis of Sou⁺ *rad52*-Δ strains heterozygous for *MTL* but exhibiting chromosome fragmentation

The presence of both *MTL* loci in some Sou⁺ strains suggests the existence of at least one additional genetic alteration responsible for sorbose utilization. The electrophoretic karyotypes indicated the presence of at least two extrabands in strain *r*-Sou⁺5 (Fig. 2Ca, lane 10, white arrows) and one extraband in strain *r*-Sou⁺6 (Fig. 2Ba). A Southern blot using the *GCD11* probe labelled Chr5 (1.190 kb) plus another extrachromosomal band slightly smaller than Chr7 (950 kb) in strain *r*-Sou⁺5 (Fig. 2Bb and 2Cb, lanes 10, lower white arrows). Similarly, the same probe labelled Chr5 and the extraband of strain *r*-Sou⁺6 but, in this case, the missing fragment was significantly larger (Fig. 2Bb, lane 11; this band was lost in the gel of Fig 2Cb, due to its small size). By contrast, the *CDC1* probe did not label any of the extrachromosomal bands (Fig. 2Bc), and the same was true when the karyotypes were probed with *ILV3*, a subtelomeric ORF telomere proximal to *CDC1*, and *HTS11*, the closest ORF to the right telomere (apart from *CTA2* that is common to fourteen out of the sixteen telomeres in *C. albicans*). In agreement with this, SNP analysis indicated that both strains were heterozygous for SNP110 (5M) but homozygous for SNP118 (5I) (Table 5). The presence of markers from both arms (SNP110 and *GCD11*) indicates that the fragments retain the centromere. However, the absence of three subtelomeric markers of the *Sfi*I fragment 5I (*CDC1*, *ILV3*, and *HTS1*) as well as the homozygosity at a polymorphic region located in the right arm (SNP118) suggest that a fragment in the neighbourhood of the right telomere had been lost (acentric fragment). Furthermore, from the sequence found in SNP118, it follows that in both strains the deletion occurred in the homologue carrying the *MTL* *a* allele (Table 5). Whether specific deletions in this region of the Chr5 homologue carrying *MTL* *a* facilitate sorbose assimilation more than deletions of its counterpart carrying *MTL* *α* will require statistical analysis. Regardless of the significance of this bias, our results suggest that the subtelomeric region of the 5I fragment is a likely candidate to carry one or more ORFs with special relevance to the mechanism of adaptation to sorbose. From the size of the extrabands derived from Chr5 (lane 10 of Fig. 2Bb and 2Cb, and lane 11 from Fig 2Bb), it follows that the lost fragment had a size of about 250 kb in strain *r*-Sou⁺5 (lane 10) and close to the size of the whole right arm of that chromosome in the strain *r*-Sou⁺6 (lane 11). This result agrees with and further supports the recent finding that a terminal deletion of Chr5 that eliminated 356 kb of the right arm caused a Sou⁺ phenotype. Within this stretch, a 209 kb fragment was shown to contain at least five regions which are critical for the Sou⁺ phenotype since deletion of one copy of certain combinations of the regions had the same effect than the loss of the entire chromosome (Kabir *et al.*, 2005). While we have not detected this kind of arrangement of Chr5 in 15 Sou⁺ mutants derived from CAF2, it might occur, although rarely, in Rad52⁺ strains. In fact, Rustchenko (2006) has pointed out that 2 out of 59 Sou⁺ mutants derived from the wild type strain 3153A carry a large deletion in the right arm of chromosome 5.

We have not identified the nature of the genetic alteration responsible for the shortening of one of the Chr5 homologues in some Sou⁺ strains. Two possibilities exist: chromosome fragmentation followed by healing or internal deletion. We favour chromosome fragmentation followed by the addition of a telomere because of both the homozygosity at SNP118 and the absence of *CDC1*, *ILV3* and, especially, *HTS1* (the closest ORF to the right telomere, except *CTA2*) (Fig. 2, bottom) in both extra bands derived from Chr5. We cannot rule out that subtelomeric regions are prone to be involved in large deletions in *C. albicans*. However, it is

unlikely that these processes, which usually rely in recombination between direct repeats, occur or are frequent in the absence of Rad52 (Paques and Haber, 1999).

It should be noted that chromosomal separation of the shorter chromosomes indicated the presence of an additional extraband of slightly lower size than Chr5 (Fig. 2C a, lane 10, upper white arrow; see below) in strain *Sor5-rad52*. However, neither the *GCD11* nor the *CDC1* probes hybridized to that band (Fig. 2Cb, lane 10), indicating that is unrelated to Chr5.

3.5. Karyotypes and SNP analysis of the *Sou*⁺ strains heterozygous for MTL carrying two, apparently normal, copies of Chr5

In the case of the *Sou*⁺ strain derived from CAF2 (Fig 2Ba, lane 4) there are apparently two copies of Chr 5 (see above), but the intensity of Chr4 also increased, suggesting the presence of at least three copies of this chromosome and accordingly of *SOU1*. Although this situation has not been described before, it would fit in the proposal that *SOU1* expression is down regulated by a repressor present in Chr5 (Janbon *et al.*, 1998; Greenberg *et al.*, 2005). The expression of *SOU1* is insufficient for growth on sorbose normally but could become sufficient from monosomy of Chr 5 or trisomy of Chr4. However, a restriction analysis of a heterozygous locus (*RBT7*) located in that chromosome indicated that the relative amount of the restriction fragments yielded by this strain was indistinguishable from that yielded by both parental CAF2 and *rad52-ΔΔ*, as well as by the rest of the *Sou*⁺ derivatives of either strain analyzed (Fig. 3A). These data suggest that either both homologues of chromosome 4 had duplicated or the increase in the intensity of that chromosome was due to the co-migration of a fragment derived from a larger chromosome. Hybridization of the electrophoretic karyotypes to a probe of chromosome 4 (*RAD17*) supported the first possibility since the intensity of the label was significantly higher than that found for the rest of the strains including CAF2 (Fig. 3Bb, lanes 3 and 4, compare the intensities of the label in Chr4 with those of the label in Chr5 in Fig. 2Bb or 2Bc).

In an effort to identify additional causes for the *Sou*⁺ phenotype of *a/α* strains displaying apparently regular karyotypic profiles, we obtained and analyzed new strains with these characteristics derived from either CAF2 or *rad52-ΔΔ*. As shown in Fig. 4A, the karyotypes of the new strains did not show significant alterations, and probes derived from Chr5 fragments 5I (*CDC1*) and 5M (*GCD11*) only labelled Chr5 (Fig. 4B). Electrokaryotypes intended to separate derivatives of Chr5 carrying small deletions (Legrand *et al.*, 2004; Selmecki *et al.*, 2005), did not indicate alterations in this chromosome either (not shown). As expected, both homologues of Chr6 and Chr7 could be distinguished under these conditions. Also, both homologues of Chr6 became closer in the *rad52-ΔΔ Sou*⁺ derivatives (not shown), but this also occurred in parental *rad52-ΔΔ Sou*⁻ strains (see Fig. 2Ca).

In order to investigate if the *Sou*⁺ phenotype of these strains could result from LOH in a region of Chr5, we analyzed the SNP118 marker (which had been deleted in one of the truncated copies of Chr5 of the *rad52-ΔΔ Sou*⁺ strains 5 and 6 shown in Fig. 2), in two additional strains (*rad52-ΔΔ Sou*⁺ 11 and 12 of Fig. 4, lanes 8 and 9). In both strains, this marker conserved the heterozygosities present in the parental CAF2. Accordingly, it is likely that, as shown before for other *rad52-ΔΔ Sou*⁺ strains (Fig. 2), the additional strains also carry truncations/translocations in other chromosomes. Supporting this possibility, a Southern blot of the karyotypes probed with a fragment of *ACT1*, located in Chr1, labelled not only this chromosome but also a truncated derivative that co-migrated with Chr3 in strain *r-Sou*⁺7 (Fig. 4C, lane 4).

4. Conclusions

rad52-ΔΔ cells of *C. albicans* produce Sou⁺ mutants earlier than the parental strain CAF2 but the total yield is significantly lower. A likely possibility is that this behaviour derives from an increased propensity of *rad52-ΔΔ* strains to lose chromosomes indiscriminately.

Fragmentation of chromosomes is quite frequent in *rad52-ΔΔ* strains of *C. albicans* when plated on sorbose plates, since four out of six *rad52-ΔΔ* Sou⁺ derivatives analyzed in some detail (Fig. 2, lanes 6, 9 10, and 11) showed at least one extrachromosomal band. By contrast, we could not detect obvious signs of fragmentation in 15 independent Sou⁺ strains derived from CAF2 (not shown; examples are given in Figs 2 and 4). In two cases, in which fragmentation caused the loss of the fragment ≥ 250 kb close to the right telomere, cells grew on sorbose. This supports previous findings on the importance of this region in the adaptation to sorbose (Kabir *et al.*, 2005). As suggested by the analysis of two chromosomal fragments derived from Chr5, each in a different Sou⁺ strain, broken chromosomes seem to be stabilized by the addition of telomere.

In addition to alterations in the number or the length of one copy of chromosome 5, there exist alternative mechanisms of adaptation to sorbose which are not infrequent in the SC5414 lineage.

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References

- Andaluz E, Ciudad T, Gómez-Raja J, Calderone R, Larriba G. Rad52 depletion in *Candida albicans* triggers both the DNA-damage checkpoint and filamentation accompanied but independent of expression of hypha specific genes. *Mol. Microbiology* 2006;59:1452–1472.
- Andaluz E, Ciudad T, Larriba G. An evaluation of the role of *LIG4* in genomic instability and adaptive mutagenesis in *Candida albicans*. *FEMS Yeast Research* 2002;2:341–349. [PubMed: 12702284]
- Bennett RJ, Johnson AD. Completion of a parasexual cycle in *Candida albicans* by induced chromosome loss in tetraploid strains. *EMBO J* 2003;22:2505–2515. [PubMed: 12743044]
- Bennett RJ, Miller MG, Chua PR, Maxon ME, Jonhson AD. Nuclear fusion occurs during mating in *Candida albicans* and is dependent on the *KAR3* gene. *Mol. Microbiol* 2005;55:1046–1059. [PubMed: 15686553]
- Chauhan N, Ciudad T, Rodríguez-Alejandre A, Larriba G, Calderone R, Andaluz E. Virulence and karyotype analyses of *rad52* mutants of *Candida albicans*: regeneration of a truncated chromosome of a reintegrant strain (*rad52/RAD52*) in the host. *Infect. Immun* 2005;73:8069–8078. [PubMed: 16299301]
- Ciudad T, Andaluz E, Steinberg-Neifach O, Lue N, Gow N, Calderone R, Larriba G. Homologous recombination in *Candida albicans*: role of CaRad52p in DNA repair, integration of linear DNA fragments and telomere length. *Mol. Microbiol* 2004;53:1177–1194. [PubMed: 15306020]
- Fonzi WA, Irwin MY. Isogenic strain construction and gene mapping in *Candida albicans*. *Genetics* 1993;134:717–728. [PubMed: 8349105]
- Forche A, Magee PT, Magee BB, May G. Genome-wide single-nucleotide polymorphism map of *Candida albicans*. *Eukaryotic Cell* 2004;3:705–714. [PubMed: 15189991]
- Forche A, May G, Magee PT. Demonstration of loss of heterozygosity by single-nucleotide polymorphism microarray analysis and alterations in strain morphology in *Candida albicans* strains during infection. *Eukaryot. Cell* 2005;4:156–165. [PubMed: 15643071]

- Gillum AM, Tsay EY, Kirsch DR. Isolation of the *Candida albicans* gene for orotidine-5'-phosphate decarboxylase by complementation of *S. cerevisiae* ura3 and *E. coli* pyF mutations. *Mol. Gen. Genetics* 1984;198:179–182.
- Greenberg JR, Price NP, Oliver RP, Sherman F, Rustchenko E. *Candida albicans* *SOU1* encodes a sorbose reductase required for L-sorbose utilization. *Yeast* 2005;22:957–969. [PubMed: 16134116] Erratum in: *Yeast* 22,1171.
- Hiraoka M, Watanabe K, Umezaki K, Maki H. Spontaneous loss of heterozygosity in diploid *Saccharomyces cerevisiae*. *Genetics* 2000;156:1531–1548. [PubMed: 11102355]
- Holton NJ, Goodwin TJD, Buttler MI, Poulter RTM. An active retrotransposon in *Candida albicans*. *Nucleic Acid Res* 2001;29:4014–4024. [PubMed: 11574684]
- Hull CM, Johnson AD. Identification of a mating type-like locus in the asexual pathogenic yeast *Candida albicans*. *Science* 1999;285:1271–1275. [PubMed: 10455055]
- Hull CM, Raisner RM, Johnson AD. Evidence for mating of the asexual yeast *Candida albicans*. *Science* 2000;289:307–310. [PubMed: 10894780]
- Janbon G, Sherman F, Rustchenko E. Monosomy of a specific chromosome determines L-sorbose utilization: a novel regulatory mechanism in *Candida albicans*. *Proc. Natl. Acad. Sci* 1998;95:5150–5155. [PubMed: 9560244]
- Janbon G, Sherman F, Rustchenko E. Appearance and properties of L-sorbose-utilizing mutants of *Candida albicans* obtained on a selective plate. *Genetics* 1999;153:653–664. [PubMed: 10511546]
- Kabir MA, Ahmad A, Greenberg IR, Wang Y-K, Rustchenko E. Loss and gain of chromosome 5 controls growth of *Candida albicans* on sorbose due to disperse redundant negative regulators. *Proc. Natl. Acad. Sci. USA* 2005;102:12147–12152. [PubMed: 16099828]
- Klein HL. Spontaneous chromosome loss in *Saccharomyces cerevisiae* is suppressed by DNA damage checkpoint functions. *Genetics* 2001;159:501–1509.
- LarribaGSan BlasGC CalderoneR Genome instability, recombination, and adaptation in *Candida albicans*. *Pathogenic Fungi: host interactions and emerging strategies for control* 2004 Chapter 8 285334 Horizon Press UK
- Legrand M, Lephart P, Forche A, Magee PT, Magee BB. Homozygosity at the *MTL* locus in clinical strains of *Candida albicans* is correlated with karyotypic rearrangements. *Mol. Microbiol* 2004;52:1451–1462. [PubMed: 15165246]
- Lephart PR, Chibana H, Magee PT. Effect of the major repeat sequence on chromosome loss in *Candida albicans*. *Eukaryotic Cell* 2005;4:733–741. [PubMed: 15821133]
- Magee PT Chibana H Calderone R The genomes of *Candida albicans* and other *Candida* species. *Candida and Candidiasis* 2002 293304 American Society for Microbiology Press, Inc. Chapter 21 Washington, D.C.
- Magee BB, Magee PT. Induction of mating in *Candida albicans* by construction of *MTL* a and *MTL* alpha strains. *Science* 2000;289:310–313. [PubMed: 10894781]
- Mortimer RK, Contopoulou R, Schild D. Mitotic chromosome loss in a radiation-sensitive strain of the yeast *Saccharomyces cerevisiae*. *Proc Natl Acad Sci U S A* 1981;78:5778–5782. [PubMed: 7029545]
- Olaiya AF, Sogin SJ. Ploidy determination of *Candida albicans*. *J. Bacteriol* 1979;140:1043–1049. [PubMed: 391796]
- Paques F, Haber JE. Multiple pathways of recombination induced by double-strand breaks in *Saccharomyces cerevisiae*. *Microbiol. Mol. Biol. Rev* 1999;63:349–404. [PubMed: 10357855]
- Perepnikhata V, Fischer FJ, Niimi M, Baker RA, Cannon RD, Wang YK, Sherman F, Rustchenko E. Specific chromosome alterations in fluconazole-resistant mutants of *Candida albicans*. *J. Bacteriol* 1999;181:4041–4049. [PubMed: 10383973]
- Rustad TR, Stevens DA, Pfaller MA, White TC. Homozygosity at the *Candida albicans* *MTL* locus associated with azole resistance. *Microbiology* 2002;148:1061–1072. [PubMed: 11932451]
- Rustchenko E. Chromosome instability in *Candida albicans*. *FEMS Yeast Res.* 2006 (in press).
- Rustchenko E, Howard DH, Sherman F. Chromosomal alterations of *Candida albicans* are associated with the gain and loss of assimilating functions. *J. Bacteriol* 1994;176:3231–3241. [PubMed: 8195078]

- Rustchenko, E.; Sherman, F. Genetic instability in *Candida albicans*. In: Howard, DH., editor. Pathogenic fungi in humans and animals. Marcel Dekker; New York, NY: 2003. p. 723-776.
- Rustchenko-Bulgac EP, Sherman F, Hicks JB. Chromosomal rearrangements associated with morphological mutants provide a means for genetic variation of *Candida albicans*. J. Bacteriol 1990;172:1276–1283. [PubMed: 2407719]
- Sanyal K, Baum M, Carbon J. Centromeric DNA sequences in the pathogenic yeast *Candida albicans* are all different and unique. Proc. Natl. Acad. Sci. USA 2004;101:11374–11379. [PubMed: 15272074]
- Selmecki A, Bergmann S, Berman J. Comparative genome hybridization reveals widespread aneuploidy in *Candida albicans* laboratory strains. Mol. Microbiol 2005;55:1553–1565. [PubMed: 15720560]
- Selmecki A, Forche A, Berman J. Aneuploidy and isochromosome formation in drug-resistant *Candida albicans*. Science 2006;313:367–370. [PubMed: 16857942]
- Tavanti A, Gow NAR, Maiden MCJ, Odds FC, Shaw DJ. Genetic evidence for recombination in *Candida albicans* based in haplotype analysis. Fungal Genet. Biol 2004;41:553–562. [PubMed: 15050544]
- Wang YK, Das B, Huber DH, Wellington M, Kabir MA, Sherman F, Rustchenko E. Role of the 14-3-3 protein on carbon metabolism of the pathogenic yeast *Candida albicans*. Yeast 2004;21:685–702. [PubMed: 15197733]
- Wu W, Pujol C, Lockhart SR, Soll D. Chromosome loss followed by duplication is the major mechanism of spontaneous mating-type homozygosis in *Candida albicans*. Genetics 2005;169:1311–1327. [PubMed: 15654090]
- Yoshida J, Umezu K, Maki H. Positive and negative roles of homologous recombination in the maintenance of genome stability in *Saccharomyces cerevisiae*. Genetics 2003;164:31–46. [PubMed: 12750319]

Fig. 1.

(A) Viability of strains on sorbose plates. About 800 cells of each strain were plated in this experiment. For details see Materials and Methods. (B) Plates corresponding to an experiment similar to that indicated in Table 1 after 20 days of incubation on sorbose plates.

Fig. 2. *MTL* analysis and karyotyping of *Sou*⁺ strains with chromosomal rearrangements

A. PCR analysis of the *MTL* locus in representative *Sou*⁺ strains derived from CAF2 and *rad52*- $\Delta\Delta$. Lanes correspond to parental CAF2 *Sou*⁻ (lane 1); its *Sou*⁺ derivatives C-*Sou*⁺1 to C-*Sou*⁺3 (lane 2 to 4, respectively); parental *rad52*- $\Delta\Delta$ *Sou*⁻ (lane 5); and its derivatives *r*-*Sou*⁺1 to *r*-*Sou*⁺6 (lane 6 to 11 respectively); B and C. Analysis of the standard electrophoretic karyotypes (left) (Ba) or the electrokaryotypes under conditions that separate homologues of the shortest chromosomes (panel Ca) of the strains indicated in A, and corresponding Southern blots (right) using the indicated probes. Arrows indicate obvious extrachromosomal bands. Bottom: Diagram of chromosome 5, showing the approximated location of the several markers or probes used in this study. The centromere is indicated (Sanyal *et al.*, 2004).

Fig. 3. Analysis of copy number and heterozygosities of chromosome 4 in selected *Sou*⁺ strains
 A) *RBT7* analysis of the indicated strains. The control used is a homozygous strain for *RBT7* isolated in our laboratory. B) Southern blot analysis of the *RAD17* locus. Karyotypes are the same of Fig. 2Ba. Lanes are as in Fig. 2.

Fig. 4. Karyotypes of selected Sou⁺ *MTL a/a* strains lacking obvious chromosomal alterations
 A) Standard electrophoretic karyotypes of new *a/a* Sou⁺ strains derived from CAF2 (C-Sou⁺4-6, lanes 1-3) or *rad52-ΔΔ* (r-Sou⁺7-12, lanes 4-9). B and C. Southern blot analysis of the karyotypes with the indicated probes of Chr5 (B) or Chr1 (C).

Table 1*C. albicans* strains used in this study

Strain	Relevant genotype	Parental	Reference
SC5314	Wild type		Gillum <i>et al.</i> , 1984
CAF2	<i>ura3Δ::imm434/URA3</i>	SC5314	Fonzi and Irwin, 1993
TCR1	<i>ura3Δ::imm434/ura3Δ::imm434</i> <i>RAD52/rad52Δ::hisG-URA3-hisG</i>	CAI4-1	Ciudad <i>et al.</i> , 2004
TCR1.1	<i>ura3Δ::imm434/ura3Δ::imm434</i> <i>RAD52/rad52Δ::hisG</i>	TCR1	Ciudad <i>et al.</i> , 2004
TCR2.1	<i>ura3Δ::imm434/ura3Δ::imm434</i> <i>rad52Δ::hisG/rad52Δ::hisG-URA3-hisG</i>	TCR1.1	Ciudad <i>et al.</i> , 2004
TCR2.2	<i>ura3Δ::imm434/ura3Δ::imm434</i> <i>rad52Δ::hisG/rad52Δ::hisG-URA3-hisG</i>	TCR1.1	Ciudad <i>et al.</i> , 2004
TCR3.2.1	<i>ura3Δ::imm434/ura3Δ::imm434</i> <i>rad52Δ::hisG/rad52Δ::RAD52_n-URA3-hisG</i>	TCR2.2	Ciudad <i>et al.</i> , 2004

Table 2Primers used in this study^{a)}

<i>RBT7</i>	F 5'-GAGTGAAAAATAAGTGACGAAC-3' R 5'-TTAGGATGTACCACACTTCATC-3'
<i>ILV3</i>	F 5'-ATGGCTATGGGAAGACACAACAGA-3' R 5'-AACGGCATTAGTAGAACCACCAGT-3'
<i>HST1</i>	F 5'-GCCATCTTCGGGACATTATCGACT-3' R 5'-CCCACATGCTTGGAAGATACCATC-3)
<i>RAD17</i>	F 5'-GTTGGGGGTTGATTTGAGTTTGAT-3' R 5'-ACCCATGCGCGTCTTTGATAA-3'

^{a)}Primers sequences were retrieved from the *C. albicans* genome database. Primers for *RBT7* were obtained from A. Forche.

Table 3
Daily appearance and final yield of Sou⁺ colonies from the indicated strains

Strain ^a	Total cells plated ^b (CFU)	Daily appearance of Sou+ colonies (days) ^c										Sou ⁺ CFUs in the indicated period (days)			% Yield	
		5	6	7	8	9	10	11	12	13	14	15	(5-9)	(10-15)		(5-15)
Str. 1	3310	2	5±1	5±1	5±1	6±2	16±3	24±2	54±14	234±85	340±90	42±3	23	710	723	22
	3120	5±1	17±4	16±1	7±1	6	17±9	27±4	63±9	290±98	288±85	36±11	51	721	772	24
	3558	15±5	24±6	18±2	13±3	9±3	14±2	12±1	3±0	2±0	0	0	79	31	110	3
	3620	4±1	11±3	12±2	10±3	7±3	21±1	20±3	76±15	386±24	353±50	15±2	44	871	915	25
Str. 2	2256	0	0	2	4±1	8±3	170±35	371±58	101±18	4±1	0	0	14	646	660	29
	3102	16±5	8±2	18±7	8±1	10±3	2±1	1	0	0	0	0	60	3	63	2

Strains are indicated in Table 1.

Quota were plated on YAPD to determine the number of viable cells added to sorbose plates

Results are means of CFU from two plates

Table 4Distribution of the Sou⁺ strains with regard to the *MTL* locus

	<i>MTLa</i>	<i>MTLa</i>	<i>MTLa/MTLa</i>
CAF2	2 (4.6%)	28 (65.1%)	13 (30.2%)
<i>rad52-ΔΔ</i> (TCR2.2)	47 (50%)	32 (33%)	16 (17%)

Table 5
Sequences of SNPs 110 and 118 in the indicated strains

	Sequence at the polymorphic sites		
	SNP110 ⁺ *	SNP118 ⁺ *	SNP68
CAF2.	YRY	RKRY	RWYRHYR
CAF2- a1 (C-Sou ⁺ 1)	CGT	AGAC	RWYRHYR
CAF2- α 1 (C-Sou ⁺ 2)	TAC	GTGT	RWYRHYR
CAF2- $\alpha\alpha$ (C-Sou ⁺ 3)	YRY	RKRY	RWYRHYR
<i>rad52</i> - $\Delta\Delta$ Sou ⁻	YRY	RKRY	RWYRHYR
<i>rad52</i> - $\Delta\Delta$ -a1 [<i>r</i> -Sou ⁺ (1-2)]	CGT	AGAC	RWYRHYR
<i>rad52</i> - $\Delta\Delta$ - α 1/ <i>r</i> -Sou ⁺ (3-4)]	TAC	GTGT	RWYRHYR
<i>rad52</i> - $\Delta\Delta$ - $\alpha\alpha$ [<i>r</i> -Sou ⁺ 5 [#]]	YRY	GTGT	RWYRHYR
<i>rad52</i> - $\Delta\Delta$ - $\alpha\alpha$ [<i>r</i> -Sou ⁺ 6 [#]]	YRY	GTGT	RWYRHYR
<i>rad52</i> - $\Delta\Delta$ - $\alpha\alpha$ [<i>r</i> -Sou ⁺ (7-12)]	YRY	RKRY	RWYRHYR

⁺ Y denotes C or T; R denotes A or G; K denotes G or T

* Only the polymorphic sites are shown. The rest of the sequence agrees with that present in the Candida database.

[#] These strains contain fragmented chromosomes and correspond to lanes 10 (Sou⁺5) and 11(Sou⁺6) of Fig. 2.