

Electrophoretic Karyotypes and Chromosome Numbers in *Candida* Species

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The electrophoretic karyotypes of five *Candida albicans* isolates and of five other *Candida* species have been determined, using orthogonal field alternating gel electrophoresis (OFAGE). None of the *C. albicans* isolates had the same electrophoretic karyotype. By comparing all five strains, we arrived at a chromosome number of nine to ten, but since the organism is diploid, we cannot distinguish genetically different chromosomes from homologues which resolve. We determined minimal chromosome numbers of 9 for *Candida stellatoidea*, 10 for *C. glabrata* and 6 for *C. guilliermondii*.

INTRODUCTION

Candida albicans is a dimorphic fungus which is the single most important fungal human pathogen (Odds, 1979). Its diploid nature (Whelan *et al.*, 1980), combined with its lack of a sexual cycle, makes it a difficult subject for genetic analysis. Nevertheless, parasexual analysis, mediated by spheroplast fusion, has led to the accumulation of a reasonable amount of information about the genetics of the organism. A number of alleles of various auxotrophic markers have been isolated (Kakar *et al.*, 1983; Poulter *et al.*, 1981; Sarachek *et al.*, 1981) and five linkage groups have been identified (Poulter & Hanrahan, 1983; Poulter *et al.*, 1981; Sarachek *et al.*, 1981; Crandall, 1982; Kakar *et al.*, 1983) using mitotic recombination and chromosome loss. The absence of meiotic segregation and the lack of cytogenetics has made assignment of the various linkage groups to chromosomes very difficult. Recently, the technique of orthogonal field alternating gel electrophoresis (OFAGE) has been developed to separate very large pieces of DNA (Carle & Olson, 1984; Schwartz & Cantor, 1982). This technique has allowed the visualization of 16 chromosomes in *Saccharomyces cerevisiae*, a number which corresponds to the genetically determined number of centromeres (Carle & Olson, 1985). We have adapted this technique to separate the chromosomes of various *Candida* species, including several strains of *C. albicans*.

METHODS

Yeast strains. Strains used in these experiments included *Sacch. cerevisiae* strains BB4 (*MATa leu2, his3, arg4*), and W66-8a (homothallic, *ura3, trp5, ade2, met4, leu1*).

C. albicans strains were clinical isolates: FC18 (Whelan *et al.*, 1980), NUM63 (T. Suzuki, Nagoya University Medical School), SG483 (a derivative of ATCC 1026), 1840 (M. Gurwith, this Department) and SC5314 (Myra Kurtz, The Squibb Institute for Medical Research).

Representative strains of Candida glabrata (81/011), *Candida tropicalis* (81/019) and *Candida parapsilosis* (81/042) were provided by Dr Frank Odds (University of Leicester, Leicester, UK). Dr Alvin Rogers of this department supplied *Candida stellatoidea* CP5Am5. A strain of *C. guilliermondii*, NUM20, came from Nagoya University and was provided by T. Suzuki. *Schizosaccharomyces pombe* (*h⁻ leu1-32*) came from Dr P. Nurse.

Abbreviation: OFAGE, orthogonal field alternating gel electrophoresis.

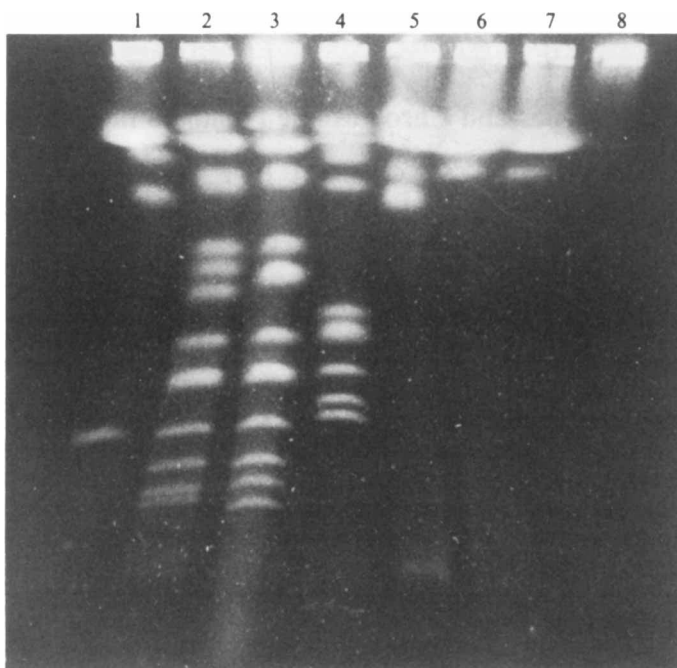


Fig. 1. DNA of various yeast strains was run on a 1.5% agarose gel, at a 60 s pulse rate at 300 V for 21 h. Lane 1, *Candida guilliermondii* NUM20; lane 2, *Sacch. cerevisiae* BB4; lane 3, *Sacch. cerevisiae* W66-8a; lane 4, *C. glabrata* 81/011; lane 5, *C. parapsilosis* 81/042; lane 6, *Candida albicans* FC18; lane 7, *C. tropicalis* 81/019; lane 8, *Schizosacch. pombe* $h^- leu1-32$.

OFAGE. Chromosomes were prepared by a modification described by Carle & Olson (1985) of the embedded-agarose procedure of Schwartz & Cantor (1982). Agarose gels (60 ml) were run on an OFAGE apparatus made by PlasLabs, Lansing, Mich., USA, according to the specifications of Carle & Olson (1984). The 1.5% (w/v) gels were run for 21 h at 300 V, 60 s switching time and the 1.2% and 1.0% gels for 21 h at 300 V, 70 s switching time. Gels were stained with 0.5 μg ethidium bromide ml^{-1} for 15 min and destained for 30 min or more.

RESULTS

Yeasts of the genus Candida show highly varied electrophoretic karyotypes

The OFAGE technique can be modified empirically by varying the pulse rate and the agarose gel concentration to allow the separation of bigger or smaller DNA molecules. We decided to get some estimate of the size of the chromosomes of several *Candida* species by comparing them to the well-studied *Sacch. cerevisiae* electrophoretic karyotype. Fig. 1 shows that with the exception of *C. glabrata*, all the *Candida* species examined had a majority of chromosomes bigger than band 10 of *Sacch. cerevisiae*. It was therefore necessary to modify the protocol described by Carle & Olson (1985). After several trials, we decided to use a 70 s pulse time and 1.2% agarose. This technique gave the results shown in Fig. 2 for several *Candida* species. *C. guilliermondii* is haploid, and it has five resolvable bands on the run shown in Fig. 2(a), lane 1. However, Fig. 2(b) shows another run in which six bands are resolvable from this organism. We conclude that six is a more plausible chromosome number than 5. *C. glabrata* (Fig. 2a, lane 2) shows 10 resolvable bands, with the fourth one up clearly a doublet. Available evidence indicates that this organism is haploid; we can thus assign a chromosome number of at least 11 to this yeast. *C. stellatoidea* (lane 3) has eight bands. Once again, band 3 appears to be a doublet, so that we arrive at a chromosome number of nine. The pattern of *C. stellatoidea* should be compared with those of *C. albicans* strains 1840 in lane 4 and SC5314 in lane 5, which have very different karyotypes from each other and from *C. stellatoidea*. Different *C. albicans* isolates vary greatly in their

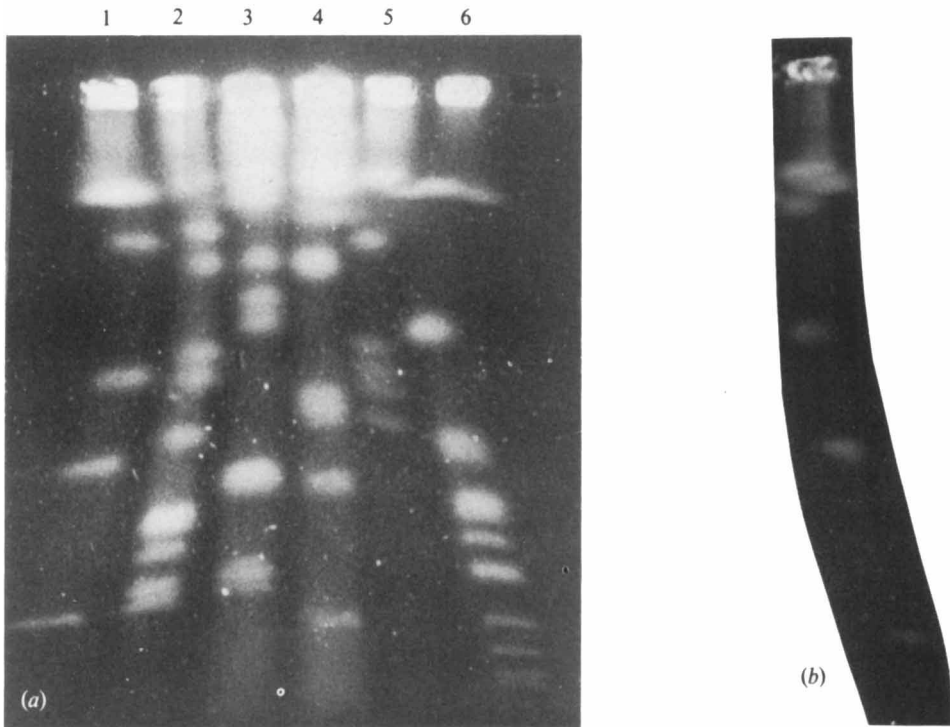


Fig. 2. (a) Electrophoretic karyotypes of several *Candida* strains. A 1.2% agarose gel was run at a 70 s pulse rate at 300 V for 21 h. Lane 1, *C. guilliermondii* NUM20; lane 2, *C. glabrata* 81/011; lane 3, *C. stellatoidea* CP5Am5; lane 4, *C. albicans* 1840; lane 5, *C. albicans* SC5314; lane 6, *Sacch. cerevisiae* BB4. (b) *C. guilliermondii* NUM20 in a different run (conditions as above).

electrophoretic karyotypes (see below), but *C. stellatoidea*, which is thought to be closely related to, if not a subspecies of, *C. albicans*, appears quite different from the general pattern. Our estimate of the number of chromosomes in the two species, however, is the same (see below). Lane 6 shows the pattern of *Sacch. cerevisiae*.

C. albicans strains show polymorphism in chromosome band size

Since *C. albicans* has no sexual cycle, the grouping of organisms into this species has been traditionally based upon morphological and nutritional characteristics. We have recently found, using restriction fragment length polymorphisms, that *C. albicans* does indeed appear to be a genetically homogeneous class for the most part (T. D'Souza, B. B. Magee & P. T. Magee, unpublished). We therefore examined the karyotypes of a number of *C. albicans* isolates. Fig. 3(a) shows the electrophoretic karyotypes of four *C. albicans* isolates, including one from Japan. The figure shows that the *C. albicans* karyotype is quite variable, at least so far as the size of the chromosomes is concerned. Lanes 1 and 2 show FC18, a strain which has been used extensively in this and other laboratories for genetic analysis. FC18 has two bright bands in the lower part of the gel where SC5314 has four (lanes 3 and 4) less bright ones; we infer that these two bands in FC18 are doublets and the FC18 has four chromosomes in this size range. Band 3 of FC18 (equivalent to band 5 of SC5314) migrates more slowly, and both strains appear to have two bands in the relatively poorly resolved upper region of the gel. Lanes 5 and 6 show the electrophoretic karyotype of NUM63; this organism contains one very bright, broad band in the fast-moving region. This band is inferred to contain the four bands seen in SC5314. There is one band in the region of band 5, migrating more rapidly than in either FC18 or SC5314. Above this band, the slowly moving region is resolved into four bands. Lanes 7 and 8 show SG483; this

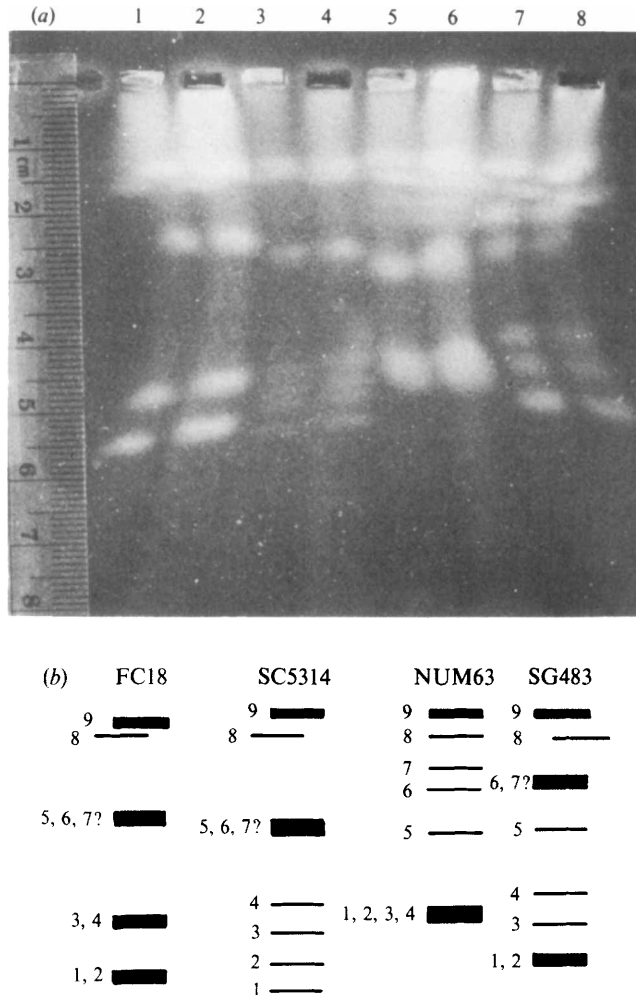


Fig. 3. (a) DNA from four strains of *C. albicans* was loaded on a 1% agarose gel, which was run at a 70 s pulse rate at 300 V for 21 h. Lanes 1 and 2 FC18; lanes 3 and 4 SC5314; lanes 5 and 6 NUM63; lanes 7 and 8 SG483. (b) A schematic representation of the gel in (a).

strain gives three bands below band 5 and three above it. We can thus, using all these strains, count nine chromosomal bands from *C. albicans*. Fig. 3(b) shows a schematic representation of the gel, with the bands numbered. The interpretation of which chromosomal bands are unresolved in some strains is, of course, tentative and will be discussed further below.

DISCUSSION

The results presented in this paper indicate that OFAGE is a useful technique for determining the electrophoretic karyotypes of a number of medically important yeasts. Of the six *Candida* species we have studied, five have, in general, very large chromosomes. *C. guilliermondii* has one of its six chromosomes in the size range of most *Sacch. cerevisiae* bands, while *C. glabrata* has a karyotype with a distribution not too different from *Sacch. cerevisiae*. In keeping with the taxonomic distinction among these species, the electrophoretic karyotypes are quite distinct.

We have not conducted a survey of multiple representatives of most of these organisms, so we cannot comment on possible polymorphisms in the karyotypes, but if the variation from strain to strain in most *Candida* species is not greater than it is in *C. albicans*, OFAGE may be a rapid and easy way of identifying clinical isolates.

The genetic studies which have located at least five linkage groups in *C. albicans* make it of considerable interest to determine the chromosome number in this organism and to try to identify physically the chromosomes to which the linkage groups correspond. Our results show that the degree of polymorphism in the electrophoretic karyotypes of *C. albicans* is such that it is important to survey several strains to determine the chromosome number, since single bands in the karyotype of one strain will sometimes resolve in another. This phenomenon has been seen in *Sacch. cerevisiae* by Carle & Olson (1985). It is also important, particularly in these yeasts with very large chromosomes, to experiment with conditions to resolve the material in the uppermost part of the gel. We have achieved enough resolution so that by comparing karyotypes of various strains, we can count nine bands.

Whether this is the definitive chromosome number for *C. albicans* is not clear at this time. There are several points which must be considered in evaluating these results. The first is that the band at the top of the gel is still bright in every case and may conceal two or more unresolved chromosomes. This, of course, would lead to an underestimate of the actual chromosome number. A second consideration is that the organism as usually isolated is diploid; thus, two bands which are resolved in one strain and unresolved in another may be chromosome homologues. Fortunately, *C. albicans* NUM63 is a haploid (or near haploid) isolate (T. Suzuki, B. B. Magee & P. T. Magee, unpublished) and thus one can be reasonably confident that all bands resolved in this organism are non-homologues. The fact that the lower four chromosomes do not resolve in NUM63 allows for the possibility that they represent two pairs of homologues in SC5314, although the extreme brightness of the band in NUM63 does not support that idea. It seems most likely, therefore, that *C. albicans* has at least nine chromosomes. The issue of homologues versus non-homologues can be resolved by blotting with cloned DNA to determine whether any two of the bands contain the same genetic information. It is important to note that these same considerations hold for the electrophoretic karyotypes of any of the normally diploid yeasts.

Fig. 3(b) shows our interpretation of the relationships between the bands in Fig. 3(a). We have tried to make the most plausible assignments, based upon brightness, of all the bands resolved in the various species. Each strain in Fig. 3(a) is an authentic *C. albicans* by the API assimilation test and by rDNA restriction enzyme analysis (T. D'Souza, B. B. Magee & P. T. Magee, unpublished). We have made the assumption that all *C. albicans* will contain the same number of chromosomes. This assumption can be tested by Southern blots, and such experiments are in progress.

These results constitute a significant step toward improving the genetic system in *C. albicans*. We now have available parasexual genetics (Kakar *et al.*, 1983; Poulter *et al.*, 1981; Sarachek *et al.*, 1981) including induced chromosome loss techniques (Hilton *et al.*, 1985), whole cell genetic exchange (Suzuki *et al.*, 1986) and transformation (Kurtz *et al.*, 1986) as genetic tools. The information derived from these systems can now be correlated with electrophoretic bands corresponding to chromosomes; this should greatly facilitate the coordination of information gained from the various systems.

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