Multiple chromosomal and phenotypic changes in spontaneous mutants of *Candida albicans*

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Previous studies have revealed the occurrence of multiple chromosomal alterations among spontaneous colony form mutants and clinical isolates of *Candida albicans*. In this report we show that such karyotype alterations are also seen in spontaneous and induced non-germinative mutants of the fungus. To determine if phenotypic changes other than colony form and microscopic morphology accompanied the rearrangements of the electrophoretic karyotype, we studied the following characteristics of the non-germinative and some of the colony form mutants: formation of pseudohyphae, chlamydospore production, germ tube formation, colony morphology, auxotrophy, growth at various temperatures, and colony morphology and pigment formation on selected media (bismuth sulphite and Phloxine B). We established that phenotypic and karyotypic variability among spontaneous, non-germinative mutants was no different than such variability among spontaneous colony form mutants. Thus, non-germination may represent another phenotypic consequence of genomic instability in *C. albicans*. The variability in different phenotypic attributes that occurred amongst the mutants was not associated with any given karyotype. Moreover, neither the low nor the high phenotypic variabilities observed were explained by the relatively high number of alterations in a limited number of chromosomes.

Introduction

The electrophoretic karyotype of clinical isolates of *Candida albicans* is highly variable (Merz *et al.*, 1988). Initially, such variation was considered to offer reasonable grounds for establishing strain differences that might be used in epidemiological work (Merz, 1990). However, it has become clear that single strains, either fresh isolates or stock cultures, may give rise to mutants with single and multiple alterations in their electrophoretic karyotypes and that the frequency of such spontaneous mutations may be high (Suzuki *et al.*, 1989; Rustchenko-Bulgac *et al.*, 1990; Rustchenko-Bulgac, 1991).

The phenotypes so far associated with multiple chromosomal aberrations in *C. albicans* are morpho-

logical mutants with changes in colony form, microscopic morphology and growth rate (Rustchenko-Bulgac *et al.*, 1990; Rustchenko-Bulgac, 1991), and germination-deficient mutants (Dabrowa *et al.*, 1990*b*). Changes in the growth rate as well as sucrose assimilation accompanying chromosome alterations were also reported in *C. stellatoidea* (Wickes *et al.*, 1991). Previously we reviewed briefly the literature on different phenotypic changes in spontaneous colony morphology mutants of *C. albicans* (Rustchenko-Bulgac *et al.*, 1990) which, one might assume because of recent work (Rustchenko-Bulgac, 1991), probably had altered electrophoretic karyotypes. No systematic study, however, was done to analyse this presumed connection. We therefore set about to determine if phenotypic alterations regularly accompanied chromosomal variability in non-germinative and colony form mutants of *C. albicans*. The phenotypic characters studied included chlamydospore production, pseudohyphal formations, germ tube formation, colony morphology, auxotrophy, growth at various temperatures, colony morphology and pigment formation on selected media.

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Abbreviations: BiGGY agar, bismuth sulphite glucose glycine yeast agar; GPA, glucose peptone agar; LBC medium, Lee, Buckley and Campbell medium; YPD agar, yeast extract peptone dextrose agar.

We observed that the phenotypic variability of individual characters was either very high or very low but that neither high nor low levels of variability corresponded to the level of karyotypic variability. Additionally, no specific pattern of chromosomal alterations was observed which could be associated with any level of phenotypic variability studied. It would appear that some attributes other than those studied, perhaps those more allied to the commensal or potentially pathogenic existence of the opportunistic, zoopathogenic fungus will be found to be more closely related to chromosomal rearrangements of *C. albicans*.

Methods

Strains of *C. albicans*. The origins of the parental strains, the colony form mutants, and the non-germinative mutants studied are given in Table 1. There are two groups of germination-deficient mutants: those that arise spontaneously (A) and those that are derived after mutagenesis with NTG or UV-irradiation (B). A group of germination-competent mutants (group C, Table 1) consisted of the clinical isolate 3153A and its spontaneous morphological mutants ml-m20 (Rustchenko-Bulgac *et al.*, 1990; Rustchenko-Bulgac, 1991).

Isolation of spontaneous non-germinative mutants. Strains 301, 302 and 303 (see Table 1 for original designations) were spontaneous mutants of strain 300 plated on glucose peptone agar (GPA; Buckley *et al.*, 1982). The strain 300-SG was a spontaneous mutant observed because of its small colony size among customary-sized colonies of strain 300 streaked on GPA and incubated at room temperature for 48 h (Howard *et al.*, 1986).

Isolation of induced non-germinative mutants. The NTG mutagenesis was performed by a method described previously (Howard & Dabrowa, 1979). Briefly, 1×10^7 yeast cells of strain 307 were suspended in 4 ml 0.1 M-citrate buffer at pH 5.5 (citric acid, 10.5 g; NaOH, 4.4 g, made up to 500 ml and adjusted to pH 5.5 with 2 M-NaOH) and exposed to 50 mg NTG (ml buffer)⁻¹. The yeast cells were shaken at 37 °C for 85 min; mutagenesis was stopped by passing the suspensions through a Millipore filter and washing the pads several times with distilled water. Mutants were enriched and selected by a germination-filtration method (Cannon, 1986; Howard & Dabrowa, 1979).

The methods used to generate the UV-induced non-germinative mutants have not been published (Table 1). However, the method of UV-irradiation used by the same group in other studies was a standard one (Oblack *et al.*, 1980). Briefly, distilled-water-suspended yeast cells (5×10^6 ml⁻¹) of strain 310 contained in an open Petri dish were exposed to a 15 W germicidal lamp (General Electric; 5.1×10^{-9} J m⁻² output at a distance of 40 cm) for 7 min at room temperature (Oblack *et al.*, 1980).

Isolation of colony form mutants. The colony form mutants arose spontaneously from strain 3153A (Rustchenko-Bulgac *et al.*, 1990) at a frequency of about 1.4%.

Reference strains. Previously analysed strains WO-1 and 3153A of *C. albicans* (Rustchenko-Bulgac *et al.*, 1990; Rustchenko-Bulgac, 1991) served as references for the positions of bands in electrophoretic karyotypes.

Establishing that the non-germinative mutants were related to the indicated parent by hybridization analyses with specific probe Ca3. Southern blot hybridization of total cellular DNA from the mutants

and their parental strains with the *C. albicans*-specific mid-repeat sequence Ca3 (Sadhu *et al.*, 1991) was performed. Genomic DNA was digested with *EcoRI* (New England Biolabs). Fragments were separated on a 0.7% agarose gel and transferred to GeneScreen Plus (Dupont, NEN Research Products). Southern blot hybridization with nick-translated Ca3 was performed according to general protocols.

Maintenance and growth of strains. Because of instabilities of the strains, the special method of maintenance (−70 °C) and growth of strains was used in these studies (Rustchenko-Bulgac, 1991). The recipes for yeast extract peptone dextrose (YPD) medium, GPA and Lee-Buckley-Campbell (LBC) synthetic medium have been published (Sherman *et al.*, 1986; Howard *et al.*, 1986). Bismuth sulphite glucose glycine yeast (BiGGY) agar was prepared in accordance with the manufacturer's instructions (Difco). Phloxine B medium was made by adding 5 mg Phloxine B (ml LBC agar)⁻¹.

Chlamydospore formation. Chlamydospore formation was determined at room temperature on corn meal agar (Difco) supplemented with 1% (v/v) Tween-80. The tests were performed in accordance with standard procedures (Cooper & Silva-Hutner, 1985). Determination of the ability to form pseudohyphae was made on the same medium.

Germ tube formation. The strains to be tested for germ tube formation were grown on GPA at room temperature for 24 h. The cells were harvested, washed in distilled water and inoculated (about 10^6 cells ml⁻¹) into 0.5 ml prewarmed calf serum (HyClone Laboratories) and incubated at 37 °C for 3 h. The percentage of cells with germ tubes was determined microscopically at the end of the incubation period.

Standard conditions for examining colonial morphologies. The protocol for studying individual colonial morphology was described previously (Rustchenko-Bulgac *et al.*, 1990; Rustchenko-Bulgac, 1991).

Growth on BiGGY agar and Phloxine B agar. Strains were plated for individual colonies (10–20 per plate) on BiGGY agar and Phloxine B agar. The inoculated plates were incubated at room temperature. Pigmentation and colony form were read after one week.

Growth at different temperatures. Strains were plated on YPD medium with a replicator and grown at 16, 22, 30, 37, 40 or 45 °C for one week. The differences in growth were recorded as: +, for good growth; ± for intermediate growth; and −, for no growth. Descriptive words for sectors and papillary growth were also employed.

Auxotrophy. Auxotrophic requirements were tested according to the standard procedure (Sherman *et al.*, 1986).

Pulsed-field gel electrophoresis. The protocol for preparing DNA to be separated in pulsed-field gel electrophoresis has been published (Carle & Olson, 1984). Orthogonal-field alteration gel electrophoresis (OFAGE) (Carle & Olson, 1984) was used to resolve *C. albicans* chromosomes. Four different running conditions of OFAGE were applied to improve separations. The first condition consisted of a constant voltage or 7.5 V cm⁻¹, approximately 100 mA at the start of the run, a 180 s pulse time, with 0.5 × Tris/borate-EDTA cooling buffer at 10 °C (Maniatis *et al.*, 1982), 1.3% (w/v) agarose and a 70 h running time. This condition was applied to resolve the shortest bands (or bottom, B, group of chromosomes). The second and third conditions were performed with the same voltage (and current) with a 1% agarose gel and two different pulse times, 160 s during a 24 h run and 250 s during a 39 h run, respectively. Both conditions were applied to the same gel and separated B and middle (M) groups of chromosomes simultaneously. The fourth condition included a constant voltage of 3 V cm⁻¹, approximately 30 mA at the start of the run, a 30-min pulse time, with 1% agarose and a 3–5 d running time,

Table 1. The origin and characteristics of the non-germinative and colony form (morphological) strains of *Candida albicans* used

Mutant group	Strain* (UCLA)	Original designation	Origin of strain	Characteristics†			Reference
				GT	Chl	Aux	
A Spontaneous non-germinative							
	<u>300</u>	3153A	Stock culture‡	+	+	P	Buckley <i>et al.</i> (1982) and Olaiya <i>et al.</i> (1980)
	301	6	Spontaneous mutants of 300‡	—	+	P	Olaiya <i>et al.</i> (1980)
	302	09		—	—	P	
	303	9		—	+	P	
	300-SG	3153-SG		—	+	P	Howard <i>et al.</i> (1986)
B Induced non-germinative							
	<u>307</u>	Dye strain (ATCC 36082)	Clinical isolate	+	+	P	Edwards <i>et al.</i> (1975)
	307D	—	NTG-induced mutant of 307§	—	—	P	Unpublished
	<u>310</u>	CA-2	Clinical isolate	+	+	Lys [—] (leaky)	Oblack <i>et al.</i> (1978)
	311	—	UV-induced	—	—	Lys [—] (leaky)	Unpublished**
	312	—	mutants of 310	—	—	Lys [—] His [—] (leaky)	
C Spontaneous morphological							
	<u>3153A</u>	3153A	Subclone of 3153A (from D. Soll)	+	+	P	Rustchenko-Bulgac (1991)
	m1–m20	—	Spontaneous mutants of 3153A	+	+	P	

* These numbers are the Stock Culture numbers of strains in the UCLA Fungus Collection. The parental strains are underlined.

† Abbreviations: GT, germ tube formation (+ indicates various percentages of cells in populations formed germ tubes, — indicates a complete inability to form germ tube); Chl, chlamydospore production; Aux, auxotrophy (P, prototrophic; Lys[—], lysine auxotroph; His[—], histidine auxotroph).

‡ The parental strain 300 (3153A) and the spontaneous non-germinative mutants (6, 09, 9) were received from S. J. Sogin (Olaiya *et al.*, 1980) but the mutants were originally isolated by Helen Buckley (Buckley *et al.*, 1982).

§ The technique used to derive this mutant was described by Howard & Dabrowa (1979); see Methods.

|| This designation is a duplication of one employed by Mattia *et al.* (1982) and is not the same as the one described by those investigators.

** These mutants were originally derived by Dr Donna Oblack, but results of work with them has not been published previously; see Methods for techniques used to derive the mutants.

providing a good separation of the three longest chromosomes (or top group, T).

Labelling with isotope, hybridization probe and hybridization. Labelling with [α -³²P]dATP by the random priming method, Southern blot hybridizations and preparation of autoradiograms were carried out by standard methods (Maniatis *et al.*, 1982). The ribosomal DNA fragment WOL-25 (M. J. McEachern and others, unpublished data) was used to assign the *C. albicans* long chromosome (VIII by our nomenclature) containing rDNA repeats.

Results

Strains used in this study

We analysed spontaneous and induced non-germinative mutants in comparison with their parental strains and 20 spontaneous morphological mutants of 3153A largely characterized previously for their electrophoretic karyotypes (Rustchenko-Bulgac, 1991). The characteristics of the three groups of mutants studied are given in Table 1. Group A consisted of non-germinative mutants that arose spontaneously from the parental strain 300 [which is a variant of 3153A (see below)]. Non-germinative mutants of the sort represented by 301, 302 and 303 have

been reported to occur at a frequency of 1–2% (Buckley *et al.*, 1982). Strain 300-SG was observed at a single instance amongst 10³ colonies of strain 300 (Howard *et al.*, 1986). Group B consisted of the NTG- and UV-induced non-germinative mutants and their parental strains 307 and 310, respectively. The colony form mutants (group C) arose spontaneously from their parental strain 3153A at a frequency of about 1.4%. Two clinical isolates, 3153A and WO-1 were used as reference strains because of their well analysed chromosomal patterns.

Establishment of the identity of clinical isolates and the fact that the mutants were related to the indicated parents

The mutants and their putative parents were analysed by Southern blot hybridization of total cellular DNA with the *C. albicans*-specific mid-repeat sequence Ca3 (Sadhu *et al.*, 1991). The results established that the germination-deficient mutants had arisen from the parents indicated in Table 1 (data not shown). Parental strains 300, 307

Fig. 1. Chromosomal patterns of spontaneous non-germinative mutants 301, 302, 303, 300-SG, their parental strain, 300, and the predecessor strain, 3153A (+). (a) The shortest chromosomes, bottom group (B), were separated under the first condition (see Methods). The remaining chromosomes are compressed in runs under the first condition. (b) The shortest and middle-sized chromosomes, or middle group (M) separated under the second and third condition on the same gel (see Methods). (c) The longest chromosomes or top group (T) separated under the fourth condition (see Methods). The chromosomes, other than the longest, are compressed into two thick bands. (d) Signals of chromosomes VIII were revealed after hybridization of the filter shown in panel (c) with probe WOL-25 (rDNA).

and 310, were not related, but 300 and 3153A had the same pattern (data not shown).

Electrophoretic karyotypes of the isolates 300, 307 and 310

The chromosomal patterns of all three isolates, 300, 307 and 310, are presented in Figs 1 and 2. The chromosomes of the three strains ran along side those of strain 3153A whose electrophoretic karyotype has been extensively examined with chromosomal markers and shown to have eight pairs of chromosomes of which five homologues ran separately in pairs and three co-migrated (Rustchenko-Bulgac, 1991; see Fig. 2). On this basis, a

direct comparison between related strains 3153A and 300 was possible. We also used 3153A as a reference strain for all parentals because we had compared earlier electrophoretic karyotypes of four clinical isolates including 3153A using chromosomal markers and found that despite certain variability in length, the basic general pattern of chromosomes was preserved (Rustchenko-Bulgac, 1991). As seen in Figs 1–3, all three strains, 300, 307 and 310, had multiple differences in band positions in comparison with 3153A and among themselves.

The differences between 3153A and 300 chromosomal patterns are of special interest. Both strains were considered to be the same (Table 1), but were maintained in different laboratories for some time. The results of

Fig. 2. Chromosomal patterns of induced non-germinative mutants 307D, 311 and 312, and their parental clinical isolates, 307 and 310, respectively. The clinical isolate 3153A (+) was used as a reference strain. Panels (a–d) are as described in the legend to Fig. 1.

testing the identity with the specific probe Ca3 confirmed that they are the same strain. Nevertheless, their electrophoretic karyotypes revealed multiple differences. Both homologues of the most frequently altered chromosome VIII (Rustchenko-Bulgac, 1991; Asakura *et al.*, 1991; Iwaguchi *et al.*, 1992) had different positions (Fig. 1 *c, d*, and see Fig. 3 for nomenclature of chromosomes). Chromosome III, which has been found to be the second most frequently altered chromosome (Rustchenko-Bulgac, 1991) is represented in strain 300 by two homologues of unequal brightness with IIIa positioned differently. Because the brightness of IIIa is lower than expected by comparison with the analogous chromosome in 3153A (the total amount of DNA loaded in both wells, for 3153A and for 300 being very close) we cannot regard this pattern to be of aneuploid origin. Taking into account that changes from separated homologues into co-migrating ones are typical for this chromosome

(Rustchenko-Bulgac *et al.*, 1990; Rustchenko-Bulgac, 1991; also see Fig. 3), one can conclude that this position on the gel indicates the presence of several different karyotypes in the population. One pattern probably contains both IIIa and b co-migrating. Other patterns could be one or other of the following: the homologues distributed in a manner similar to these of 3153A but with IIIa running more slowly; homologues co-migrating in an unusual position, in a manner similar to those of 300-SG (Fig. 3); and, finally, chromosome III is monosomic with only chromosome IIIa present and unusually positioned. Rare instances of spontaneous monosomy have been reported (Rustchenko-Bulgac, 1991). We do not know which of the several possible karyotypes are present in populations of strain 300 and contribute to the pattern of chromosomes displayed. The assumption of a mixed composite karyotype is also supported by the diversity of its colony forms in the

Fig. 3. Schematic representation of the electrophoretic karyotypes of parental strains 300, 307 and 310, and their progenies: spontaneous non-germinative mutants 301, 302, 303, 300-SG; UV-induced non-germinative 307D; and NTG-induced non-germinative 311 and 312, respectively. Parental strains are in boxes. The chromosomal pattern of clinical isolate 3153A is presented as a reference. The assignments of chromosomes from I to VIII and the homologous pairs, a and b, are indicated on the left. Dotted, thin and thick lines correspond, respectively, to one, two or more than two chromosomes, the thickness of the lines being related to the number of chromosomes. Assignments of individual chromosomes with their markers and approximate sizes have been published previously (Rustchenko-Bulgac, 1991). When the intensity of ethidium bromide staining was lower than expected for a single homologue, the corresponding band was marked with an asterisk. Groups of unresolved bands are marked with brackets.

population, an observation that correlates with our earlier finding that different colonial forms from the same population reveal different and altered electrophoretic karyotypes (Rustchenko-Bulgac, 1991). An additional difference in the karyotypes of strains 300 and 3153A is that there is a group of unresolved bands in the area of the homologues of the second chromosome (bottom group) of strain 300 (Fig. 1a), which are well separated in strain 3153A. This situation clearly reflects a number of differently sized but closely running bands which probably represent various alignments of chromosome II in different cells in the population, a situation which is similar to that displayed by highly unstable mutant m500 reported previously (Rustchenko-Bulgac, 1991). Some other differences in the karyotypes of strains 300 and 3153A are shown in the schematic presentation of band separations (Fig. 3). The condition of chromosome III together with a group of unresolved bands around the position of chromosome II represent the composite electrophoretic karyotype of strain 300 and indicates the presence of different karyotypes in the population (i.e. a mixed population). As was reported (Rustchenko-Bulgac, 1991), the customary way of subculturing by picking one subclone (colony) from a plate leads to selection of an altered electro-karyotype as well as colonial phenotype of 3153A. In contrast, maintaining *Candida* strains as populations leads to the proliferation and accumulation of the spontaneous variants with various changes in electrophoretic karyotypes which are the products of only a few unstable cells and thus, any observable chromosomal alterations

escape detection. However maintaining unstable variants of natural isolates, which strain 300 apparently is, would lead to the observation of a mixture of karyotypes.

Electrophoretic karyotypes of spontaneous and induced non-germinative progenies

(i) Spontaneously altered electrophoretic karyotypes of strains 301, 302, 303 and 300-SG

Observation of the spontaneous mutants of 300, 301, 302, 303 and 300-SG, revealed several peculiarities in their karyotypes that reflected components of the collective karyotype of strain 300 (see for example, chromosome II; Figs 1–3). Outstanding differences were found in the banding pattern of 300-SG. Whereas mutants 301, 302 and 303 had bands in slightly different positions, some of the bands of 300-SG occurred in completely different positions. The banding pattern of B and M groups of chromosomes of 300-SG was compared with that of WO-1 (Fig. 4), which is remarkably altered in comparison with other clinical isolates and has multiple translocations (Rustchenko-Bulgac, 1991). Possible hybrid chromosomes in 300-SG similar to those in WO-1 remain to be uncovered by using chromosomal markers (Rustchenko-Bulgac, 1991). Besides the pronounced alterations in both karyotypes, an additional similarity between WO-1 and 300-SG lies in the fact that both strains roughly maintain the range of sizes and a basic structure of the genome which consists of three groups of chromosome sizes (Fig. 3). A faint band in an unusual position under the top group is probably an

Fig. 4. Chromosomal patterns of clinical isolate WO-1, strain 300 and its spontaneous non-germinative mutant 300-SG. B and M group chromosomes were well-separated under the second and third conditions (see Methods), the T group chromosomes are compressed.

indication of a mixed karyotype as suggested above for chromosome IIIa.

The chromosomal patterns of 301, 302 and 303, which have been reported to be haploids (Olaiya *et al.*, 1980) under different designations 6, 09 and 9, respectively (Table 1), resembled typical diploids with each chromosome represented by two homologues as compared to the related reference strain 3153A. Again, this disagreement with the earlier report could be due to the prolonged period of maintenance of the mutants which may have been isolated as haploids (or aneuploids with multiple monosomia) and subsequently restored to the diploid

state during subculture. However, it would be difficult to explain in this way the large number of similarities in chromosomal patterns of 301, 302 and 303 because such similarities could only have resulted from a large number of independent events occurring during diploidization.

(ii) *Induced altered electrophoretic karyotypes of 307-D, 311 and 312*

The electrophoretic karyotypes of all three induced mutants (see Table 1) were altered in many ways from their parental strains 307 and 310 (Figs 2 and 3). If a thick band in the position of chromosome II contains more than three chromosomes, then 307D would be an aneuploid for the $2n+x$ type. Mutant 311 has a weak band (marked with asterisks, in the B group), an indication of a mixed population in which not all cells possess bands in these positions. Also, mutant 312 has some unresolved bands in the middle and bottom groups, a feature of collective karyotypes described previously for the unstable mutant m500 (Rustchenko-Bulgac, 1991) and discussed above for strain 300.

Altered phenotypes of spontaneous morphological mutants and spontaneous and induced non-germinative mutants

(i) *Colonial morphology*

The three parental strains, 300, 307 and 310, had individual colony morphologies under standard conditions. The spontaneous and induced progenies showed altered and individual colonial morphologies in comparison with their parents under the same standard conditions. An example of colonies of one non-germinative mutant from each parental strain, as well as parental forms are shown in Fig. 5. All three parental strains and some of their progenies displayed mixtures of

Fig. 5. Colonial morphologies of clinical isolates 307, 310, 300 (the unstable variant of 3153A) and some selected spontaneous and induced non-germinative progenies. See Table 1 for the relationship between strains.

Table 2. *Summary of colonial morphology on LBC medium*

Each strain has an individual colony appearance or is represented by a mixture of several different and individual forms (see representative colonial forms in Fig. 5). Spontaneous morphological mutants m1–m20 were characterized previously (Rustchenko-Bulgac *et al.*, 1990; Rustchenko-Bulgac, 1991).

Strain no.*	State of colonial population†
<u>3153A</u>	Uniform with small mycelium
<u>300</u>	Mixed
301	Uniform with sectoring
302	Uniform with sectoring
303	Uniform with sectoring
300-SG	Uniform with sectoring
307	Uniform with huge mycelium
307D	Uniform smooth
310	Some slight non-uniformity
311	Mixed
312	Mixed

* Parental strains are underlined. See Table 1 for relationship between strains.

† Uniform, colonies with the same morphological appearance; mixed, colonies of various forms; mycelium, colonies with filamentous edges; sectoring, colonies with areas of morphology different from overall appearance.

different colonial forms (Table 2). Mixed colonial populations usually corresponded to mixed electrophoretic karyotypes, e.g. in parental strain 300 or induced mutants, 311 or 312. Spontaneous mutant 300-SG was initially identified by its small colony size and was described as a slow-growing mutant (Howard *et al.*, 1986), but over time during maintenance at 4 °C in a refrigerator both normal growth rate and colonial size have been restored (Fig. 5).

The colonial forms of spontaneous morphological mutants m1–m20, as well as the uniformity of their populations were reported previously (Rustchenko-Bulgac *et al.*, 1990; Rustchenko-Bulgac, 1991).

(ii) *Germ tube formation and chlamydospore production*

Each of the germination-deficient mutants has retained the non-germination phenotype for many years (Howard *et al.*, 1986). Although each of the non-germinative mutants formed chlamydospores and pseudohyphae when studied initially (unpublished observation), strains 302, 307D, 311 and 312 have subsequently lost the ability to form chlamydospores (Table 1), though they all still form pseudohyphae. All of the colony form mutants (m1–m20) formed germ tubes (although the percentage of yeast cells germinating ranged from 27 to 91 % among the mutants), chlamydospores and pseudohyphae (Table 1).

(iii) *Growth on BiGGY agar and on LBC agar with Phloxine B*

Strains were plated for individual colonies (10–20 per

plate) on BiGGY agar and on LBC agar with Phloxine B. There was a great deal of variation in colonial appearance, not only among the strains but also between the colonies of a single strain. On BiGGY agar variations among strains and between colonies were seen with regard to colour (tan, brown or dark brown), texture (smooth, umbonate or wrinkled) and edge of colony (entire or filamentous). Variations also occurred among strains and between colonies on the Phloxine B-containing medium. Colour ranged from light pink to red, sometimes in a streaked (sectored) pattern. Colony surfaces were variously sized, usually smooth and occasionally umbonate. The edges of the colonies were entire, i.e. filamentation was not seen. The results of testing strains on BiGGY and on LBC agar with Phloxine B are presented in Table 3.

(iv) *Amino-acid deficiency*

All strains were tested for their auxotrophy as described in Methods. Parental strain 310 and its induced progeny, 311, had leaky requirements for lysine. Another induced progeny, 312, had an additional leaky requirement for histidine. The remaining 28 strains (3 parental, 24 spontaneous mutants and 1 induced mutant) were prototrophs.

(v) *Temperature tolerance of non-germinative and morphological mutants*

All the parental strains, 3153A, 300, 307 and 310, grew over a broad temperature range of 16–45 °C (Table 4). Spontaneous non-germinative mutants (Group A) grew almost as well except for two instances of diminished growth at elevated temperatures (301 and 300-SG). Nine of the spontaneous morphological mutants showed no difference from their parental strain, except for slightly diminished growth of three of them (m1, m3 and m11) at 45 °C. The growth of 10 of the morphological mutants was inhibited at 45 °C and some strains also grew poorly at 40 °C. The growth of some morphological mutants was slightly diminished at 16 °C, showing a narrower range of tolerable temperatures than the parental strain. In this respect, they were similar to the induced non-germinative mutant 307D which also grew over a somewhat restricted range of temperatures. The induced mutants 311 and 312 could not grow normally at any of the temperatures and often grew in the form of papillae which indicated mixed populations of cells with mixed responses to growth conditions.

Probing with rDNA

As demonstrated previously, the chromosome that most frequently changed in natural isolates and spontaneous morphological mutants was chromosome VIII, which contains rDNA repeats (Rustchenko-Bulgac, 1991; Asa-

Table 3. Colonial morphology of germination-deficient and morphological mutants, and their parental strains on BiGGY agar and Phloxine B agar

Strain*	Morphology on BiGGY agar		
	Colour	Surface	Edge†
3153A, 300	Brown	Umbonate‡	Entire
m1, m2	Tan to brown§	Umbonate or wrinkled	Entire or filament
m3, m15	Brown	Umbonate	Filament
m4	Brown	Umbonate or wrinkled	Filament
m5, m6, m9, m11, 301, 302, 303, 310	Brown	Umbonate	Entire
m7, m19	Brown	Umbonate or wrinkled	Entire or filament
m8, m17	Brown to dark brown	Umbonate or wrinkled	Entire or filament
m10	Brown	Umbonate to wrinkled	Entire to filament
m12	Dark brown	Wrinkled	Filament
m13	Light tan	Umbonate	Entire
m14	Brown to tan	Papillate to wrinkled	Entire or filament
m16	Brown	Umbonate	Entire or filament
m18	Brown	Wrinkled	Entire
m20	Tan	Umbonate	Entire
300-SG	Dark brown	Umbonate or wrinkled	Entire
307	Tan	Wrinkled	Filament
307D	Tan	Wrinkled	Entire
311	Tan	Smooth	Entire
312	Dark brown	Umbonate	Entire
	Morphology on Phloxine B agar		
	Colour	Surface	Edge†
3153A, 300, 300-SG, 301, 302, 303, 307, 307D, 310	Light pink with dark pink rim	Smooth	Entire
m1, m2, m3, m4, m5, m6, m7, m15, m16, m17	Pink (sector) with dark pink rim	Smooth	Entire
m8, m9, m10, m11	Pink to white with dark pink rim§	Smooth	Entire
m12	Pink to white	Smooth	Entire
m13	Pink	Smooth	Entire
m14	Pink (sector)	Smooth	Entire
m18, m19, m20	Pink with dark pink rim	Smooth	Entire
311	Pink with dark pink rim	Tiny colony smooth	Entire
312	Dark pink with dark pink rim	Tiny colony smooth	Entire

* For the relationship between strains, see Table 1.

† Entire edge indicates no peripheral filamentation.

‡ The umbonate centre was a darker shade of brown than the rest of the colony.

§ Mixture of colonies over the range indicated.

|| Many shades with sectoring.

kura *et al.*, 1991; Iwaguchi *et al.*, 1992). Probing the non-germinative mutants and their parents with rDNA (Figs 1*d* and 2*d*) served to distinguish among bands in group T and revealed a similar situation with regard to chromosome VIII as reported for morphological mu-

tants. Thus, all three parental strains, 300, 307 and 310, had different patterns for chromosome VIII and their progenies had different alterations in chromosomes VIII in comparison with the parental strains and among themselves.

Table 4. Growth of germination-deficient and morphological mutants at different temperatures in 1 week on YPD medium

Group	Strain*	Growth at:†				
		16 °C	22 °C	30 and 37 °C	40 °C	45 °C
A Spontaneous non-germinative						
	<u>300</u>	+	+	+	+	+
	301	+	+	+	+	±
	302	+	+	+	+	+
	303	+	+	+	+	+
	300-SG	+	+	+	±	—
					Opaque‡	
B Induced non-germinative						
	<u>307</u>	+	+	+	+	+
	307D	±	+	+	±	Some pap
					Opaque	
	<u>310</u>	+	+	+	+	+
	311	pap	pap	±	±	Few pap
				big sectors	Opaque	
					white sectors	
	312	pg	±	pg	pap	—
		pap	pap			
C Spontaneous morphological						
	<u>3153A</u> , m4–m6, m9, m15, m17	+	+	+	+	+
	m1, m3, m11	+	+	+	+	±
						pap
	m2	±	+	+	pap	—
	m7, m10, m16	+	+	+	+	pg
	m8, m12	±	+	+	+	—
	m13, m18, m19	+	+	+	pg	—
	m14	±	+	+	f micro.	—
					pap	
	m20	+	+	+	±	—

* See Table 1 for relationship between strains.

† Symbols: +, growth; —, no growth; ±, diminished growth; pg, poor growth; pap, papillae of different sizes instead of confluent growth; f micro. pap, a few microscopic papillae instead of confluent growth; opaque, creamy or grey colour, clearly different from usual white.

‡ The change in colour in our experiment is not necessarily the same as the white–opaque transition described for strain WO-1 (Soll *et al.*, 1991); also the opaque phenotype is induced at an elevated temperature, whereas the white–opaque transition of WO-1 is repressed at 37 °C.

Discussion

Many authors have described spontaneous and induced variations in the form of colonies in laboratory strains and natural isolates of *C. albicans* (for a review, see Odds, 1988). Brown-Thompson (1968) reported an unstable variant that produced high frequencies of different colonial forms. Also, Slutzky *et al.* (1985) reported that five UV-induced colonial variants 'switched' from one phenotype to another at various rates. In addition, a high frequency of reversible switching between white and opaque phenotypes has been studied extensively and reviewed recently (Soll *et al.*, 1991). In a systematic study, Rustchenko-Bulgac demonstrated an association between stable and unstable spontaneous morphological mutants and electrophoretic karyotypes (Rustchenko-Bulgac *et al.*, 1990; Rustchenko-Bulgac, 1991).

A distinct feature of *C. albicans* is that different clinical isolates have various electrophoretic karyotypes, as described by Merz *et al.* (1988) and others (for a review, see Merz, 1990), and investigated in detail for the four clinical isolates, 3153A, WO-1, C9 and FC18 (Rustchenko-Bulgac, 1991). In addition to having varied electrophoretic karyotypes, these four clinical isolates also had distinct colonial morphologies, consistent with the result with spontaneous morphological mutants. We assumed that the variable colonial morphology was due to genetic differences, some of which were reflected in chromosomal patterns. In accordance with this assumption is the present observation that isolates 307 and 310 have different chromosomal patterns and display individual colonial morphologies under our standard conditions (Figs 1–3 and 5). A third strain, 300, is a subclone of clinical isolate 3153A having an altered electrophoretic karyotype. It displays a mixture of several

colonial morphologies in its population (Fig. 5) and its electrophoretic karyotype is collective, reflecting apparently individual alterations of several different karyotypes recovered from populations of its cells (Figs 1 and 3).

In this work, we expanded our previous analyses of chromosomal alterations to include a group of spontaneous and UV- and NTG-induced non-germinative mutants (see Table 1), which have remained stable for more than 12 years despite repeated laboratory manipulations (e.g. Dabrowa *et al.*, 1990a). We initiated a more complete study of the relationship between multiply altered phenotypes and chromosomal changes (see below). Overall, induced and spontaneous non-germinative mutants revealed the following chromosomal patterns: mixed or collective electrophoretic karyotypes (e.g. 311, 312 and possibly 300-SG, Figs 2a, b and 1b, respectively); high variability of chromosome VIII homologue patterns (Figs 1c, d and 2c, d); same lengths of previously deviated homologues, probably duplications of one and the loss of the other (e.g. chromosomes III and VIII in 300-SG, or II in 311, Fig. 1b, d and 1a, respectively); relatively small deletions and insertions which slightly changed the positions of two homologues (e.g. note the pattern of chromosome II in 301, 302 and 303, Fig. 1a, b); and, rarely, some significant changes in positions of bands (300-SG, Figs 1 and 4). The mechanism(s) of chromosomal changes encountered in our work remains unknown. Nevertheless, it is clear that they did not result from reciprocal translocations between ectopically located copies of Ca3, because all the related strains preserved the same hybridization pattern with Ca3 in the *EcoRI* digest (see Results). All electrophoretic karyotypes are schematically summarized in Fig. 3 and analysed in more detail in Results. All of these changes have been observed for the 20 spontaneous morphological mutants, m1–m20, of 3153A included in this study, as well as for clinical isolates (Rustchenko-Bulgac, 1991). The fact that all seven non-germinative mutants showed individually altered colonial forms (Fig. 5) indicates a parallel between the 20 spontaneous colony form mutants and the non-germinative ones. Moreover, strains with mixed colonial populations, 300, 311 and 312 (Table 2), have cumulative, collective karyotypes (Fig. 3). These facts taken together are in agreement with the previous observation that altered karyotype and colonial morphology are associated. The fact that all spontaneous non-germinative mutants displayed altered colonial morphology and had patterns of low and high phenotypic variabilities (see below) indicates that non-germinative mutants represent another phenotypic consequence of genomic instability in *C. albicans*.

The connection between altered colonial form and

altered electrophoretic karyotype is a well-established fact (Rustchenko-Bulgac, 1991). We have also obtained a substantial amount of data on altered microscopic morphology of cells and rates of growth associated with altered karyotypes (Rustchenko-Bulgac *et al.*, 1990; Rustchenko-Bulgac, 1991). To investigate further the dependence of *Candida* phenotypes upon changing chromosomal patterns, we compared in this study several morphological and physiological properties of spontaneous and induced non-germinative mutants as well as spontaneous morphological mutants with their parents.

C. albicans is known to grow as a budding yeast (blastoconidia), as a pseudohyphae of elongated yeast cells, and as a true septate hypha (Meyer *et al.*, 1984; Odds, 1988). The true hyphal form of growth is initiated by germination of the blastoconidia (Dabrowa & Howard, 1983; Szaniszlo *et al.*, 1983) and interest in this germination event centres in the developmental biology of the fungus and in the potential relevance to pathogenicity, though there is no evidence that germ tube formation is an essential virulence factor (Cutler, 1991). A large number of factors are known to control blastoconidial germination by *C. albicans* (Dabrowa & Howard, 1983; Odds, 1988) and clinical isolates are often variable in their response to inductive conditions. For example, proline is a strong inducer of germ tubes, yet in one study the percentage germination, among 15 randomly selected isolates in the presence of proline, ranged from 16–86% (Dabrowa *et al.*, 1976). In the present study the percentage of the spontaneous morphologic mutants (m1–m20) that germinated in the presence of serum varied from 27–91%. These recorded variations are associated with altered electrokaryotypes as is colonial morphology, but there is neither an obvious alliance of a given karyotype with a certain percentage germination (McEachern & Hicks, 1991), nor with a certain type of colonial appearance.

Stable non-germinative isolates whose inability to germinate is not related to the methods employed are fairly uncommon, but have been consistently recorded in the literature (Torosantucci & Cassone, 1983). The frequency of occurrence in one instance was 1–2% (Buckley *et al.*, 1982), a frequency of the same order as that for spontaneous colony form mutants (1.4%; Rustchenko-Bulgac *et al.*, 1990). We believe that in recording the frequency of a given phenotype, one ought to be particular about the parental strain used to obtain the variant. For example, the frequency of recovery of spontaneous mutants from an unstable variant of a clinical isolate can be expected to be much higher, generally speaking, than from the more stable 3153A. Also, the frequency can probably vary from experiment to experiment because of the fact that the strain which is studied is a changing mixture of different phenotypes and

electrokaryotypes of the cells in the population. Indeed, 300-SG was observed in one instance among 10^3 cells of the same parental strain, 300, which, in the laboratory of H. Buckley, produced spontaneous non-germinative mutants with 10-times higher frequency (see above). Interestingly filamentation of a different type, namely pseudohyphae formation, was a very stable feature observed in all of the parental strains and the various mutants derived from them.

A study of the pigmentation and colonial morphology that resulted from cultivation of strains on bismuth sulphite- and Phloxine B-containing media uncovered an overwhelming amount of variability among the strains (Table 3). The growth of the strains at different temperatures revealed that all clinical isolates grew well within a broad range of temperatures and that nine of the spontaneous morphological mutants and the spontaneous non-germinative mutants, 301, 302 and 303, also grew over a broad range of temperatures (Table 4, Groups C and A, respectively). However, 10 of the spontaneous morphological mutants and the NTG-induced non-germinative 307D did not grow at 45 °C and grew poorly at 16 and 40 °C. Induced mutants 311 and 312, which were remarkable for the diversity of their populations of cells as manifested in mixed colonial forms (Fig. 5, Table 2) and collective electrophoretic karyotypes (Figs 2 and 3), grew consistently in sectors and papillae. Moreover, the range of temperature for growth of strains 311 and 312 was much narrower and strain 312 grew better at room temperature (22 °C) than at 37 °C, optimal for *C. albicans*.

In contrast to the experiments discussed above, the tests for chlamydospore formation and auxotrophy did not show appreciable variability. All four non-germinative mutants (302, 307D, 311 and 312) that originally formed chlamydospores lost this ability during laboratory maintenance. Only the UV-induced mutant, 312, revealed any induced auxotrophic requirements. In addition, approximately 80 spontaneous colony morphology mutants checked recently were all prototrophic (Rustchenko-Bulgac, unpublished data). It appears that spontaneous mutations in chlamydospore production and auxotrophic requirements are rare events, despite multiple chromosomal alterations.

The many different combinations of colonial morphologies, textures and colours observed, as well as other highly variable features exceed the number of changes in karyotype among the strains. It is also important to note that these types of variability were not associated with a given karyotype. For example, chromosome III, which is frequently altered (Rustchenko-Bulgac, 1991, and Fig. 3), was nevertheless represented by co-migrating homologues in similar positions amongst non-related strains 300-SG, 307 and 310 and their progeny. However, a

feature which was similar for all of them was not seen. There was no specific change common to all of the altered banding patterns that could be related to the inability to germinate (McEachern & Hicks, 1991), nor was the pattern of spontaneous compared to induced non-germinative karyotypes distinctive. In previous work we probed the spontaneously altered chromosomal patterns with gene *ADE2* and observed that the *Candida* genome can undergo more changes than reflected by electrophoretic karyotype (Rustchenko-Bulgac *et al.*, 1990). The inconsistency between phenotypic and chromosomal variability presented here provides additional support to this observation.

Finally, we wish to conclude that multiple new phenotypes among the spontaneous mutants of *C. albicans* cannot be explained solely through chromosomal aberrations detectable by electrophoretic karyotyping, despite the fact that the phenotypic variation and chromosomal changes occurred in the same population of cells.

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References

- ASAKURA, K., IWAGUCHI, S.-I., HOMMA, M., SUKAI, T., HIGASHIDE, K. & TANAKA, K. (1991). Electrophoretic karyotypes of clinically isolated yeasts of *Candida albicans* and *C. glabrata*. *Journal of General Microbiology* **137**, 2531–2538.
- BROWN-THOMPSON, J. (1968). Variability in *Candida albicans* (Robin Berkout I. Studies on morphology and biochemical activity. *Hereditas* **60**, 355–398.
- BUCKLEY, H. R., PRICE, M. P. & DANEQ-MOORE, L. (1982). Isolation of a variant of *Candida albicans*. *Infection and Immunity* **37**, 1209–1217.
- CANNON, R. R. (1986). Isolation of a mycelial mutant of *Candida albicans*. *Journal of General Microbiology* **132**, 2405–2407.
- CARLE, G. F. & OLSON, M. V. (1984). Separation of chromosomal DNA molecules from yeast by orthogonal-field alternation gel electrophoresis. *Nucleic Acid Research* **12**, 5647–5664.
- COOPER, B. H. & SILVA-HUTNER, M. (1985). Yeast of medical importance. In *Manual of Clinical Microbiology*, pp. 526–541. Edited by E. H. Lennette, A. Balows, W. J. Hausler, Jr & H. J. Shadomy. Washington, DC: American Society for Microbiology.
- CUTLER, J. E. (1991). Putative virulence factors of *Candida albicans*. *Annual Review of Microbiology* **45**, 187–218.
- DABROWA, N. & HOWARD, D. H. (1983). Blastocnidium germination. In *Fungi Pathogenic for Humans and Animals, Part A: Biology*, pp. 525–545. Edited by D. H. Howard. New York: Marcel Dekker.
- DABROWA, N., TAXER, S. S. & HOWARD, D. H. (1976). Germination of *Candida albicans* induced by proline. *Infection and Immunity* **13**, 830–835.
- DABROWA, N., ZEUTHEN, M. L. & HOWARD, D. H. (1990a). Nutritional stress proteins in *Candida albicans*. *Journal of General Microbiology* **136**, 1387–1391.
- DABROWA, N., RUSTCHENKO-BULGAC, E. P., HICKS, J. B. & HOWARD, D. H. (1990b). Karyotype variations among morphological mutants of *Candida albicans*. In *Abstracts of the 3rd Symposium, Topics in*

- Mycology, Mycoses in AIDS Patients, 1989*, P. 13, p. 147. Janssen Pharmaceutica.
- EDWARDS, J. E., JR., MONTGOMERY, J. Z., ISHADA, K., MORRISON, J. O. & GUZE, B. (1975). Experimental hermatogenous endophthalmitis due to *Candida*: species variation in ocular pathogenicity. *Journal of Infectious Diseases* **135**, 294–297.
- HOWARD, D. H. & DABROWA, N. (1979). Mutants of *Arthroderma benhamiae*. *Sabouraudia* **17**, 35–50.
- HOWARD, D. H., ZEUTHEN, M. L. & DABROWA, N. (1986). Phenotypic characteristics of a slow-growing, non-germinative variant of *Candida albicans*. *Journal of General Microbiology* **132**, 2359–2366.
- IWAGUCHI, S.-I., HOMMA, M. & TANAKA, K. (1992). Clonal variation in chromosome size derived from the rDNA cluster region in *Candida albicans*. *Journal of General Microbiology* **138**, 1177–1184.
- MCEACHERN, M. J. & HICKS, J. B. (1991). Dosage of the smallest chromosome affects both the yeast–hyphal transition and the white–opaque transition of WO-1. *Journal of Bacteriology* **173**, 7436–7442.
- MANIATIS, T., FRITSCH, E. F. & SAMBROOK, J. (1982). *Molecular Cloning: A Laboratory Manual*. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press.
- MATTIA, E., CARRUBA, G., ANGIOLELLA, L. & CASSONE, A. (1982). Induction of germ tube formation by *N*-acetyl-D-glucosamine in *Candida albicans*: uptake of inducer and germinative response. *Journal of Bacteriology* **142**, 555–562.
- MERZ, W. G. (1990). *Candida albicans* strain delineation. *Clinical Microbiology Reviews* **3**, 321–334.
- MERZ, W. G., CONNELLY, C. & HEITER, P. (1988). Variation of electrophoretic karyotypes among clinical isolates of *Candida albicans*. *Journal of Clinical Microbiology* **26**, 842–845.
- MEYER, S. A., AHEARN, D. G. & YARROW, D. (1984). Genus 4. *Candida* Berkout. In *The Yeasts, A Taxonomic Study*, pp. 585–844. Edited by N. J. W. Kregar-van Rij. Amsterdam: Elsevier.
- OBLACK, D., SCHWARZ, J. & HOLDER, I. A. (1978). Biochemical examination of sera during systemic *Candida* infection in mice. *Infection and Immunity* **19**, 992–998.
- OBLACK, D., SCHWARZ, J. & HOLDER, I. A. (1980). *In vivo* muscle damage during *Candida* infection. *Sabouraudia* **18**, 21–31.
- ODDS, F. C. (1988). *Candida and Candidosis*. London: Balliere Tindall.
- OLAIYA, A., STEED, J. & SOGIN, S. (1980). Deoxyribonucleic acid-deficient strains of *Candida albicans*. *Journal of Bacteriology* **141**, 1284–1290.
- RUSTCHENKO-BULGAC, E. P. (1991). Variations of *Candida albicans* electrophoretic karyotypes. *Journal of Bacteriology* **173**, 6586–6596.
- RUSTCHENKO-BULGAC, E. P., SHERMAN, F. & HICKS, J. B. (1990). Chromosomal rearrangements associated with morphological mutants provide a means for genetic variation of *Candida albicans*. *Journal of Bacteriology* **172**, 1276–1283.
- SADHU, S., MCEACHERN, M. J., RUSTCHENKO-BULGAC, E. P., SCHMID, J., SOLL, D. R. & HICKS, J. B. (1991). Telomeric and dispersed repeat sequences in *Candida* yeasts and their use in strain identification. *Journal of Bacteriology* **173**, 842–850.
- SHERMAN, F., FINK, G. R. & HICKS, J. B. (1986). *Laboratory Course Manual for Yeast Genetics and Molecular Biology*. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press.
- SLUTSKY, B., BUFFO, J. & SOLL, D. R. (1985). High frequency switching of colony morphology in *Candida albicans*. *Science* **230**, 666–669.
- SLUTSKY, B., STAEBELL, M., PFALLER, M. & SOLL, D. R. (1987). The 'White–opaque transition': a second switching system in *Candida albicans*. *Journal of Bacteriology* **169**, 189–197.
- SOLL, D. R., ANDERSON, J. & BERGEN, M. (1991). The developmental biology of the white–opaque transition in *Candida albicans*. In *Candida albicans, Cellular and Molecular Biology*, pp. 20–45. Edited by R. Prasad. Berlin: Springer-Verlag.
- SUZUKI, T., KOBAYASHI, I., KANBE, T. & TANAKA, K. (1989). High frequency variation in colony morphology and chromosome reorganization in the pathogenic yeast *Candida albicans*. *Journal of General Microbiology* **135**, 425–434.
- SZANISZLO, P. J., JACOBS, C. W. & GEIS, P. A. (1983). Dimorphism: morphological and biochemical aspects. In *Fungi Pathogenic for Humans and Animals, Part A: Biology*, pp. 323–436. Edited by D. H. Howard. New York: Marcel Dekker.
- TOROSANTUCCI, A. & CASSONE, A. (1983). Induction and morphogenesis of chlamydospores in an agerminative variant of *Candida albicans*. *Sabouraudia* **21**, 49–57.
- WICKES, B. L., GOLIN, J. E. & KWON-CHUNG, K. J. (1991). Chromosomal rearrangement in *Candida stellatoidea* results in a positive effect on phenotype. *Infection and Immunity* **59**, 1762–1771.