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Isolation of the *Candida albicans* Histidinol Dehydrogenase (*HIS4*) Gene and Characterization of a Histidine Auxotroph

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Genetic studies were done with *Candida albicans* CBS 562. Various auxotrophs were isolated following mutagenesis with *N*-methyl-*N*′-nitro-*N*-nitrosoguanidine. SAG5 (*his4C*), a stable histidine auxotroph defective in histidinol dehydrogenase activity, was characterized and chosen for further molecular studies. Therefore, the *C. albicans HIS4* gene was isolated. The gene was obtained from a genomic library of the wild-type strain, which was constructed in plasmid YEp24. The *HIS4* gene was isolated by transformation of a *Saccharomyces cerevisiae* strain that carried a *his4* mutation. The isolated *C. albicans* HIS4 gene complemented *S. cerevisiae his4A*, *his4B*, *his4C*, and *his4ABC* mutant strains, which indicates that the clone contains the entire *HIS4* gene. The gene was isolated on plasmid pSTC7, whose physical map was constructed with *Bam*HI, *Sal*I, and *Eco*RV restriction endonucleases, locating the *HIS4* gene on a 14-kilobase-pair DNA fragment. Hybridization experiments with *HIS4* and *C. albicans* genomic DNA showed correspondence between the restriction patterns of the gene with that of the chromosomal DNA, indicating that the gene originates from *C. albicans* and appears in a single copy. Chromosomes of *C. albicans* CBS562 and four other strains were resolved by orthogonal-field alteration gel electrophoresis. The electrokaryotyping results showed heterogeneity in chromosomal sizes. The electrokaryotyping of CBS 562 showed a resolution of six chromosomal bands, three of which seemed to be doublets. The *C. albicans HIS4* gene was located on the largest resolvable chromosome in all of the strains.

Candida albicans, a common opportunistic pathogen in humans, is a dimorphic fungus (29). The clinical importance of *C. albicans* requires the development of a genetic system in this organism for better understanding of its mechanisms of pathogenesis and eventually to develop improved therapies for candidal infections. *C. albicans* has a diploid genome and no sexual cycle, characteristics which hamper genetic studies of the fungus (17). Nevertheless, recent genetic studies of *C. albicans* resulted in the isolation of nutritional auxotrophs (12, 24), determination of complementation groups by parasexual techniques (12, 27, 28, 39), isolation of specific genes (11, 13, 14, 30, 37), construction of expression vectors (16), and electrokaryotyping (21).

Our previous studies focused on attachment of the fungus to host mammalian tissues as an expression of one of the fungal pathogenic determinants (32). Because those studies involved a specific *C. albicans* isolate (CBS 562), we considered it important to develop a genetic system in this strain.

The present study, therefore, concentrated on development of a genetic system in *C. albicans* CBS 562 involving isolation of auxotrophic mutants, construction of a gene bank (carried in *Escherichia coli*-*Saccharomyces cerevisiae* shuttle vector YEp24), and isolation of *C. albicans* genes by complementation of an *S. cerevisiae* strain. Herewith, we describe specifically the isolation, characterization, and chromosomal location of the *HIS4* gene of this *C. albicans* isolate.

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MATERIALS AND METHODS

Strains. The strains used in this study are listed in Table 1. *C. albicans* CBS 562 (ATCC 18804) was obtained from the Centraal Bureau voor Schimmelcultures, Delft, The Netherlands. It is the type species strain and was originally isolated from a skin disorder (19). The strain was characterized as serotype A (33) and was extensively used in previous studies in our laboratory (31, 32). Other *C. albicans* isolates were used for comparative electrophoretic karyotyping. The *S. cerevisiae* strains were used to identify the *C. albicans HIS4* gene. *E. coli* HB101, a *recA* strain (25), was the recipient strain for bacterial transformation and was used for amplification of plasmids.

Media. A rich medium containing yeast extract (1%), peptone (2%), and glucose (2%) (YPD) was used to grow *C. albicans* and *S. cerevisiae*. A synthetic medium (SD) was prepared by addition of the appropriate amino acids to 0.67% yeast nitrogen base (without amino acids) and 2% glucose (35). The medium was used for growth and characterization of auxotrophs. *E. coli* strains were grown in Luria Bertani (LB) medium (1% tryptone, 0.5% yeast extract, 0.5% NaCl) supplemented with appropriate antibiotics for plasmid maintenance (22).

Mutagenesis of *C. albicans*. *C. albicans* CBS 562 grown in SD medium to 4×10^7 cells per ml was harvested and suspended in 0.2 M sodium acetate buffer (pH 7.0) at approximately 10^8 cells per ml. Freshly dissolved *N*-methyl-*N*′-nitro-*N*-nitrosoguanidine (NTG) was added to a final concentration of 0.15 mg/ml. The culture was incubated for 90 min at 37°C with constant shaking, centrifuged, and suspended in distilled water. The cells were then spread on complete medium (YPD) and incubated for 3 days at 28°C. The growing colonies were replica plated to SD plates. Putative auxotrophic mutants that failed to grow on SD

TABLE 1. Strains used in this study

Strain	Genotype	Reference or source
C. albicans		
CBS 562 (ATCC 18804)		31–33
SAG5	*his4-1*	This work
3153-A (ATCC 32354)		18
WO-1		21, 38
CA-8[a]		
S. cerevisiae		
8534-15C	α *his4-34 ura3-52 leu2-112*	P. R. Schimmel[b]
DBY 1034	**a** *his4-539*am *lys2-89*am *ura3-52*	P. R. Schimmel
X1651/7	**a** *his4-25*	G. R. Fink[c]
4772-17A	α *his4-15 ade2* Can^r	G. R. Fink
7023-12D	**a** *his4-331 leu2-1 MAL2*	G. R. Fink
4619-1C	**a** *his4-301*	G. R. Fink
A2393A	α *his4-280 ade2*	G. R. Fink
8984-6D	**a** *his4-763 ura3-52*	G. R. Fink
5799-4D	**a** *his4-39 his4-260*	G. R. Fink
5799-2A	α *his4-39 his4-260*	G. R. Fink

[a] Clinical isolate from the Department of Clinical Microbiology, Hadassah University Hospital, Jerusalem, Israel.
[b] Department of Biology, Massachusetts Institute of Technology, Cambridge.
[c] Whitehead Institute for Biomedical Research, Cambridge, Mass.

medium were transferred to SD plates containing pools of various amino acids, purines, and pyrimidines and other metabolites for specific characterization (35).

Assay of histidinol dehydrogenase activity. Crude histidinol dehydrogenase was extracted from 2 g (wet weight) of toluene-treated cells as described by Sherman et al. (35). This enzyme catalyzes the reaction L-histidinol + $2NAD^+ \rightarrow 2NADH + H^+$ + L-histidine. Enzymatic activity was measured at 340 nm by monitoring the reduction of NAD essentially as described by Fink (10). The reaction mixture contained 50 μg of cell extract protein (determined by the method of Lowry et al. [20]), 50 μmol of Tris hydrochloride (pH 9.0), 0.4 μmol of NAD, and 2 μmol of L-histidinol in a final volume of 0.35 ml.

Preparation of cell extracts. *C. albicans* cells were grown to the stationary phase in YDP at 30°C. Five grams (wet weight) of washed cells was suspended in 10 mM Tris hydrochloride (pH 7.5) and disrupted mechanically with glass beads (0.45- to 0.5-mm diameter) in a Braun MSK cell homogenizer (31). Cell debris was removed from the disrupted cell mass by centrifugation (10,000 × *g* for 20 min). For enzymatic activity assays, the cell extract was dialyzed against 10 mM Tris hydrochloride (pH 7.5). Protein concentration was determined by the method of Lowry et al. (20).

Accumulation studies. The method used for accumulation studies detects accumulation of an intermediate product in histidine-deficient strains. The reactions were performed essentially as described by Fink (9). Cell extracts were spotted on Whatman no. 1 papers, and chromatography was performed with a mixture of isopropanol-water-ammonia (70:40:10) for 4 h at room temperature. Accumulation of histidinol, which contains imidazole, was detected after spraying the dried filters with a diazo reagent (2), 5% nitrous acid, and 5% sodium carbonate. A redness characteristic of imidazole was developed in cell extracts of histidinol-accumulating strains. Bound BBMII [*N*-(5′-phospho-D-ribosylformimino)-5-amino-1-(5″-phosphoribosyl)-4-imidazolecarboxamide] was detected by the Bratton-Marshall method for determination of diazotizable amine (36). Cell extracts were spotted on Whatman no. 1 papers and treated with 1 N HCl at 100°C–5% nitrous acid, and the diazo products were detected with a 0.1% solution of *N*-(1-naphthyl)ethylenediamine–2HCl.

Assay of PR-AMP pyrophosphohydrolase and PR-AMP 1,6-cyclohydrolase. The activities of phosphoribosyl-AMP pyrophosphohydrolase (PR-AMP pyrophosphohydrolase) and phosphoribosyl-AMP 1,6-cyclohydrolase (PR-AMP 1,6-cyclohydrolase) can be measured together by the following reactions: 5-phosphoribosyl-1-pyrophosphate + ATP→PR-ATP (reaction 1), PR-ATP→PR-AMP (reaction 2), and PR-AMP→BBMII (reaction 3). Reactions 2 and 3 are performed by the enzymes PR-AMP pyrophosphohydrolase and PR-AMP 1,6-cyclohydrolase, respectively. Dialyzed cell extracts (approximately 5 mg/ml) were assayed by the methods described by Ames et al. (1). The assay mixture contained 20 μmol of Tris hydrochloride (pH 8.5), 4 μmol of $MgCl_2$, 0.18 μmol of 5-phosphoribosyl-1-pyrophosphate, 2 μmol of ATP, and various concentrations of cell extracts in a 0.5 ml solution. The increase in optical density at 290 nm, which is a result of the hydrolysis of the 1-6 bond of the purine ring of PR-AMP, was recorded. The specificity of the reaction was confirmed by omission of either ATP or 5-phosphoribosyl-1-pyrophosphate from the reaction mixtures, which resulted in no change in the optical density at 290 nm.

Plasmid DNA preparation and gel electrophoresis. Plasmid DNA was prepared from *E. coli* by the alkaline lysis method (3) or the CsCl gradient procedure (22). Yeast plasmids were prepared by the method described by Denis and Young (7). Agarose gel electrophoresis of DNA fragments was performed in 89 mM Tris–89 mM boric acid–2 mM EDTA (22).

Isolation of *C. albicans* genomic DNA. Total genomic DNA from *C. albicans* was isolated from a 200-ml culture by the spheroplast method (35). Spheroplasts were produced by treatment of *C. albicans* cells with Zymolase T100 (Miles Scientific, Div. Miles Laboratories, Inc., Naperville, Ill.). The spheroplasts were lysed by sodium dodecyl sulfate, and the nucleic acids were extracted with a phenol-chloroform-isoamyl alcohol (50:50:1) mixture and precipitated by ethanol. RNA was removed by RNase treatment, and the DNA was reextracted and reprecipitated.

Construction of a *C. albicans* genomic library. A genomic library of *C. albicans* CBS 562 was constructed in *S.*

cerevisiae-E. coli shuttle vector YEp24 (4). The plasmid contains the yeast *URA3* gene and sequences from the yeast 2 μm plasmid, which is responsible for autonomous high-copy-number propagation in *S. cerevisiae*. The purified genomic DNA from *C. albicans* was partially or totally digested with restriction endonuclease *Bam*HI. The pooled DNA fragments were fractionated at 10 to 40% sucrose density gradients. Fractions containing fragments in the 5- to 15-kilobase-pair size range were pooled and used for construction of the library. Plasmid YEp24 was linearized by cleavage at the unique *Bam*HI site located within the tetracycline resistance gene, treated with alkaline phosphatase, and ligated (by T4 DNA ligase) with the pooled *C. albicans* *Bam*HI fragments. The ligated mixture was used to transform *E. coli* HB101 to ampicillin resistance. Approximately 3,000 ampicillin-resistant, tetracycline-sensitive transformants were isolated, pooled, and grown, and their plasmid DNA was isolated.

Transformation. Yeast cells were transformed by the lithium acetate method and spheroplast procedure (35). *E. coli* HB101 was transformed by the $CaCl_2$ procedure (22).

Southern blot analysis. DNA was extracted from a 20-ml culture by the method described above. Five micrograms of DNA was digested with restriction enzymes, run on agarose gels, blotted, and hybridized to the *C. albicans HIS4* gene probe. The probe was labeled with [α-^{32}P]dATP by the nick translation procedure to a specific activity of 5×10^8 cpm/μg of DNA (22). Hybridization was performed at 68°C for 24 h with 5×10^6 cpm in a solution consisting of 6× SSC (1× SSC in 0.15 M sodium chloride plus 0.015 M sodium citrate), 0.02% Ficoll, 0.02% polyvinylpyrrolidone, 0.02% bovine serum albumin, and 100 μg of sonicated and denatured salmon sperm DNA per ml. The filters were washed with 0.1× SSC–0.1% sodium dodecyl sulfate at 37°C and exposed to X-ray films.

Electrophoretic karyotyping. Orthogonal-field alternating gel electrophoresis (OFAGE) was performed essentially as described by Polacheck and Lebens (25). Cells were grown to the early-exponential phase at 37°C in YPD medium. Approximately 10 ml of the culture was washed twice in 50 mM EDTA (pH 7.5) and suspended in 1 ml of 20 mM citrate phosphate buffer (pH 5.6) containing 50 mM EDTA, 0.9 M sorbitol, and 0.3 mg of Zymolase T100 per ml. This suspension was incubated at 37°C for 45 to 60 min until 90 to 100% protoplasts were formed. One milliliter of protoplasts was mixed with 1 ml of 1% low-melting-point agarose (Bethesda Research Laboratories, Inc., Gaithersburg, Md.) made up in 0.125 M EDTA (pH 7.5). One milliliter of this mixture was placed in each of two wells of a 24-well tissue culture plate and allowed to set. Each block was then cut into smaller pieces, and these were mixed with 5 ml of 0.25 M EDTA (pH 7.5) containing 1% (wt/vol) sodium dodecyl sulfate and incubated at 60°C for 2 h. Following incubation, the gel pieces were transferred to 3 to 5 ml of 0.5 M EDTA (pH 9.0) containing 1% (wt/vol) Sarkosyl and 1 mg of proteinase K (Sigma Chemical Co., St. Louis, Mo.) per ml and incubated for 24 h at 50°C. This step was repeated with fresh solution and used in OFAGE analysis. The separations were performed on 1.5% agarose gels (agarose NA; Pharmacia, Piscataway, N.J.) in an LKB Pulsaphos system at 350 V with a pulse time of 120 s for 44 h. Following electrophoresis, the gels were stained with ethidium bromide and the DNA bands were transferred to Nytran NY13 filters (Schleicher & Schuell, Inc., Keene, N.H.) by the Southern blot technique.

TABLE 2. Characterization of auxotrophic mutants of *C. albicans* CBS 562 induced by NTG treatment

Strain	Phenotype	Genotype	Growth requirement(s)
CBS 562		Wild type	
SAG5	His$^-$	*his4-1*	Histidine
SAG1	Arg$^-$	*arg*	Arginine
SAG2	Met$^-$	*met*	Methionine
SAG9	Met$^-$ Thr$^-$	*hom*	Homoserine or methionine + threonine

RESULTS

Isolation of auxotrophic mutants. *C. albicans* was mutagenized with NTG as described in Materials and Methods. Of 1,050 colonies screened, four auxotrophs were isolated and characterized (Table 2). Of the four strains, SAG2 was an unstable Met$^-$ mutant and SAG1 (Arg$^-$) and SAG9 (Met$^-$ Thr$^-$) showed a low frequency of spontaneous reversion to prototrophy. SAG5 (His$^-$) was a stable mutant and did not revert to prototrophy spontaneously or following exposure to NTG or UV irradiation.

Characterization of strain SAG5 (His$^-$). Since SAG5 was a stable His$^-$ strain, we chose to characterize its mutation further. SAG5 had an absolute requirement for L-histidine and failed to grow on SD plates supplemented with either L-histidinol or L-histidinol phosphate, which are the last two metabolites in the histidine biosynthetic pathway (10). This may indicate that SAG5 is defective in histidinol dehydrogenase activity, which is the last enzyme in histidine biosynthesis. This was verified by determination of histidinol dehydrogenase activity in crude extracts of SAG5 in comparison with *C. albicans* CBS 562 (Table 3). The results showed that SAG5 essentially lacks histidinol dehydrogenase activity, retaining less than 0.92% of the activity of the wild-type strain, which may explain the failure of SAG5 to grow on minimal medium without histidine. This phenomenon is similar to that of a *his4*-carrying strain, *S. cerevisiae* DBY 1034, whose histidinol dehydrogenase activity (Table 3) was less than 9.5% of that of wild-type strain 288C.

The *HIS4* gene in *S. cerevisiae* encodes a single multifunctional protein which catalyzes three steps in the pathway of histidine biosynthesis. The enzymes are PR-AMP pyrophosphohydrolase (*HIS4B*), PR-AMP 1,6-cyclohydrolase (*HIS4A*), and histidinol dehydrogenase (*HIS4C*). *HIS4A* and *HIS4B* encode steps 2 and 3 in the histidine biosynthetic pathway, while *HIS4C* encodes enzyme 10. To test whether SAG5 is also defective in *HIS4A* and *HIS4B* enzyme activity, two assays were performed: (i) detection of the synthesis of BBMII, which is intermediate 3 in the histidine biosynthetic pathway, and (ii) accumulation tests to define the

TABLE 3. Determination of histidinol dehydrogenase activity

Strain	Relevant genotype	Histidinol dehydrogenase activity[a]
C. albicans		
CBS 562	Wild type	5.83
SAG5	*his4C*	0.054
S. cerevisiae		
288C	Wild type	10.14
DBY 1034	*his4C*	0.97

[a] Change in optical density at 340 nm per minute per milligram of protein.

TABLE 4. Plasmid-curing experiments with *S. cerevisiae* strains carrying *C. albicans* cloned genes

Strain	Growth characteristics (no. of colonies)[a]			Plasmid-curing rate (%)[b]
	SD−Ura−Leu−His	SD−His	SD−Leu	
STC7	24		18	25.0
STC1	70	41		41.4
STC2	96	51		46.8
STC6	75	47		37.3

[a] Number of colonies that grew on SD plates supplemented with uracil (Ura), leucine (Leu), and histidine (His).
[b] Percentage of colonies that lost the cloned *HIS4* or *LEU2* gene.

nature of the accumulated intermediate. *his4C* strains accumulate histidinol, whereas *his4A*, *his4B*, *his4AB*, and *his4ABC* strains fail to accumulate histidinol. The mean (± the standard deviation) enzymatic activities (changes in optical density at 290 nm per minute per milligram of protein) of PR-AMP pyrophosphohydrolase and PR-AMP 1, 1,6-cyclohydrolase in strains CBS 562 and SAG5, respectively, were 0.103 ± 0.060 and 0.090 ± 0.025. CBS 562 had no accumulation product, but SAG5 accumulated histidinol. These results show that SAG5 and CBS 562 have the same enzymatic activities, which indicates that SAG5 is not defective in its *HIS4A* and *HIS4B* genes. This was further confirmed by the accumulation test; SAG5 accumulated histidinol, which indicates that the strain is a *his4C* strain defective in histidinol dehydrogenase activity.

Isolation of the *C. albicans HIS4* gene. Since it was found that *C. albicans* genes can be functional in *S. cerevisiae* (30), we used this approach to isolate the *HIS4* gene. We therefore constructed a genomic library of *C. albicans* CBS 562 in vector YEp24. This gene bank was used to complement *S. cerevisiae* 8534-15c, whose *his4-34* marker carries two point mutations. The strain was transformed with the gene bank by using protoplast and lithium acetate techniques (35). Since the *S. cerevisiae* strain carried two mutations (*his4* and *leu2*), we were able to complement both mutations with *C. albicans* genes. His^+ Ura^+ (designated STC5 and STC7) and Leu^+ Ura^+ (STC1, STC2, and STC6) cotransformants were isolated, indicating that these transformants apparently carry the *C. albicans HIS4* and *LEU2* genes.

Linkage between the YEp24 *URA3* gene and the *C. albicans LEU2* and *HIS4* genes was established in plasmid-curing experiments. The strains were grown under nonselective conditions (YPD medium), and individual colonies were toothpicked onto selective medium to score for growth requirements. The results (Table 4) show that 25% of the STC7 colonies tested had a concomitant loss of the ability to grow without histidine and uracil. Similarly, about 41% of STC1, 47% of STC2, and 37% of STC6 strains, respectively, were unable to grow without leucine and uracil. The inability to grow without uracil-leucine or uracil-histidine was associated with loss of the plasmids carrying the corresponding genes. STC7 carried the *HIS4* gene, and strains STC1, STC2, and STC6 carried the *LEU2* gene. Cloning of the *C. albicans LEU2* gene was also reported recently by Jenkinson et al. (13) and Kelly et al. (14).

Following the linkage experiments, a plasmid was isolated from strain STC7. Plasmid pSTC7 was introduced into *E. coli* HB101 for amplification and purification. Transformation of *S. cerevisiae* 8534-15c and DBY 1034 with pSTC7 resulted in a high frequency of Ura^+ His^+ transformants.

The *HIS4* region in *S. cerevisiae* encodes a trifunctional protein, with each of the functions specified by a subregion of the gene, i.e., *HIS4A*, *HIS4B*, or *HIS4C*. To determine whether the cloned *C. albicans HIS4* gene contains genetic information that can complement the entire *his4* region, various *S. cerevisiae his4* strains carrying identified mutations were transformed with pSTC7. The following *his4* strains were used: *his4A* mutants X1651/7 (carries a missense mutation) and 4772-17A (in-frame deletion); his4B mutants 7023-12D (missense mutation) (10, 34) and 4619-1C (missense mutation); *his4C* mutants A2393A (missense mutation) (10, 34) and 8984-6D (missense mutation); and *his4ABC* mutant 5799-49 (carries two different nonsense mutations in *his4A*, causing a polar effect that inactivates the *HIS4A*, *HIS4B*, and *HIS4C* functions) (8, 10, 34). All of the strains tested were successfully transformed to His^+ by pSTC7. These results indicate that pSTC7 contains the entire *HIS4* gene.

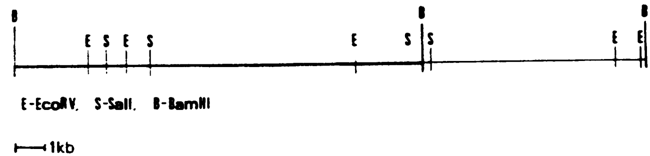

FIG. 1. Linear restriction cleavage map of pSTC7. The thick line contains the cloned *C. albicans HIS4* gene. The thin line shows the vector YEp24.

Restriction endonuclease map of the *HIS4* gene. To identify the DNA fragment containing the *HIS4* gene, plasmid pSTC7 was digested with several restriction enzymes. The DNA fragments were separated in agarose gels, and their mobilities were compared with those of YEp24 and λ *Hin*dIII and φX174 *Hae*III markers. The total length of pSTC7 was estimated as 21.92 kilobases, and it contained a 14.15-kilobase-pair *Bam*HI DNA fragment cloned in YEp24. A linear restriction cleavage map of the plasmid is presented in Fig. 1.

Hybridization of the *HIS4* gene with *C. albicans* genomic DNA. To confirm that the *HIS4* gene originated from the *C. albicans* genome, hybridization experiments were performed with the gene and *C. albicans* genomic DNA. The genomic and pSTC7 DNAs were digested with several restriction enzymes, and the digested DNAs were electrophoresed on agarose gel (Fig. 2A) and transferred to nitrocellulose filters. The isolated *Bam*HI DNA fragment containing the *HIS4* gene was labeled with [α-^{32}P]dATP by nick translation and hybridized with the *C. albicans* genomic DNA fragments. The results (Fig. 2B) indicate that the *HIS4* gene hybridized to the *C. albicans* genome. In addition, the restriction pattern of the gene was colinear with that of the chromosomal DNA, which indicates that the *C. albicans* genome contains a single copy of the *HIS4* gene.

Chromosomal location of the *HIS4* gene. Chromosomes of *C. albicans* CBS 562 were prepared as described in Materials and Methods. The chromosomal bands were resolved by the OFAGE technique. The electrokaryotype (Fig. 3A) showed a resolution of six bands, of which bands 1, 3, and 5 (numbered from the top down) seemed to contain more than one chromosomal band. The chromosomes were blotted and probed with the *HIS4* gene. The results (Fig. 3B) revealed that the gene hybridized to the largest resolvable chromosome.

Four different additional *C. albicans* strains were analyzed for electrophoretic karyotyping by the OFAGE technique. The strains varied in chromosomal pattern (Fig. 4A). Hy-

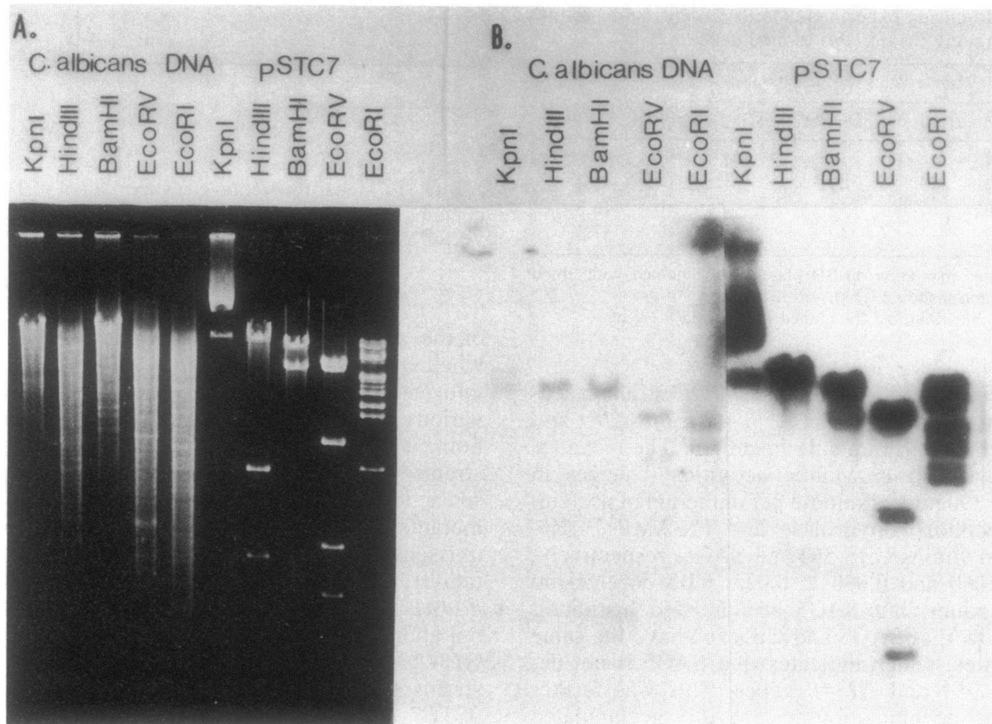

FIG. 2. Hybridization of the *HIS4* gene with pSTC7 and *C. albicans* genomic DNAs. (A) Agarose gel electrophoresis of restriction endonuclease-digested *C. albicans* genomic DNA and pSTC7. The digested pSTC7 DNA fragments are as follows: *Kpn*I, no restriction site; *Hin*dIII, 18.6, 2.1, and 1.1 kilobases; *Bam*HI, 14.1 and 7.7 kilobases; *Eco*RV, 9.0, 8.0, 2.7, 1.3, and 0.9 kilobases; *Eco*RI, partial digest. (B) Hybridization of the ^{32}P-labeled *C. albicans HIS4* gene to a Southern transfer of the gel in panel A.

bridization of these chromosomes with the *C. albicans HIS4* gene (Fig. 4B) showed that the *HIS4* gene was located on the largest resolvable chromosome in all four strains.

DISCUSSION

The results presented in this report describe the development of a genetic system which will enable gene manipulation in *C. albicans* CBS 562. This strain was used previously in numerous studies focusing on experimental infection, fungal pathogenesis, and immune responses, as summarized in two reviews (31, 32). Following mutagenesis with NTG, several auxotrophs requiring histidine, methionine, arginine, and methionine-threonine were isolated. In addition, temperature-sensitive mutants and strains defective in mitochondrial metabolism were isolated (data not shown).

The relative ease of isolating mutants from *C. albicans* although it contains a diploid genome was also noted by other investigators (27). His$^-$, Arg$^-$, Met$^-$, and Met$^-$ Thr$^-$ mutants were isolated by Poulter and Hanrahan (26) in different *C. albicans* strains. The growth characteristics of their mutants resemble those of our Met$^-$, Arg$^-$, and Met$^-$

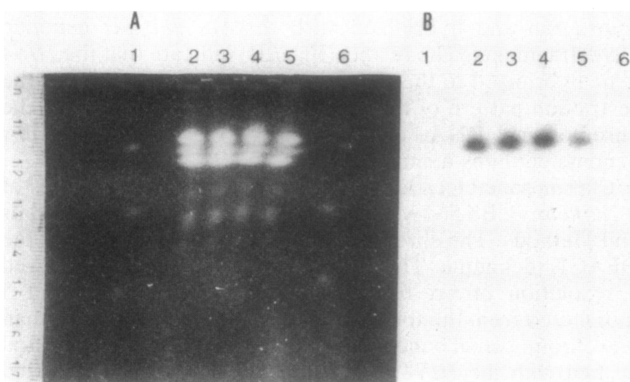

FIG. 3. Chromosomal location of the *HIS4* gene in *C. albicans* CBS 562. (A) Electrophoretic karyotypes of *C. albicans* CBS 562 (lanes 2 to 5) and *S. cerevisiae* 2012 (lanes 1 and 6) resolved by OFAGE. (B) Hybridization of the ^{32}P-labeled *C. albicans HIS4* gene to a Southern blot of the chromosomes in panel A.

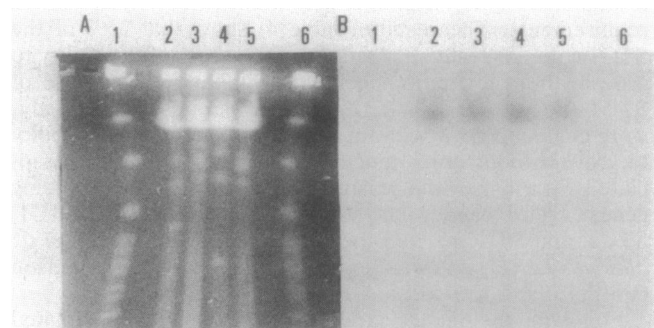

FIG. 4. Chromosomal location of the *HIS4* gene in various *C. albicans* isolates. (A) Electrophoretic karyotypes of *C. albicans* CA-8 (lane 2), 252 (lane 3), WO1 (lane 4), and 3253a (lane 5) in comparison with that of *S. cerevisiae* 2012 (lanes 1 and 6), resolved by OFAGE. (B) Hybridization of ^{32}P-labeled *C. albicans HIS4* gene to a Southern blot of the chromosomes in panel A.

Thr$^-$ mutants, thus establishing their genotypes as *met* (SAG2), *arg* (SAG1), and *hom* (SAG9), respectively.

Strain SAG5 has an absolute requirement for L-histidine for growth and lacks histidinol dehydrogenase activity, an enzyme involved in conversion of histidinol to histidine. In analogy to *S. cerevisiae*, in which this mutation is identified as *his4C* (10), we designated the genotype of SAG5 as *his4C*. Since SAG5 represented a stable mutant, we chose to isolate the homologous *HIS4* gene to construct a vehicle for gene manipulation in that strain.

On the basis of the observation that *C. albicans* genes can complement *S. cerevisiae* genetic lesions (30), various investigators isolated the *HIS3*, *LEU2*, *ADE1*, *ADE2*, *URA3*, *TRP1*, *LYS2*, and *GAL1* genes of *C. albicans* (13, 14, 17). We used this approach to clone the *C. albicans HIS4* gene. Thus, a genomic library from wild-type *C. albicans* CBS 562 was constructed in shuttle vector YEp24 and used to transform *S. cerevisiae* 8534-15C (*ura3 his4 leu2*). Numerous Ura$^+$ transformants were obtained, from which two Ura$^+$ His$^+$ and three Ura$^+$ Leu$^+$ cotransformants were isolated. Plasmid pSTC7 contained the *C. albicans HIS4* gene, as shown by plasmid-curing experiments, high-frequency transformation of *S. cerevisiae his4A*, *his4B*, *his4C*, and *his4ABC* strains to His$^+$, and hybridization of the gene to the *C. albicans* genome. The restriction enzyme pattern of the *HIS4* gene and that of the genomic DNA were colinear, indicating that the origin of the gene is indeed *C. albicans* and that the gene is present in a single copy. A restriction map of the cloned DNA fragment was obtained by cleavage with *Bam*HI, *Sal*I, and *Eco*RV, which determined that the size of the fragment was about 14 kilobase pairs.

In *S. cerevisiae* (8, 10) and *Neurospora crassa* (23), the *HIS4* gene encodes a single multifunctional protein which catalyzes three steps in the pathway of histidine biosynthesis. The *HIS4*-encoded protein is divided into three functional domains, and each of these domains is encoded by a subregion of the *HIS4* gene. The coding sequence of the *S. cerevisiae HIS4* gene extends for 2,397 base pairs and codes for a 95,000-dalton protein (8). The complementation results of the different *S. cerevisiae his4* mutant strains indicate that the cloned *C. albicans HIS4* gene carries genetic information for PR-AMP 1,6-cyclohydrolase (*HIS4A*), PR-AMP pyrophosphohydrolase (*HIS4B*), and histidinol dehydrogenase (*HIS4C*). The results indicate that it is possible that the *C. albicans HIS4* gene codes for a similar multifunctional protein.

Electrokaryotyping of *C. albicans* CBS 562 by the OFAGE technique revealed six separate chromosomal bands, three of which seemed to be doublets. These findings are similar to the results obtained by DeJonge et al. (6) and are compatible with the observation of Magee et al. (21). It should be noted that various *C. albicans* isolates may differ in their chromosomal patterns. However, it is believed that the number of chromosomes in *C. albicans* is seven (21). The *HIS4* gene was found to be located on the largest chromosome in all of the *C. albicans* strains.

In summary, the present work describes the isolation and characterization of a histidine auxotroph (*his4C*) of *C. albicans* and the isolation of the *HIS4* gene. This investigation constitutes the initiation of a defined genetic system in a *C. albicans* isolate whose pathogenic properties have been studied previously. This system will be used in future research to investigate molecular aspects of the pathogenic characteristics of this fungus.

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