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Lysine Biosynthesis in Selected Pathogenic Fungi: Characterization of Lysine Auxotrophs and the Cloned *LYS1* Gene of *Candida albicans*

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The α-aminoadipate pathway for the biosynthesis of lysine is present only in fungi and euglena. Until now, this unique metabolic pathway has never been investigated in the opportunistic fungal pathogens *Candida albicans*, *Cryptococcus neoformans*, and *Aspergillus fumigatus*. Five of the eight enzymes (homocitrate synthase, homoisocitrate dehydrogenase, α-aminoadipate reductase, saccharopine reductase, and saccharopine dehydrogenase) of the α-aminoadipate pathway and glucose-6-phosphate dehydrogenase, a glycolytic enzyme used as a control, were demonstrated in wild-type cells of these organisms. All enzymes were present in *Saccharomyces cerevisiae* and the pathogenic organisms except *C. neoformans* 32608 serotype C, which exhibited no saccharopine reductase activity. The levels of enzyme activity varied considerably from strain to strain. Variation among organisms was also observed for the control enzyme. Among the pathogens, *C. albicans* exhibited much higher homocitrate synthase, homoisocitrate dehydrogenase, and α-aminoadipate reductase activities. Seven lysine auxotrophs of *C. albicans* and one of *Candida tropicalis* were characterized biochemically to determine the biochemical blocks and gene-enzyme relationships. Growth responses to α-aminoadipate- and lysine-supplemented media, accumulation of α-aminoadipate semialdehyde, and the lack of enzyme activity revealed that five of the mutants (WA104, WA153, WC7-1-3, WD1-31-2, and A5155) were blocked at the α-aminoadipate reductase step, two (STN57 and WD1-3-6) were blocked at the saccharopine dehydrogenase step, and the *C. tropicalis* mutant (X-16) was blocked at the saccharopine reductase step. The cloned *LYS1* gene of *C. albicans* in the recombinant plasmid YpB1078 complemented saccharopine dehydrogenase (*lys1*) mutants of *S. cerevisiae* and *C. albicans*. The *Lys1*$^+$ transformed strains exhibited significant saccharopine dehydrogenase activity in comparison with untransformed mutants. The cloned *LYS1* gene has been localized on a 1.8-kb *Hin*dIII DNA insert of the recombinant plasmid YpB1041RG1. These results established the gene-enzyme relationship in the second half of the α-aminoadipate pathway. The presence of this unique pathway in the pathogenic fungi could be useful for their rapid detection and control.

Candida albicans, *Cryptococcus neoformans*, and *Aspergillus fumigatus* are among the most important fungal pathogens of humans (4, 20, 21, 23). Among the yeasts, *C. albicans* is able to colonize rapidly and take advantage of the loss of host resistance. Candidiasis can be cutaneous or systemic and is often associated with immunocompromised patients (cancer, transplant, or AIDS patients) undergoing treatment with steroids, immunosuppressive drugs, or cytotoxic agents. Most cases of *C. neoformans* infections are also associated with patients having some degree of immunodeficiency. Cryptococcosis may spread through various organs and the central nervous system (23). The organ most frequently affected by *A. fumigatus* is the lung, but the organism can cause diseases of the skin, ear, central nervous system, and hemopoietic system (20). Serious aspergillosis affects primarily patients suffering from leukemia who have undergone bone marrow transplantation. Diagnosis of fungal pathogens is time consuming and often requires a complete cultural identification protocol (20). Most genetic, biochemical, and molecular studies of fungi have been performed on model organisms such as the yeast *Saccharomyces cerevisiae* (6, 19) and the mold *Neurospora crassa* (8, 22). It is important to utilize this knowledge for the investigation of pathogenic fungi. Some success has been achieved in cloning and molecular studies of *C. albicans* genes (15–17, 28). However, to date, only a few biosynthetic genes have been cloned, and even fewer mutants have been biochemically characterized (1, 16, 24). It is highly desirable to identify unique genes and metabolic processes as targets for rapid detection and possible control of fungal pathogens. The genes and enzymes for the biosynthesis of lysine may be exploited for this purpose.

Lysine is an essential amino acid (obtained from protein in the diet) for humans and animals. Lysine is synthesized via the α-aminoadipate pathway in fungi (Fig. 1) (2). Bacteria and plants use the diaminopimelic acid pathway for the synthesis of lysine (34, 35). The pathways for the synthesis of other amino acids are similar in bacteria and fungi (34). Because the α-aminoadipate pathway is unique, being present only in fungi, its enzymes and cloned genes can be used as molecular probes for the rapid detection of fungal pathogens. Also, the selective inhibition of the enzyme(s) of the α-aminoadipate pathway by an appropriate substrate analog may control the growth of fungal pathogens in vivo. The genes and enzymes of the α-aminoadipate pathway in fungal pathogens, including *C. albicans*, have not been investigated until now.

The presence of the α-aminoadipate pathway for the biosynthesis of lysine has been demonstrated in several yeasts and molds, including *S. cerevisiae* (2, 3), *Yarrowia lipolytica* (9), *Schizosaccharomyces pombe* (39), *Rhodotorula glutinis* (10), *Candida maltosa* (15), *N. crassa* (7), and *Penicillium chrysogenum* (12). We have performed exten-

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FIG. 1. Intermediates for the conversion of aminoadipate to lysine (second half of the pathway) and the biochemical block of lysine auxotrophs of *C. albicans* and *C. tropicalis*. AAR, aminoadipate reductase; SRD, saccharopine reductase; SDH, saccharopine dehydrogenase.

sive genetic, enzymological, regulatory, and gene cloning studies of lysine biosynthesis in *S. cerevisiae*. Eight enzyme steps and more than 12 nonlinked genes are responsible for the biosynthesis of lysine in *S. cerevisiae* (2, 5, 36). Although the α-aminoadipate pathway is present only in fungi, it is not known whether the genes and the enzymes are completely conserved in all organisms. A detailed comparative study of the lysine biosynthetic pathway is essential to gain basic knowledge of the metabolism and molecular genetics of the pathogenic fungi. Lysine mutants of *C. albicans* have been procured by several investigators (17, 24, 27), but none has been characterized biochemically. We report here for the first time the presence of lysine biosynthetic enzymes in prototrophic strains of *C. albicans*, *C. neoformans*, and *A. fumigatus*, characterization and determination of biochemical blocks of 12 lysine auxotrophs of *C. albicans*, and characterization of the cloned *LYS1* gene of *C. albicans*.

MATERIALS AND METHODS

Strains, plasmids, and media. Strains of pathogenic fungi (*C. albicans* 18804, *A. fumigatus* 36607, *C. neoformans* 6352 serotype A, *C. neoformans* 32269 serotype B, and *C. neoformans* 32608 serotype C) were obtained from the American Type Culture Collection. Lysine auxotrophs of *C. albicans* and *Candida tropicalis* were obtained from A. Sarachek (27) and B. Magee (17). Plasmid vector YpB1041, the cloned *LYS1* gene of *C. albicans* in YpB1078, and YpB1113 (subclone) were obtained from S. Scherer (28). Recombinant plasmid YpB1041RG1 was constructed by ligation of a 1.8-kb *Hin*dIII DNA fragment of YpB1113 into the unique *Hin*dIII site of vector YpB1041 (Fig. 2). Prototrophic and mutant strains were maintained in YEPD medium (yeast extract [10 g], peptone [20 g], dextrose [20 g], agar [20 g], double-distilled water [to 1 liter]). Enzyme assays, growth response studies, and accumulation studies were performed on cultures grown in minimal medium (dextrose [10 g], Difco yeast nitrogen base without amino acids [6.7 g], distilled water [to 1 liter]; lysine [20 μg/ml] or α-aminoadipic acid [30 μg/ml] was added when appropriate).

Growth response and accumulation studies. Growth responses of wild-type strains and lysine auxotrophs to minimal medium or to minimal medium supplemented with α-aminoadipic acid or lysine were determined on the basis of optical density at 550 nm following incubation of cultures at 30°C with shaking. Accumulation of α-aminoadipic δ-semialdehyde, the intermediate between α-aminoadipic acid and saccharopine (Fig. 1), in the culture supernatant was determined on the basis of the *p*-dimethylaminobenzaldehyde assay (5, 26).

Enzyme assays. Extracts were prepared from pre-stationary-phase cells of wild-type strains and lysine auxotrophs grown, respectively, in minimal medium and minimal medium supplemented with lysine. Homocitrate synthase (EC 4.1.21.93) (33), homoisocitric dehydrogenase (EC 1.1.1.87) (32), α-aminoadipate reductase (EC 1.2.1.31) (26), saccharopine reductase (EC 1.5.1.10) (13), and saccharopine dehydrogenase (EC 1.5.1.7) (13) of the lysine biosynthetic pathway, as well as glucose-6-phosphate dehydrogenase (EC 1.1.1.49) (14), a control enzyme, were assayed by published procedures (7, 14, 37). Each enzyme was assayed in three different concentrations of protein. The values obtained were averaged and determined to be within 1 standard error of the mean.

Molecular biology technique. Vector YpB1041, plasmid YpB1078, subclone YpB1113, and YpB1041RG1 carrying the cloned *LYS1* gene of *C. albicans* were amplified through transformation of *Escherichia coli* DH5α Amp^s to Amp^r. Plasmids were isolated and purified by density gradient centrifugation (18). Functional complementation of an *S. cerevisiae lys1* to $Lys1^+$ transformant by plasmids YpB1078, YpB1113, and YpB1041RG1 was performed as described by Ito et al. (11). Transformation of the *C. albicans* saccharopine dehydrogenase mutant STN57 with plasmid YpB1078 was performed as described by Kurtz et al. (16). Restriction enzyme digestions, electrophoretic analysis, and construction of restriction maps of the vector and clones carrying the cloned *LYS1* gene were performed by published procedures (18, 36).

RESULTS

Enzymes of the α-aminoadipate pathway and glucose-6-phosphate dehydrogenase in wild-type organisms. Five enzymes of the α-aminoadipate pathway and glucose-6-phosphate dehydrogenase, a constitutive glycolytic enzyme used as a control, were assayed in all six wild-type organisms (Table 1). Enzyme activities of wild-type *S. cerevisiae* also served as positive controls. The remaining three enzymes for lysine biosynthesis (Fig. 1) were not assayed because of the lack of availability of substrates. The results demonstrate the presence of homocitrate synthase, homoisocitrate dehydrogenase, α-aminoadipate reductase, saccharopine reductase, and saccharopine dehydrogenase activities in all organisms tested except for the lack of saccharopine reductase activity in *C. neoformans* 32608 serotype C. Although the enzymes for lysine biosynthesis were present, considerable differences were observed from organism to organism. Variation among organisms was also observed for the control enzyme, glucose-6-phosphate dehydrogenase. Among the pathogenic fungi, *C. albicans* exhibited significantly higher levels of homocitrate synthase, homoisocitrate dehydrogenase, and α-aminoadipate reductase.

Biochemical characterization of lysine auxotrophs of *C.*

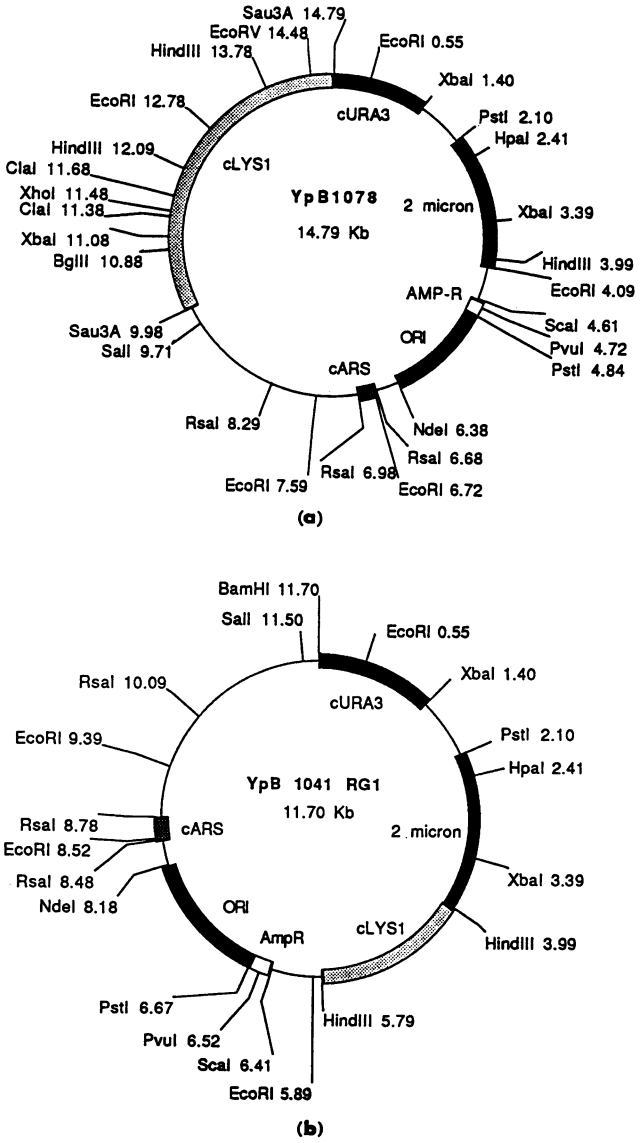

FIG. 2. Physical map of recombinant plasmid YpB1078 (a) and subclone YpB1041 RG1 (b) depicting the 1.8-kb *Hin*dIII DNA insert which contains the *LYS1* gene of *C. albicans*.

albicans. Table 2 shows growth responses of lysine auxotrophs to minimal medium and to α-aminoadipate- and lysine-supplemented media, accumulation of α-aminoadipate semialdehyde, and appropriate enzyme activities. All 12 mutants grew well in lysine-supplemented medium but not in minimal medium or α-aminoadipate-supplemented medium. Wild-type strains (WA132, WT792, WT207, WT526, and WT9517) grew in all three media. Lack of growth in the α-aminoadipate-supplemented medium indicated the biochemical blocks of mutants between α-aminoadipate and lysine (Fig. 1). Five of the mutants (WA104, WA153, WC7-1-3, WD1-31-2, and A5155) lacked α-aminoadipate reductase activity, did not accumulate α-aminoadipate semialdehyde, and exhibited saccharopine reductase activity. These mutants are blocked at the α-aminoadipate reductase step (Fig. 1). Three of the mutants (*C. tropicalis* X-16 and *C. albicans* WD1-3-6 and STN57) exhibited accumulation of α-aminoadipate semialdehyde and α-aminoadipate reductase activities, indicating that these mutants are blocked between α-aminoadipate semialdehyde and lysine. One of these strains (X-16) lacked saccharopine reductase activity, and the other two (STN57 and WD1-3-6) lacked saccharopine dehydrogenase activity and are blocked in the respective steps. Two of the strains (WC5-44-6 and WD18) were revertants, and the biochemical blocks of four strains (WB7-23, WCR1-69, WC5-36-1, and WC7-1-8) remain to be determined.

Functional and physical characterization of the cloned *LYS1* gene of *C. albicans*. The *LYS1* gene of *C. albicans* has been cloned by Scherer and Magee (28) in plasmid YpB1078 from a *C. albicans* YpB1041 (vector) genomic library by functional complementation of the *C. albicans* STN57 *lys1* (saccharopine dehydrogenase) mutant. Plasmid YpB1078 and subclone YpB1041RG1 carrying the *LYS1* DNA insert also transformed STX4B and STX4A *lys1* mutants of *S. cerevisiae* to *Lys1*$^+$ prototrophs (data not presented and Table 3). Wild-type, *lys1* mutant, Lys1$^+$ transformed, and plasmid-cured strains were analyzed for saccharopine dehydrogenase activity (Table 4). Wild-type and Lys1$^+$ transformed strains grown in minimal medium exhibited significant saccharopine dehydrogenase activity. One *S. cerevisiae* Lys1$^+$ transformant, STX4B (1078), and one *C. albicans Lys1*$^+$ transformant, STN57 (1078), exhibited significantly higher enzyme activities than did wild-type strains. Plasmid-cured strains and *lys1* mutants grown in lysine-supplemented minimal medium exhibited little or no activity. Curing experiments with *C. albicans Lys1*$^+$ transformants were not successful, indicating a possible integrative transformation of *lys1* mutants (16).

Physical characterization of the cloned *LYS1* gene was done by restriction digestions, electrophoretic analysis, and comparison of the restriction maps of vector YpB1041,

TABLE 1. Activities of five enzymes of the lysine biosynthetic pathway and glucose-6-phosphate dehydrogenase in wild-type organisms

Organism	Enzyme activity[a] (SE)					
	HSY	HDH	AAR	SRD	SDH	GPD
C. albicans 18804	1.3 (±0.34)	1.4 (±0.36)	0.6 (±0.12)	0.2 (±0.08)	0.14 (±0.04)	1.4 (±0.5)
C. neoformans 6352	0.4 (±0.16)	0.1 (±0.04)	0.1 (±0.03)	1.2 (±0.14)	0.09 (±0.03)	0.6 (±0.4)
C. neoformans 32608	0.3 (±0.12)	0.1 (±0.04)	0.1 (±0.03)	0.0	0.05 (±0.01)	0.7 (±0.4)
C. neoformans 32269	0.5 (±0.05)	0.3 (±0.12)	0.1 (±0.03)	0.4 (±0.11)	0.20 (±0.06)	1.0 (±0.4)
A. fumigatus	0.5 (±0.22)	0.1 (±0.01)	0.1 (±0.03)	0.2 (±0.14)	0.09 (±0.02)	0.8 (±0.3)
S. cerevisiae	0.4 (±0.16)	1.2 (±0.2)	0.7 (±0.28)	0.7 (±0.3)	0.49 (±0.01)	0.8 (±0.2)

[a] Expressed as follows: HSY (homocitrate synthase), change in A_{412} per 30 min per milligram of protein; HDH (homoisocitrate dehydrogenase), change in A_{520} per 2 h per milligram of protein; AAR (aminoadipate reductase), change in A_{460} per h per milligram of protein; SRD (saccharopine reductase), change in A_{460} per hour per milligram of protein; SDH (saccharopine dehydrogenase), change in A_{340} per minute per milligram of protein; GPD (glucose-6-phosphate dehydrogenase), change in A_{340} per minute per milligram of protein.

TABLE 2. Biochemical and physiological properties of lysine auxotrophs of *C. albicans*

Strain and genotype for lysine	A_{550}[a]			Accumulation of ASA[b]	Enzyme activity (ΔA/mg of protein)[c]		
	MM	Lys	AA		AAR	SRD	SDH
WA104 *lys*	0.05	0.37	0.06	−	0.00	0.37	ND
WA153 *lys*	0.01	0.72	0.03	−	0.00	0.64	ND
WA132 *LYS*	0.72	0.70	0.69	−	0.26	0.22	ND
WT792 *LYS*	0.46	0.74	0.40	−	0.19	0.42	ND
WB7-23 *lys*	0.03	0.51	0.03	−	0.11	0.19	0.79
WT207 *LYS*	0.78	0.72	0.69	−	0.30	0.29	ND
WCR1-69 *lys*	0.04	0.72	0.04	−	0.41	1.21	0.72
WC5-36-1 *lys*	0.07	0.48	0.07	−	0.39	0.13	0.87
WC5-44-6 *lys*	0.19	0.73	0.36	−	0.39	0.27	ND
WC7-1-3 *lys*	0.04	0.57	0.04	−	0.02	0.32	ND
WC7-1-8 *lys*	0.03	0.06	0.00	−	0.70	0.67	0.48
WT526 *LYS*	0.72	0.75	0.69	−	0.19	0.13	0.86
WD18 *lys*	0.38	0.79	0.69	−	0.80	0.56	ND
WD1-3-6 *lys*	0.27	0.72	0.03	−	0.19	0.19	0.08
WD1-31-2 *lys*	0.03	0.71	0.03	−	0.00	0.58	ND
WT9517 *LYS*	0.81	0.77	0.73	−	0.29	0.18	ND
X-16 *lys*	0.04	0.67	0.05	+	0.19	0.03	ND
STN57 *lys*	0.03	1.39	0.03	+	0.27	1.16	0.08
A5155 *lys*	0.03	1.24	0.10	−	0.01	0.75	ND

[a] Growth after 48 h in minimal medium (MM) or lysine (Lys)- or α-aminoadipate (AA)-supplemented medium.
[b] Accumulation (+) or lack of accumulation (−) of α-aminoadipate semialdehyde (ASA) in the culture of lysine-supplemented medium.
[c] AAR, aminoadipate reductase; SRD, saccharopine reductase; SDH, saccharopine dehydrogenase; ND, not determined.

original clone YpB1078, and subclones YpB1113 and YpB1041RG1. The sizes of the plasmids were determined to be as follows: vector YpB1041, 9.9 kb; clone YpB1078, 14.79 kb; subclone YpB113, 12.9 kb; and subclone YpB1041RG1, 11.7 kb. The recombinant plasmid subclone YpB1041RG1 was constructed by ligating the 1.8-kb *Hin*dIII *LYS1* DNA fragment into the *Hin*dIII site of the 2μm segment of vector YpB1041 (Fig. 2b). The cloned *LYS1* gene is localized in a 5-kb *Eco*RI-*Sal*I DNA insert of YpB1078, a 3-kb *Sau*3a-*Cla*I DNA insert of YpB1113, and a 1.8-kb *Hin*dIII-*Hin*dIII DNA insert of subclone YpB1041RG1 (Fig. 2). Restriction digestion of the original clone and subclone YpB1041RG1 with *Hin*dIII resulted in a common 1.8-kb DNA band, confirming the homology of the subclone with the DNA insert of plasmid YpB1078. This 1.8-kb *Hin*dIII-*Hin*dIII DNA fragment is not present in the vector (Fig. 3). Plasmid YpB1078 contained three *Hin*dIII sites (one within the 2μm segment and two within the *LYS1* DNA segment), resulting in three bands upon *Hin*dIII digestion (Fig. 3, lane 3).

TABLE 3. Functional complementation of a *lys1* mutant with *C. albicans* DNA in a plasmid

Plasmid (DNA insert)	Complementation of *lys1* auxotroph of[a]:	
	S. cerevisiae	*C. albicans*
YpB1041 (no insert)	−	−
YpB1078 (5-kb *Sau*3A-*Sau*3A insert)	+	+
YpB1113 (3-kb *Sal*I-*Sau*3A insert)	+	+
YpB1041RGI (1.8-kb *Hin*dIII-*Hin*dIII insert)	+	ND

[a] +, transformed lysine auxotroph to prototrophy; −, did not transform lysine auxotroph; ND, not determined.

DISCUSSION

Determination of five enzymes of the α-aminoadipate pathway established the presence of this pathway for lysine biosynthesis in the pathogenic fungi *C. albicans*, *C. neoformans*, and *A. fumigatus*. Differences in the levels of activity

TABLE 4. Saccharopine dehydrogenase activity of wild-type, *lys1* mutant, and *Lys1*$^+$ transformed strains of *C. albicans* and *S. cerevisiae*

Organism and strain	Lysine genotype	Saccharopine dehydrogenase activity (ΔA/min/mg of protein)
C. albicans		
WT207	*LYS*	0.41
STN57	*lys1*	0.08
STN57-J_2(YpB 1078)[a]	*Lys1*$^+$	0.34
STN57-J_2(YpB 1078)[a]	*Lys1*$^+$	0.43
STN57-J_3(YpB 1078)[a]	*Lys1*$^+$	0.97
S. cerevisiae		
STX4B	*lys1*	0.06
STX4B-4(YpB 1078)[a]	*Lys1*$^+$	0.51
STX4B-8(YpB 1078)[a]	*Lys1*$^+$	1.37
STX4B-4(YpB 1078)[b]	*lys1*	0.04
STX4B-8(YpB 1078)[b]	*lys1*	0.05
STX4A	*lys1*	0.08
STX4A-8(YpB 1078)[a]	*Lys1*$^+$	0.26
STX4A-8(YpB 1078)[b]	*lys1*	0.06
STX4A	*lys1*	0.08
STX4A-10(YpB 1041RGI)[a]	*Lys1*$^+$	0.23
STX4A-10(YpB 1041RGI)[b]	*lys1*	0.06
STX4A-16(YpB 1041RGI)[a]	*Lys1*$^+$	0.27
STX4A-16(YpB 1041RGI)[b]	*Lys1*	0.06
Wild type	*LYS*	0.49

[a] Mutant strain transformed with stated plasmid.
[b] Transformed strain which lost the plasmid after serial transfer on YEPD medium; cells from selected colonies were grown in lysine-supplemented minimal medium.

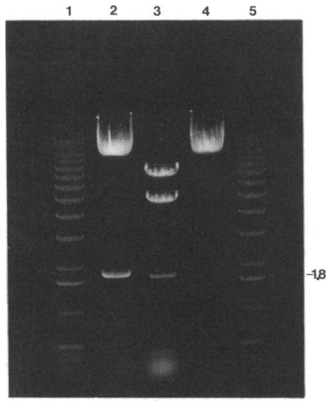

FIG. 3. Electrophoretic analysis of restriction enzyme (*Hin*dIII)-digested plasmids YpB1078 and YpB1041RG1 containing the *LYS1* gene of *C. albicans*. Lanes: 1 and 5, 1-kb DNA standard ladders (Bethesda Research Laboratories); 4, vector YpB1041; 3, YpB1078; 2, YpB1041RG1.

in different wild-type organisms may indicate transcriptional or posttranslational regulation of the enzymes. Higher levels of homocitrate synthase, homoisocitrate dehydrogenase, and α-aminoadipate reductase in *C. albicans* may be the result of a gene dosage effect due to homozygous dominant genes responsible for these enzymes in this diploid organism (17, 24, 27). Such high enzyme activity, however, may offer a detection probe for *C. albicans* in clinical specimens. The lack of saccharopine reductase activity in *C. neoformans* 32608 serotype C is unexplainable, particularly because the organism grew in minimal medium. Inactivation of this enzyme during cell homogenization is not a satisfactory explanation because the enzymes of cells from other two serotypes were prepared by an identical procedure.

Biochemical characterization of lysine auxotrophs established the biochemical blocks for one *C. tropicalis* mutant and seven mutants of *C. albicans* (Fig. 1). Results of growth response, accumulation, and enzyme studies indicated that all of the mutants were blocked in the second half of the pathway (after the α-aminoadipate step). The lack of α-aminoadipate reductase activity in five mutants, saccharopine reductase activity in one mutant, and saccharopine dehydrogenase activity in two mutants demonstrates unequivocally the physiological and biosynthetic role of these enzymes in the α-aminoadipate pathway. The lack of enzyme activity also confirms that the mutants are stable and homozygous recessive. The majority of the mutants are blocked in the α-aminoadipate reductase step. A large number of lysine mutants of *S. cerevisiae* arising from two distinct loci, *lys2* and *lys5*, are blocked in this step (30, 31). There are also two genes, *LYS1* and *LYS7*, responsible for the production of α-aminoadipate reductase of *S. pombe* (39). It is not certain whether the α-aminoadipate reductase mutants of *C. albicans* arise from one or two loci. Similarly, there are two distinct loci, *lys9* and *lys14*, responsible for saccharopine reductase in *S. cerevisiae* (2, 5). The status of X-16, whether isogenic to *lys9* or *lys14*, is not known. Since *C. albicans* is not amenable to genetic complementation, heterologous transformation with cloned lysine genes could be used to identify each locus.

Homologous transformation of the STN57 *lys* (saccharopine dehydrogenase) mutant of *C. albicans* and heterologous transformation of *lys1* mutants STX4B and STX4A of *S. cerevisiae* by the cloned *LYS1* gene in plasmids YpB1078 and YpB1041RG1 indicate the isofunctional nature of these mutants. A significant saccharopine dehydrogenase activity in homologous and heterologous transformants indicates efficient expression of the cloned gene in two different hosts. The cloned *LYS1* gene is localized in the 5-kb *Eco*RI-*Sal*I DNA insert of the original clone YpB1078 and the 3-kb *Sau*3A-*Cla*I and 1.8-kb *Hin*dIII-*Hin*dIII DNA inserts of subclones YpB1113 and YpB1041RG1. The isofunctional gene for saccharopine dehydrogenase (designated as *LYS5*) of *Y. lipolytica* has been cloned in a 4.8-kb *Bgl*II-*Sph*I DNA insert of plasmid pINA127 (38), and *S. cerevisiae LYS1* has been cloned in a 5-kb *Bgl*II-*Bgl*II DNA insert of plasmid pFRI (25). The restriction sites of the *S. cerevisiae* gene are not known. However, the restriction sites of the *Y. lipolytica* and *C. albicans* genes exhibit no homology. Complete molecular characterization of such isofunctional genes would provide important insights into the evolution of lysine genes for the α-aminoadipate pathway in fungi. This study represents biochemical characterization of the largest number of amino acid auxotrophs of *C. albicans* (24). Lysine and α-aminoadipate are used as sole nitrogen sources by *C. albicans* (40); however, these amino acids when used as sole nitrogen sources are growth inhibitory and cause morphological alterations (pseudohypha formation) of *S. cerevisiae* (37). Finally, studies of lysine mutants indicate that lysine is synthesized by the α-aminoadipate pathway. Since the α-aminoadipate pathway is not present in the host and auxotrophs are nonpathogenic (29), any impairment of this pathway may be used to control the growth and pathogenesis of *C. albicans*. Also, well-characterized enzymes and genes and the ability to use lysine and α-aminoadipate as sole nitrogen sources (40) may be used for rapid detection of *C. albicans*.

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