D-Arabitol Metabolism in *Candida albicans*: Studies of the Biosynthetic Pathway and the Gene That Encodes NAD-Dependent D-Arabitol Dehydrogenase

BRIAN WONG,* JEFFREY S. MURRAY, MIGUEL CASTELLANOS,
AND KENNETH D. CROEN

*Division of Infectious Diseases, Department of Internal Medicine, University of Cincinnati
College of Medicine, Cincinnati, Ohio 45267-0560*

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Candida albicans produces large amounts of the pentitol D-arabitol in culture and in infected mammalian hosts, but the functional and pathogenic significance of D-arabitol in *C. albicans* is not known. In this study, we sought to elucidate the pathway by which *C. albicans* synthesizes D-arabitol and to identify and characterize key enzymes in this pathway. *C. albicans* B311 produced D-[¹⁴C-1]arabitol from [¹⁴C-2]glucose; this finding implies on structural grounds that D-ribulose-5-PO₄ from the pentose pathway is the major metabolic precursor of D-arabitol. NAD- or NADP-dependent pentitol dehydrogenases catalyze the final steps in D-arabitol biosynthesis in other fungi; therefore, lysates of *C. albicans* B311 were tested for enzymes of this class and were found to contain a previously unknown NAD-dependent D-arabitol dehydrogenase (ArDH). The ArDH structural gene was cloned by constructing a new D-arabitol utilization pathway in *Escherichia coli*. The *C. albicans* ArDH gene expressed in *E. coli* and *Saccharomyces cerevisiae* an enzyme that catalyzes the reaction D-arabitol + NAD = D-ribulose + NADH; this gene was present as a single copy per haploid genome, and its deduced peptide sequence was homologous with sequences of several members of the short-chain dehydrogenase family of enzymes. These results suggest that (i) *C. albicans* synthesizes D-arabitol by dephosphorylating and reducing the pentose pathway intermediate D-ribulose-5-PO₄ and (ii) ArDH catalyzes the final step in this pathway.

Acyclic polyols are produced in large amounts by many fungi, and several functions have been ascribed to these compounds. For example, it has been proposed that fungi utilize polyols as storage or transport carbohydrates, as intracellular osmoregulatory solutes, and as metabolic reducing equivalents. Also, it has been suggested that fungal polyol metabolic pathways participate in the regulation of intracellular NAD(P)/NAD(P)H ratios and pH levels (14, 16). In 1979, Kiehn et al. (19) showed that cultures of the human pathogen *Candida albicans* produced large amounts of the acyclic pentitol D-arabitol and that people with invasive candidiasis had higher concentrations of arabitol in the blood than did normal or superficially colonized controls. Subsequent studies established that tissue and body fluid arabitol concentrations rose directly in proportion to fungal load in animals with *C. albicans* infection (42), that the excess arabitol in infected animals and humans was D-arabitol (the enantiomer produced by *Candida* spp.) (2, 43), that most humans with invasive candidiasis had high serum D-arabitol levels (11, 44), and that these levels declined with effective therapy (40). Thus, D-arabitol is a quantitative diagnostic marker for invasive *Candida* infections.

Several groups have investigated the clinical diagnostic implications of D-arabitol metabolism by *C. albicans* (9, 31, 36), but more basic aspects of D-arabitol metabolism have not been studied. Consequently, the metabolic pathway by which *C. albicans* synthesizes D-arabitol is not known, nor have the functional and pathogenetic significances of D-arabitol metabolism in *C. albicans* been examined. The goals of this study were to elucidate the D-arabitol biosynthetic pathway in *C. albicans* and to identify and characterize key

enzymes in this pathway. This report describes (i) metabolic labeling studies that suggest that the pentose pathway intermediate D-ribulose-5-PO₄ is the major metabolic precursor of D-arabitol in *C. albicans*, (ii) the presence in *C. albicans* lysates of a previously unknown NAD-dependent D-arabitol dehydrogenase (ArDH), and (iii) molecular cloning and analysis of the gene that encodes ArDH.

MATERIALS AND METHODS

Strains and media. *C. albicans* B311 (from H. Buckley, Temple University), WO-1 (from B. B. Magee, University of Minnesota), 1006 (from S. Scherer, University of Minnesota), and 1001 (ATCC 64385) were cultured in Trypticase soy broth (BBL, Cockeysville, Md.), yeast nitrogen base (Difco, Detroit, Mich.)–1% glucose (YNB-glucose), or 0.5% yeast extract–1% peptone–1% glucose (YPD).

Saccharomyces cerevisiae BWG 1-7A (*MATa his4-119 leu2-112 ura3-52 ade1-100*; from J. Loper, University of Cincinnati) was cultured in YPD or in 0.5% yeast extract–1% peptone–1% D-arabitol (YP-D-arab). Uracil prototrophs were selected on synthetic complete medium lacking uracil (12).

Escherichia coli JM109 (45) and DH5- α (GIBCO BRL, Gaithersburg, Md.) were grown in LB medium (31) supplemented as needed with 50 to 100 μ g of ampicillin per ml (LB-Ampicillin), 1% D-arabitol, and/or 2% agar. The *araC*(Con) mutant strain *E. coli* BW31 was isolated by selecting for growth of *E. coli* JM109 on minimal L-arabinose–D-fucose plates; the *araC*(Con) phenotype was verified by demonstrating L-arabinose isomerase in extracts of uninduced (LB-grown) cells (35). *E. coli* BW31M is a mucoid variant of *E. coli* BW31; its derivation is described below. *E. coli* strains were tested for the ability to utilize D-arabitol on

* Corresponding author.

MacConkey agar base (Difco)–1% D-arabitol (MacConkey–D-arab) and eosin-methylene blue agar base (Difco)–1% D-arabitol (EMB–D-arab); D-arabitol utilizers (D-arab⁺) produce pink or red colonies on MacConkey–D-arab plates and darkly pigmented colonies on EMB–D-arab plates, whereas D-arabitol nonutilizers (D-arab[–]) produce nonpigmented colonies on both media. *E. coli* strains were tested for the ability to utilize D-xylose on minimal D-xylose plates.

Lactobacillus pentosus (ATCC 8041) was used to ferment D-xylulose to acetate and lactate (10).

Metabolic labeling of D-arabitol. ¹⁴C labeling studies were used to study D-arabitol biosynthesis in *C. albicans*. One milliliter of a saturated overnight culture of *C. albicans* B311 in YNB-glucose was diluted into 9 ml of fresh medium containing 10 μ Ci of [¹⁴C-2]glucose (Dupont NEN, Boston, Mass.). The culture was shaken at 37°C for 8 h (late logarithmic phase), and the cell-free filtrate was deionized with mixed ion exchangers and lyophilized. D-arabitol in the lyophilized filtrate was purified by high-pressure liquid chromatography (HPLC) (HPX-87P column [Bio-Rad, Hercules, Calif.], elution with H₂O at 80°C), and one half of the purified D-arabitol was oxidized to D-xylulose with 2 U of purified *Klebsiella pneumoniae* D-arabitol dehydrogenase (43), 100 U of rabbit muscle lactic dehydrogenase (Sigma Chemical Co., St. Louis, Mo.), 1 mg of NAD, and 25 mg of sodium pyruvate (all in 1 ml of 0.1 M potassium phosphate [pH 7.3] for 4 h at 21°C). The reaction was stopped by precipitating the proteins with acetone, and D-xylulose in the supernatant was purified by HPLC. The D-xylulose was fermented to acetate and lactate by *L. pentosus*, and the amount of ¹⁴C in each carbon atom was measured by carbon degradation methods (10).

Lysis of cells and enzyme assays. *C. albicans* B311 was cultured in Trypticase soy broth for 24 h at 37°C, and the cells were washed and disrupted by vortexing with glass beads in 20 mM phosphate (pH 7)–10 mM 2-mercaptoethanol. *E. coli* transformants were grown in LB–ampicillin–1% D-arabitol at 37°C, and the cells were washed and disrupted by sonication in 0.1 M Tris (pH 7.3). *S. cerevisiae* transformants were grown in YP–D-arab at 30°C, and the cells were washed and disrupted by vortexing with glass beads in 0.1 M Tris (pH 7.3). Cell-free supernatants of the disrupted *C. albicans*, *E. coli*, or *S. cerevisiae* cells were prepared by centrifugation (20,000 $\times$ g, 30 to 60 min, 4°C) and analyzed for enzymes.

Polyol dehydrogenases in *C. albicans* lysates were assayed by monitoring NADH or NADPH appearance at A_{340} when the polyol of interest (10 mM) was added to 35 mM glycine (pH 9), 0.7 mM 2-mercaptoethanol, 0.34 mM NAD or NADP (all final concentrations), and the diluted cell supernatant. Polyol dehydrogenases in lysates of *E. coli* or *S. cerevisiae* were assayed by monitoring reduction of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide (MTT) at A_{578} when 12.5 mM polyol was added to 35 mM *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid (HEPES; pH 8.5), 0.2 mM NAD or NADP, 0.2 mM phenazine methosulfate, 0.4 mM MTT, and the diluted cell supernatant. Aldose and ketose reductases were assayed by monitoring NADH or NADPH disappearance (A_{340}) when 5 mM aldose or ketose was added to 35 mM glycine (pH 7), 7 mM 2-mercaptoethanol, 0.34 mM NADH or NADPH, and the diluted cell supernatant. In all enzyme assays, controls containing no carbohydrate substrates were analyzed in parallel. Specific activities were defined as micromoles of NAD(P) reduced or NAD(P)H oxidized per minute (units) per milligram of protein (4).

Construction of a D-arabitol utilization pathway in *E. coli*. Our strategy for cloning the *C. albicans* ArDH gene was to construct a new D-arabitol utilization pathway in *E. coli* K-12. In *Klebsiella aerogenes* (5, 6) and *E. coli* C (33), the four enzymes encoded by the pentitol operon catalyze D-arabitol and ribitol utilization. These enzymes are (i) D-arabitol (to D-xylulose) dehydrogenase, (ii) D-xylulokinase (which converts D-xylulose to the pentose pathway intermediate D-xylulose-5-PO₄), (iii) ribitol (to D-ribulose) dehydrogenase, and (iv) D-ribulokinase (which converts D-ribulose to the pentose pathway intermediate D-ribulose-5-PO₄). *E. coli* B and K-12 cannot utilize D-arabitol or ribitol because they lack the pentitol operon (30). However, *E. coli* K-12 mutants that are constitutive producers of either L-ribulokinase from the L-arabinose pathway [*araC*(Con) mutants] or L-fuculokinase from the L-fucose pathway (*fucR* mutants) can utilize D-ribulose for growth (21). Since our preliminary studies suggested that *C. albicans* ArDH interconverts D-arabitol with D-ribulose (see below), we reasoned that expression of functional *C. albicans* ArDH in an *E. coli* K-12 *araC*(Con) mutant should complete a D-arabitol utilization pathway.

Therefore, the *araC*(Con) *E. coli* strain BW31 was transformed with a library of *C. albicans* WO-1 genomic DNA fragments in the *E. coli*-*S. cerevisiae* shuttle plasmid pEM-BLYe23 (23) (kindly provided by B. B. Magee). A total of 3.3×10^4 ampicillin-resistant transformants were obtained; these were expanded on LB–ampicillin plates, pooled, and screened for D-arabitol utilizers on EMB–D-arab and MacConkey–D-arab plates. Mucoid dark colonies were first noted on the EMB–D-arab plates after 6 days of incubation at 37°C. When purified, the bacterial clones of interest produced large mucoid colonies on LB–Ampicillin, mucoid dark colonies on EMB–D-arab, and mucoid pink colonies on MacConkey–D-arab. Similar plasmids were isolated from several D-arab⁺ transformants; one of these (pE2A) was analyzed in detail.

Molecular genetics methods. The CaCl₂ (32) and colony transformation (13) methods were used to transform *E. coli* with plasmid DNA, and the lithium acetate method was used to transform *S. cerevisiae* (12). Standard methods were used to extract plasmid DNA from *E. coli* and to analyze the DNA by restriction enzyme digestion and agarose gel electrophoresis (32). The insert in plasmid pE2A was mapped with restriction enzymes, various restriction fragments were subcloned into pBluescript II SK⁺ (Stratagene, La Jolla, Calif.) or pGEM3Z (Promega, Madison, Wis.), and the subclones were tested for the ability to transform *E. coli* BW31M to D-arab⁺.

Genomic DNA was extracted from *C. albicans* WO-1 spheroplasts (12), and it was digested with *Eco*RI, *Bam*HI, *Hind*III, *Xba*I, or *Xho*I. The digests were separated by electrophoresis in 0.9% agarose, they were transferred to a nylon filter (Magnagraph; Micron Separations Inc., Westboro, Mass.), and the filter was hybridized (overnight, 65°C) with a 1.7-kb gel-purified *Bam*HI-*Hind*III fragment from the pE2A subclone pJB16 (see below) that had been labeled with digoxigenin-labeled dUTP by the random-primer method. The filter was washed in 2 $\times$ SSC (300 mM NaCl, 30 mM sodium citrate [pH 7])–0.1% sodium dodecyl sulfate (SDS) (twice from 5 min each time, 21°C) and then in 0.5 $\times$ SSC (75 mM NaCl, 7.5 mM sodium citrate [pH 7])–0.1% SDS (twice for 15 min each time, 65°C), and the probe was detected with alkaline phosphatase-conjugated antidigoxigenin antibody (Genius system; Boehringer Mannheim, Indianapolis, Ind.). *C. albicans* 1006, 1001, and WO-1 chromosomes were sep-

TABLE 1. Polyol dehydrogenase activities in *C. albicans* lysates

Substrate	Activity (μ mol reduced/min/mg of protein)	
	NAD	NADP
D-Arabitol	0.056	0
L-Arabitol	0	0.042
Xylitol	0.11	0
Ribitol	0.029	0
Glucitol	0.013	0
Mannitol	0	0.035
Galactitol	0	0.040

arated by pulsed-field gel electrophoresis (22), transferred to a nylon filter, and probed as described above.

The dideoxy method (32) was used for DNA sequencing; synthetic oligonucleotide primers were purchased as needed. DNA and deduced protein sequences were analyzed with the PC/Gene program (IntelliGenetics, Mountain View, Calif.). The FASTA program (26) was used to search the EMBL and SWISS-PROT data bases for homologous gene and protein sequences, and homologous sequences were aligned by using PC/GENE.

Nucleotide sequence accession number. The nucleotide sequence of the ArDH gene has been deposited in the GenBank data base under accession number L16227.

RESULTS

D-arabitol biosynthesis by *C. albicans*. The cell-free filtrate of the 10-ml *C. albicans* B311 culture contained 490 μ g of D-arabitol, and this D-arabitol contained 0.45 μ Ci of 14 C. Most (69.5%) of the D- 14 C]arabitol was labeled at position C-1, and the remainder was distributed among the other four carbons (9.4% at C-2, 3.7% at C-3, 10.2% at C-4, and 7.2% at C-5).

Polyol dehydrogenases of *C. albicans*. Since NAD- or NADP-dependent polyol dehydrogenases catalyze the final steps in D-arabitol biosynthesis in several other fungi (3, 15, 23, 39), we examined a lysate of *C. albicans* B311 for enzymes of this class. The lysate reduced NAD in the presence of xylitol, D-arabitol, ribitol, or glucitol, and it reduced NADP in the presence of L-arabitol, galactitol, or mannitol (Table 1). Also, the lysate oxidized NADH in the presence of D-xylulose (0.065 U/mg of protein) or D-ribulose (0.019 U/mg of protein), but it oxidized no measurable NADH in the presence of D-arabinose, L-arabinose, D-xylose, D-mannose, or D-fructose. *C. albicans* is known to have (i) an NAD-dependent dehydrogenase that interconverts xylitol with D-xylulose, ribitol with D-ribulose, and glucitol with D-fructose (38) and (ii) an NADP-dependent dehydrogenase that interconverts xylitol with D-xylose, ribitol with D-ribose, and L-arabitol with L-arabinose (37). D-Arabitol is not a substrate for the NAD-dependent dehydrogenase (38); therefore, the fact that the *C. albicans* lysate reduced NAD in the presence of D-arabitol implies the presence of a previously unknown ArDH.

Properties of plasmid pE2A. Efforts to purify ArDH from *C. albicans* have not succeeded; therefore, we sought to clone the ArDH structural gene. Plasmid pE2A was isolated from a D-arab⁺ *E. coli* BW31 transformant, and the phenotypic consequences of its loss and reacquisition were assessed as follows. First, the transformant was expanded in

liquid LB without ampicillin, an aliquot was streaked on MacConkey-D-arab medium, and a nonpigmented colony was identified and purified. This strain (*E. coli* BW31M) produced large mucoid colonies on LB agar, did not grow on LB-ampicillin, and produced nonpigmented colonies on MacConkey-D-arab and EMB-D-arab media. In addition, lysates of *E. coli* BW31M contained no plasmid DNA that could be visualized in agarose gels stained with ethidium bromide. When *E. coli* BW31M was transformed with pE2A, the ampicillin-resistant transformants were D-arab⁺; conversely, *E. coli* BW31M cells transformed with the control plasmid pEMBLye23 were D-arab⁻. Thus, the D-arab⁺ phenotype was mediated by plasmid pE2A.

Properties of recombinant ArDH. To determine whether pE2A transformed *E. coli* BW31M to D-arab⁺ by expressing functional ArDH, we tested lysates of pE2A- and pEMBLye23-transformed *E. coli* BW31M and *S. cerevisiae* BWG 1-7A cells for ArDH activity. The pE2A-transformed bacteria contained 0.17 U of ArDH per mg of protein, compared with 0.003 U/mg of protein in the pEMBLye23-transformed controls. Similarly, pE2A-transformed yeast cells contained 0.37 U of ArDH per mg of protein, compared with 0.046 U/mg of protein in pEMBLye23-transformed controls.

To identify the ketopentose substrate of recombinant ArDH, lysates of *E. coli* BW31M cells that had been transformed with a functional subclone of pE2A (pJB4; see below) were tested for NADH-dependent D-ribulose and D-xylulose reductase activities. The lysates contained 0.22 U of NADH-dependent D-ribulose reductase activity per mg of protein and no measurable D-xylulose reductase activity. In contrast, lysates of control cells transformed with pBlue-script II SK⁺ contained no measurable NADH-dependent D-ribulose or D-xylulose reductase activity.

Further evidence that ArDH interconverts D-arabitol with D-ribulose was obtained by comparing pE2A-transformed *E. coli* BW31M and JM109 for the ability to utilize D-arabitol. The *E. coli* BW31M transformants were D-arab⁺, whereas *E. coli* JM109 transformants were D-arab⁻. Both *E. coli* host strains grew well on minimal D-xylose medium; therefore, both have a D-xylulose-inducible D-xylulokinase and can grow on D-xylulose (41). In contrast, only strain BW31M expresses L-ribulokinase constitutively and is able to grow on D-ribulose. Therefore, the fact that pE2A transformed *E. coli* BW31M (but not *E. coli* JM109) to D-arab⁺ implies that the ketopentose substrate of ArDH is D-ribulose (not D-xylose).

Hybridization results. The 1.7-kb *Bam*HI-*Hind*III pJB16 probe hybridized to single bands in *Eco*RI, *Bam*HI, *Hind*III, *Xba*I, and *Xho*I digests of *C. albicans* WO-1 genomic DNA (Fig. 1). Thus, the gene of interest was derived from *C. albicans*, and there is a single copy of this gene per haploid genome.

The same probe hybridized to (i) chromosome 6 of *C. albicans* 1001, (ii) chromosome 6 of *C. albicans* 1006, and (iii) chromosomes 4 and 6 of *C. albicans* WO-1 (Fig. 2). *C. albicans* WO-1 has an electrophoretic karyotype more complex than those of most other *C. albicans* strains (34), and Chu and Magee (8) have recently shown that one of the homologs of chromosome 4 in *C. albicans* WO-1 is the result of a reciprocal translocation between *Sfi*I restriction fragment O of chromosome 6 and *Sfi*I fragment M of chromosome 5. Therefore, our results show that the ArDH gene is located on *Sfi*I fragment O of *C. albicans* chromosome 6.

Restriction mapping and subcloning. Restriction maps of the 5.5-kb insert from pE2A and the inserts of several pE2A

FIG. 1. Genomic Southern hybridizations. *EcoRI*, *BamHI*, *HindIII*, *XbaI*, and *XhoI* digests of *C. albicans* WO-1 genomic DNA were separated by agarose gel electrophoresis, transferred to a nylon filter, and probed with a digoxigenin-labeled 1.7-kb *BamHI*-*HindIII* fragment derived from plasmid pE2A. The probe hybridized to single bands in all five restriction digests, indicating that *C. albicans* has one copy of the ArDH gene per haploid genome. Molecular sizes in kilobases are shown at the left.

subclones are shown in Fig. 3. The smallest subclone that transformed *E. coli* BW31M to D-arab⁺ was pJB4.0, which consists of a 3.6-kb *HindIII*-*ClaI* restriction fragment in pBluescript II SK⁺. In contrast, plasmids containing all of the insert DNA in pJB4.0 to the left or to the right of the internal *SalI* or *KpnI* restriction sites did not transform *E. coli* BW31M to D-arab⁺. This finding suggested that the *SalI*

FIG. 2. Chromosome blot. Chromosome-size DNA from *C. albicans* 1001, WO-1, and 1006 spheroplasts was separated by contour-clamped homogeneous electric field electrophoresis, transferred to a nylon filter, and probed with a digoxigenin-labeled 1.7-kb *BamHI*-*HindIII* fragment derived from plasmid pE2A. (A) Ethidium bromide-stained agarose gel; (B) hybridized filter. The probe hybridized to chromosome 6 of *C. albicans* 1001 and 1006 and to chromosomes 4 and 6 of *C. albicans* WO-1.

FIG. 3. Restriction maps and functional analyses of pE2A and its subclones. Plasmids pE2A, pJB4, and pJB4.0 transformed *E. coli* BW31M to D-arab⁺. In contrast, plasmids containing all of the pJB4.0 insert DNA to the left (pJB5) or to the right (pSL2) of the *SalI* site or to the left (pJB6) or to the right (pSL4) of the *KpnI* site did not. This finding suggested that the *SalI* and *KpnI* sites are within the essential domain of the ArDH gene, and this possibility was confirmed by deleting the *SalI*-*KpnI* fragment and also by the DNA sequence. The arrow shows the ArDH gene coding region and the direction of transcription. Abbreviations: A, *AccI*; B, *BamHI*; C, *ClaI*; H, *HindIII*; K, *KpnI*; S, *SalI*.

and *KpnI* restriction sites are within the essential domain of the gene of interest. Therefore, pJB4.1 was constructed by replacing the 225-bp *SalI*-*KpnI* fragment in pJB4.0 with a short *SalI*-*XhoI*-*KpnI* linker (from the multiple cloning site of pBluescript II SK⁺). *E. coli* BW31M cells transformed with pJB4.1 were D-arab⁺, thus verifying that the *SalI*-*KpnI* restriction fragment is essential for expression of functional ArDH.

Sequence of the ArDH gene. Once the ArDH gene had been localized as described above, the 1,614 bp of DNA to the left of the rightmost *AccI* site in Fig. 3 was sequenced in both directions. This region contains an 843-bp open reading frame with no introns, several potential TATA elements in the 5' flanking region, and an *S. cerevisiae*-type transcription termination signal (46) in the 3' flanking region (Fig. 4). The open reading frame encodes 281 amino acids, and the molecular mass of the deduced protein is 30,643 Da. To verify that we had sequenced the correct portion of the pE2A insert, nucleotides 4 through 1304 were amplified by the polymerase chain reaction (32) and cloned into the *EcoRV* site of pBluescript II SK⁺; the resulting plasmid (pJB14) transformed *E. coli* BW31M to D-arab⁺.

The EMBL data base contained no nucleotide sequences that were closely homologous to the ArDH gene. The mean $\pm$ standard deviation Initn homology score (26) for all proteins in the SWISS-PROT data base was 21.9 ± 3.70 . Twenty-five proteins had Initn scores of >80 (i.e., >15 standard deviations above the mean), and 10 of these proteins had optimized homology scores of >175 . All 10 of these proteins are members of the extended superfamily of short-chain alcohol-polyol-sugar dehydrogenases (17, 27). Figure 5 shows an alignment of ArDH with two of the highest-scoring proteins; there was considerable amino acid homology in the N-terminal region (which contains the cofactor binding domain [17]) and in a central region that contains the absolutely conserved pentapeptide Y-x-x-x-K (26).

DISCUSSION

Two D-arabitol biosynthetic pathways have been described in fungi. In the osmophilic yeast species *Saccharomyces*

1 AAAGAAACGACGACCTATTCACATTTTCAAAAAAATTCATACCTGATTTTAGCAAAACCAAGCTCGATGACGTTAGATGCTAGGCCATGTGT
 101 ACACAAACATCTTTGGCTACGATATATGCAAAACACGATGATCTATTAAGAAATTTCAACCAACATGAGGCTCTTGATAAAGCACATTGG
 201 TTAATAAATTTTCCGCGGACCTGTATATAGCCCACTTTTATTAATTTCTCCGTAGAACTAATTTCTCGGTGCATTATACATAACAAT
 301 TTATATCTTCCGAAAGAAACATTGAGCGGCTCATCTCTCCGAAAAATATATAACACACTCATATCACCTTTTTCGATTTTCTCTCCC
 401 CCTACAACCTACTCTTTATTCTCACTACACATAATGGATTCGCATACGTAGTAACTAGTCCCAAGCTTCCGTTGGATGGAAACATAGT
 501 I L T G G S G G L A A V V S R A L L A K G A D V A L V D M N L E R T
 ATATTAACCGGTGGCTGGTGGTTGGCTGCCGTGATCAAGAGCTTTATAGCCAAAGGTGCCGATGTCATTAGTCGATATGAATCGGAAGAA
 601 Q Q A A R D V L Q W G E E Q M K G K Y E S P I G Q V S A W S C N I
 CACAACAAGCTGTAGAGACGCTTACAATGGGCGAAGAGCAATGAAAGTAAATACGAATCACCACCGTCAGGTGAGTCTGGTCATGTAATAT
 701 G D A E A V D L T F K A I N E H H G K I S S V L V N T A G Y A E N
 TGGCGATGCTGAAGCTGTGACATTCAAGCCATCAACGAACACACGCGAAAAATCTCAAGTGTCTTGGTCAACACTGCCGTTACGCTGAAAC
 801 F P A E E Y P A K N A E N L M K V N G L G S F Y V S Q A F A R P L I
 TTCCAGCTGAAGAGTACCCAGCAAGACGCTGAAACCTTATGAAAGTAAACGGGTGGGGTCATTACGTTTCCCAAGCTTTGCTAGACCATTA
 901 Q N N M T G S I I L I G S M S G T I V N D P Q P Q C M Y N M S K A
 TCCAAAACACATGACCGGATCGATCATTTGATCGGTCATGTCGGTACCATCGTCAACGACCCACCAACCAATCATGTACACATGTCCAAAGC
 1001 G V I H L A R S L A C E W A K Y N I R V N T L S P G Y I L T P L T
 CGGTGTCATTATTAGCCAGATCATGGCTGTGAATGGCTAAATACAATCATGAGTCAACACATTGTGCCAGGGTACATCTTAACCCATTGACA
 1101 R N V I S G H T E M K T E W E S K I P M K R M A E P K E F V G S I L
 AGAAACGTTATTAGTGGACACCGAAATGAAGACAGATGGGAATCAAGATCCCAATGAAGAGATGGCAACCAAAAGAGTTTGTGGTCTATCT
 1201 Y L A S E S A S S Y T T G H N L V D G G Y E C W
 TATACCTGGCTCAGAATCTGCTCATACACTACTGGTCAACCTTGGTGTGACGGGGTTACGAGTGTGTTAAACCCACTGTTTACCAATA
 1301 TTTATTGGTTTATGCTGTATATTAGAGTTTAGGGTATTAATGAAAGTCGCGTGTTTTCAAGAAATAGCGTCTATTGTCCACCAAAAGGCTT
 1401 GCAGATTCTTTTATAGCCAGCACGCTGCGAAAAAGAGTATATAGCACCACCAAAATTCCTTTTACAAGTACACCATACTTTTATCAC
 1501 CCAAGTCACTGTTAAATCGTTATCTACTGTTTACCTTTATGCTTTTGTACGCCAACTACACCACATGGTCTTTAGTCACAGACTATACCACTAC
 1601 TGTCCACAGTCTAC

FIG. 4. Nucleotide sequence of the *C. albicans* ArDH gene and deduced amino acid sequence of ArDH. The functionally important domain in plasmid pE2A contained a single long open reading frame of 843 bp. This open reading frame encodes a deduced protein of 281 amino acid residues and molecular mass 30,643 Da. The 5' flanking region contains several potential TATA elements (underlined), and the 3' flanking region contains an *S. cerevisiae*-type transcription termination signal (also underlined).

rouxii (15), *Saccharomyces mellis* (39), and *Debaryomyces hansenii* (18), glucose is converted to D-ribulose-5-PO₄ via the pentose phosphate pathway, and then D-ribulose-5-PO₄ is dephosphorylated and reduced to D-arabitol. The final step in this pathway is catalyzed by an NADP-dependent pentitol dehydrogenase. In contrast, another strain of *S. rouxii* (3) and the marine fungus *Dendryphiella salina* (23) convert glucose to D-xylulose-5-PO₄ via the pentose pathway, and then D-xylulose-5-PO₄ is dephosphorylated and reduced to D-arabitol; the final step in this pathway is catalyzed by an NAD-dependent pentitol dehydrogenase.

In the present study, *C. albicans* converted [¹⁴C-2]glucose to D-[¹⁴C-1]arabitol. This labeling pattern implies that C-1 of glucose was lost; thus, *C. albicans* (like the fungi discussed above) synthesizes D-arabitol via the pentose pathway. Also, since oxidation of the C-2 hydroxyl group of D-arabitol yields D-ribulose, the observed labeling pattern implies on structural grounds that D-ribulose-5-PO₄ is the major metabolic precursor of D-arabitol. Since oxidation of the C-4 hydroxyl of D-arabitol yields D-xylulose, D-arabitol synthesized via D-xylulose-5-PO₄ would have been labeled at C-5. The most likely explanation for the small amounts of ¹⁴C in positions C-2 to C-5 of D-arabitol is that ¹⁴C was incorporated into other compounds that were subsequently recycled into the pentose pathway; however, the possibility of additional D-arabitol pathways cannot be excluded.

We also found in a lysate of *C. albicans* B311 a previously undescribed ArDH. To characterize this enzyme, we cloned its structural gene by taking advantage of (i) the ability of L-ribulokinase from *E. coli* to convert D-ribulose to D-ribulose-5-PO₄ (22), (ii) constitutive expression of L-ribulokinase by *E. coli* *araC*(Con) mutants (35), and (iii) the absence of

ARDH CA	MDSAYWSYDNIVPSFRLDGKLVLTGGSSGLAAVVSRLAKGADVALVD	50
FABG EC	M-----N-----FEKGLALVTGASRGIGRAIAETLAARGGKVI---	33
DHGB BM	M-----YKD-----LEGKVVVITGSSTGLGKSMARFATEKAKV---	35
	*	
ARDH CA	MNLTERTQQAARDVLQWGEQMKGYESPIGQVSAWSNIGDAEAVDLTFK	100
FABG EC	GT-ATSENGAQALSDY----LGANGKGLMLNVT-----DPASIESVLE	71
DHGB BM	VNYRSKEDANSVLEEIKKVGGEAIAVKGDVT-----VESDVINLVQ	78
		
ARDH CA	AINEHHGKISSVLNTAGYAENFPAEYPAKNAENLMKVNGLSGFYVSQA	150
FABG EC	KIRAEFGVD--LVNNAIGITRDNLMRMKDEEWNDIETNLSVFRLSKA	120
DHGB BM	SAIKEFGKLD--VMINNAGMENPVSSHEMSLDWNKVIDTNLTGAFGLSRE	127
		
ARDH CA	FARPLIQNNMTGSIILIGSMGTVNDPQPQCMYNSKAGVIHLARSLAC	200
FABG EC	VIRAMMKR--HGRIITIGSVVG--TWNGNG--QANYAAKAGLIGFSKSLAR	167
DHGB BM	AIKYFVENDIKGTVINMSSVHEMKIPWPL--FVHYAASKGGMKMTETLAL	176
		
ARDH CA	EWAKYNIRVNTLSPGYILTLPLTRNVISGHEMTEWESKIPMKRMAEPKE	250
FABG EC	EVASRGITVNVVAPGFIEDM--TRALSD--DQAGILAQVPAGRLGGAQE	214
DHGB BM	EYAPKGIKIRVNNIGPGAINTPINAEPADP--EQRADVESMIPMGYIGPEE	225
	*	
ARDH CA	FVGSILYLASESASSYTTGHNLVVDG---YECW-----	281 (1.00)
FABG EC	IANAVAFLASDEAA--YITGETLTVNGGM--Y-----MV	244 (0.26)
DHGB BM	IA-AVAVLASSEAS--YVTGITLFDAGGGTQYPSFQARG	262 (0.28)
		

FIG. 5. Alignment of ArDH and two other short-chain dehydrogenases. The amino acid sequences of ArDH (ARDH CA), 3-oxo-acyl-acyl carrier protein reductase from *E. coli* (FABG EC), and glucose dehydrogenase B from *Bacillus megaterium* (DHGB BM) are homologous in several regions, including the N-terminal cofactor binding domain (ArDH residues 22 to 40) and a central region that includes the highly conserved pentapeptide Y-x-x-x-K (ArDH residues 174 to 178). Positions marked by asterisks are perfectly conserved within the group, and those marked by periods are highly conserved. The proportion of each protein's amino acids that are identical to those in ArDH is shown in parentheses.

introns (which would preclude expression in *E. coli*) in most known *C. albicans* genes. Several independent lines of evidence indicate that (i) plasmid pE2A and its derivatives encode ArDH and (ii) ArDH catalyzes the reaction D-arabitol + NAD $\rightleftharpoons$ D-ribulose + NADH. First, lysates of pE2A-transformed *E. coli* and *S. cerevisiae* reduced NAD in the presence of D-arabitol, and lysates of pJB4-transformed *E. coli* oxidized NADH in the presence of D-ribulose. Second, pE2A transformed the *araC*(Con) mutant *E. coli* BW31M (but not its parent *E. coli* JM109) to D-arab⁺. Third, the deduced protein encoded by the ArDH gene has substantial sequence homology with many members of the extended family of short-chain alcohol-polyol-sugar dehydrogenases (17, 27). Enzymes in this family catalyze diverse metabolic oxidations and reductions in bacteria, fungi, plants, and animals; of particular relevance to ArDH is that NAD-dependent ribitol (to D-ribulose) dehydrogenase of *K. aerogenes* is a member of this family (17). Lastly, Quong et al. (28) have purified and characterized an enzyme from *Candida tropicalis* that also catalyzes the interconversion of NAD plus D-arabitol with NADH plus D-ribulose; the subunit molecular mass of this enzyme (31 kDa) is similar to the deduced molecular mass of *C. albicans* ArDH (30.6 kDa).

In summary, our results suggest that *C. albicans* synthesizes D-arabitol as follows. First, glucose is metabolized to D-ribulose-5-PO₄ via the pentose pathway; *C. albicans* is known to have all of the pentose pathway enzymes required to accomplish these steps (29). Next, D-ribulose-5-PO₄ is dephosphorylated to D-ribulose; the enzyme that catalyzes this step has not been demonstrated directly, but the cytoplasmic acid phosphatase of *C. albicans* is known to dephosphorylate several other sugar phosphates (7). Lastly, D-ribulose and NADH are converted to D-arabitol and NAD by ArDH. Most strains of *C. albicans* can also utilize D-arabitol for growth (1); thus, ArDH may also catalyze D-arabitol utilization when preferred substrates are absent.

Definitive proof that *C. albicans* synthesizes and/or utilizes D-arabitol via the proposed pathways would require specific enzyme inhibitors or enzyme-deficient *C. albicans* mutants, and neither of these is available. However, it has recently been shown that *C. albicans* mutants can be constructed by sequential disruption of both chromosomal homologs of single-copy genes (20). Since *C. albicans* has a single ArDH gene per haploid genome, we are currently endeavoring to use this approach to construct ArDH-deficient *C. albicans* mutants. We plan to use these mutants to assess directly the role of ArDH in D-arabitol biosynthesis and to study the functional and pathogenetic significance of D-arabitol in *C. albicans*.

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