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Contribution of Horizontal Gene Transfer to the Evolution of *Saccharomyces cerevisiae*†

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The genomes of the hemiascomycetes *Saccharomyces cerevisiae* and *Ashbya gossypii* have been completely sequenced, allowing a comparative analysis of these two genomes, which reveals that a small number of genes appear to have entered these genomes as a result of horizontal gene transfer from bacterial sources. One potential case of horizontal gene transfer in *A. gossypii* and 10 potential cases in *S. cerevisiae* were identified, of which two were investigated further. One gene, encoding the enzyme dihydroorotate dehydrogenase (DHOD), is potentially a case of horizontal gene transfer, as shown by sequencing of this gene from additional bacterial and fungal species to generate sufficient data to construct a well-supported phylogeny. The DHOD-encoding gene found in *S. cerevisiae*, *URA1* (YKL216W), appears to have entered the *Saccharomycetaceae* after the divergence of the *S. cerevisiae* lineage from the *Candida albicans* lineage and possibly since the divergence from the *A. gossypii* lineage. This gene appears to have come from the *Lactobacillales*, and following its acquisition the endogenous eukaryotic DHOD gene was lost. It was also shown that the bacterially derived horizontally transferred DHOD is required for anaerobic synthesis of uracil in *S. cerevisiae*. The other gene discussed in detail is *BDS1*, an aryl- and alkyl-sulfatase gene of bacterial origin that we have shown allows utilization of sulfate from several organic sources. Among the eukaryotes, this gene is found in *S. cerevisiae* and *Saccharomyces bayanus* and appears to derive from the alpha-proteobacteria.

In eukaryotes, a few examples of horizontal gene transfer have been well documented. Eukaryote-to-eukaryote horizontal transfer of mitochondrial genes has been recently reported between parasitic plants and their plant hosts (33). Two examples of interkingdom horizontal transfer from prokaryotes to eukaryotes are also well known: the transfer of genetic information by *Agrobacterium tumefaciens* into its plant symbiont (59) and the transfer of genetic information from mitochondria and chloroplasts to the nuclear genome (19). Beyond these two special cases, however, the frequency of transfer of genetic information from bacteria into eukaryotes, though postulated in a number of cases, is still a matter of debate. Prokaryotic genomic sequencing has revealed horizontal gene transfer as an important evolutionary mechanism among these organisms (30, 37). As the number of available sequenced eukaryotic genomes increases, these data can be used to determine the existence and/or frequency of horizontal gene transfer in specific lineages. Several cases of horizontal gene transfer from prokaryotes to microbial eukaryotes have been previously postulated including the 3-hydroxy-3-methylglutaryl–coenzyme A class 2 reductase found in *Giardia* (6), iron hydrogenase found in *Nyctotherus* (20), and the fungal catalases (see references 24 and 27 for reviews). A recent report by Dujon et al. (13) suggests that eight genes from *Yarrowia lipolytica*, five genes from *Kluyveromyces lactis*, and one gene from *Debaryomyces hansenii* are horizontally transferred. The phylogeny of horizontally transferred genes is characteristically different from the species phylogeny; these phylogenetic differences can also arise by means other than horizontal gene transfer such as accelerated gene evolution, gene loss, or horizontal transfer from a eukaryote to a prokaryote. Differences can also result from species misassignment of sequences due to DNA contamination. Cases of accelerated gene evolution in *Saccharomyces cerevisiae*, such as the gene for kinesin (31), and of gene loss (7) have been documented. Thus, to demonstrate that a gene has entered the *S. cerevisiae* or *A. gossypii* lineages by horizontal gene transfer, it is necessary to construct an extensive gene phylogeny to rule out potential alternative explanations such as those listed above.

In order to determine a broader estimate of horizontal gene transfer in a specific eukaryotic lineage, we employed a genome-wide comparative screen to determine the extent of horizontal gene transfer in the *S. cerevisiae* and *A. gossypii* lineages. This comparison allows us to identify potential cases of horizontal gene transfer since the divergence of these species; we expect these recent transfer events to be more readily identified and more easily experimentally supported than more ancient events.

In addition to being the best-studied eukaryotic model system, *S. cerevisiae* is the first eukaryote whose genome was completely sequenced (16). The *S. cerevisiae* genome encodes about 5,570 proteins (56) and is an ideal system in which to try to detect horizontal gene transfer from prokaryotes to eukaryotes. For comparative purposes a fungal genome sequence separated from *S. cerevisiae* by an appropriate evolutionary distance is needed. Closely related *Saccharomyces* species such as the *Saccharomyces* sensu stricto species (10, 26) contain a very similar gene set to that of *S. cerevisiae*, whereas in more distantly related fungi such as *Neurospora crassa* (15) homo-

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† Supplemental material for this article may be found at http://ec.asm.org/.

logues of only around 50% of *S. cerevisiae* genes can be identified. More useful evolutionary distances are represented by the recently published *A. gossypii* (11) and *Kluyveromyces waltii* (25) genomes, where approximately 95% of the genes have identifiable homologs with *S. cerevisiae*. Genes horizontally transferred since the divergence of these species with *S. cerevisiae* are to be found among the remaining 5% of genes lacking homologs between these species. In this work we focused on identifying genes horizontally transferred since the divergence of the *S. cerevisiae* and *A. gossypii* lineages, as we expect more recent transfer events to be more clearly identifiable. The work of Gojkovic et al. (17) has previously shown that *URA1* (GenPept accession no. P28272) is of bacterial origin. We show additional evidence supporting this claim and also that the *BDS1* (GenPept Q08347) gene of *S. cerevisiae* is of bacterial origin. *URA1* is the best-supported horizontal gene candidate in *S. cerevisiae*, and *ura1*Δ cells present an identifiable phenotype. Previous work by Nara et al. speculated that *URA1* might be horizontally transferred (36); as *URA1* from *S. cerevisiae* was the only fungal sequence included in their analysis insufficient data were provided to support this speculation. Further supporting evidence for a bacterial origin of *URA1* has been reported based on sequences from *Saccharomyces kluyveri* (17, 58). *URA1* encodes the 315-amino-acid protein dihydroorotate dehydrogenase (DHOD) (46). This enzyme catalyzes the conversion of dihydroorotate to orotate, the fourth step of the de novo pyrimidine biosynthetic pathway (22, 35). DHOD enzymes are grouped into families 1a, 1b, and 2. These groupings are based on nucleotide and biochemical characteristics (22). Eukaryotes typically have the family 2 DHOD enzyme. In this work we demonstrate that the family 2 DHOD from *A. gossypii* can complement the uracil auxotrophy of a *S. cerevisiae ura1*Δ; however, it is unable to do so under anaerobic conditions.

BDS1 is a 1,941-bp open reading frame located in a subtelomeric position on chromosome XV. Though previously of unknown function, *BDS1* has high sequence identity at the protein level to bacterial alkyl-sulfatases. Sulfatases catalyze hydrolytic cleavage of sulfate ester bonds, liberating sulfate and the corresponding alcohol (41). They are present in a wide variety of species, ranging from bacteria to humans. Sulfatases are involved in a wide range of lineage-specific biological activities. In mammals, sulfatases are involved in the desulfation of sulfated glycolipids, glycosaminoglycans, and steroids. The aryl-sulfatase gene *ars-1* of *N. crassa* has been extensively studied (39). In *N. crassa* aryl-sulfatase is up regulated by sulfur starvation and appears to function as a mechanism for sulfur scavenging. The primary roles of bacterial sulfatases are in assimilation of sulfur and in the provision of carbon (23, 41).

Alkyl-sulfatases hydrolyze organic sulfate esters of primary or secondary alkyl alcohols. Most of the work on alkyl-sulfatases has been carried out with bacterial species, particularly of the genus *Pseudomonas*. In this work we show that the *BDS1* gene of *S. cerevisiae* is a gene of bacterial origin encoding a sulfatase with a broad substrate range, including primary alkyl-sulfates and aryl-sulfates. Wild-type *S. cerevisiae* cells are capable of utilizing the alkyl-sulfates sodium dodecyl sulfate (SDS) and sodium octyl sulfate as sources of sulfur. A disruption of the *BDS1* open reading frame abolishes this activity. The same result was obtained using an aryl-sulfate as a sole sulfur source. A photometric assay for aryl-sulfatase activity shows that *BDS1* is the primary aryl-sulfatase of *S. cerevisiae*.

These findings clearly demonstrate that the horizontal transfer of a DHOD from a lactic acid bacterial lineage and a sulfatase from α-proteobacteria contributed to the evolution of the *S. cerevisiae* lineage. The bacterially derived DHOD facilitates anaerobic growth of *S. cerevisiae*, and the bacterial sulfatase allows for the utilization of organic sulfur compounds previously not available to the lineage of *S. cerevisiae*.

MATERIALS AND METHODS

Identification of potentially horizontally transferred genes. The *S. cerevisiae* protein set (16), *A. gossypii* protein set (11), and a database of all available bacterial protein sequences were compared to each other by tFASTA (40). To determine whether *S. cerevisiae* genes with better alignments to bacterial genes than *A. gossypii* genes were present in other eukaryotes, all *S. cerevisiae* genes with better alignments to bacterial genes were compared by BLAST (1) to general and fungal databases as described in Results. BLAST results were evaluated by comparison of relative E values with a minimal E-value cutoff of −10.

Strains. Strains used in this analysis were *A. gossypii* ATCC 10895, *Candida albicans* MMRL2010, *Candida glabrata* CBS138, *Enterococcus faecalis* ATCC 6055, *K. lactis* CBS6003, *Kluyveromyces marxianus* NRRL Y-8281, *Lactococcus lactis* subsp. *cremoris* NCK436, *Lactococcus lactis* subsp. *hordniae* ATCC 29071, *Leuconostoc mesenteroides* LA81, *Saccharomyces bayanus* CBS424, *Saccharomyces castellii* Y-12630, *S. cerevisiae* S288C and BY4741, *Saccharomyces kluyverii* CBS3082, *Saccharomyces kudriavzevii* IFO1802, *Saccharomyces mikatae* IFO1815, and *Saccharomyces paradoxus* CBS2980.

Phylogenetic methods. Accession numbers for all sequences used in this analysis can be found in Table S1 in the supplemental material. Ribosomal small-subunit ribosomal (SSU) DNA sequences used in this analysis were acquired from the European database on small-subunit rRNA (57).

Ribosomal SSU sequences were aligned by primary structure using ClustalX (51). Amino acid sequences for dihydroorotate dehydrogenase (DHOD) and sulfatase proteins were aligned by primary structure using ClustalX. Alignments were manually refined. All alignments used in this analysis have been submitted to TreeBASE (32). Coding DNA sequences of DHOD and sulfatase genes were aligned from protein alignments. Estimates of phylogenetic relatedness among species were determined using neighbor-joining (NJ) (47) analysis of SSU sequences. NJ trees were constructed in ClustalX using the IUB matrix. NJ trees were bootstrapped in ClustalX using 1,000 replicates.

Estimates of phylogenetic relatedness among DHOD and sulfatase genes were determined using NJ and Bayesian analyses of protein sequences and maximum likelihood (ML) analysis of coding DNA sequences for DHOD genes. NJ trees were constructed in ClustalX using the method of Saitu and Nei (47) and the Gonnet matrix (18). NJ trees were bootstrapped in ClustalX using 1,000 replicates.

Bayesian analyses were performed with MRBAYES 3.0 (21). The Whelan–Goldman protein matrix was used as a substitution model (54). Markov-chain Monte Carlo chain length was 1,000,000 generations run with four chains, with every 100th tree saved. The first 1,000 trees were discarded as "burn-in." The remaining trees were used to construct a majority-rule consensus tree.

ML trees were constructed in PAUP* 4.0b (50). Likelihood settings were estimated using Modeltest (42). A general time reversible model of sequence evolution was used with the gamma distribution with invariants in all cases. Tree searching was performed using 100 random-addition-sequence replicates. ML tree searches for DHOD-coding genes were carried out both unconstrained and with a constraint forcing all fungal sequences to be monophyletic in the resulting trees. To assess the significance of the difference in likelihood between the constrained and unconstrained ML trees, the Shimodaira-Hasegawa test (48) was implemented in PAUP* 4.0b, using 1,000 bootstrap replicates.

Sequencing of DHOD-encoding genes. Total DNA was isolated, and the DHOD-encoding gene was amplified by standard or degenerate PCR using standard methods (34). The amplified DNA was sequenced using ABI dye terminator chemistry on an ABI 310 genetic analyzer using standard methods.

Genetic transformation. A PCR-generated (2, 55) deletion strategy was used to replace *URA1* from its start to stop codon with a KanMX module whose expression confers resistance to G418 to *S. cerevisiae* (53). The deletion "cassette" was constructed using PCR. Replacement was conducted by homologous recombination using lithium acetate-mediated transformation by standard methods (2). Successful homologous recombination was confirmed by PCR. We

TABLE 1. Gene candidates for horizontal transfer in *S. cerevisiae* and *A. gossypii*

Species and locus name/gene name	Deletion phenotype[a]	In sibling species[b]	Function	Percent identity/best bacterial alignment
Saccharomyces cerevisiae				
YKL216W/*URA1*	Uracil auxotroph	Yes	Dihydroorotate dehydrogenase	71/*Lactococcus lactis*
YJL217W	Viable	Yes	Unknown	64/*Xanthomonas axonopodis*
YFR055W	Viable	Yes	Cystathionine beta-lyase	60/*Yersinia pestis*
YJL218W	Viable	Yes	Acetyltransferase	59/*Methanosarcina mazei*
YOL164W/*BDS1*	Alkyl-, aryl-sulfatase negative	Yes	Alkyl-, aryl-sulfatase	55/*Rhodopseudomonas palustris*
YDR540C	Viable	Yes	Unknown	54/*Enterococcus faecalis*
YNR058W/*BIO3*	Biotin auxotroph	Yes	7,8-Diamino pelargonic acid amino transferase	49/*Yersinia enterocolitica*
YPL245W	Viable	Yes	Unknown	44/*Lactobacillus plantarum*
YMR090W	Viable	Yes	Unknown	42/*Lactobacillus plantarum*
YNR057C/*BIO4*	Biotin auxotroph	Yes	Dethiobiotin synthesis	40/*Magnetospirillum magnetotacticum*
Ashbya gossypii				
AGL264W	IS605-TnpB	Not known	Transposase	25/*Helicobacter pylori*

[a] Results of open reading frame deletion by the *Saccharomyces* Genome Deletion Project (55).
[b] *Saccharomyces* sensu stricto species (10, 26) except where indicated.

transformed *ura1*Δ strains with an *S. cerevisiae* shuttle vector, pRS416 (49), containing DNA from an *A. gossypii* genomic library covering the DHOD gene. Transformed cells were plated on medium lacking uracil to confirm the presence of *A. gossypii* DHOD.

A PCR-generated disruption strategy was used to insert a KanMX module into the coding region of *BDS1* in *S. cerevisiae* by homologous recombination using lithium acetate-mediated transformation. Successful disruption was confirmed by PCR.

Determination that *URA1* is required for anaerobic biosynthesis of uracil. Plates of synthetic minimal medium (8), yeast nitrogen base lacking uracil, were plated with *S. cerevisiae ura1*Δ, *S. cerevisiae ura1*Δ plus *A. gossypii* pAG, and the wild type (S288C). Plates were incubated either in atmospheric air or in a Mitsubishi Gas Chemical Company AnaeroPack rectangular jar using ascorbic acid gas generators to catalytically remove oxygen.

Assay for alkyl-sulfatase activity. Cells of *K. lactis*, *S. kluyveri*, *C. glabrata*, *S. bayanus*, *S. kudriavzevii*, *S. mikatae*, *S. paradoxus*, and *S. cerevisiae* were grown overnight at 30°C in yeast-peptone-dextrose medium. The total cell number for each culture was determined by counting in a hemocytometer, and each culture diluted to 200,000 cells/μl. Cells were washed twice and suspended in high-purity water. Five microliters of culture was spotted onto B medium (a sulfur-free minimal medium) (9) plates (20% agarose) supplemented with 0.3 mM SDS. Plates were incubated at 25°C.

To test whether the *bds1*::*KanMX* strain shows a growth defect on SDS media, wild-type and mutant cells were inoculated in a total volume of 300 μl in five 96-well plates (four experiments per plate) in B medium supplemented with SDS with a concentration range of 0.3 M to 30 nM. Cells were grown with agitation at 30°C for 48 h. Cell growth was determined by measuring the optical density at 600 nm.

To test whether the *bds1*::*KanMX* strain shows a growth defect in octyl sulfate media, wild-type and mutant cells were inoculated in a total volume of 2 ml in B medium supplemented with sodium octyl sulfate with a concentration range of 0.3 M to 30 nM. Cells were grown with agitation at 30°C for 60 h. Cell growth was determined by measuring the optical density at 600 nm.

To verify that the alkyl-sulfate metabolism defect demonstrated by the *bds1*::*KanMX* strain is linked to the mutation, *bds1*::*KanMX* MATα cells were mated to strain BY4741 (MAT**a** *his3 leu2 met15 ura3*). Diploids were sporulated, and 11 tetrads dissected. Spores were replica plated onto yeast-peptone-dextrose plus G418, synthetic defined (SD)-histidine, SD-leucine, SD-methionine, SD-uracil, and B medium plus 0.3 mM SDS.

Assay for aryl-sulfatase activity. To test whether *S. cerevisiae* also possesses an *BDS1*-dependent aryl-sulfatase activity, cells were grown using 4-nitrocatechol sulfate as a sole source of sulfur. Wild-type and mutant cells were inoculated in a total volume of 2 ml in B medium supplemented with 4-nitrocatechol sulfate with a concentration range of 0.3 M to 30 nM. Cells were grown with agitation at 30°C for 60 h. Cell growth was determined by measuring the optical density at 600 nm. As we were unable to acquire high purity 4-nitrocatechol sulfate, initial growth experiments produced very high background growth of wild-type and mutant cells (data not shown). In order to limit the background, contaminating sulfur was exhausted biologically. Mutant cells were grown in 2× B medium supplemented with 4-nitrocatechol sulfate for 60 h. The medium was then filtered, reinoculated with wild-type or mutant cells, and incubated as described above.

Aryl-sulfatase activity was assayed photometrically as release of 4-nitrocatechol from 4-nitrocatechol sulfate (44). After 60 h of growth, the medium was diluted 1/10 in water and the relative level of 4-nitrocatechol was determined by measuring the optical density at 516 nm.

RESULTS

Identification of horizontal gene transfer candidates. The tFASTA program (40) was used to compare the *S. cerevisiae* and *A. gossypii* protein sets both to the genomic DNA of each other and also to the complete set of bacterial sequences found in GenBank (3) as of October 2001. *S. cerevisiae* genes with homologs in bacteria but not *A. gossypii* and *A. gossypii* genes with homologs in bacteria but not *S. cerevisiae* were identified as putative horizontally transferred genes. The approximately 100 genes that satisfied these criteria were then compared to other available fungal sequences, and genes with homologs in other fungal species were eliminated, as these genes most likely represent cases of gene loss and not horizontal gene transfer. The 10 *S. cerevisiae* genes and the 1 *A. gossypii* gene identified as potential horizontal gene transfer candidates are shown in Table 1. The *URA1* and *BDS1* genes were selected for further study in the work reported here. These genes were chosen because of their high sequence identity to bacterial genes (Table 1) and their phylogenetic relationships to prokaryotic and eukaryotic homologs, both of which support horizontal gene transfer. Both genes present an identifiable phenotype when mutated, and *URA1* was chosen because of previous reports that it might be horizontally transferred.

***URA1* gene phylogeny is consistent with horizontal transfer from lactic acid bacteria.** The *S. cerevisiae URA1* gene appears to be much more similar to bacterial homologs than to any gene present in the *A. gossypii* genome. Further examination of available sequence data confirmed that the *URA1* gene has an unexpected phylogeny. The topology of the DHOD phylogeny appears to be quite similar to the ribosomal DNA phylogeny with the exception of *S. cerevisiae* and related species. Horizontal gene transfer into the *S. cerevisiae* lineage could

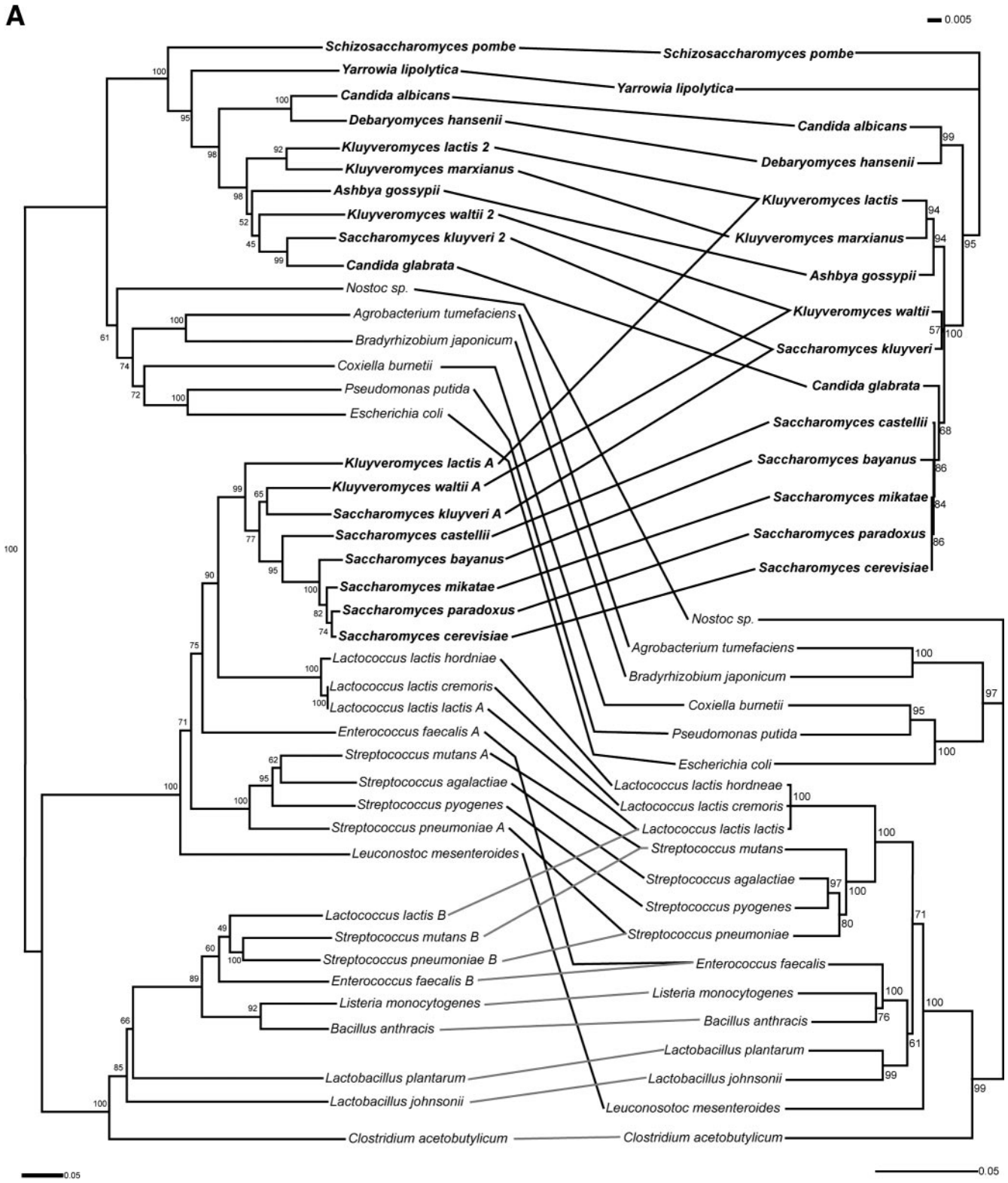
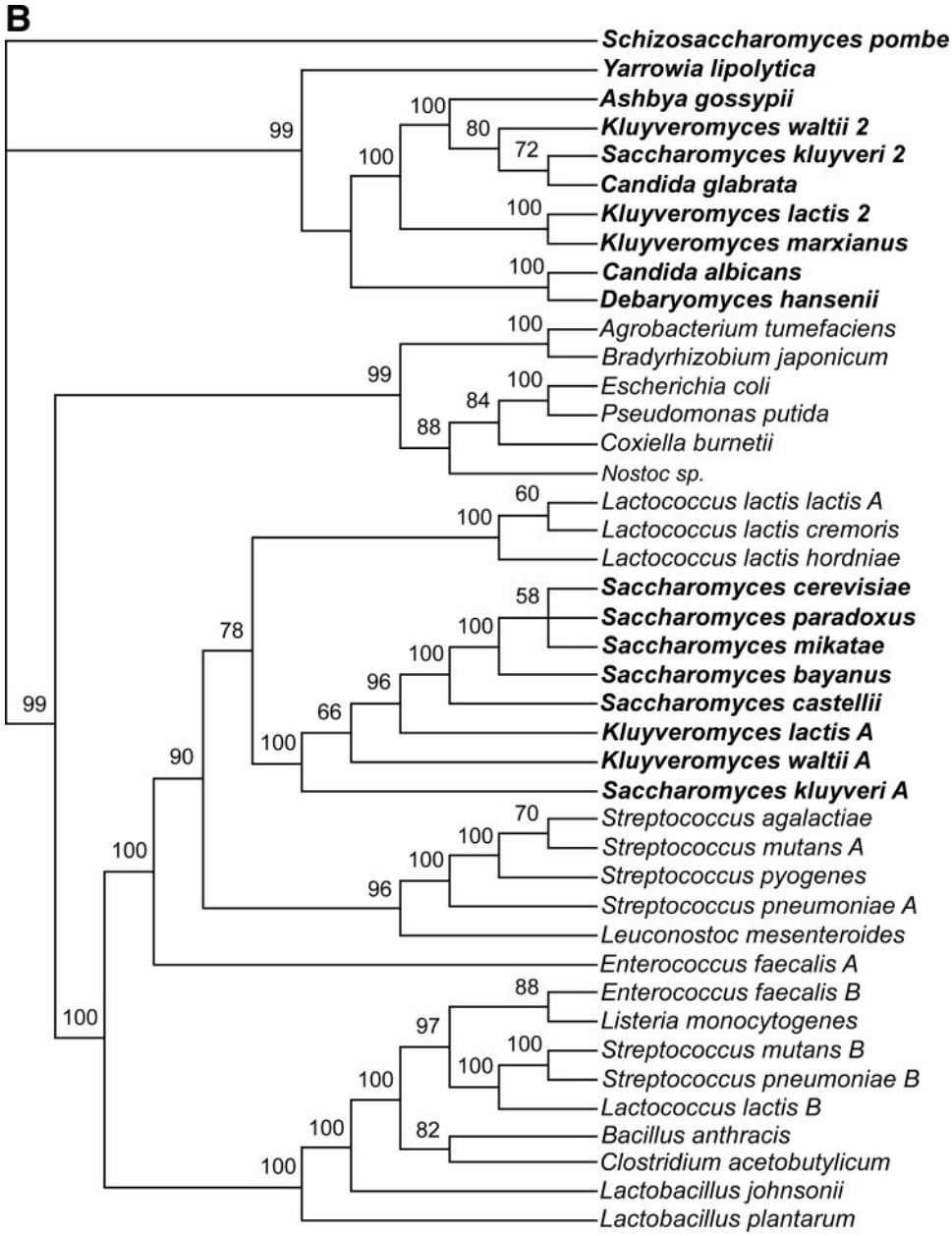


FIG. 1. The phylogeny of dihydroorotate dehydrogenase supports horizontal gene transfer from bacteria to fungi. (A) Phylogenetic tree constructed from the DHOD amino acid sequence (on left) shows a topology generally similar to a tree constructed from small-subunit (SSU) rRNA (on right). The main exception is that the DHOD (*URA1*) gene of members of the *Saccharomycetaceae* clusters with the DHOD sequences from *Lactobacillales*. Fungal species are shown in bold. Lines connect taxa between trees. On the DHOD phylogeny, 2 indicates type 2 DHOD, *A* indicates type 1a DHOD, and *B* indicates type 1b DHOD. *K. lactis*, *K. waltii*, and *S. kluyveri* have both a bacterially derived family 1a and a eukaryotic family 2 DHOD. A complete genome sequence of *K. marxianus* is not yet available, and thus it is possible this species may have a type 1a DHOD as well. Type 1a DHOD genes are shown from *L. lactis* subsp. *hordniae*, *L. lactis* subsp. *cremoris*, and *L. mesenteroides*; no attempt was



made to identify type 1b DHOD genes in these species. Based on complete genome sequences, *B. anthracis*, *L. plantarum*, and *L. johnsonii* genomes carry type 1b but not type 1a DHOD genes and *S. agalactiae* and *S. pyogenes* carry type 1a but not type 1b DHOD genes. Both trees constructed with neighbor joining (47) in ClustalX (51). Numbers indicate bootstrap support for nodes from 1,000 NJ bootstrap replicates. Scale bar, changes per amino acid or nucleotide. (B) Bayesian tree phylogeny of dihydroorotate dehydrogenase (DHOD) proteins. Majority-rule consensus tree of 9,000 Bayesian trees. Numbers above branches represent the posterior probability of each clade. Tree searching done with MrBayes3 (45). Consensus trees and posterior probabilities were determined in PAUP* 4.0b (50). Fungal species are shown in bold. (C) Maximum likelihood phylogenetic analyses of dihydroorotate dehydrogenase (DHOD) coding regions. Trees were constructed in PAUP* 4.0b (50). Likelihood settings were estimated from a previously generated NJ tree. A general time-reversible model of sequence evolution was used with the gamma distribution with invariants. ML tree searches were carried out both unconstrained and with a constraint forcing all fungal sequences to be monophyletic in the resulting trees. To assess the significance of the difference in likelihood between the constrained and unconstrained ML trees, the Shimodaira-Hasegawa (SH) test (48) was implemented in PAUP* 4.0b. Tree scores ($-\ln L$) are 29,424.7 for the unconstrained tree, and 29,646.8 for the constrained tree, giving a ΔL of 194 and a P value of <0.0001 for the SH test. Fungal species are shown in bold.

explain these results, though other explanations are also feasible, such as sequence contamination or species misassignment, selective evolutionary rates, gene loss, or horizontal transfer from fungi to bacteria. To rule these out, the DHOD gene was sequenced from *E. faecalis* (GenPept AAP04498), *L. mesenteroides* (GenPept AAQ01774), *L. lactis* subsp. *cremoris* (GenPept AAQ01776), and *L. lactis* subsp. *hordniae* (GenPept AAQ01775). In order to rule out possible species misassignment or sequence contamination, we resequenced the DHOD gene based on several publicly available sequences, including

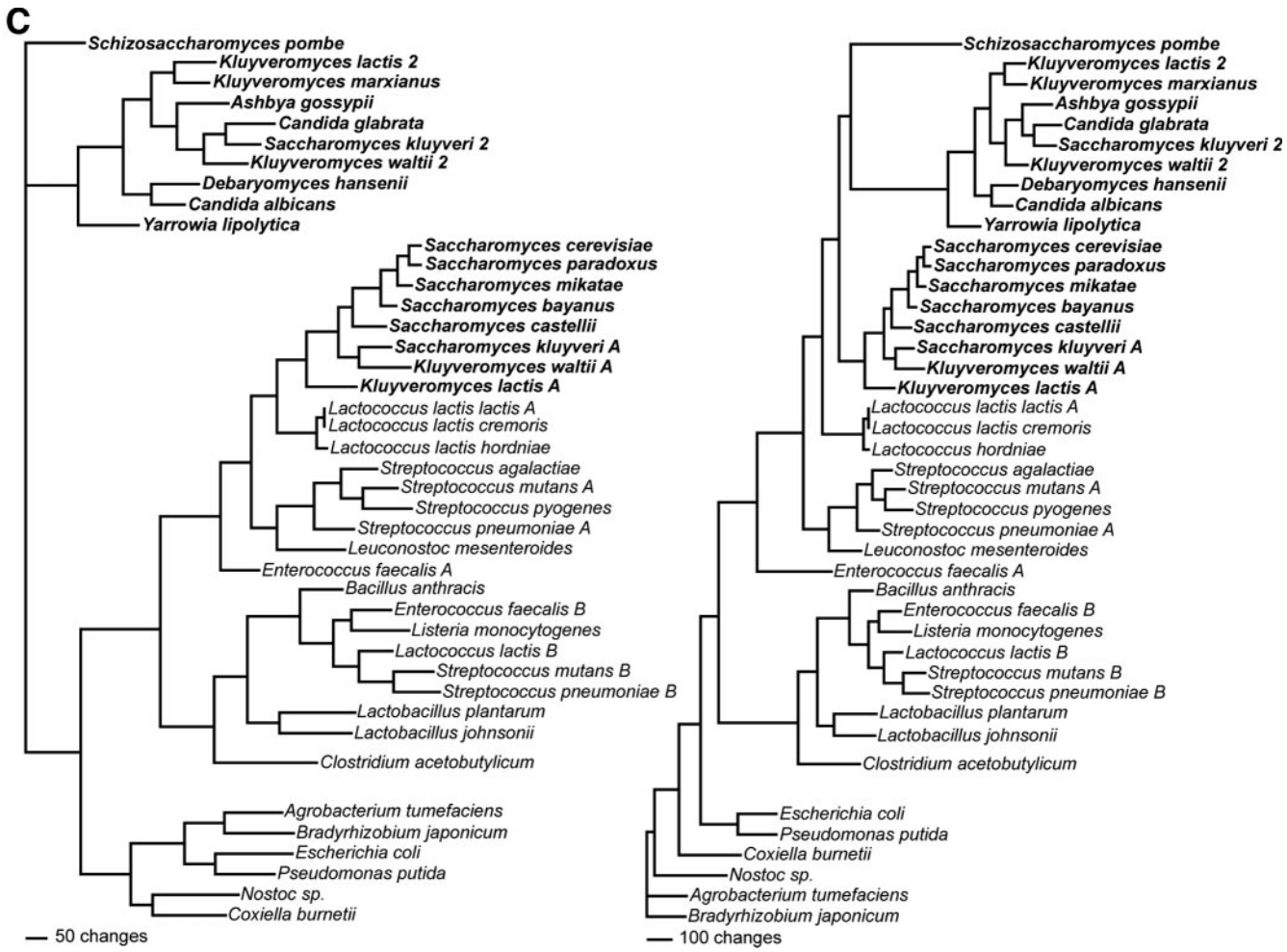


FIG. 1—*Continued*.

those from *C. albicans* (GenPept AAP39962), *S. bayanus* (GenPept AAQ01777), *S. castellii* (GenPept AAQ57200), *S. kluyveri* (GenPept AAQ01779 [*URA1*], GenPept AAQ57201 [*URA9*]), *S. mikatae* (GenPept AAQ01778), and *S. paradoxus* (GenPept AAQ01780), confirming their sequence and including them in this analysis. Partial sequences from *C. glabrata* (GenPept AAR17523), *K. lactis* (GenPept AAR1773), and *K. marxianus* (GenPept AAQ017522) were sequenced for the full length of the gene and included in the analysis. A phylogeny was then constructed on this expanded set of DHOD genes by NJ (47), Bayesian (21), and ML methods (14) (Fig. 1). Trees constructed by all three methods showed a similar topology. By all three methods the *S. cerevisiae* type 1a DHOD sequence and those of closely related fungal species are a branch within a larger type 1a DHOD tree consisting of bacterial sequences. Closely related *Saccharomyces* species including *S. bayanus*, *S. paradoxus*, and *S. castellii* have the type 1a gene, whereas *K. marxianus* and *C. glabrata* have the type 2 enzyme and *K. lactis*, *K. waltii*, and *S. kluyveri* have both family 1- and family 2-type enzymes. This more complete data support the horizontal transfer hypothesis. Using a constraint of eukaryotic monophyly, the constrained ML search results in a tree that is less likely than that resulting from an unconstrained ML search (Fig. 1C). To assess the significance of the difference in likelihood between the constrained and unconstrained ML trees, the Shimodaira-Hasegawa likelihood ratio test (SH) was performed as implemented in PAUP* 4.0b. The SH test rejected the constrained topology with a P value < 0.0001.

Analysis of gene order. The presence of two DHOD genes in *K. lactis*, *K. waltii*, and *S. kluyveri* supports the argument that *URA1* is horizontally transferred and suggests that an ancestor of *S. cerevisiae* had both DHOD genes. It is likely that the family 2 DHOD was lost at some time after the divergence of *C. glabrata* and *S. cerevisiae*. An alignment of the family 2 DHOD regions of *A. gossypii, S. kluyveri*, and *S. cerevisiae* and the family 1a regions in *S. kluyveri* and *S. cerevisiae* is shown in Fig. 2. In *A. gossypii* and *S. kluyveri* the family 2 DHOD and its flanking genes show a conserved gene order. Local synteny is also preserved in *S. cerevisiae*, except this region is lacking the family 2 DHOD. No evidence or residual trace of the ancestral type 2 DHOD gene could be identified at this location, nor could it be found elsewhere in the genome. The *URA1* gene is at a different position in the genome. This is consistent with loss of the family 2 DHOD from the lineage of *S. cerevisiae*.

It is interesting that the *URA1* gene in *S. cerevisiae* is located near the chromosome XI telomere. Recent work (10, 26) has

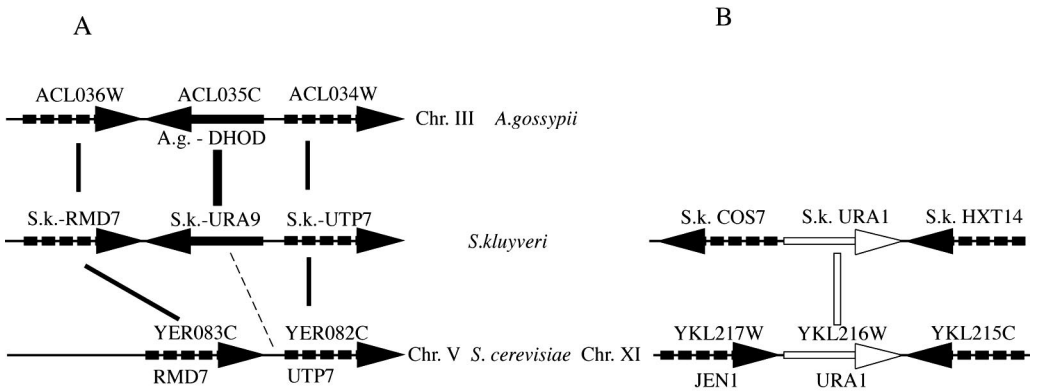


FIG. 2. Synteny identifies the location from which the family 2 DHOD gene was lost in the *Saccharomyces cerevisiae* lineage. (A) Synteny is conserved in the region of the family 2 DHOD in *A. gossypii* and *S. kluyveri*. DHOD genes are shown as solid arrows; adjacent genes are shown with dashed arrows. Vertical bars indicate homologues. In *S. cerevisiae* the region containing the family 2 DHOD is conserved, though the DHOD gene is not present, as indicated by the dashed line. This is consistent with a deletion of the family 2 DHOD in the lineage leading to *S. cerevisiae*. (B) Synteny in the region of the family 1a DHOD genes, indicated by hollow arrows, is not conserved between *S. kluyveri* and *S. cerevisiae*, possibly due to genomic rearrangements since the divergence of these two species.

shown that among the *Saccharomyces* sensu stricto species most of the genes that differ between species are located near telomeres. This location of the *S. cerevisiae URA1* gene may thus be a reflection of its recent acquisition. Of the 10 *S. cerevisiae* genes proposed to have been acquired by horizontal gene transfer, all except YMR090W are located near telomeres.

Growth in anaerobic conditions. As the horizontally transferred *URA1* gene replicated a function already present in the host organism, the family 1a enzyme probably provided some selective advantage to become fixed in the population. Based upon the biochemical characteristics of family 1a and family 2 enzymes (family 1a enzymes do not require oxygen as an electron acceptor as is the case with family 2 [22]), we hypothesized that possession of a family 1a-type enzyme may facilitate growth in anaerobic environments. In order to test this hypothesis, a *ura1*Δ *S. cerevisiae* strain was constructed by disrupting *URA1* by homologous recombination (2). This strain was transformed with a plasmid containing a genomic fragment from *A. gossypii* including the DHOD gene. This strain was grown in parallel anaerobic and aerobic conditions; the results are shown in Fig. 3. The *A. gossypii* DHOD gene fully complements the uracil auxotrophy of the *S. cerevisiae ura1*Δ strain. The *A. gossypii* DHOD gene was however unable to complement the uracil auxotrophy under anaerobic conditions. This is consistent with horizontal transfer of a bacterial family 1a-type DHOD gene into the *S. cerevisiae* lineage. It is possible that such a transfer facilitated the exploitation of anaerobic environments. Similar experiments carried out by Gojkovic et al. and Zameitat et al. using the family 1a and 2 DHOD enzymes from *S. kluyveri* (17, 58) showed similar results indicating that the failure of the *A. gossypii* family 2 DHOD to complement under anaerobic conditions is not species specific. *A. gossypii* is unable to grow under anaerobic conditions even when supplemented with uracil (data not shown), indicating that while the type 1a DHOD gene is necessary for anaerobic growth in the absence of uracil it is only one of a number of modifications necessary for anaerobic growth. The majority of fungal species are restricted to aerobic growth (52), and the acquisition of the bacterially derived *URA1* gene could have been one of the adaptations necessary for anaerobic growth.

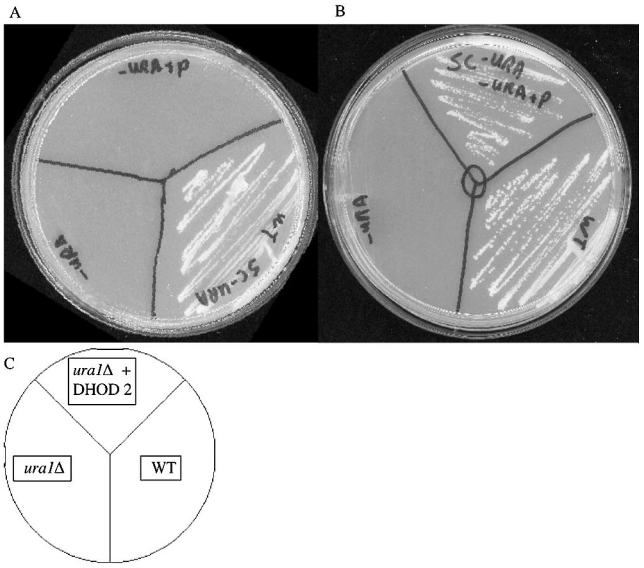


FIG. 3. The *Ashbya gossypii* type 2 DHOD gene is unable to fully complement a *Saccharomyces cerevisiae ura1*Δ strain under anaerobic conditions. Plasmid pAG containing the *A. gossypii* DHOD gene fails to complement *S. cerevisiae ura1*Δ under anaerobic conditions (A) but complements under aerobic conditions (B). (C) Arrangement of strains on plates; all plates contained synthetic complete medium without uracil (8).

The *BDS1* gene phylogeny is consistent with horizontal transfer from α-proteobacteria. The *S. cerevisiae* BDS1 protein has higher sequence identity to a family of bacterial sulfatases than to any genes found in eukaryotes. Further examination of available sequence data confirmed that BDS1 is a member of a family of bacterial alkyl-sulfatase (Fig. 4). A sulfatase phylogeny was constructed including bacterial and eukaryotic alkyl- and aryl-sulfatase genes by NJ and Bayesian methods (Fig. 5). Trees constructed by all methods showed a similar topology. By both methods the *S. cerevisiae BDS1* sequence

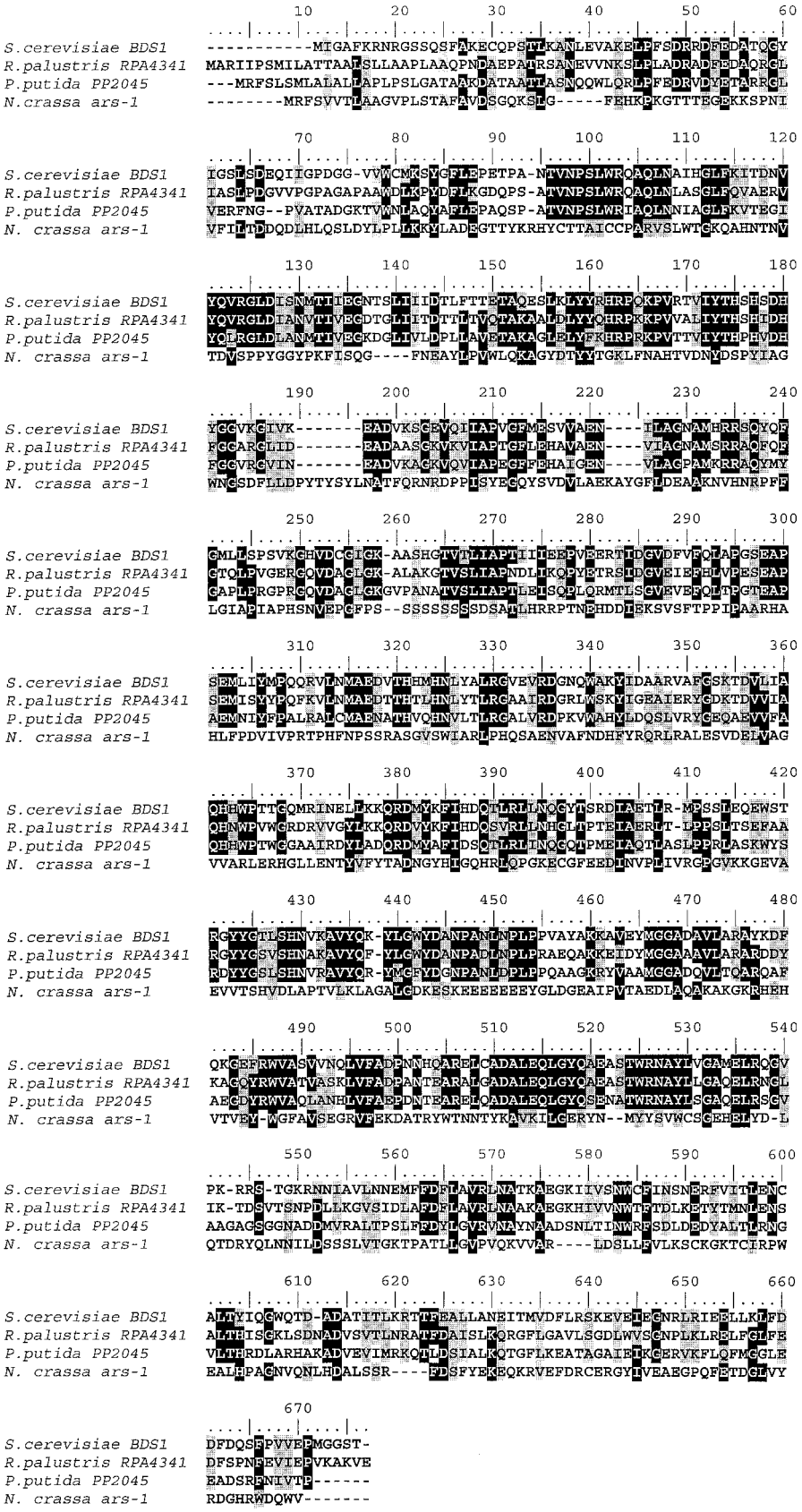


FIG. 4. Alignment of BDS1 of *S. cerevisiae* and *S. bayanus* with sulfatases from *Rhodopseudomonas palustris*, *Pseudomonas putida*, and the *ars-1* gene product *of N. crassa*. For all species, the full length of the protein is shown.

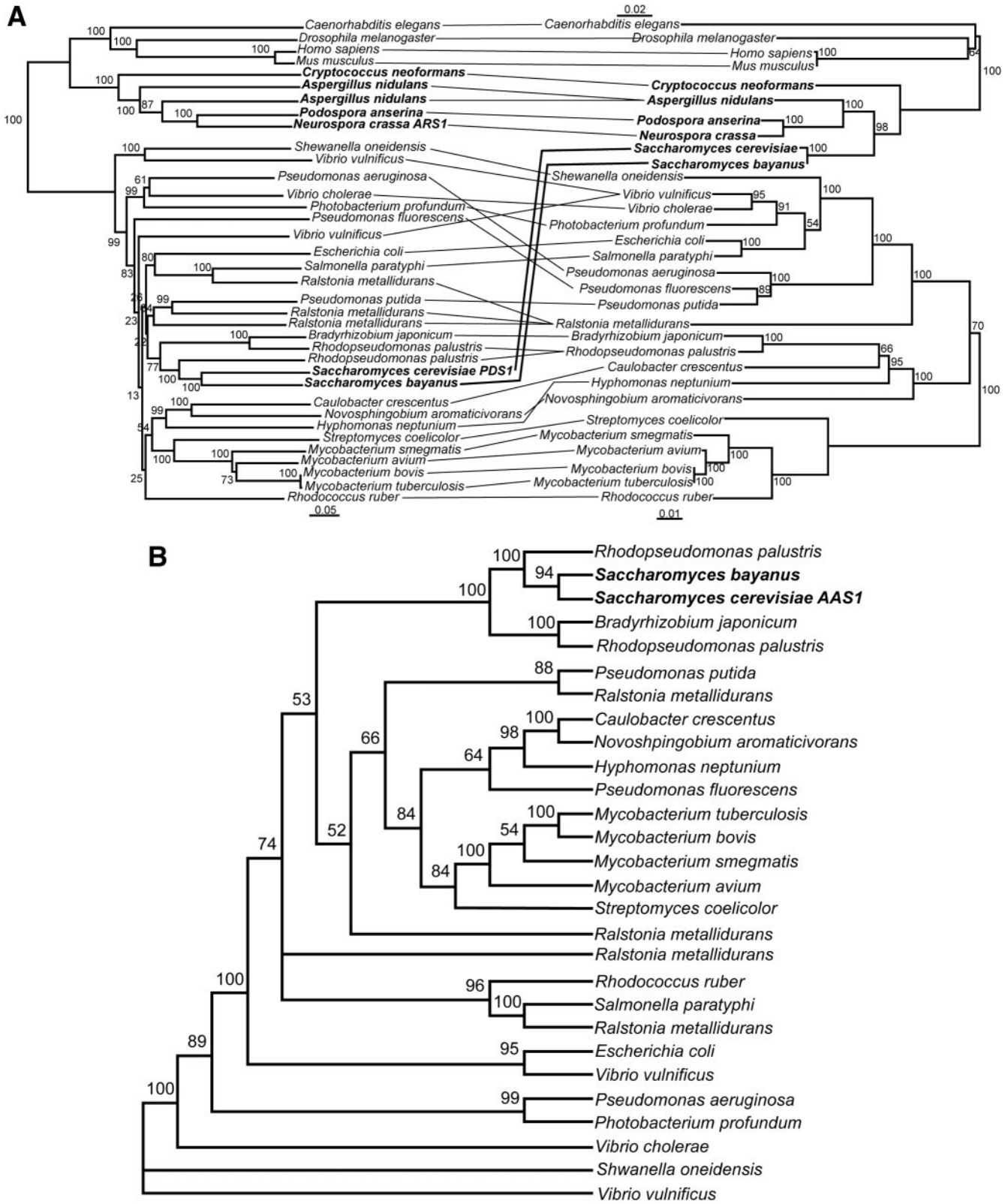


FIG. 5. A phylogeny of bacterial and eukaryotic sulfatases supports horizontal gene transfer from bacteria to fungi. (A) Phylogenetic tree constructed from alkyl- and aryl-sulfatase amino acid sequences (on left) shows a topology generally similar to a tree constructed from small-subunit (SSU) rRNA (on right). The *BDS1* genes of *S. cerevisiae* and *S. bayanus* are members of a family of bacterial sulfatase genes and not closely related to the aryl-sulfatase genes of eukaryotes. Fungal species are shown in bold. Lines connect taxa between trees. Both trees constructed with neighbor joining (47) in ClustalX (51). Numbers indicate bootstrap support for nodes from 1,000 NJ bootstrap replicates. Scale bar, changes per amino acid or nucleotide. (B) Bayesian tree phylogeny of sulfatase proteins. Majority-rule consensus tree of 9,000 Bayesian trees. Numbers above branches represent the posterior probability of each clade. Tree searching done with MrBayes3 (45). Consensus trees and posterior probabilities were determined in PAUP* 4.0b (50). Fungal species are shown in bold.

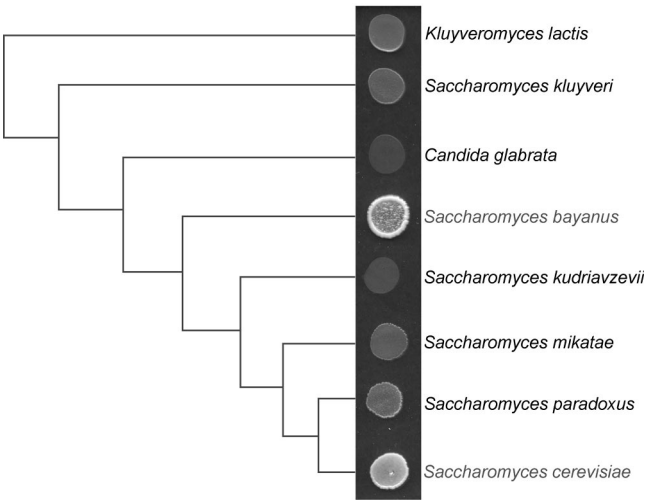


FIG. 6. *S. cerevisiae* and *S. bayanus* possess alkyl-sulfatase activity Cells of *K. lactis*, *S. kluyveri*, *C. glabrata*, *S. bayanus*, *S. kudriavzevii*, *S. mikatae*, and *S. paradoxus* plated on sulfur-free medium supplemented with 0.3 mM SDS. Only *S. cerevisiae* and *S. bayanus* grew vigorously, strongly indicating alkyl-sulfatase activity. Trees show evolutionary relationships between species (29).

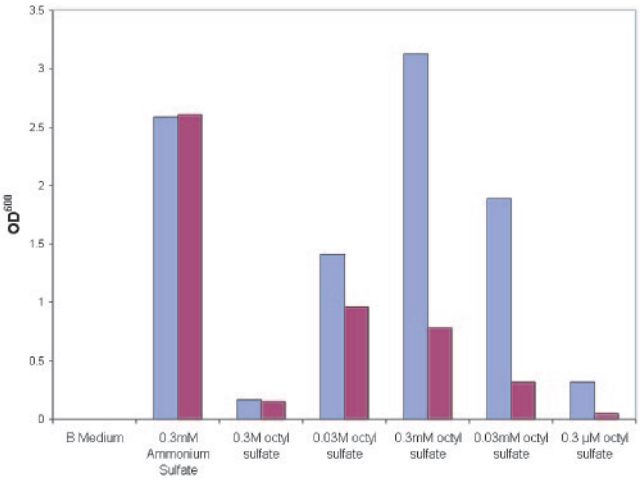


FIG. 8. Growth on octyl sulfate. Mutant *bds1*::*KanMX* cells (red bars) and the wild type (blue bars) were grown in liquid B medium supplemented with octyl sulfate. Experimental condition is plotted versus optical density at 600 nm (OD^{600}). B medium unsupplemented is sulfur-free medium with no added sulfur (negative control). The positive control is 0.3 mM ammonium sulfate.

and that of *S. bayanus* are a branch within a larger tree consisting of bacterial alkyl-sulfatase gene sequences. The *S. cerevisiae BDS1* gene does not appear to share homology with the *ars-1* gene of *N. crassa* nor to any other known eukaryotic aryl-sulfatase gene.

BDS1 is an alkyl-sulfatase. Alkyl-sulfatases similar to BDS1, particularly from *Pseudomonas* spp., are capable of cleaving SDS. The products of this reaction are dodecanol and sulfate. The genomes of the *A. gossypii*, *D. hansenii*, *K. lactis*, *K. waltii*, *S. kluyveri*, *C. albicans*, *C. glabrata*, *S. bayanus*, *S. mikatae*, *S. paradoxus*, and *Y. lipolytica* have been sequenced and were examined for the presence of a *BDS1* homolog. Of these, only *S. bayanus* has a *BDS1* gene homologous to that of *S. cerevisiae*. *K. lactis, S. kluyveri, C. glabrata, S. bayanus, S. kudriavzevii, S. mikatae, S. paradoxus*, and *S. cerevisiae* were plated on sulfur-free minimal medium supplemented with 0.3 mM SDS. Only *S. cerevisiae* and *S. bayanus* grew vigorously, strongly indicating alkyl-sulfatase activity (Fig. 6). All species grew well on sulfur-free minimal medium supplemented with 0.3 mM ammonium sulfate (data not shown). As *BDS1* is the only gene in the *S. cerevisiae* genome with significant homology to alkyl-sulfatase genes, we hypothesized that *BDS1* is the gene responsible for the observed alkyl-sulfatase activity. In order to test this hypothesis, a *bds1 S. cerevisiae* strain was constructed by inserting a G418 resistance marker into the *BDS1* open reading frame by homologous recombination (2).

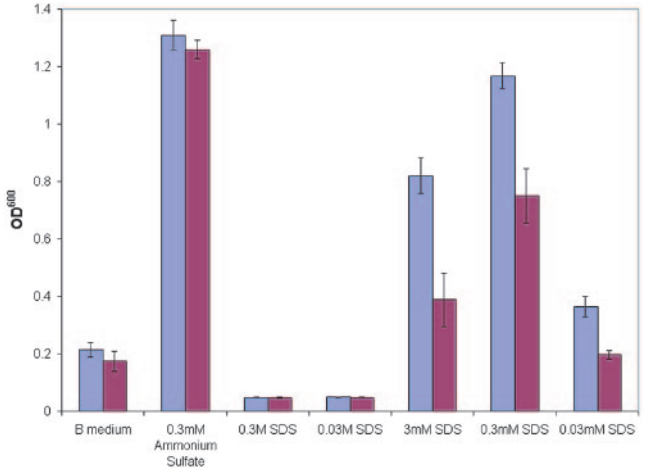


FIG. 7. Growth on SDS. Mutant *bds1*::*KanMX* cells (red bars) and the wild type (blue bars) were grown in liquid B medium supplemented with SDS. Columns represent averages of 21 measurements. Experimental condition is plotted versus optical density at 600 nm (OD^{600}). B medium, unsupplemented B medium (9). Error bars represent one standard deviation. B medium unsupplemented is sulfur-free medium with no added sulfur (negative control). The positive control is 0.3 mM ammonium sulfate.

Mutant *bds1*::*KanMX* cells and the wild type were grown in liquid B medium supplemented with SDS (Fig. 7). Wild-type *S. cerevisiae* grows significantly better than the *bds1*::*KanMX* strain. The same result was achieved with growth on octyl sulfate (Fig. 8), indicating a substrate range of compounds with 8 to 12 carbon atoms, with higher activity for 8-carbon compounds.

To verify that the alkyl-sulfate metabolism defect demonstrated by the *bds1*::*KanMX* strain is linked to the mutation, *bds1*::*KanMX* MATα cells were mated to strain BY4741. In the progeny resulting from this cross, loss of the ability to metabolize SDS cosegregated with the marked deletion (Fig. 9).

BDS1 is an aryl-sulfatase. In 1974 J. Reiss observed aryl-sulfatase activity in *S. cerevisiae* cells by cytochemical methods (43). *S. cerevisiae* has no gene in its genome with significant homology to known eukaryotic aryl-sulfatase genes. We hypothesized that BDS1 may be a dual-function enzyme with alkyl- and aryl-sulfatase activity encoded by the gene responsible for the aryl-sulfatase activity reported by Reiss. Cells were grown using 4-nitrocatechol sulfate as a sole source of sulfur to

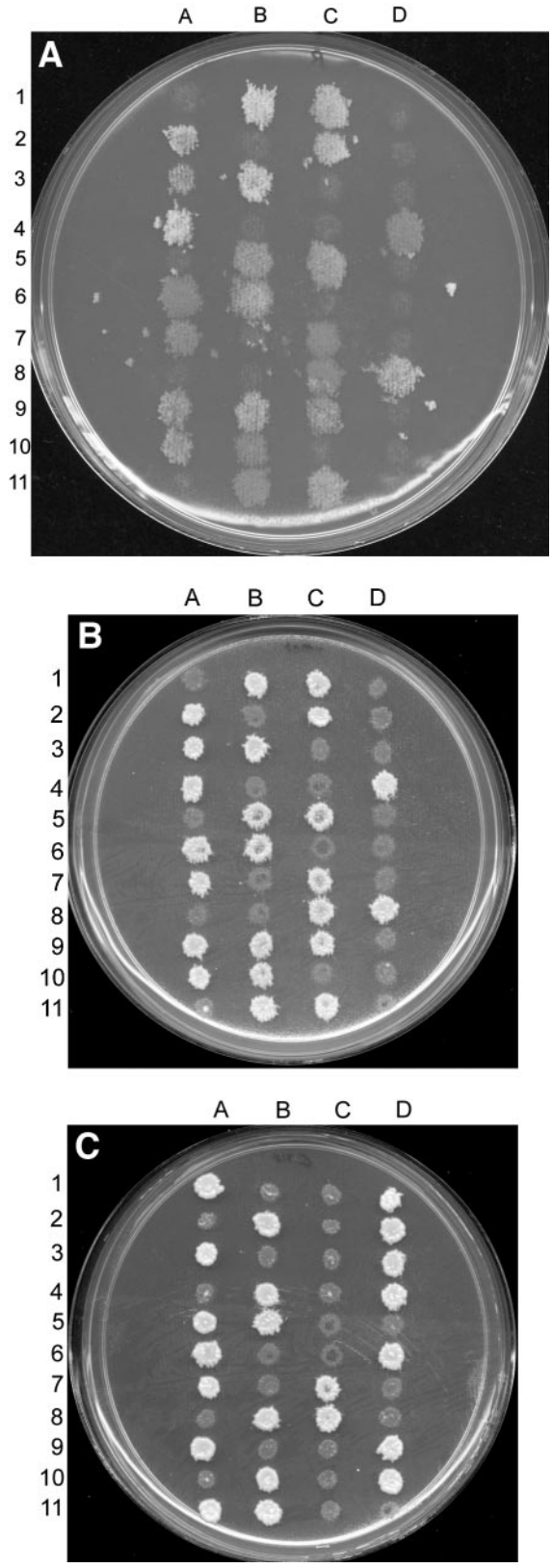


FIG. 9. Tetrad analysis. To verify that the alkyl-sulfate metabolism defect demonstrated by the *bds1*::*KanMX* strain is linked to the mutation, *bds1*::*KanMX* MATα cells were mated to strain BY4741. The progeny resulting from this cross were plated on B medium (9) supplemented with 0.3 mM SDS. Two progeny from each tetrad were unable to grow in medium lacking methionine and represent *met15* cells. These cells were also unable to grow on medium supplemented with SDS. G418-sensitive *BDS1* cells grow more vigorously on SDS as a sulfur source. Tetrads are shown horizontally. (A) B medium supplemented with 0.3 mM SDS. All tetrads show two growth:two non-growth, identical to results with synthetic complete medium without methionine (panel B). Seven tetrads (tetrads 3, 4, 5, 6, 8, 10, and 11) show one vigorous:one weak growth, dependent on *BDS1*. (B) Synthetic complete medium without methionine. All tetrads (except tetrad 9—possible recombination event) show 2:2 segregation of vigorous growth, independent of *BDS1*. (C) Yeast-peptone-dextrose medium with G418. All tetrads show 2:2 segregation of knockout.

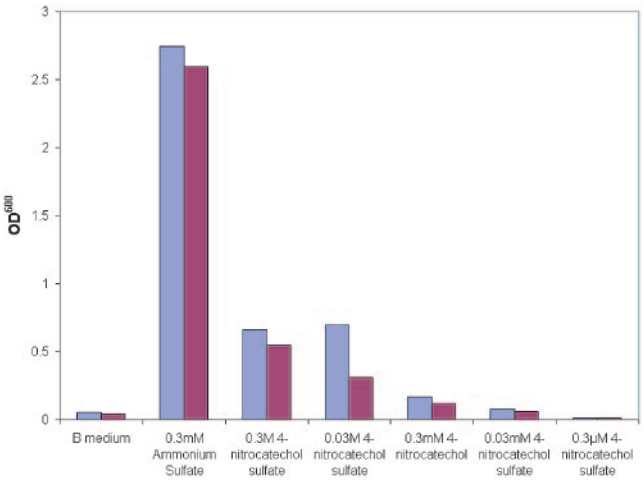


FIG. 10. Growth on the aryl-sulfate 4-nitrocatechol sulfate. Mutant *bds1*::*KanMX* cells (red bars) and the wild type (blue bars) were grown in liquid B medium supplemented with 4-nitrocatechol sulfate. Experimental condition is plotted versus optical density at 600 nm (OD^{600}). B medium unsupplemented is sulfur free-medium with no added sulfur (negative control). The positive control is 0.3 mM ammonium sulfate.

test this possibility. Wild-type and mutant cells were inoculated in a total volume of 2 ml in B medium supplemented with 4-nitrocatechol sulfate. Cell growth was determined by measuring the optical density at 600 nm. As shown in Fig. 10, wild-type *S. cerevisiae* cells grow more vigorously with the aryl-sulfate 4-nitorcatechol sulfate as a sulfur source than do mutant cells. Aryl-sulfatase activity was also assayed photometrically for release of 4-nitrocatechol from 4-nitrocatechol sulfate (Fig. 11). The supernatant of wild-type cells contains over 10 times the level of 4-nitrocatechol than that of mutant cells or controls, indicating that mutant cells lacking functional *BDS1* are negative for aryl-sulfatase activity.

DISCUSSION

Recent horizontally transferred genes appear to be quite rare in *S. cerevisiae*, with 10 cases proposed here, less than 0.2% of the gene set of this species. Comparison of *A. gossypii* and *S. cerevisiae* revealed nearly complete conservation of their gene sets, with most genes found in only one of these species appearing to result from gene loss.

In this study, we found phylogeny, synteny, and high sequence identity across entire proteins useful in assessing whether a gene is potentially found in *S. cerevisiae* as a result of hor-

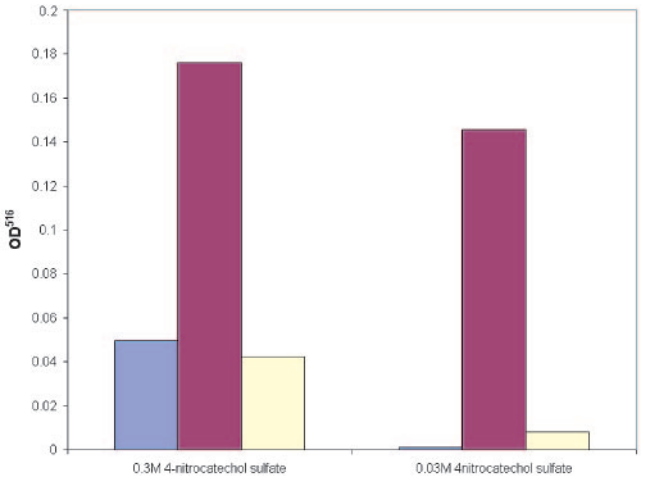


FIG. 11. Photometric assay for aryl-sulfatase activity with mutant *bds1*::*KanMX* cells (white bars) and the wild type (red bars). Aryl-sulfatase activity was assayed photometrically as release of 4-nitrocatechol from 4-nitrocatechol sulfate. Optical density at 516 nm (OD^{516}) is plotted on the *y* axis. Blue bars represent B medium supplemented with 4-nitrocatechol sulfate with no cells.

izontal gene transfer. While in bacterial cases it has been reported that GC content is a useful indicator of horizontally transferred genes (38), we have found this not to be the case in *S. cerevisiae*. For all 10 of the genes potentially of bacterial origin reported here, the GC content falls within 2 standard deviations of the mean (see Fig. S1 in the supplemental material). This is as expected, as the GC content of the hemiascomycetes differs significantly from species to species. For *S. cerevisiae* the GC content is 38%, whereas for *A. gossypii* it is 52% (11). Thus the GC content of genes in these fungi appears to be malleable, and genes acquired by horizontal gene transfer would be expected to rapidly come under the influence of the factors causing these overall shifts in GC content.

Horizontal gene transfer provides a mechanism for genomic innovation and plasticity. In bacteria, horizontal gene transfer is well known as an adaptive mechanism. Horizontal transfer events can be classified into three distinct categories: acquisition of new genes, acquisition of paralogs of existing genes, and gene displacement whereby a gene is displaced by a horizontally transferred ortholog from another lineage (28). All three categories appear to be present in the genome of *S. cerevisiae*. *BDS1* is a member of a class of bacterial sulfatase genes. Among currently sequenced species, *S. cerevisiae* and *S. bayanus* are the only eukaryotic organisms with a gene of this class. Therefore, horizontal transfer is clearly a mechanism for the acquisition of new genes in eukaryotes. Three hemiascomycete yeasts, *K. lactis*, *K. waltii*, and *S. kluyveri*, possess a family 2 DHOD optimized for aerobic conditions and a horizontally transferred family 1a DHOD optimized for anaerobic conditions. In these organisms horizontal transfer has functioned as a mechanism for acquisition of paralogs with novel functions. *S. bayanus*, *S. castellii*, *S. cerevisiae*, and *S. paradoxus* all possess only a horizontally transferred family 1a DHOD. These species present a clear case of gene displacement as they have lost the eukaryotic family 2 DHOD of their ancestors and kept the bacterially derived family 1a DHOD. *BDS1* also presents a variation of gene displacement. The *ars-1* gene of *N. crassa* is a good example of the class of experimentally characterized aryl-sulfatase genes found in many fungi and animals, though not the *Saccharomycetacea* (12) or the "*Saccharomyces* complex" (29), which appears to have lost this eukaryotic aryl-sulfatase gene. Some species of hemiascomycetes, including *C. albicans, D. hansenii, K. lactis*, and *Y. lipolytica*, contain genes of a family related to *ars-1*. These sulfatase-like genes are of unknown function, however, and appear to be distantly related to the eukaryotic aryl-sulfatase genes (see Fig. S2 in the supplemental material). This sulfatase-like gene was also lost in the *S. cerevisiae* lineage after the divergence of the *K. lactis* and *S. cerevisiae* lineages. Neither the eukaryotic aryl-sulfatase gene nor the sulfatase-like gene appears in the genome of *A. gossypii*, *C. glabrata, K. waltii, S. castellii, S. kluyveri*, or any of the *Saccharomyces* sensu stricto species. The *ars-1*-encoded aryl-sulfatase of *N. crassa* is up-regulated by sulfur starvation and appears to function as a mechanism for sulfur scavenging (39). *BDS1* shows higher expression in sulfur-limited chemostat cultures (4). It is possible that the acquisition of *BDS1* was beneficial in that it restored aryl-sulfatase activity or was beneficial in that it provided the novel (for a eukaryote) activity of an alkyl-sulfatase. As is well known, horizontal gene transfer appears to be a mechanism for the acquisition of novel traits. Interestingly, however, horizontal transfer also appears to be a mechanism of genomic plasticity, allowing lineages to reacquire traits and capabilities lost by their ancestors. Curiously, assuming that the established phylogeny of the *Saccharomyces* sensu stricto is correct, as *BDS1* is found in *S. cerevisiae* and *S. bayanus* but does not appear in *S. paradoxus* and *S. mikatae*, it seems likely that this gene was lost in these species.

Horizontal gene transfer can facilitate the adaptation of an organism to a particular niche. While in *S. cerevisiae* the horizontally transferred genes identified in this study make up 0.2% of the genome, horizontally transferred genes can help us understand the life-style of a particular organism. To become fixed in the population of a species, a horizontally transferred gene most likely provided a selective benefit. This selective benefit requires a selective pressure. In the case of *URA1*, the transfer of a DHOD gene optimized for anaerobic conditions indicates that adaptation to anaerobic conditions and anaerobic environments has been an important part of the evolution of hemiascomycete yeasts. In the case of *BDS1*, the transfer of a multifunction sulfatase gene means that these species can inhabit a niche where organic sulfur is predominant. The prokaryotic donor can also provide information about the environment in which a particular organism lives (or lived). The family 1a DHOD was transferred from a *Lactococcus* species. Lactococci are found on plant and animal surfaces and in the animal gastrointestinal tract. *L. lactis* is thought to be dormant on plant surfaces and to multiply in the gastrointestinal tract of ruminants (5). Though *S. cerevisiae* shares plant environments with *L. lactis*, *URA1* is a gene optimized for anaerobic conditions and this strongly indicates that adaptation to survival in the gastrointestinal tracts of animals has been important in the evolution of some hemiascomycete yeasts. This conclusion is supported by the observation that of the 10 candidate genes listed in Table 1, 7 appear to have come from anaerobic prokaryotes that either permanently or transiently inhabit animal gastrointestinal tracts (*URA1*, *L. lactis*;

YFR055W, *Yersinia pestis*; YJL218W, *Methanosarcina mazei*; YDR540C, *E. faecalis*; *BIO3*, *Yersinia enterocolitica*; YPL245W, *Lactobacillus plantarum*; YMR090W, *Lactobacillus plantarum*). *BDS1* appears to have been transferred from α-proteobacteria of the family *Bradyrhizobiaceae*. These bacteria are primarily found in soils. *Rhodopseudomonas palustris* is commonly found in soils and water. A sulfatase transferred from a soil-dwelling bacterium suggests that survival in the harsh soil environment has also been an important evolutionary pressure in the recent evolution of *Saccharomyces* spp. (the likely donor lineage of *BIO4*, *Magnetospirillum* sp., is also a soil- and water-dwelling α-proteobacterium).

Mechanism of DNA transfer. Horizontal gene transfer requires that foreign DNA enter a cell. Bacterium-to-fungus conjugation and natural transformation are possible explanations for how DNA from a lactic acid bacterium could be taken up by a *Saccharomyces*-like yeast. Cell-to-cell conjugation requires that bacterial and yeast cells physically interact with each other such that cellular components such as DNA may be transferred from the donor to the new host. Heinemann and Sprague showed that conjugative plasmids of *Escherichia coli* could mobilize DNA transmission to *S. cerevisiae* (19a). Though neither *E. coli* nor other members of its lineage is the donor of the 1a-type DHOD gene found in *S. cerevisiae*, it is possible that DNA can be transferred from lactic acid bacteria to fungi by a similar mechanism. Two *S. cerevisiae* horizontal gene transfer candidates, YFR055W and YNR058W/*BIO3* (Table 1), appear to be transferred from enteric bacteria related to *E. coli*.

Natural transformation, the uptake of free DNA from the surrounding environment, has been found among some prokaryotic lineages, where it often facilitates horizontal gene transfer (12a). While no dedicated DNA uptake mechanism has been discovered in *S. cerevisiae*, it has been shown by Nevoigt et al. that *S. cerevisiae* cells incubated in 1 M sucrose with plasmid DNA at a minimum concentration of 25 μg/ml can become transformation competent (36a). Also the well-known methods for yeast transformation using polyethylene glycol and anions, as well as electrical damage or mechanical damage, are conditions conceivable in environments encountered by *S. cerevisiae* under nonlaboratory conditions. Thus, there are mechanisms by which the ancestor of *S. cerevisiae* could have taken up foreign DNA. It is not known why such apparently foreign DNA is often seen near telomeres, though it is tempting to speculate that telomerase might have a role in this by adding telomeric sequence to the foreign DNA and thus providing a site for homologous recombination with a chromosomal telomere.

Further work will be required to either confirm or disprove that the other nine genes listed in Table 1 are present in these fungal lineages as the result of horizontal gene transfer.

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