

Histidine Degradation via an Aminotransferase Increases the Nutritional Flexibility of *Candida glabrata*

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The ability to acquire nutrients during infections is an important attribute in microbial pathogenesis. Amino acids are a valuable source of nitrogen if they can be degraded by the infecting organism. In this work, we analyzed histidine utilization in the fungal pathogen of humans *Candida glabrata*. Hemiascomycete fungi, like *C. glabrata* or *Saccharomyces cerevisiae*, possess no gene coding for a histidine ammonia-lyase, which catalyzes the first step of a major histidine degradation pathway in most other organisms. We show that *C. glabrata* instead initializes histidine degradation via the aromatic amino acid aminotransferase Aro8. Although ARO8 is also present in *S. cerevisiae* and is induced by extracellular histidine, the yeast cannot use histidine as its sole nitrogen source, possibly due to growth inhibition by a downstream degradation product. Furthermore, *C. glabrata* relies only on Aro8 for phenylalanine and tryptophan utilization, since ARO8, but not its homologue ARO9, was transcriptionally activated in the presence of these amino acids. Accordingly, an ARO9 deletion had no effect on growth with aromatic amino acids. In contrast, in *S. cerevisiae*, ARO9 is strongly induced by tryptophan and is known to support growth on aromatic amino acids. Differences in the genomic structure of the ARO9 gene between *C. glabrata* and *S. cerevisiae* indicate a possible disruption in the regulatory upstream region. Thus, we show that, in contrast to *S. cerevisiae*, *C. glabrata* has adapted to use histidine as a sole source of nitrogen and that the aromatic amino acid aminotransferase Aro8, but not Aro9, is the enzyme required for this process.

As an opportunistic fungal pathogen, *Candida glabrata* is one of the leading causes of candidiasis (1, 2) and is responsible for several thousand systemic infections and deaths worldwide each year (3), yet *C. glabrata* is much more closely related to the nonpathogenic yeast *Saccharomyces cerevisiae* than to other pathogenic fungi, like *Candida albicans*. Identifying differences in the genetic, phenotypic, and metabolic setups of *S. cerevisiae* and *C. glabrata* is therefore highly relevant for understanding the basis of *C. glabrata* virulence (4, 5).

C. glabrata can cause several types of infections, ranging from superficial to systemic. During the course of these infections, *C. glabrata* cells face many different and rapidly changing host environments and have to adapt to each of these microniches. The ability to use as many nutrient sources as possible is vital for this kind of flexibility. Thus, a detailed knowledge of the fungal metabolic pathways involved can help in understanding, and consequently in fighting, fungal infections (6). Examples of the relevance of nutrient uptake in the host are trace elements, like iron or zinc (7–10), but also carbon and nitrogen compounds (11, 12). For the nitrogen supply of infecting fungi, amino acids are especially important (13). They are present in free form in low millimolar concentration at different host sites, like blood or urine (14, 15), or can be liberated from proteins and peptides by the actions of fungal hydrolases (16). For their uptake, fungi like *S. cerevisiae* and *C. glabrata* possess large families of different small and broad-spectrum permeases (17). In *C. albicans* and some of its pathogenic close relatives, these permease families are significantly expanded compared to other related, nonpathogenic species (18), indicating a role for flexibility of nitrogen utilization in pathogenicity. This flexibility includes the ability to use proteins, peptides, amino acids, and other low-molecular-weight nitrogen sources.

For humans, histidine is an essential amino acid. In the body, it

can be converted to histamine by a histidine decarboxylase or to urocanic acid and ammonia by a histidine ammonia-lyase (HAL), also known as histidinase (19). In this work, we investigate the initiation of histidine degradation in *C. glabrata* and show that it uses an aromatic aminotransferase instead of a histidinase for histidine utilization.

MATERIALS AND METHODS

Strains and growth conditions. The following strains were used in the experiments: *C. glabrata* ATCC 2001, *S. cerevisiae* ATCC 9763, *C. albicans* SC5314, and *Saccharomyces kluyveri* CBS3082. The fungi were maintained on solid YPD medium (2% glucose, 1% yeast extract, 1% peptone, 2% agar) and grown at 30°C. The *aro8Δ* deletion mutant has been described previously (20).

Growth curve experiments were performed in 1× YNB medium (BD Biosciences, CA, USA) with 2% glucose (Roth, Germany), 100 mM phosphate buffer (pH 5.8), and 10 mM the respective amino acid (Roth) or 0.5% NH₄SO₄ (Roth). For competitive-inhibition experiments, 10 mM (aminoxy)acetate (AOA) (Sigma) was added to these media. In selected experiments, 5 mM histidine was combined with 5 mM other amino acids as indicated. Fungi were inoculated from a YPD overnight culture at an optical density at 600 nm (OD₆₀₀) of 0.2. Growth was measured every 15 min in a 1-ml volume in a 24-well plate (TPP, Switzerland) using a Tecan Infinite M200 microplate reader at 30°C with intermittent shaking.

Received 17 March 2014 Accepted 4 April 2014

Published ahead of print 11 April 2014

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doi:10.1128/EC.00072-14

In vitro transaminase assays. The *C. glabrata* Aro8 (CgAro8) protein was expressed heterologously in *Escherichia coli* and purified via a His tag, as described previously (20). The transaminase activity of the protein was measured spectrophotometrically in an assay solution consisting of 100 mM Tris buffer, pH 7.5, 1 μ M pyridoxal phosphate (Sigma), 5 μ g purified protein, and different amounts of the amino acid to be tested (0.75 to 36 mM) (all from Roth, Germany). To start the reaction, the amino acceptor α -ketoglutarate (10 mM) was added. The formation of indolepyruvate (from tryptophan; molar extinction coefficient [ϵ] = 9,300 M⁻¹ cm⁻¹ at 338 nm), phenylpyruvate (from phenylalanine; ϵ = 5,900 M⁻¹ cm⁻¹ at 300 nm), and imidazol-5-yl-pyruvate (from histidine; ϵ = 11,000 M⁻¹ cm⁻¹ at 293 nm) was followed in an Amersham Ultrospec 3100pro spectrophotometer (21). As a negative control for intrinsic transaminase activity of the expression system, LacZ protein expressed and purified under the same conditions was used. K_m and V_{max} values were calculated from the data using GraphPad Prism software version 5.03 (GraphPad Software, San Diego, CA, USA).

Histidine uptake assay. Yeast cells were cultivated in 1 \times YNB medium supplemented with 10 mM histidine as the sole nitrogen source at 30°C. Samples of cultivation broths were harvested after 1, 2, 4, and 24 h of cultivation and kept at -80°C until further analysis. For histidine quantification, samples were diluted 20-fold in high-performance liquid chromatography (HPLC)-grade water before analysis on a Biochrom 30+ amino acid analyzer (Biochrom) according to the manufacturer's protocol for standard amino acid determination in physiological fluids. Briefly, the diluted samples were deproteinized with sulfosalicylic acid, and amino acids were HPLC separated, followed by postcolumn derivatization using ninhydrin and photometrical detection at 570 nm.

Transcription analyses. Yeast cells were grown in media containing 10 mM the appropriate amino acid, as described previously. Samples were taken at 0 min (YPD preculture) and after 1, 4, and 8 h of growth. RNA was isolated using an RNeasy kit (Qiagen) after glass bead disruption in a Precellys homogenizer (PeqLab). RNA quality was ensured with the Bioanalyzer instrument (Agilent). Expression of *ARO8*, *ARO9*, and the reference *ACT1* genes was detected in *C. glabrata* and *S. cerevisiae* using an EvaGreen quantitative-PCR (qPCR) mix (Bio&Sell) with appropriate primers in a CFX96 real-time detection system (Bio-Rad). The expression levels of three biological replicates were normalized to *ACT1* mRNA levels.

Alignment of the genomic locus. The nucleotide alignment of selected regions of the *C. glabrata* and *S. cerevisiae* genomes was performed with the GATAligner tool, version 1.0, and displayed using GATAPlotter version 0.7 (22). The parameters used were as follows: window size, 7; cutoff score, 5 bits; and standard BLASTN parameters. Genome sequence data were downloaded from the *Candida* and yeast genome databases (www.candidagenome.org and www.yeastgenome.org, respectively).

RESULTS

***C. glabrata* and *C. albicans* can use histidine as the sole nitrogen source.** We measured the ability of the hemiascomycetes *C. glabrata*, *C. albicans*, *S. cerevisiae*, and *S. kluyveri* to use different amino acids as their sole nitrogen source (Fig. 1). When grown in minimal medium with glucose as a carbon source and 10 mM single amino acids as a nitrogen source, *C. glabrata* and *S. cerevisiae* were able to use most of the standard proteinogenic amino acids. Only cysteine and lysine cannot support the growth of either yeast. Glycine and histidine enable good growth of *C. glabrata*, but not of *S. cerevisiae*. The yeast *S. kluyveri* is similar to *S. cerevisiae* in its growth profile, with the exception of lysine, which can be used by *S. kluyveri* but not by baker's yeast. In contrast, *C. albicans* was able to use all 20 classical proteinogenic amino acids as its sole source of nitrogen. As expected, all fungi grew best in YPD complex medium or in minimal medium with ammonium sulfate as the nitrogen source.

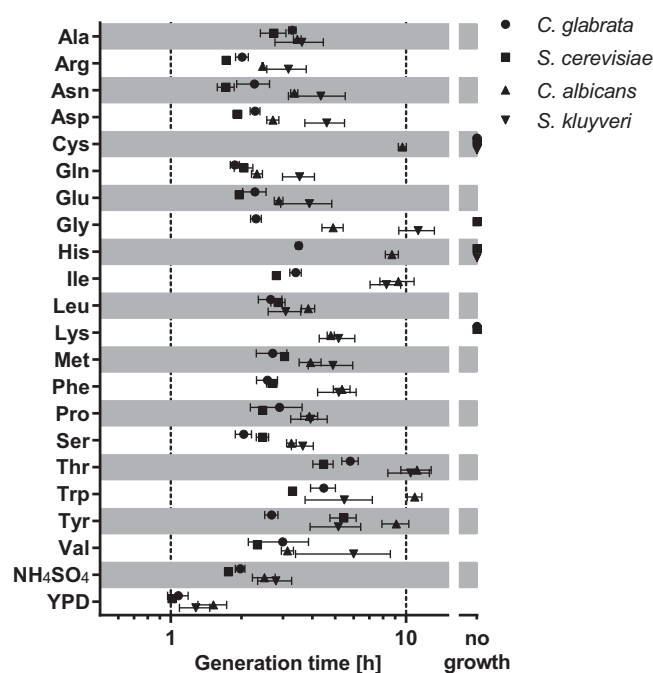


FIG 1 Growth of selected hemiascomycetes at 30°C with amino acids as sole nitrogen sources. Generation times of >12 h were generally considered absence of growth. Both *C. glabrata* and *C. albicans* were able to grow with histidine as the sole nitrogen source. Only *C. albicans* was able to use any amino acid for growth, while *C. glabrata* and *S. cerevisiae* generally grew faster than *C. albicans* or *S. kluyveri* under the conditions tested. Ammonium sulfate (0.5% NH₄SO₄) and YPD complex medium served as controls. All data are means \pm standard deviations (SD).

Furthermore, the overall growth pattern of *C. glabrata* resembles that of *S. cerevisiae* more than that of *C. albicans*, with the exceptions noted above. Compared to the other species, *C. albicans* grows much more slowly with aromatic amino acids (phenylalanine, tryptophan, tyrosine, and, for both *Candida* species, histidine). Importantly, histidine supports the growth of *C. glabrata*, with a generation time of 3.5 h, but both *S. cerevisiae* and the more basal *S. kluyveri* cannot grow, while the more distantly related *C. albicans* multiplies with a generation time of 8.7 h.

The HAL gene is absent in the hemiascomycetes. Histidine ammonia-lyases (HALs), or histidinases (EC 4.3.1.3), catalyze the conversion of histidine into ammonium and urocanic acid, enabling organisms to use histidine as a nitrogen source. Histidine ammonia-lyases are ubiquitous enzymes and can be found in archaea, bacteria, and eukaryota: a pfam search (23) for the conserved phenylalanine and histidine ammonia-lyase family (PF00221) detected HALs in 573 species from all three kingdoms (data not shown). Among the fungi, HALs can be found in many of the ascomycota and the basidiomycota. However, no conserved HAL sequences can be found in the hemiascomycete branch of the ascomycota, which contains (among other fungi) the fungal species investigated here, *S. cerevisiae*, *C. albicans*, *C. glabrata*, and *S. kluyveri*. In contrast, HALs can be found in the Pezizomycotina, as a related euascomycete phylum, for example, in *Aspergillus* and *Penicillium* spp. Evidently, the histidine ammonia-lyase gene was lost in the evolution of the Saccharomycotina. A TBLASTN search of fungal DNA sequences with the *Aspergillus clavatus* phenylalanine and histidine ammonia-lyase protein sequences as the

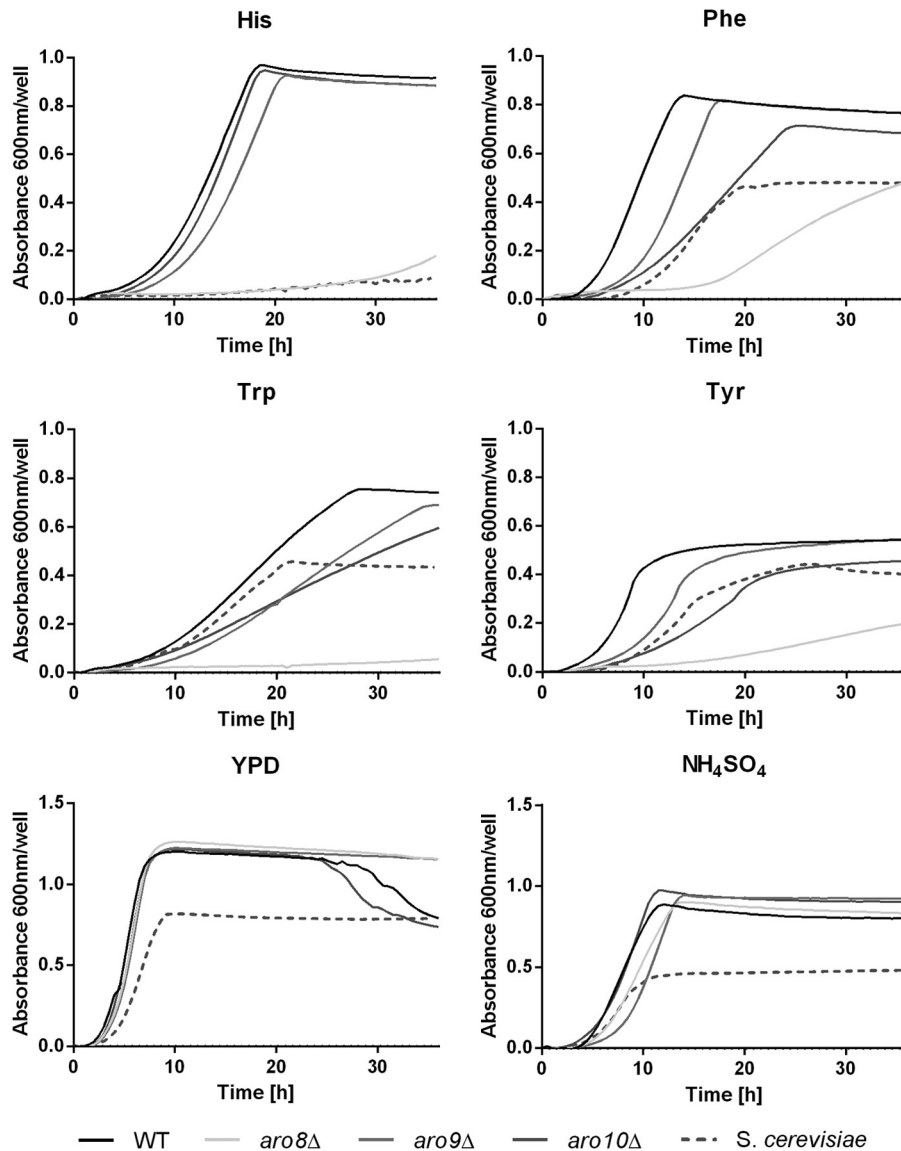


FIG 2 Growth curves of different mutants of *C. glabrata* and the *S. cerevisiae* wild type, used for comparison. Deletion of *ARO8* nearly abolished growth of *C. glabrata* with histidine, tryptophan, or tyrosine and strongly reduced growth with phenylalanine as the sole nitrogen source. Deleting the aromatic aminotransferase gene *ARO9* or the decarboxylase gene *ARO10* had much less effect on growth. All mutant strains grew nearly identically to the *C. glabrata* wild type in YPD complex medium.

query further supported the pfam results (data not shown). While this absence of a histidine ammonia-lyase would explain the lack of growth of *S. cerevisiae* and *S. kluyveri* with histidine as the sole source of nitrogen, both *C. albicans* and *C. glabrata* must have other means available to obtain the nitrogen from histidine. Thus, we propose that these fungi have evolved alternative metabolic pathways in order to use histidine as a potential nitrogen source.

Deletion of *ARO8* in *C. glabrata*. Recently, we created a mutant of *C. glabrata* that lacks the homologue of the yeast *S. cerevisiae* aromatic aminotransferase I gene, *ScARO8* (20). In *S. cerevisiae*, aromatic aminotransferases are known to catalyze the transfer of the amino group from phenylalanine, tyrosine, and tryptophan to amino acceptors like 2-oxoglutarate (24). As anticipated, growth with the three aromatic amino acids was strongly

reduced in the *C. glabrata aro8Δ* mutant (20), highlighting the broad substrate spectrum of the aminotransferase CgAro8. Unexpectedly, we found that the mutant was also nearly unable to use histidine as a nitrogen source, a phenotype resembling that of wild-type *S. cerevisiae* (Fig. 2). Therefore, it seems that the CgAro8 aminotransferase contributes significantly to the growth of *C. glabrata* on histidine as the sole nitrogen source.

In contrast, deleting the *C. glabrata* homologue of the yeast aromatic aminotransferase II gene, *ScARO9*, or of the downstream decarboxylase gene *ScARO10*, had only a negligible effect on growth with histidine as the sole nitrogen source (Fig. 2). Growth with the aromatic amino acid tryptophan, phenylalanine, or tyrosine was moderately reduced in the *C. glabrata aro9Δ* and *aro10Δ* mutants. In YPD complex medium, none of the deletion mutants displayed a reduced growth rate, while a minor growth

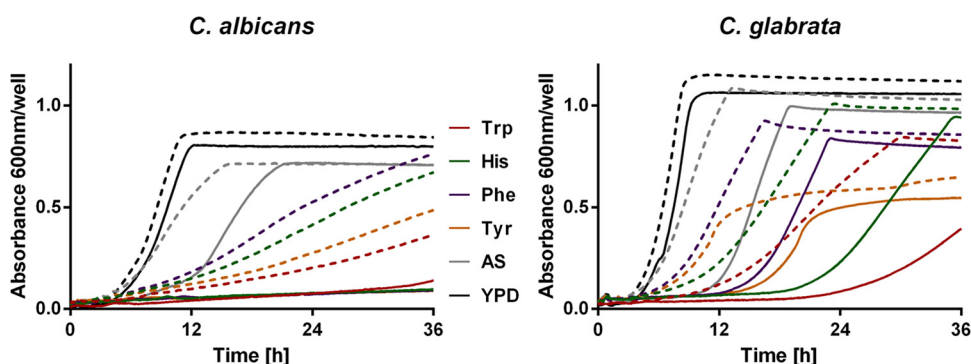


FIG 3 Growth of *C. albicans* and *C. glabrata* with 10 mM the aromatic aminotransferase inhibitor AOA. The presence of AOA reduces growth with all tested aromatic amino acids and ammonium sulfate (AS). Growth in YPD complex medium is nearly unaffected. Dashed lines, without inhibitor; solid lines, with the inhibitor AOA.

defect was observed with ammonium sulfate as the nitrogen source.

Addition of the competitive aromatic aminotransferase inhibitor AOA to *C. glabrata* cultures produced an effect similar to that of deletion of *CgARO8*. AOA is a broad-spectrum inhibitor of pyridoxal phosphate-dependent reactions like transaminations (25) and has been shown to inhibit, among others, fungal transaminases (26). In our hands, it strongly inhibited the growth of *C. glabrata* on histidine and all other aromatic amino acids at a concentration of 10 mM (Fig. 3). Growth with ammonium sulfate in the presence of the inhibitor was also reduced (as in the *aro8Δ* deletion mutant). There was no general growth reduction by addition of up to 10 mM AOA to YPD complex medium, showing that the reduced growth is likely not due to a general toxicity of AOA at these concentrations. Stronger growth inhibition was also observed for *C. albicans*. After a long lag phase, growth mostly resumed for *C. glabrata*. For *C. albicans*, which grew significantly more slowly even in the absence of the inhibitor (Fig. 1), no significant growth with any single aromatic amino acid was detected even after 36 h in the presence of AOA (Fig. 3). Overall, these results, in combination with the data from the deletion mutants, demonstrate that, like the utilization of other aromatic amino acids, histidine utilization is dependent on the aminotransferase activity of *CgAro8* in *C. glabrata*.

Finally, uptake of histidine was measured by HPLC analyses of the supernatants from growing yeast cultures (Fig. 4). After 24 h,

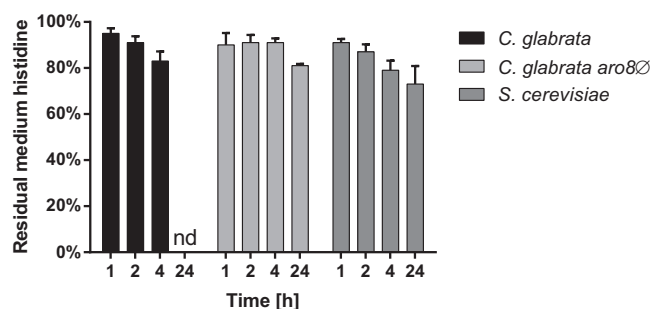


FIG 4 Residual histidine in the medium after incubation with the *C. glabrata* wild type, the *S. cerevisiae* wild type, or the *C. glabrata aro8Δ* mutant. The *C. glabrata* wild type used up the available histidine to levels below the detection threshold (nd), while most histidine remained in the medium in cultures with *S. cerevisiae* or the *aro8Δ* mutant.

complete removal (a concentration below the detection limit of 34 μ M) of the initial 10 mM histidine was observed from the supernatant of *C. glabrata* wild-type cultures. In contrast, nearly all of the histidine (9.8 mM) remained in the supernatant of the *aro8Δ* mutant. The remaining histidine level of the *S. cerevisiae* supernatant (7.4 mM) showed that the yeast can take up histidine, albeit at significantly lower levels than *C. glabrata*. However, since growth of *S. cerevisiae* is not supported by histidine as a sole nitrogen source, the exact fate of histidine in *S. cerevisiae* remains to be elucidated.

Aro8 and Aro9 regulation in *C. glabrata* and *S. cerevisiae*.

Previous microarray analyses indicated differences in regulation of *ARO8* and *ARO9*, the genes for aromatic aminotransferases I and II, respectively, between *C. glabrata* and *S. cerevisiae* (20). We investigated this regulation on the transcriptional level in more detail using qRT-PCR on RNA isolated from liquid cultures of *S. cerevisiae* and *C. glabrata* cells exposed to histidine, tryptophan, or ammonium sulfate as the sole nitrogen source (Fig. 5A).

ScARO8 was nearly 10-fold upregulated already at 1 h when histidine or tryptophan was offered as a nitrogen source. An early, lower upregulation was also observed with ammonium sulfate in the medium, but the transcript levels of *ARO8* decreased over time again in both *C. glabrata* and *S. cerevisiae*. This likely reflects the catabolic and anabolic activities of the enzymes, respectively, in the different media.

As described previously (27), the main aromatic aminotransferase gene, *ScARO9*, was strongly upregulated in *S. cerevisiae* when tryptophan was provided as the source of nitrogen but downregulated with ammonium sulfate initially at 1 h compared to the preculture (as opposed to *ScARO8*). With histidine, an increase of *ScARO9* mRNA levels was observed, but to a much lesser extent.

For *C. glabrata*, the most striking difference from *S. cerevisiae* was the complete lack of induction of the *CgARO9* gene under any of our experimental conditions. Even with tryptophan, the strongest inducer of *ScARO9* transcription in *S. cerevisiae*, there was no detectable difference from the preculture RNA levels in *C. glabrata*. In contrast, the transcription of *CgARO8* was upregulated in histidine- and in tryptophan-containing media, in agreement with our previous data (20). With these data, we confirmed the differences in *ARO* gene regulation between *S. cerevisiae* and *C. glabrata* and found that the *CgARO9* gene seems to be largely nonresponsive to external aromatic amino acids or histidine.

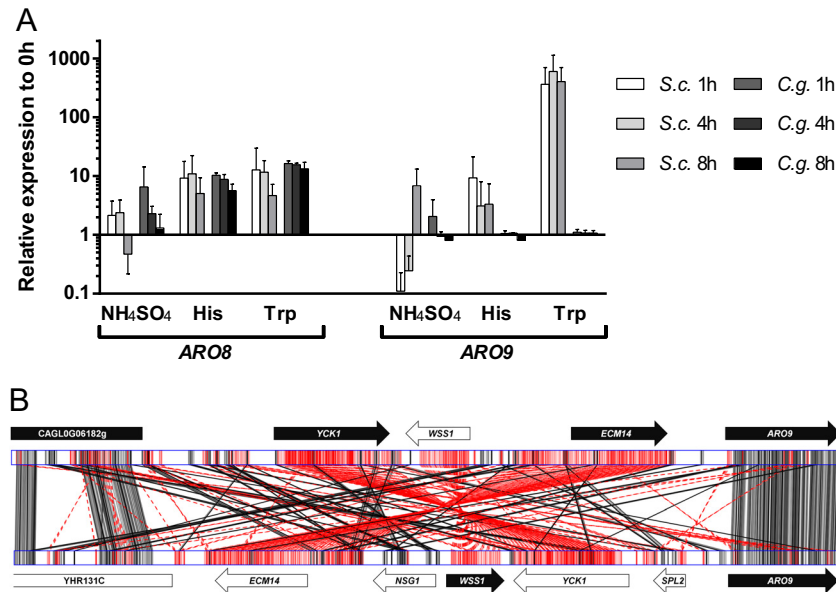


FIG 5 (A) Relative expression of *ARO8* and *ARO9* after transfer from complex medium to minimal medium containing ammonium sulfate, histidine, or tryptophan as the sole source of nitrogen. In both *S. cerevisiae* (*S.c.*) and *C. glabrata* (*C.g.*), *ARO8* expression was upregulated compared to its expression in the preculture at all time points when the amino acids were offered. There was no regulation observable for *ARO9* in *C. glabrata*, while *S. cerevisiae* very strongly upregulated *ARO9* when tryptophan was present. The error bars indicate standard deviations. (B) Genomic-locus alignment of the *ARO9* gene and 10-kbp upstream regions in *C. glabrata* (top) and *S. cerevisiae* (bottom). An inversion event was detected encompassing the upstream genes *YCK1*, *WSS1*, and *ECM14*. The genes *CAGL0G06182g* and *YHR131C* are homologues with identical orientations with respect to the *ARO9* genes. The solid arrows indicate ORFs with the same orientation as *ARO9*, and the open arrows indicate opposite orientations. Alignment was performed with GATA (22). Black lines indicate alignments in the same orientation (+/+), and red lines indicate those in inverse orientation (+/-). Darker shadings indicate better alignment scores.

Genomic differences between the *ARO8* and *ARO9* loci in *C. glabrata* and *S. cerevisiae*. To find an explanation for the differences in the regulation of these aromatic aminotransferase genes, we examined the structures of their respective genomic loci. Using YGOB, the yeast gene order browser (28), we found that *CgARO8* and the next gene located upstream, *CAGL0G01232g* (an *ScKEX1* homologue), form a pair with synteny to its counterpart in *S. cerevisiae* and the predicted common ancestor. Downstream of *CgARO8* and upstream of the *KEX1* homologue, the synteny is broken.

For *CgARO9*, there is an inversion upstream of the open reading frame (ORF) compared to the genome of *S. cerevisiae* and all other available genomes of the YGOB. Sequence analysis showed that this inversion encompasses several kilobases and includes the neighboring predicted genes *YCK1*, *WSS1*, and *ECM14* (Fig. 5B). In addition, the inversion may also include one or more transcription factor binding sites of the immediate *CgARO9* upstream region. Such a removal of the binding sites from *CgARO9* gives a possible explanation for its lack of regulation in the presence of aromatic amino acids.

In vitro activity of the Aro8 proteins. For further confirmation of the role of Aro8 in histidine degradation, we heterologously expressed a histidine-tagged *CgAro8* protein in *E. coli* and affinity purified the enzyme. As we have shown before (20), the *CgAro8* protein converted tryptophan into indole pyruvate. The K_m of this reaction was 14.9 ± 2.5 mM, and the V_{max} was 593.3 ± 48.5 $\text{nmol} \cdot \text{min}^{-1} \cdot \text{mg}^{-1}$ under our *in vitro* assay conditions (with α -ketoglutarate as the amino acceptor). In addition, the protein was also able to convert phenylalanine into phenylpyruvate under the same conditions, with a K_m of 1.39 ± 0.16 mM and a V_{max} of

196.7 ± 3.9 $\text{nmol} \cdot \text{min}^{-1} \cdot \text{mg}^{-1}$. This reaction is similar to that of the *S. cerevisiae* Aro8 protein (29). However, most interestingly, a test with L-histidine as the amino donor revealed that *CgAro8* is also able to transfer the amino group of this amino acid to α -ketoglutarate, producing imidazol-5-yl-pyruvate (Fig. 6A), with a K_m of 8.80 ± 1.41 mM and a V_{max} of 274.9 ± 19.8 $\text{nmol} \cdot \text{min}^{-1} \cdot \text{mg}^{-1}$. Interestingly, a heterologously expressed Aro8 protein from *S. cerevisiae* had a similar ability to catalyze the aminotransferase reaction with L-histidine as a donor (data not shown), implying a principal ability of *S. cerevisiae* for histidine transamination.

Interaction of histidine with other nitrogen sources in *S. cerevisiae*. The Aro8 proteins of both species were able to catalyze the histidine aminotransferase reaction *in vitro*, and in both species, the *ARO8* gene was upregulated in response to histidine. We therefore investigated the effect of adding histidine to the medium on the growth of *S. cerevisiae*. To this end, we mixed histidine 1:1 (5 mM each) with different nitrogen sources: alanine as an amino acid with an independent degradation pathway, phenylalanine as an aromatic amino acid that induces *ARO8* expression, and ammonium sulfate as a non-amino-acid nitrogen source (Fig. 6B).

As before, *S. cerevisiae* was unable to grow with histidine as the sole nitrogen source. Strikingly, the comparatively good growth with 5 mM phenylalanine or tryptophan (generation times, 2.8 h and 4.9 h, respectively) was completely abolished when 5 mM histidine was added. Growth with 5 mM alanine (3.2 h) in the presence of additional histidine still took place but was slowed down significantly (9.9 h). Interestingly, addition of the inhibitor AOA partly rescued the growth in medium with alanine and histidine (to 7.2 h), while it reduced the growth rate in medium with

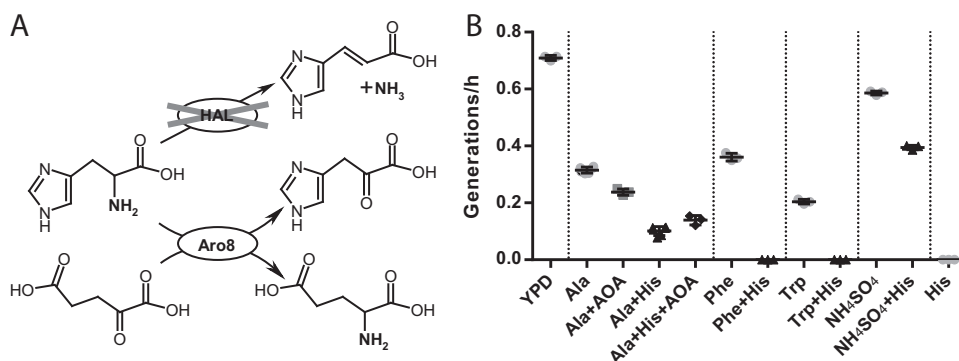


FIG 6 (A) Proposed aromatic amino acid aminotransferase reaction by Aro8 with α -ketoglutarate and histidine as substrates, producing glutamate and imidazol-5-yl-pyruvate. The pathway from histidine to urocanic acid via the HAL does not take place in *C. glabrata* or *S. cerevisiae* due to the absence of the HAL gene. The transferred amine group is marked in boldface. (B) Effects of histidine supplementation on *S. cerevisiae* growth with different nitrogen sources. Addition of 5 mM histidine abolishes growth with the aromatic amino acids phenylalanine and tryptophan and reduces growth with alanine or ammonium sulfate as the nitrogen source. Addition of the aromatic amino acid aminotransferase inhibitor AOA slightly increases the growth rate when alanine is supplemented with histidine.

alanine as a sole nitrogen source (to 4.2 h). Finally, growth with ammonium sulfate (1.7 h) was only slightly reduced (2.5 h) by addition of histidine. This indicates that histidine is indeed taken up by *S. cerevisiae* and transaminated under conditions of *ScARO8* or *ScARO9* induction. However, while histidine degradation can be initiated, downstream processing of the transaminated product seems to be disturbed or interferes with other metabolic pathways.

DISCUSSION

The comparatively close phylogenetic relationship between the normally nonpathogenic yeast *S. cerevisiae* and the pathogenic yeast *C. glabrata* allows the detection of specific factors and traits that are associated with fungal pathogenesis (4). These include, for example, the ability of *C. glabrata* to grow efficiently at body temperature, but also its ability to use nutrients from particular host niches, which likely differ significantly from *S. cerevisiae*'s normal habitats.

Both *S. cerevisiae* and *C. glabrata* were able to grow with most of the proteinogenic amino acids as sole nitrogen sources. For baker's yeast, the only exceptions were cysteine, glycine, histidine, and lysine, in accordance with previous observations (30). The pathogenic yeast *C. glabrata*, on the other hand, was additionally able to grow with either glycine or histidine as the sole nitrogen source. Evidently, this pathogen is even more versatile than *S. cerevisiae* in its ability to use different nitrogen sources, though not as flexible as *C. albicans*, which can utilize all 20 proteinogenic amino acids.

We hypothesized that the inability of *S. cerevisiae* to use histidine as a nitrogen source was due to the loss of the histidine ammonia-lyase gene. Since this loss is common to all hemiascomycetes, it should also affect *C. albicans* and *C. glabrata*, yet these two species can utilize histidine as their sole nitrogen source. Comparison with *S. kluyveri*, which also cannot use histidine for a nitrogen supply, indicates that at least *C. glabrata*, and probably *C. albicans*, has independently gained this ability in a lineage that is unable to use histidine. As the pathogenic yeasts in this study, *C. albicans* and *C. glabrata*, were most flexible with regard to the utilization of amino acids, it is tempting to speculate that a correlation may exist between the ability to use a broad range of nitrogen sources and the pathogenic potential of these fungi.

Having a broad set of catabolic pathways for the specific nutrient sources in the host is clearly advantageous for pathogenic fungi (6). On the other hand, unused metabolic pathways are quickly lost. One striking example of this is the absence of the kynurenine pathway in *C. glabrata* (31). In baker's yeast, the aromatic amino acid tryptophan can be degraded by two distinct pathways, via the kynurenine pathway to NAD or via the Ehrlich pathway to indole-3-ethanol (32). *C. glabrata* has lost six of the seven *BNA* genes of the kynurenine pathway (31, 33) and is therefore auxotrophic for niacin, as opposed to *S. cerevisiae* and *C. albicans*. Due to these losses, the only remaining pathway for tryptophan degradation in *C. glabrata* is via the Ehrlich pathway, and therefore via the CgAro8 aromatic amino acid aminotransferase (20).

Here, we show that degradation of histidine in *C. glabrata* also occurs via the Ehrlich pathway. The aromatic amino acid aminotransferase Aro8 is both sufficient for *in vitro* aminotransferase activity and required for growth with histidine as the sole nitrogen source. The K_m values of *C. glabrata* Aro8 for the transamination reactions of phenylalanine and tryptophan seem high for its biological function but are similar to those published for its *S. cerevisiae* counterpart (1.4 versus 0.3 mM and 14.9 versus 6 mM, respectively [24]). Thus, a similar biological role for CgAro8 in the degradation of external aromatic amino acids seems likely, and the histidine transamination observed *in vitro* should be sufficient to enable *in vivo* growth with histidine.

Like the deletion of *CgARO8*, inhibition of the aromatic aminotransferase with the substance AOA led to a severe growth defect of *C. glabrata* (and *C. albicans*) with tryptophan and histidine as the sole nitrogen sources. The growth defect with ammonium sulfate is likely due to the general inhibition of aminotransferase activities that are also required in amino acid anabolic processes. In *S. cerevisiae*, deletion of both *ScARO8* and *ScARO9* leads to phenylalanine and tryptophan auxotrophy of the double mutant (34). Thus, with only ammonium sulfate as a nitrogen source in the medium, AOA likely blocked the biosynthesis of aromatic and other amino acids by inhibition of the respective aminotransferases, which could lead to a limitation of building blocks for anabolic purposes. Alternatively, inhibition of aminotransferases could lead to accumulation of α -ketoacids that provoke general

toxic effects and may thus cause reduced growth rates. However, the normal growth rate in YPD medium in the presence of AOA makes the latter scenario rather unlikely, although the possibility that differences in AOA uptake depending on the applied growth medium result in reduced toxicity cannot be excluded. Nevertheless, the strong growth-inhibitory effect of any of the aromatic amino acids used as a sole nitrogen source further indicates that, despite its low specificity, aromatic aminotransferases of *C. glabrata* were indeed inhibited by AOA treatment.

An aminotransferase reaction for histidine degradation has already been proposed to occur in other organisms. In the rat's liver, for example, this reaction has been reported to take place, but it is most likely not used for catabolic purposes. Instead, it serves a function during mitochondrial transport (35). No gene has been found so far to code for the enzyme. In *E. coli* and other bacteria, like *Pseudomonas testosteroni* (36), a similar histidine transaminase reaction has been described, but the well-investigated *E. coli* K-12 strain lacks this reaction and is unable to catabolize histidine. Other fungi, like *Aspergillus* spp., possess the standard histidine ammonia-lyase, and the protein has been shown to be active, for example, in *A. nidulans* when histidine is the sole nitrogen source (37). Therefore, to our knowledge, catabolism via an aromatic aminotransferase instead of a histidinase presents a novel route for fungal growth with histidine as a nitrogen source.

Interestingly, *S. cerevisiae* Aro8 also catalyzes the aminotransferase reaction from histidine, although the fungus is unable to grow with histidine as the sole nitrogen source. Our data indicate, however, that histidine degradation may lead to an inhibiting metabolic intermediate in yeast, while histidine did not support growth, it also abolished growth under conditions where the Ehrlich pathway was active (in the presence of phenylalanine or tryptophan as a nitrogen source). Under these conditions, histidine will likely be deaminated by the aromatic amino acid aminotransferases. Growth with ammonium sulfate was only slightly reduced in the presence of histidine, since ammonium sulfate is a preferred nitrogen source and does not lead to the activation of the Ehrlich pathway. Additionally, high expression of amino acid permeases like Gap1 for histidine uptake is unlikely (38). Finally, with alanine, the growth rate was reduced to an intermediate level. Alanine belongs to the amino acids with intermediate growth support (Fig. 1), and it appears likely that general amino acid permeases are expressed and allow uptake of histidine. Since inhibition of aromatic amino acid aminotransferases increased the growth rate under these conditions, Aro8 and Aro9 appear to be active and to produce an intermediate that causes the growth inhibition. In summary, these effects would explain why Aro8 transaminase activity is not sufficient for use of histidine as a nitrogen source in *S. cerevisiae*: at least one of the histidine degradation products itself likely inhibits growth.

Besides the observation that aromatic amino acid aminotransferases in *S. cerevisiae* are able to transaminate histidine but cannot support its utilization, it is interesting that the gene product of ScARO9 is the main aromatic aminotransferase in *S. cerevisiae*, and the gene is accordingly upregulated when tryptophan is offered as the sole source of nitrogen (27). In contrast, *C. glabrata* shows no upregulation of the corresponding gene; however, CgARO8 expression is increased in the presence of external aromatic amino acids (reference 20 and this work). A possible explanation for this phenomenon is an inversion event in the direct upstream region of the CgARO9 gene, which may have caused the

loss of some or all regulatory genetic elements. Most of the aromatic amino acids, including histidine, will therefore likely be processed by CgAro8, since CgARO8 is the only aromatic amino acid transferase gene of *C. glabrata* upregulated in the presence of aromatic amino acids. Thus, the inversion event in the phylogenetic lineage of *C. glabrata* likely contributed to the observed differences between *C. glabrata* and *S. cerevisiae*.

The presence of an unusual nitrogen metabolic pathway has precedents in the hemiascomycetes. These yeasts catabolize urea via a urea amidolyase instead of the urease of other higher fungi (39). Deleting the urea amidolyase gene, *DUR1,2*, in *C. albicans* leads to a reduction in virulence and, importantly, of the fungal burden in organs with high urea content (40). This can, at least in part, be explained by the dependency of fungi on external nitrogen sources during infections (40). Hence, like the Dur1,2 pathway in *C. albicans*, the unusual histidine metabolism of *C. glabrata* may be considered a possible target for drugs that could reduce growth of the fungus in the host.

ACKNOWLEDGMENTS

We thank Markus Gressler for his valuable input.

This work was supported by the German Federal Ministry of Education and Health (BMBF), FKZ 01EO1002 from the Integrated Research and Treatment Center, Center for Sepsis Control and Care (CSCC), and the ERA-NET PathoGenoMics Program (Candicol; BMBF 0315 901 B). S.R. was supported by the graduate school, Jena School for Microbial Communication (JSMC) (www.jsmc.uni-jena.de).

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