The Fungal Pathogen *Candida albicans* Autoinduces Hyphal Morphogenesis by Raising Extracellular pH

Slavena Vylkova, Aaron J. Carman,* Heather A. Danhof, John R. Collette, Huaijin Zhou, and Michael C. Lorenz

Department of Microbiology and Molecular Genetics, The University of Texas Health Science Center, Houston, Texas, USA

*Present address: Department of Molecular Pharmacology and Chemistry, Memorial Sloan-Kettering Cancer Center, New York, New York, USA.

**ABSTRACT** pH homeostasis is critical for all organisms; in the fungal pathogen *Candida albicans*, pH adaptation is critical for virulence in distinct host niches. We demonstrate that beyond adaptation, *C. albicans* actively neutralizes the environment from either acidic or alkaline pHs. Under acidic conditions, this species can raise the pH from 4 to >7 in less than 12 h, resulting in autoinduction of the yeast-hyphal transition, a critical virulence trait. Extracellular alkalinization has been reported to occur in several fungal species, but under the specific conditions that we describe, the phenomenon is more rapid than previously observed. Alkalinization is linked to carbon deprivation, as it occurs in glucose-poor media and requires exogenous amino acids. These conditions are similar to those predicted to exist inside phagocytic cells, and we find a strong correlation between the use of amino acids as a cellular carbon source and the degree of alkalinization. Genetic and genomic approaches indicate an emphasis on amino acid uptake and catabolism in alkalinizing cells. Mutations in four genes, STP2, a transcription factor regulating amino acid permeases, *ACH1* (acetetyl-coenzym A [acetyl-CoA] hydrolase), *DUR1,2* (urea amidolyase), and *ATO5*, a putative ammonia transporter, abolish or delay neutralization. The pH changes are the result of the extrusion of ammonia, as observed in other fungi. We propose that nutrient-deprived *C. albicans* cells catabolize amino acids as a carbon source, excreting the amino nitrogen as ammonia to raise environmental pH and stimulate morphogenesis, thus directly contributing to pathogenesis.

**IMPORTANCE** *Candida albicans* is the most important fungal pathogen of humans, causing disease at multiple body sites. The ability to switch between multiple morphologies, including a rounded yeast cell and an elongated hyphal cell, is a key virulence trait in this species, as this reversible switch is thought to promote dissemination and tissue invasion in the host. We report here that *C. albicans* can actively alter the pH of its environment and induce its switch to the hyphal form. The change in pH is caused by the release of ammonia from the cells produced during the breakdown of amino acids. This phenomenon is unprecedented in a human pathogen and may substantially impact host physiology by linking morphogenesis, pH adaptation, carbon metabolism, and interactions with host cells, all of which are critical for the ability of *C. albicans* to cause disease.
lus nidulans, and C. albicans, where the central mediators are the Rim101p/PacC transcription factors, which are activated by a proteolytic cleavage event at neutral-alkaline pH (8–10). In both C. albicans and Aspergillus fumigatus, Rim101p/PacC is required for full virulence, and in C. albicans it is required for hyphal induction by neutral pH (8, 11). A compelling example of the importance of host niche pH in C. albicans is found in Phr1p and Phr2p, homologous glycosidases that are inversely regulated by pH (12, 13). Mutants lacking the acid-expressed PHR2 gene are unable to grow in vitro at pHs of <5 and are avirulent in a rodent model of vaginitis, a body site with a low ambient pH, yet remain fully virulent in a bloodstream sepsis model (12, 14). PHR1, induced at neutral pH by Rim101p, is required for bloodstream infections but not during growth at low pH or in the vagina (8, 12, 15).

A much less well understood aspect of pH regulation is the ability to actively alter extracellular pH. Helicobacter pylori tolerates the highly acidic pH of the stomach by expressing a urease, found in part on the cell surface, that produces ammonia to create microenvironments of more hospitable pH; mutants that lack urease activity are unable to colonize the stomach and are avirulent (16, 17). Several fungal species have limited ability to alkalize the extracellular milieu, including Neurospora crassa, A. fumigatus, Mortierella anisopliae, and S. cerevisiae (18, 19). Dermatophytic fungi are identified based on a slow alkalization phenomenon assaysed over 10 to 14 days (20). More significantly, phytopathogenic species of Colletotrichum elevate both the local ammonia concentration and the pH in plant tissue, and this regulates expression of pectin lyase, a key virulence factor (21, 22). In some of these species, the rise in pH has been associated with the release of ammonia, a highly basic compound (18, 19, 21, 22). The mechanisms behind ammonia production are mostly unknown, but in Colletotrichum cocodes it has been associated with nitrogen catabolite repression and reduction of nitrate/nitrite to ammonia (23).

C. albicans has also been reported to raise extracellular pH over the course of 10 to 14 days in a minimal medium containing keratin (24). In contrast, we report here that C. albicans has a remarkable ability to alter extracellular pH, creating a neutral environment from either acidic or alkaline starting conditions, with changes of ≥3 pH units in 12 h. This phenomenon occurs in both solid and liquid media, is glucose repressible, and requires exogenous amino acids. The rise in pH induces hyphal morphogenesis, a key virulence trait of this species, and is correlated with release of volatile ammonia from the cells. By using a combination of genetic and genomic approaches, we have identified several components required for alkalization, including regulators of amino acid uptake (Stp2p and Csh3p), putative ammonia transporters (the Ato family), and amino acid catabolic enzymes (Dur1p, Acr11p). Our findings link carbon metabolism, hyphal growth, and pH responses, each important contributors to virulence in C. albicans.

RESULTS
Candida albicans neutralizes the environmental pH under glucose-limiting conditions. The wide range of pH values in which C. albicans can grow has prompted numerous studies on how this species adapts to such extreme conditions (reviewed in references 25 and 26), often using medium 199, an amino acid-rich, glucose-poor tissue culture medium with phenol red as a colorimetric readout of pH. C. albicans grows well in this medium over a wide range of initial pHs, from 2 to 10 (Fig. 1A). In unbuffered medium, we observed a dramatic increase in the culture pH when the wild-type strain SC5314 was incubated in aerated (shaking) cultures at either 37 or 30°C (Fig. 1B and data not shown). From an initial pH of 4.0, the pH was noticeably higher within 6 h (pH 4.9), reached 7.2 within 24 h (Fig. 1B), and peaked at 7.5 by 48 h (data not shown). The ability to alter pH was limited: from pH 3 there was only a small rise in culture pH by 24 h (to 3.7) (Fig. 1B), and no change was seen from pH 2 after extended incubation, despite overall growth similar to that obtained with the other pH conditions (Fig. 1A).

From alkaline pH (pH 8 to 10), the pH dropped rapidly and then slowly rose again to 8.15 to 8.70 by 24 h (Fig. 1B). Medium 199 contains 0.1% glucose, and we believe that the transient acidification is related to the breakdown of this limited glucose, a well-known effect of glycolysis, though we have been unable to definitively prove this, as C. albicans grows very poorly at very alkaline pH in the presence of nonfermentable carbon sources. We subsequently focused on the alkalization of low-pH environments.

GM-BCP medium, containing glycerol, yeast extract, and the pH indicator bromocresol purple, has been used to study alkalization in S. cerevisiae (18). C. albicans alkalizes this medium very rapidly (see Fig. S1 in the supplemental material; see also Fig. 3C); the process is essentially complete in C. albicans after 2 to 3 days, while S. cerevisiae strain BY4742 has been reported to require ~2 weeks for significant change to be shown (29) (in fact, we have not observed BY4742 to alkalize even after 4 weeks of incubation, but we do see alkalization with an ancestor, EM93; data not shown). Substantial pH changes are seen in both GM-BCP and medium 199 inoculated with other Candida species as well (Fig. S1 and data not shown). C. albicans, Candida tropicalis, Candida dubliniensis, and Candida parapsilosis all alkalized to similar degrees, as did the related species Lodderomyces elongisporus and Debaromyces Hansenii; Candida guilliermondii and Candida lusitaniae show a delay in alkalization. The taxonomically distinct Candida glabrata and S. cerevisiae showed little or no pH change under these conditions (Fig. S1).

Environmental alkalization in C. albicans is glucose-repressible. We sought to better understand the environmental conditions that stimulate the extracellular pH changes we observed. One key difference between both medium 199 and GM-BCP and more standard media used to propagate C. albicans (e.g., yeast extract-peptone-dextrose [YPD]) is the level of glucose: most yeast media have 2% (wt/vol) glucose, while medium 199 has 0.1% glucose and GM-BCP has no added sugars. Supplementing medium 199 (pH 4.0) to give a final concentration of 0.3 to 2.0% glucose promoted more-rapid growth (Fig. 1C) but either greatly slowed (0.5%) or abolished (>0.5%) alkalization (Fig. 1D). Addition of 2% glucose also inhibited alkalinization on medium 199 (pH 4.0) to give a final concentration of 0.3 to 2.0% glucose promoted more-rapid growth (Fig. 1C) but either greatly slowed (0.5%) or abolished (>0.5%) alkalization (Fig. 1D). Addition of 2% glucose also inhibited alkalization on GM-BCP medium (see Fig. 3C). These results indicate that alkalization is glucose repressible.

Amino acids promote alkalization. To further understand the environmental conditions that promote alkalization, we developed an assay based on the minimal defined medium YNB, which contains salts, trace minerals and cofactors, and ammonium sulfate but no source of carbon (see Materials and Methods). The addition of glucose, ethanol, or glycerol supported growth without any rise in pH (Fig. 2A). In contrast, addition of...
1% Casamino Acids (an acid hydrolysate of casein) permitted growth accompanied by a significant alkalinization, from pH 4 to pH ~6.3, over 24 h (Fig. 2A). Alkalinization was also seen on synthetic complete (SC) medium (YNB plus a mixture of most of the amino acids), and this was repressed by the addition of glucose (data not shown). Thus, alkalinization is stimulated by the presence of exogenous amino acids.

We assayed growth and pH changes in YNB medium at pH 4 containing 1 mM each amino acid individually as the sole carbon source (Fig. 2B). At this level, only a subset of amino acids allowed any increase in culture density (Ala, Arg, Asn, Gln, Glu, Pro, and Ser), and there was a strong correlation between the ability of *C. albicans* to metabolize individual amino acids as a carbon source and the degree of alkalinization (Fig. 2B), suggesting that metabolism of amino acids is required for the change in pH.

**Alkaline domains autoinduce hyphal morphogenesis.** *C. albicans* cells shifted to neutral pH media rapidly initiate germ tube formation and hyphal growth. We hypothesized that the gradual neutralization under these conditions would also promote this morphological transition. We tested this by growing cells of the wild-type strain SC5314 in liquid YNB medium with 2% (wt/vol) Casamino Acids as the sole carbon source. In this medium, the pH rises gradually from 4 to 7.2 over 12 h (Fig. 3B). By 7 h, when the pH of the medium was 5.9, 78% of cells were hyphal, compared to 4.2% of cells in the same medium supplemented with 2% glucose, where the pH was 3.8 (Fig. 3A; representative images are shown in Fig. S2 in the supplemental material). Buffering the medium with 0.1 M Tris and 0.1 M MOPS (morpholinepropanesulfonic acid) greatly slowed the rise in pH (4.3 at 7 h) and reduced the proportion of hyphal cells (13%). By 24 h, the pH of the medium containing Casamino Acids as the sole carbon source was 7.3, and 60% of the cells remained in the hyphal state; no hyphae were observed in the glucose or buffered control cultures, despite small rises in pH (to 5.5 and 5.4, respectively). Initial germ tube growth was observed in all cultures at an early time point (3 h), as has been previously reported following dilution into fresh medium (30), but hyphal induction is maintained only in the alkalinizing culture.

Alkaline domains on solid GM–BCP medium also switched to hyphal growth. As shown in Fig. 3C, the alkalinizing colony had a border of highly filamentous cells, and both alkalinization and filamentous growth were blocked by the addition of glucose. Thus,
C. albicans appears to induce its own morphogenesis via raising extracellular pH.

**Alkalization is not controlled by the Rim101p or Mnl1p transcription factors.** In *C. albicans*, two transcription factors have been linked to pH responses: the Rim101p/PacC pathway is required for growth at alkaline pH (reviewed in reference 25), and Mnl1p mediates adaptation to the presence of several weak acids, such as acetic and formic acids (31). Strains lacking *RIM101* were obtained from D. Davis (8, 11), and *mnl1Δ* mutants were derived from a transcription factor mutant library (a gift from D. San- 
glard; see below). Neither of these mutants affected the alkalization phenomenon (see Fig. S3 in the supplemental material). We also tested strains lacking several other components of the Rim101p pathway or the ESCRT complex, and none altered alkalization (data not shown). The pH changes that we observe are therefore independent of the previously identified pH adaptation machinery.

![Image of pH changes](image_url)

** FIG 2** Amino acids are required for alkalization. (A) Wild-type strain SC5314 was grown in liquid YNB medium at pH 4.0 (see Materials and Methods), with the indicated compound as the sole carbon source, present at 1% (wt/vol) (for Casamino Acids) or 2% (wt/vol) (for other compounds). Growth (blue bars) and pH (red bars) were measured after 24 hours at 37°C. The horizontal black line is present to indicate the starting pH of the cultures. (B) The wild-type strain (SC5314) was grown in liquid YNB medium at pH 4.0, supplemented with the indicated amino acid present at 1 mM as the sole carbon source. Growth (blue bars) and pH (red bars) were measured after 24 hours at 37°C. The horizontal black bar indicates the starting pH and OD_{600} of the cultures for reference.

![Image of pH changes](image_url)

** FIG 3** Alkalization induces hyphal morphogenesis. The wild-type strain (SC5314) was grown overnight in YPD and then washed in water and diluted to an OD_{600} of ~0.2 in YNB plus 2% Casamino Acids at pH 4.0 or in this medium supplemented with 2% glucose or 0.1 M Tris and 0.1 M MOPS to block alkalization. At the time points indicated, samples were collected and photographed (see Fig. S2 in the supplemental material). (A) Hyphal induction was quantified by counting cells from the photomicrographs. (B) The pH of the same cultures was measured at the same time points. (C) *C. albicans* wild-type cells were grown in YPD, washed in water, and diluted to an OD_{600} of 1.0, and then 2 μl was spotted onto GM-BCP, pH 4.0, medium with or without 2% glucose. The alkalization of the medium and the morphology of the colony were observed at 48 h.
Identification of *C. albicans* genes involved in environmental alkalinization. To identify factors that mediate extracellular alkalinization, we performed a genetic screen for *C. albicans* mutants unable to change the pH of the medium using libraries containing ~500 strains enriched for mutations in transcription factors, signaling components, and cell wall proteins generated in the laboratories of A. Mitchell (32, 33) and D. Sanglard (personal communication). The mutants were screened in 96-well plates containing medium 199, pH 4.0, to identify wells in which the medium remained yellow (acidic) but abundant yeast growth was observed. This screen identified two alkalinization-deficient mutants: ach1, encoding acetyl-coenzyme A (acetyl-CoA) hydrolase, and stp2, encoding a transcription factor that regulates the expression of amino acid permeases.

We confirmed the results from the library screen using previously published mutants of *ACH1* and *STP2*. First, we plated serial dilutions of both strains in 96-well plates in medium 199, pH 4.0, and incubated them for 24 h at 37°C. Neither mutant alkalinized the medium, while the wild-type (WT) strain and complemented versions of both mutants did (Fig. 4A). The *stp2*/H9004 strain was also unable to alkalinize solid GM-BCP medium (Fig. 4B), while the *ach1*/H9004 mutant did alkalinize this medium, albeit with a variable delay relative to the level for wild-type controls (data not shown). Similarly, we observed no pH change in cultures of the *stp2*/H9004 mutant in aerated medium 199 (Fig. 4C). The *ach1*/H9004 mutant retarded the rise in pH but eventually reached the same pH as the wild-type or complemented strains (Fig. 4D). The *ach1Δ* mutants grew at the same rate as the wild-type strain in medium 199, while...
the stp2Δ mutant had a moderate growth retardation, presumably as a result of impaired amino acid uptake (data not shown).

Stp2p is a transcription factor that activates amino acid permeases in response to extracellular amino acids (34). Many of these permeases require Csh3p, an endoplasmic reticulum (ER)-resident chaperone, for proper folding and sorting to the cell surface (35); a csh3Δ mutation also blocked alkalinization (Fig. 4B), reinforcing the role of amino acid import and metabolism in this phenomenon. Conversely, Stp1p, homologous to Stp2p, has no effect (Fig. 4B). Stp1p regulates the protease Sap2p and the oligopeptide permease Opt1p when protein is present in the medium (34); we were unable to detect alkalinization when protein (bovine serum albumin [BSA]) was the sole source of carbon. Collectively, these results suggest that general amino acid uptake and amino acid catabolism are aspects of intermediate metabolism essential for the extracellular pH changes we observe.

Microarray analysis of neutralization in C. albicans. In order to further understand the molecular basis for alkalinization, we assayed transcript profiles of cells actively alkalinizing the medium by growing wild-type cells in medium 199, initially at pH 4.0, until the pH of the medium reached pH 5.0. This profile was compared to two control conditions that do not alkalinize (the wild-type strain in the same medium supplemented with 2% glucose or the Δaht1 mutant in medium 199), focusing on genes whose expression was increased or decreased under the alkalinizing conditions but not under the nonalkalinizing conditions (see Materials and Methods). Using a significance cutoff of 3-fold change and a P value of less than 0.01 (Student’s t test), we identified 60 genes, 34 overexpressed and 26 underexpressed, under the alkalinizing conditions. Uncharacterized open reading frames (ORFs) comprised the largest set of regulated genes (22/60). None of the genes identified genetically, STP2 or CSH3 (under both conditions) or ACH1 (versus the glucose culture), were differentially regulated in a statistically significant manner. The remainder of the data showed an emphasis on amino acid metabolism and transmembrane transport, as described below and in Table 1.

Differential regulation of amino acid metabolism was apparent in the microarray data, particularly for arginine and methionine. Genes for arginine synthesis, such as ARG1 and ARG3, are repressed in alkalinizing cultures (Table 1). Conversely, alkalinizing cells induce arginine import via genes encoding basic amino acid permeases (CAN1 and CAN2) and degradation via genes for arginase (CAR1) and ornithine transaminase (CAR2). One product of arginase is urea, and the urea amidolase gene DUR1,2 is strongly repressed in the alkalinizing culture relative to the level for the high-glucose conditions (Table 1). Together, there is a strong transition during alkalinization from synthesis to catabolism of arginine. Methylamine synthesis is also repressed during alkalinization: MET1, -2, -3, -4, and -14 are each repressed in the alkalinizing culture, and two other MET genes (MET10 and MET15) were underexpressed in comparison to the level for only one nonalkalinizing condition (Table 1).

Other transmembrane transporters were also affected during alkalinization (Table 1). Alkalinizing cultures upregulated genes encoding transporters for glucose (HGT2, HGT13, and HGT16), lactate (JEN1), phospholipids (CDR3), phosphoinositol (GIT2), and water (AQY1). Genes for two ammonia permeases, MEP1 and MEP2, were significantly induced in the nonalkalinizing WT-glucose cells but not in the aht1Δ culture. Four other putative transporters, homologs of the S. cerevisiae Ato proteins (see Table S1 in the supplemental material), are discussed below.

Finally, we observed several notable changes in carbon metabolism (Table 1). Genes encoding the glyoxylate cycle components Icl1p and Ms1p, acetyl-CoA synthase Acslp, and the carnitine acetyltransferases Ctn1p and Ctn3p and several putative β-oxidation genes (FOX2, PXP2, and FAA2) were expressed at lower levels under one or both of the nonalkalinizing conditions.

These data suggested additional candidate effectors of the pH changes we observe. We assayed alkalinization on both medium 199 and GM–BCP medium of mutant strains lacking ICL1, FOX2, CTN1, INO1, MET15, MEP1, MEP2, and DUR1,2 either generated in our laboratory or generously provided by others (28, 36–40). We also constructed strains lacking ARG1, ARG3, JEN1, or JEN2 (see Materials and Methods). Only a strain with a mutation in DUR1,2, which degrades urea to CO₂ and ammonia, showed a defect in alkalinization, with a slower pH change than its wild-type strain AT72 (see Fig. S4 in the supplemental material). It is notable that while most of the genes tested are involved in either transport or anabolic functions, DUR1,2 and FOX2 are the only genes in this set that are catabolic and dur1,2Δ has an alkalinization phenotype under these conditions.

In Colletotrichum coccodes, a pathogen of tomatoes, three genes have been associated with alkalinization (23). One of these, ArcA, encodes a transcription factor that mediates nitrogen catabolite repression. Its homolog in C. albicans, GAT1, was upregulated 4.2-fold relative to the level in one nonalkalinizing culture, but a gat1Δ mutant (41) had no effect on alkalinization in C. albicans (data not shown). The other C. coccodes genes, NIT1 and NIT3, encode nitrate and nitrite, respectively, reductases, and have no C. albicans homologs. It is interesting to note that extracellular pH changes in C. coccodes appear tightly linked to nitrogen metabolism, while our data indicate that it is associated with carbon metabolism in C. albicans.

Role of Ato proteins in environmental alkalinization by C. albicans. Four of the genes highly induced during alkalinization relative to the levels for both control conditions encode homologs of a family of fungus-specific transmembrane proteins that have been associated with the limited extracellular pH changes observed in S. cerevisiae. It was proposed that they actively extrude ammonia and hence were named Ato, for "ammonia transport outward" (29). The precise function of these proteins is not clear, but in addition to pH changes, they have been associated with acetate utilization in S. cerevisiae, A. nidulans, and Yarrowia lipolytica (29, 42–45) and sporulation in S. cerevisiae (46) and have a variety of aliases. Most fungal species have two or three predicted homologs (A. J. Carman and M. C. Lorenz, unpublished observations) and we were surprised to identify 10 ATO homologs in C. albicans (see Table S1 in the supplemental material). The regulation of ATO1 was particularly striking; it is expressed 79- to 177-fold higher in alkalinizing cells than in the aht1 or glucose-repressed cultures, respectively (Table S1). Quantitative reverse transcription-PCR (qRT-PCR) on independent RNA samples indicated that this was actually an underestimate of the magnitude of regulation (data not shown).

S. cerevisiae mutants lacking any one of the ATO homologs show modest reductions in alkalinization (29). We attempted to test the role of the larger C. albicans ATO family in a similar manner, by constructing several ATO mutants using the UAU-mediated gene disruption method (47) using constructs created as
Alternative carbon utilization

Transporters/channels

Methionine/cysteine/sulfate metabolism

TABLE 1 Genes differentially regulated during alkalinizationa

| Annotation and gene | Expression (fold) | Function of encoded protein |
|---------------------|-------------------|----------------------------|
| **Arginine metabolism** |                  |                           |
| CAN2                | 111               | 8.9                       | 66.6 | Basic amino acid permease |
| CAR1                | 3,934             | 8.1                       | 3.3  | Arginase (Arg → urea-ornithine) |
| CAR2                | 5,641             | 10.3                      | 3.1  | Ornithine transaminase (Orn → Glu) |
| CAN1                | 97                | 4.2                       | 3.0  | Basic amino acid permease |
| ARG1                | 7,469             | 0.18                      | 0.31 | Argininosuccinate synthetase |
| ARG2                | 6,689             | 0.63                      | 0.31 | Argininosuccinate lyase |
| ARG3                | 5,610             | 0.11                      | 0.14 | Ornithine carbamoyltransferase |
| DUR1,2              | 780               | 1.3                       | 0.12 | Urea amidolase |
| DUR3                | 781               | 0.97                      | 0.03 | Putative urea transporter |
| **Methionine/cysteine/sulfate metabolism** |                  |                           |
| MET2                | 2,618             | 0.15                      | 0.29 | Homoserine acetyltransferase |
| MET10               | 4,076             | 0.59                      | 0.28 | Sulfite reductase |
| MET4                | 5,312             | 0.28                      | 0.28 | Transcription factor for MET/CYS |
| MET15               | 5,645             | 1.1                       | 0.19 | Acetylhomoserine aminocarboxypropyl transferase |
| MET14               | 946               | 0.33                      | 0.18 | Adenyl-sulfate kinase |
| MET1                | 5,842             | 0.26                      | 0.12 | Uroporphyrin methyltransferase |
| MET3                | 5,025             | 0.25                      | 0.10 | ATP sulfurylase |
| **Transports/channels** |                  |                           |
| JEN1                | 7,447             | 15.7                      | 99.9 | Lactate transporter |
| HGT13               | 7,093             | 3.4                       | 12.2 | Glucose transporter |
| HIP1                | 3,195             | 2.1                       | 10.3 | Similar to amino acid permeases |
| CDR3                | 1,313             | 17.7                      | 9.4  | ABC transporter of phospholipids |
| HGT2                | 3,668             | 3.3                       | 5.6  | Glucose transporter |
| DIP5                | 2,942             | 0.94                      | 4.0  | Dicarboxylic acid permease |
| GIT2                | 1,978             | 5.6                       | 4.0  | Putative glycerophosphoinositol permease |
| HGT16               | 6,141             | 2.7                       | 3.2  | Glucose transporter |
| AQR1                | 2,849             | 22.7                      | 3.1  | Aquaporin water channel |
|                  | 3,120             | 0.38                      | 0.27 | Putative PDR family transporter |
| HAK1                | 6,249             | 0.35                      | 0.19 | Putative potassium transporter |
| MEP1                | 1,614             | 1.8                       | 0.12 | Ammonium permease |
| MEP2                | 5,672             | 0.49                      | 0.05 | Ammonium permease |
| **Alternative carbon utilization** |                  |                           |
| ACS1                | 1,743             | 49.7                      | 19.1 | Acetyl-CoA synthetase |
| FDH1                | 638               | 0.82                      | 11.7 | Formate dehydrogenase |
| PKP2                | 1,655             | 15.1                      | 9.5  | Acyl-CoA oxidase (β-oxidation) |
| FAA2                | 7,379             | 4.6                       | 6.8  | Fatty acyl-CoA ligase (β-oxidation) |
| ICL1                | 6,844             | 0.88                      | 5.3  | Isocitrate lyase (glyoxylate cycle) |
| MLS1                | 4,833             | 0.90                      | 4.4  | Malate synthase (glyoxylate cycle) |
| FOX2                | 1,288             | 0.69                      | 3.3  | 3-Hydroxyacyl-CoA epimerase (β-oxidation) |

a Ratios given are the expression levels in alkalinizing cultures relative to the levels for the nonalkalinizing ach1Δ mutant (ach1Δ column) or the nonalkalinizing high-glucose condition (WT-Glu column). Shown are >3-fold changes up (bold) or down (italic).

part of a large-scale project for three of these genes. Surprisingly, given the potential for redundancy, mutation of ATO5 (orf19.6997) significantly retarded alkalinization in all three of our assays (Fig. 5; see also Fig. S5 in the supplemental material), while insertional mutations in ATO1 and ATO8 had no effect. The ato5Δ mutant strain grows at the same rate as the wild-type control DAY286 (data not shown). In a parallel approach, we constructed an ATO1 allele carrying a mutation in a highly conserved motif (FGGTLN) in the amino-terminal part of the protein that has previously been identified as critical for some Ato functions and was predicted to be dominant negative (42). This allele, ATO1* (Gly53Asp, a mutation of the second glycine in the FGGTLN motif), was expressed under the control of the strong, constitutive ACT1 promoter in a wild-type strain, where it mildly retarded alkalinization in medium 199 (Fig. S5). Expression of ATO8, which is naturally nonconsensus in this region (Table S1), also slightly reduced alkalinization. Further, we find a correlation between the number of ATO homologs and the degree of alkalinization in the Candida genus: the six species that alkalinize effectively (Fig. S1) have 4 to 10 ATO homologs each (median = 6.5), while the three that do not have 2 to 4 (median = 3). Our results are consistent with a role for the Ato family in ammonia extrusion, but the phenotypes that we have observed are moderate, probably due to redundancy in this large gene family.

Alkalination is associated with extrusion of ammonia. Localized alkalination in several fungal species has been associated with the release of volatile ammonia, a strong base, from the cells. To track the release of ammonia during this pH change, we grew strains on GM-BCP plates under alkalinizing and nonalkalinizing conditions (with or without 2% glucose) and placed an “acid trap” containing 10% citric acid below the cells in the beginning of this process (see Materials and Methods). Over 24 to 72 h, the pH rose (Fig. 6A), and we analyzed the acid trap for the presence of ammonia, released as volatile ammonia from the cells, via a color-
shown). A second, independent measured at the indicated time points. Culture density was also measured (not pH 4. These cultures were incubated at 30°C with aeration, and the pH was grown overnight in YPD and then diluted to an OD600 of 0.1 in medium 199 at to of acidic media, which occurs rapidly, with the pH changing from partially at pH 4 to 10. We have focused mostly on the alkalinization environment to a remarkable degree, neutralizing media ini-

...hyphal form; this is blocked by buffering the medium or addition hyphal morphogenesis, and cells in alkalinizing media shift to the 49], and excretion of the excess ammonia would both detoxify the cytosol and raise the extracellular pH. The Ato family of proteins has been proposed to be ammonia exporters—the name comes from “ammonia transport outward” (29)—and we find that deletion of one of these genes, ATO5, slows alkalinization. The Ato family is signifi-

Figure 7 presents a model for the metabolic functions that contribute to alkalinization, based on the utilization of amino acids as the primary source of carbon. Amino acid uptake via transmembrane permeases is required, and while there is significant redundancy in permease specificity, a deletion of the Stp2p transcription factor reduces expression of multiple permeases (34), and this ablates alkalinization. The close correlation of pH changes and growth indicates that catabolism of these amino acids is required. Amino acids are catabolized through several different routes, resulting in acetyl-CoA, succinyl-CoA, α-ketoglutarate, or oxaloacetate, each of which involves deamination steps. Under carbon-rich conditions nitrogen is generally stored in the form of glutamate and glutamine, but the capacity for doing so when amino acids are needed as a carbon source is presumably limited. High cytosolic ammonia concentrations are toxic (49), and excretion of the excess ammonia would both detoxify the cytosol and raise the extracellular pH. The Ato family of proteins has been proposed to be ammonia exporters—the name comes from “ammonia transport outward” (29)—and we find that deletion of one of these genes, ATO5, slows alkalinization. The Ato family is signifi-

...FIG 5 Mutation of ATO5 retards alkalinization. The ato5Δ strain (HDC1), its congenic His+ control (P 17), and the prototrophic wild-type (SC5314) were grown overnight in YPD and then diluted to an OD600 of 0.1 in medium 199 at pH 4. These cultures were incubated at 30°C with aeration, and the pH was measured at the indicated time points. Culture density was also measured (not shown). A second, independent ato5Δ strain behaved identically (not shown).

imetric assay based on Nessler’s reagent, as previously described (48). Detectible ammonia increased significantly (Fig. 6B) over the 72-h period, correlating with the pH change observed on the plates (Fig. 6A). Very little volatile ammonia was released by non-

...alkalinizing cultures with the use of either the stp2Δ mutant or a glucose-repressed wild-type strain. We attempted to measure amon-

...ria release from liquid cultures, aerated or not, and were un-

...successful using several approaches, possibly as a result of the vol-

...atile nature of this compound. On solid media, however, extrusion of ammonia correlates with the observed pH changes, providing a probable explanation for extracellular alkalinization, consistent with reports of ammonia release from Colletotrichum during its alkalinization in planta (23).

DISCUSSION

We show here that C. albicans is able to actively modulate the environmental pH to a remarkable degree, neutralizing media ini-

...ially at pH 4 to 10. We have focused mostly on the alkalinization of acidic media, which occurs rapidly, with the pH changing from 4 to >7 in less than 24 h in aerated liquid cultures. This glucose-repressible phenotype occurs on both solid and liquid media of several compositions but requires the presence of exogenous amino acids. Neutral pH has been long recognized as an inducer of hyphal morphogenesis, and cells in alkalinizing media shift to the hyphal form; this is blocked by buffering the medium or addition of glucose. Thus, C. albicans effectively autoinduces morphogenesis under these conditions. The rise in pH is associated with the release of ammonia, a highly basic compound, as has been ob-

...erved in other fungi.

Genetic and genomic approaches have begun to identify the mechanism behind this phenomenon, and these have confirmed the importance of amino acid import and metabolism: the amino acid permease regulators Stp2p and Csh3p have strong phenotypes, while the catabolic enzymes ACh1p and Dur1,2p have more mild effects. This is consistent with the microarray analysis of alkalinizing cells in which induction of amino acid uptake and catabolism and repression of amino acid biosynthesis were gen-

...eral trends.

Figure 7 presents a model for the metabolic functions that contribute to alkalinization, based on the utilization of amino acids as the primary source of carbon. Amino acid uptake via transmembrane permeases is required, and while there is significant redundancy in permease specificity, a deletion of the Stp2p transcription factor reduces expression of multiple permeases (34), and this ablates alkalinization. The close correlation of pH changes and growth indicates that catabolism of these amino acids is required. Amino acids are catabolized through several different routes, resulting in acetyl-CoA, succinyl-CoA, α-ketoglutarate, or oxaloacetate, each of which involves deamination steps. Under carbon-rich conditions nitrogen is generally stored in the form of glutamate and glutamine, but the capacity for doing so when amino acids are needed as a carbon source is presumably limited. High cytosolic ammonia concentrations are toxic (49), and excretion of the excess ammonia would both detoxify the cytosol and raise the extracellular pH. The Ato family of proteins has been proposed to be ammonia exporters—the name comes from “ammonia transport outward” (29)—and we find that deletion of one of these genes, ATO5, slows alkalinization. The Ato family is signifi-

...cantly enlarged in Colletotrichum species relative to species that alkalinize poorly. We note that the most pathogenic CUG Candida species (C. albicans, C. tropicalis, and C. parapsilosis) have large Ato families, reminiscent of the expansion of gene families for a number of virulence-related traits, particularly those on the cell surface or secreted, such as secreted aspartyl proteases, lipases, and phospholipases, and GPI-anchored cell wall proteins such as the ALS agglutinins (27).

The model in Fig. 7 is based on simple metabolic activities and should occur in any fungal cell capable of using amino acids to satisfy cellular carbon requirements (which is true for at least most fungi) and, indeed, has been observed in several other species. Ammonia has been reported to occur in alkalinized halos sur-

...rounding colonies of Metarhizium anisopliae, A. fumigatus, N. crassa, Coccidioides immitis, and S. cerevisiae (19, 29, 50, 51), and alkalinization by several dermatophyte species also occurs (20). One previous report has also shown ammonia-dependent pH changes in C. albicans in media containing keratin; as with the other species mentioned, this process occurred slowly over the course of ~2 weeks (24). Under these defined conditions of glucose limitation and amino acid excess, C. albicans alters the pH much more rapidly than previously seen. Of the other fungal species examined, only Colletotrichum species raise pH to a similar degree in the tissues of decaying fruit, though the process is slower than in Candida (21, 22). The metabolic basis for the pH change in Colletotrichum appears linked to nitrogen metabolism, as opposed to amino acid catabolism in C. albicans.

The rise in pH resulting from Colletotrichum in planta induces expression of the key virulence factor pectin lyase (22). Similarly, we show that the pH change caused by C. albicans induces hyphal morphogenesis, a key virulence trait in this species (2, 3). Indeed, the conditions that promote alkalinization are at least superficially similar to those encountered within phagocytes, as determined by transcriptional profiling (52–54), and it has been suggested that C. albicans might interfere with normal phagosomal trafficking such that it is not always in an acidified phagolysosome (55, 56). If C. albicans were to neutralize the phagolysosome, it would
promote fungal survival both by inhibiting acid hydrolases and by enabling escape via hyphal growth. It is intriguing to speculate that the activity we have identified here contributes to fungal pathogenesis in this manner, though a conclusive proof of this idea poses several technical and biological challenges.

*C. albicans* has a uniquely intimate association with the mammalian host amongst fungi. Virulence in these species appears to be the result of a gradual adaptation of common aspects of fungal physiology to the host environment rather than the acquisition of specific virulence factors such as toxins or secondary metabolites. Amongst these adaptations are a robust response to changes in extracellular pH, rapid metabolic shifts after changes in nutritional status, and, of course, avid hyphal morphogenesis; all of these are required for virulence in *C. albicans* (2, 3, 11, 57–59). Our findings link these phenomena, as the extracellular pH changes require a carbon-limited environment and result in hyphal growth. In conclusion, we suggest that the ability to control extracellular pH by *Candida* species may be an evolutionary adaptation that contributes to fitness within the host.

### MATERIALS AND METHODS

**Strains and media.** The *C. albicans* strains used in this study are presented in Table S2 in the supplemental material. Strains were routinely maintained on YPD (1% yeast extract, 2% peptone, 2% glucose) or YNB (1.7% yeast nitrogen base without amino acids, 0.5% ammonia sulfate, 2% glucose) medium, as previously described (60). Neutralization assays with liquid media were performed either with medium 199 (Invitrogen), prepared according to the manufacturer’s instructions, without buffering, or with YNB prepared without glucose. Amino acids or other carbon sources were added to this medium as indicated in the text and figures. For neutralization on solid media, strains were grown on GM-BCP (1% yeast extract, 30 mM CaCl2, 3% glycerol, 0.01% bromocresol purple, 2% agar) (29). The media were adjusted to the desired pH using HCl or NaOH.

*C. albicans* mutant libraries were obtained from A. Mitchell (32, 33) and D. Sanglard (unpublished) and contain mutations in approximately 500 unique genes. Strain and plasmid construction. Strains lacking *ATO1*, *ATO5*, *ATO8* (see Table S1 in the supplemental material), *JEN1*, or *JEN2* were generated via the UAU disruption method (47) using transposon insertion constructs created by Q. Zhao and W. Nierman (Institute for Genomic Research) and provided by F. Smith and A. Mitchell (Columbia University) as part of a large-scale project. Linearized constructs were used to transform strain BWP17 by electroporation (61), selecting for arginine prototrophy. Candidates were passaged twice in YPD at 30°C and then plated to SD-Arg-Ura medium to select for mutants generated by gene

FIG 6 Ammonia extrusion by alkalinizing *C. albicans* colonies. (A) The wild-type strain, the *stp2Δ* mutant, and its complement were prepared as described in the legend to Fig. 4 and spotted to GM-BCP, pH 4.0 (plus 2% glucose where indicated). Plates were photographed at the indicated time points. At 4 days, a stereomicroscope was used to photograph the colony borders at ×20 magnification. Hyphal growth is indicated in the alkalinizing samples by the brush border at the colony edge. (B) Ammonia release from the same strains was measured as described in Materials and Methods at the indicated time points. A strong correlation between medium color (indicating alkalinization) and ammonia concentration was observed. The measurements in panel B are not from the same plates shown in panel A, as the arrangement of the acid trap to collect ammonia does not facilitate manipulation of the plates, but the time course in panel A is representative.
conversion and recombination. Loss of the wild-type allele was confirmed by PCR.

Mutations in ARG1 and ARG3 were generated using the SAT-flipper method, as previously described (61). Briefly, 5′ and 3′ untranslated regions of the two genes were cloned to flank the FRT-SAT1-FLP-FRT cassette. These were used to transform strain SC5314, with selection on YPD plus 100 μg/ml nourseothricin (Werner Bioagents, Jena, Germany). Integration was confirmed by PCR, and the SAT-FLP was excised as previously described (61) to generate nourseothricin-sensitive colonies. The second allele was disrupted in the same manner.

Constitutive expression of ATO1 alleles was accomplished by cloning the ORF (or a point mutant generated by overlap PCR) under the control of the ACT1 promoter and integrating it at the RPS10 locus. Briefly, ~1,000 bp of the ACT1 promoter from pAUA34 (62) was subcloned between the KpnI and XhoI sites in Clp10 (63) to generate pHZ116. Next, the ATO1 ORF was PCR amplified and cloned as the XhoI-HindIII fragment into pHZ116 to generate ACT1p-ATO1 (pML342) plasmids. The ATO1* mutant was generated by site-directed overlap PCR using complementary oligonucleotides with a single mismatch to change Gly-53 to Asp, analogous to the Y. lipolytica GPR1-1 mutant identified by Barth and colleagues (42), and cloned into pHZ116 to generate pML341. Both plasmids, plus pHZ116, were digested with Stul and used to transform CAI4-F2 to uridine prototrophy. Accurate integration at the RPS10 locus was verified by PCR.

Neutralization assays. Three forms of neutralization assays were used. For assays with aerated (shaking) cultures, strains were grown overnight in YPD, collected by centrifugation, washed in water, and transferred at a final optical density at 600 nm (OD600) of 0.1 in medium 199, pH adjusted as indicated. Cells were incubated at 37°C with aeration and growth, and pH measurements were taken at the indicated time points by drawing 5-ml samples. Glucose or amino acids were added in some experiments as described in the text and figure legends. Assays were also performed with 96-well plates (nonaerated), in which cells were grown in YPD overnight, collected by centrifugation, washed in water, and transferred to medium 199, pH 4, at a final OD600 of 1.0 and then serially diluted 1:5.

Assays with solid media were performed using 12-well plates to isolate strains from potential intercolony communication as has been described for S. cerevisiae (18), in which each well contained ~2 ml of GM-BCP or SC-BCP, supplemented or not supplemented with glucose, as indicated. Strains were grown overnight in YPD, collected by centrifugation, washed in water, and diluted to an OD600 of 1.0 in distilled water (dH2O), and 7 μl of this dilution was spotted into the wells, one strain/well. Cells were incubated at 30°C, and alkalinization observed as a purple halo around the colonies was documented after 48 to 72 h unless otherwise indicated.

The effects of individual amino acids on alkalinization were tested using YNB medium, pH 4.0, supplemented with individual amino acids at 1 mM. C. albicans strain SC5314 was grown as previously described and added to the medium to give a final OD600 of 0.2. Cells were incubated for 24 h, at which time point the pH and OD600 of the medium were recorded.

Assessment of hyphal induction in alkalinizing cultures. Wild type SC5314 cells were grown into YPD medium overnight, washed, and diluted to give a final OD600 of 0.2 in YNB medium supplemented with 2% Casamino Acids as the sole carbon source, and the pH was adjusted to 4.0. For the control conditions, this medium was either supplemented with 2% glucose or buffered with 0.1 M Tris-0.1 M MOPS, pH 4.0. Cells were incubated at 37°C, and samples were drawn at 0 h, 3 h, 5 h, 7 h, or 24 h. Cellular morphology was assessed by counting cells in photomicrographs; at least 150 cells were counted per time point. The pH of the medium was taken using a standard pH meter.

Screen for genes involved in neutralization in C. albicans. Mutant libraries of C. albicans generously provided by A. Mitchell and D. Sanglard were screened to identify nonalkalinizing mutants. The libraries, in 96-well-plate format, were grown in YPD at 30°C for 2 days, and then 5 μl from each well was transferred to unbuffered medium 199, pH 4.0, in 96-well plates. Strains were incubated at 37°C, and neutralization was detected colorimetrically after 24 h. Candidate nonalkalinizing mutants were retested individually in triplicate for neutralization on medium 199 and GM-BCP medium as described above.

Microarray analysis of C. albicans during neutralization. To determine the transcript profiles of C. albicans cells as cultures alkalinize, strains SC5314 and ACC229 (ach1Δ/ach1Δ) were grown overnight in YPD, collected by centrifugation, washed with water, and diluted to an OD600 of 1.0 in 50 ml unbuffered medium 199 with or without 2% glucose at pH 4.0. Cells were incubated at 37°C to the point where SC5314 cultures had reached pH 5.0. The pH of the ach1Δ cultures remained ~4.0. Cells were collected and RNA was extracted using the hot acidic phenol method as described previously (64). Duplicate cultures were prepared for all conditions.

RNA samples were converted to labeled cDNA via oligo(dT) priming using an aminoalyl protocol (53) and hybridized to C. albicans complete genome microarrays generated from a 70-nucleotide (nt) oligonucleotide set (Qiagen) and printed on glass slides (Microarrays, Inc., Huntsville, AL). Experimental samples were hybridized against a reference RNA pool composed of a mixture of all the samples. We used technical replicates as dye-swap pairs for all RNA samples. Arrays were scanned with an Axon Instruments 4000B scanner, and data were extracted using the Axon Instruments GenePix program. Data processing and analysis basically followed our standard protocols (53, 65). The data from each array were normalized such that the median spot intensity from both the Cy3 and the Cy5 channels were equalized, the data were floored to a minimum value of 5% of the slide median, and then the ratio of the level for the experimental condition to that for the reference RNA pool was calculated. Genes with a P value of >0.01 as determined by Student’s t test were discarded.

Three analyses were conducted, each involving a neutralizing condition compared to a nonneutralizing one: WT versus ach1Δ, WT versus WT-glucose, and ach1Δ versus WT-glucose. Analyses included rank ordering genes by fold regulation, identification of overrepresented gene ontology (GO) annotations using the term finder tool at the Candida Genome Database (http://www.candidagenome.org), and hierarchical
clustering using the Cluster and TreeView algorithms (66). The complete data set can be downloaded at http://www.lorenzlab.org.

Colorimetric analysis of volatile NH₃ during alkalization. Ammonia released by C. albicans cells during alkalization was collected using acid traps as previously described (48). Briefly, cells were grown in YPD medium overnight, washed in water, and resuspended to an OD₆₀₀ of 1.0. Cells were spotted onto GM-BCP plates or GM-BCP plates containing 2% glucose, and 100 µl of 10% citric acid was placed underneath the colonies. After cells were incubated for 24 to 72 h at 37°C to allow alkalization to occur, the acid trap was removed and the NH₃ concentration evaluated colorimetrically. For NH₃ quantification, 20 µl of the samples was diluted with H₂O to 200 µl and combined with 800 µl Nessler’s reagent. Samples were incubated at room temperature for 30 min, and the OD₆₅₅ was measured. NH₃ quantity in the samples was calculated based on an ammonia standard.

ACKNOWLEDGMENTS
We are grateful to P. Ljungdahl, J. Morschhauser, K. Nickerson, T. Reynolds, W. Fonzi, and R. Contreras for providing strains and plasmids and D. Garsin and M. Gustin for comments on the manuscript. We particularly thank A. Mitchell and D. Sanglard for providing the mutant libraries used in this study.

This work was supported by NIH awards R01 AI075091 and R21 AI071134 to M.C.L.

SUPPLEMENTAL MATERIAL
Supplemental material for this article may be found at http://mbio.asm.org/lookup/suppl/doi:10.1128/mBio.00055-11/-/DCSupplemental.

Table S1, PDF file, 0.060 MB.

REFERENCES
1. Biswas S, Van Dijck P, Datta A. 2007. Environmental sensing and signal transduction pathways regulating morphophenotypic determinants of Candida albicans. Microbiol. Mol. Biol. Rev. 71:348–376.
2. Lo HJ, et al. 1997. Nonfilamentous C. albicans mutants are avirulent. Cell 90:939–949.
3. Saville SP, Lazzell AL, Monteagudo C, Lopez-Ribot JL. 2003. Engineered control of cell morphology in vivo reveals distinct roles for yeast and filamentous forms of Candida albicans during infection. Eukaryot. Cell 2:1053–1060.
4. Merrell DS, Camilli A. 2002. Acid tolerance of gastrointestinal pathogens. Curr. Opin. Microbiol. 5:51–55.
5. Cotter PD, Emerson N, Gahan CG, Hill C. 1999. Identification and disruption of lisRK, a genetic locus encoding a two-component signal transduction system involved in stress tolerance and virulence in Listeria monocytogenes. J. Bacteriol. 181:6840–6843.
6. Merrell DS, Hava DL, Camilli A. 2002. Identification of novel factors involved in colonization and acid tolerance of Vibrio cholerae. Microbiol. 43:1471–1491.
7. Wilmes-Riesenberg MR, Foster JW, Curtiss R III. 1997. An altered rpoS allele contributes to the avirulence of Salmonella typhimurium LT2. Infect. Immun. 65:203–210.
8. Davis D, Wilson RB, Mitchell AP. 2000. RIM101-dependent and -independent pathways govern pH responses in Candida albicans. Mol. Cell. Biol. 20:971–978.
9. Su SS, Mitchell AP. 1993. Molecular characterization of the yeast mitotic regulatory gene RIM1. Nucleic Acids Res. 21:3789–3797.
10. Tilburn J, et al. 1995. The Aspergillus PacC zinc finger transcription factor mediates regulation of both acid- and alkaline-expressed genes by ambient pH. EMBO J. 14:779–790.
11. Davis D, Edwards JE Jr, Mitchell AP, Ibrahim AS. 2000. Candida albicans RIM101 pH response pathway is required for host-pathogen interactions. Infect. Immun. 68:5953–5959.
12. De Bernardis F, Mühlcheldeg FA, Cassone A, Fonzi WA. 1998. The pH of the host niche controls gene expression in and virulence of Candida albicans. Infect. Immun. 66:3317–3325.
13. Fonzi WA. 1999. PHR1 and PHR2 of Candida albicans encode putative glycosidases required for proper cross-linking of beta-1,3- and beta-1,6-glucans. J. Bacteriol. 181:7070–7079.
14. Sparatore-Irwin SM, Birse CE, Sypherd PS, Fonzi WA. 1999. PHR1, a pH-regulated gene of Candida albicans, is required for morphogenesis. Mol. Cell. Biol. 19:601–613.
15. Mühlcheldeg FA, Fonzi WA. 1997. PHR2 of Candida albicans encodes a functional homolog of the pH-regulated gene PHR1 with an inverted pattern of pH-dependent expression. Mol. Cell. Biol. 17:5960–5967.
16. Eaton KA, Brooks CJ, Morgan DR, Krakowka S. 1991. Essential role of urease in pathogenesis of gastritis induced by Helicobacter pylori in gnotobiotic piglets. Infect. Immun. 59:2470–2475.
17. Tsuda M, Karita M, Morshed MG, Okita K, Nakazawa T. 1994. A urease-negative mutant of Helicobacter pylori constructed by allelic exchange mutagenesis lacks the ability to colonize the nude mouse stomach. Infect. Immun. 62:3586–3589.
18. Palková Z, et al. 1997. Ammonia mediates communication between yeast colonies. Nature 390:532–536.
19. St. Leger RJ, Nelson JO, Scorn SE. 1999. The entomopathogenic fungus Metarhizium anisopliae alters ambient pH, allowing extracellular protease production and activation. Microbiology 145(Pt 6):2691–2699.
20. Taplin D, Zaia N, Rebbl G, Blank H. 1969. Isolation and recognition of dermatophytes on a new medium (DTM). Arch. Dermatol. 99:203–209.
21. Prusky D, McEvoy JL, Leverentz B, Conway WS. 2001. Local modulation of host pH by Colletotrichum species as a mechanism to increase virulence. Mol. Plant Microbe Interact. 14:1105–1113.
22. Yakoby N, Kobiler I, Dinoor A, Prusky D. 2000. pH regulation of pectate lyase secretion modulates the infection of Colletotrichum gloeosporioides on avocado fruits. Appl. Environ. Microbiol. 66:1026–1030.
23. Alkan N, Fluhr R, Sherman A, Prusky D. 2008. Role of ammonia secretion and pH modulation on pathogenicity of Colletotrichum coccodes on tomato fruit. Mol. Plant Microbe Interact. 21:1058–1066.
24. Tsuboi R, Matsuda K, Koj IJ, Ogawa H. 1989. Correlation between culture medium pH, extracellular proteinase activity, and cell growth of Candida albicans in insoluble stratum corneum-supplemented media. Arch. Dermatol. Res. 281:342–345.
25. Davis D. 2003. Adaptation to environmental pH in Candida albicans and its relation to pathogenesis. Curr. Genet. 44:1–7.
26. Davis DA. 2009. How human pathogenic fungi sense and adapt to pH: the link to virulence. Curr. Opin. Microbiol. 12:365–370.
27. Butler G, et al. 2009. Evolution of pathogenicity and sexual reproduction in eight Candida genomes. Nature 460:567–572.
28. Chen YL, Kaufmann S, Reynolds TB. 2008. Candida albicans uses multiple mechanisms to acquire the essential metabolite inositol during infection. Infect. Immun. 76:2793–2801.
29. Palková Z, et al. 2002. Ammonia pulses and metabolic oscillations guide yeast colony development. Mol. Biol. Cell 13:3901–3914.
30. Kadosh D, Johnson AD. 2005. Induction of the Candida albicans filamentous growth program by relief of transcriptional repression: a genome-wide analysis. Mol. Biol. Cell. 16:2903–2912.
31. Ramsdale M, et al. 2008. MNL1 regulates weak acid-induced stress responses of the fungal pathogen Candida albicans. Mol. Biol. Cell 19:4393–4403.
32. Davis DA, Bruno VM, Loza L, Filler SG, Mitchell AP. 2002. Candida albicans Mds3p, a conserved regulator of pH responses and virulence identified through insertional mutagenesis. Genetics 162:1573–1581.
33. Nobile CJ, et al. 2006. Critical role of Bcr1-dependent adhesins in C. albicans biofilm formation in vitro and in vivo. PLoS Pathog. 2:e63.
34. Martinez P, Ljungdahl PO. 2005. Divergence of Stp1 and Stp2 transcription factors in Candida albicans places virulence factors required for proper nutrient acquisition under amino acid control. Mol. Cell. Biol. 25:9435–9446.
35. Martinez P, Ljungdahl PO. 2004. An ER packaging chaperone determines the amino acid uptake capacity and virulence of Candida albicans. Mol. Microbiol. 56:649–669.
37. Ghosh S, et al. 2009. Arginine-induced germ tube formation in Candida albicans is essential for escape from murine macrophage line RAW 264.7. Infect. Immun. 77:1596–1605.
38. Ramirez MA, Lorenz MC. 2007. Mutations in alternative carbon utilization pathways in Candida albicans attenuate virulence and confer pleiotropic phenotypes. Eukaryot. Cell 6:280–290.
39. Viaene J, et al. 2000. MET15 as a visual selection marker for Candida albicans. Yeast 16:1205–1215.
40. Zhou H, Lorenz MC. 2008. Carnitine acetyltransferase are required for growth on non-fermentable carbon sources but not for pathogenesis in Candida albicans. Microbiology (Reading, Engl.) 154:500–509.
41. Limjindaporn T, Khalaf RA, Fonzi WA. 2003. Nitrogen metabolism and virulence of Candida albicans require the GATA-type transcriptional activator encoded by GAT1. Mol. Microbiol. 50:993–1000.
42. Augstein A, Barth K, Gentsch M, Kohlwein SD, Barth G. 2003. Characterization, localization and functional analysis of Gpr1p, a protein affecting sensitivity to acetic acid in the yeast Yarrowia lipolytica. Microbiology (Reading, Engl.) 149:589–600.
43. Paiva S, Devaux F, Barbosa S, Jacq C, Casal M. 2004. Ady2p is essential for the acetate permease activity in the hyphal fungus Pichia pastoris. Yeast 21:201–210.
44. Robellet X, Flippin M, Pégot S, Maccabe AP, Velot C. 2008. AcpA, a member of the GPR1/FUN34/YaaH membrane protein family, is essential for acetate permease activity in the hyphal fungus Aspergillus nidulans. Biochem. J. 412:485–493.
45. Tschoppe K, Augstein A, Bauer R, Kohlwein SD, Barth G. 1999. Trans-dominant mutations in the GPR1 gene cause high sensitivity to acetic acid and ethanol in the yeast Yarrowia lipolytica. Yeast 15:1645–1656.
46. Rabitsch KP, et al. 2001. A screen for genes required for meiosis and spore formation based on whole-genome expression. Curr. Biol. 11:1001–1009.
47. Enloe B, Diamond A, Mitchell AP. 2000. A single-transformation gene function test in diploid Candida albicans. J. Bacteriol. 182:5730–5736.
48. Gori K, Mortensen HD, Arneborg N, Jespersen L. 2007. Ammonia production and its possible role as a mediator of communication for Debaryomyces hansenii and other cheese-relevant yeast species. J. Dairy Sci. 90:5032–5041.
49. Hess DC, Lu W, Rabinowitz JD, Botstein D. 2006. Ammonium toxicity and potassium limitation in yeast. PLoS Biol. 4:e351.
50. Mansour S, Beckerich JM, Bonnarme P. 2008. Lactate and amino acid catabolism in the cheese-ripening yeast Yarrowia lipolytica. Appl. Environ. Microbiol. 74:6505–6512.
51. Mirbod-Donovan F, et al. 2006. Urease produced by Coccidioides posadasii contributes to the virulence of this respiratory pathogen. Infect. Immun. 74:504–515.
52. Fradin C, et al. 2005. Granulocytes govern the transcriptional response, morphology and proliferation of Candida albicans in human blood. Mol. Microbiol. 56:397–415.
53. Lorenz MC, Bender JA, Fink GR. 2004. Transcriptional response of Candida albicans upon internalization by macrophages. Eukaryot. Cell 3:1076–1087.
54. Rubin-Bejerano I, Fraser I, Grisafi P, Fink GR. 2003. Phagocytosis by neutrophils induces an amino acid deprivation response in Saccharomyces cerevisiae and Candida albicans. Proc. Natl. Acad. Sci. U. S. A. 100:11007–11012.
55. Fernández-Arenas E, et al. 2009. Candida albicans actively modulates intracellular membrane trafficking in mouse macrophage phagosomes. Cell. Microbiol. 11:560–589.
56. Mor N, Goren MB. 1987. Discrepancy in assessment of phagosome-lysosome fusion with two lysosomal markers in murine macrophages infected with Candida albicans. Infect. Immun. 55:1663–1667.
57. Barelle CJ, et al. 2006. Niche-specific regulation of central metabolic pathways in a fungal pathogen. Cell. Microbiol. 8:961–971.
58. Lorenz MC, Fink GR. 2001. The glyoxylate cycle is required for fungal virulence. Nature 412:83–86.
59. Piekarska K, et al. 2006. Peroxisomal fatty acid beta-oxidation is not essential for virulence of Candida albicans. Eukaryot. Cell 5:1847–1856.
60. Sherman F. 1991. Getting started with yeast, p. 3–21. In Guthrie C, Fink GR (ed). Methods in enzymology, vol. 194. Academic Press, San Diego, CA.
61. Reuss O, Vik A, Kolter R, Morschhäuser J. 2004. The SAT1 flipper, an optimized tool for gene disruption in Candida albicans. Gene 341:119–127.
62. Uhl MA, Johnson AD. 2001. Development of Streptococcus thermophilus lacZ as a reporter gene for Candida albicans. Microbiology (Reading, Engl.) 147:1189–1195.
63. Murad AM, Lee PR, Broadbent ID, Barelle CJ, Brown AJ. 2000. CLP10, an efficient and convenient integrating vector for Candida albicans. Yeast 16:325–327.
64. Ausubel FM, et al. 2000. Current protocols in molecular biology. John Wiley & Sons, Edison, NJ.
65. Agarwal AK, et al. 2008. Role of heme in the antifungal activity of the azaoxoaporphine alkaloid sampangine. Eukaryot. Cell 7:387–400.
66. Eisen MB, Spellman PT, Brown PO, Botstein D. 1998. Cluster analysis and display of genome-wide expression patterns. Proc. Natl. Acad. Sci. U. S. A. 95:14863–14868.