MINIREVIEW

Nitrogen regulation of virulence in clinically prevalent fungal pathogens

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Abstract

The habitats of fungal pathogens range from environmental to commensal, and the nutrient content of these different niches varies considerably. Upon infection of humans, nutrient availability changes significantly depending on the site and pathophysiology of infection. Nonetheless, a common feature enabling successful establishment in these niches is the ability to metabolise available nutrients including sources of nitrogen, carbon and essential metals such as iron. In particular, nitrogen source utilisation influences specific morphological transitions, sexual and asexual sporulation and virulence factor production. All these physiological changes confer selective advantages to facilitate fungal survival, proliferation and colonisation. The three most well-studied components of the nitrogen regulatory circuit that commonly impact fungal pathogenesis are the ammonium permeases (the nitrogen availability sensor candidate), ureases (a nitrogen-scavenging enzyme) and GATA transcription factors (global regulators of nitrogen catabolism). In certain species, the ammonium permease induces a morphological switch from yeast to invasive filamentous growth forms or infectious spores, while in others, urease is a bona fide virulence factor. In all species studied thus far, transcription of the ammonium permease and urease-encoding genes is modulated by GATA factors. Fungal pathogens therefore integrate the expression of different virulence-associated phenotypes into the regulatory network controlling nitrogen catabolism.

Background on fungal nitrogen metabolism

Microorganisms are constantly challenged with stressors such as nitrogen deprivation in their ever-changing local environment, and various regulatory mechanisms have evolved to ensure that genes are appropriately expressed according to their cellular state. In eukaryotes, studies into gene regulation in response to nitrogen availability were first carried out in the model ascomycetes, Saccharomyces cerevisiae, Aspergillus nidulans and Neurospora crassa. Unlike diazotrophic bacterial species, the free-living fungi are unable to fix gaseous nitrogen and therefore have to obtain reduced forms of nitrogenous compounds from alternative sources.

Pioneering research in the model fungi led to the understanding of nitrogen catabolite/metabolite repression, a transcriptional regulatory mechanism that enables preferential utilisation of easily assimilated nitrogen sources such as ammonium and glutamine in order to conserve energy (Wong et al., 2008). When metabolically favoured sources are lacking or exhausted, nonpreferred/secondary/complex nitrogenous compounds are then selectively consumed through the expression of permease and catabolic enzyme-encoding genes of their specific catabolic pathways. This metabolic response to nitrogen limitation typically necessitates two distinct signals: (1) the absence of preferred nitrogen sources leading to the activation of globally acting GATA transcription factor(s), and (2) the presence of a particular intermediate or substrate of the secondary nitrogen pathway leading to activation of pathway-specific transcription factor(s). Thus, both global- and pathway-specific factors are generally required to induce the transcription of nonpreferred nitrogen catabolic genes.
Over the past decade, interest in nitrogen metabolic gene regulation has expanded to encompass a number of pathogens including clinical *S. cerevisiae* isolates, *Candida albicans*, *Aspergillus fumigatus*, *Coccidioides posadasii* and *Cryptococcus neoformans*, a few of the several dozen fungal species that consistently cause disease in the human population. A common feature enabling these pathogenic fungi to survive in the environment and to cause disease in a host is inextricably linked to their ability to forage nitrogen from the surroundings. This article focuses explicitly on our latest understanding of how nitrogen regulation influences specific morphological changes, sporulation, virulence factor expression and catabolism-related pathogenesis activity of human pathogenic fungi. Such physiological responses are critical for successful colonisation of a host during infection as they help evade immune defence systems, facilitate attachment or invasion of tissues, promote dissemination or cause clinical symptoms of the disease.

**Fungal pathogens of humans**

**Saccharomyces cerevisiae**

The hemiascomycete *S. cerevisiae* is one of the most intensively studied eukaryotic model organisms in molecular and cell biology and has recently emerged as an opportunistic pathogen. This model yeast shares a relatively close phylogenetic relationship with the more clinically prevalent *Candida* spp. Its natural habitat is on Oak trees and fruits and can be widely found commercially in breweries, bakeries and scientific laboratories. Infection with *S. cerevisiae* presents with a variety of symptoms and is believed to be acquired nosocomially, through sexual contact or by oral ingestion (McCusker, 2006).

Under harsh conditions such as nitrogen starvation, *S. cerevisiae* a/α yeast diploid cells can undergo a dimorphic transition to an invasive filamentous pseudohyphal growth form (Gimeno *et al.*, 1992). The high-affinity ammonium permease Mep2, but not the two other ammonium permeases Mep1 and Mep3, is necessary for differentiation into a pseudohyphal growth form during ammonium-limiting conditions (Lorenz & Heitman, 1998). The mechanism of ammonium sensing and the signal transduction that activates filamentous growth in *S. cerevisiae*, however, remains obscure. In a human host, ammonium is present at varying concentrations in all the major tissue locations (Supporting Information, Table S1).

Activation of *MEP2* expression requires at least one of the GATA factors Gln3 or Gat1, depending on the nitrogen source present (Marini *et al.*, 1997). Mutations to Gln3 or its cytoplasmic interaction partner Ure2 abrogates *MEP2* expression and stability and results in pseudohyphal deficiency (Lorenz & Heitman, 1998). Heterologous expression of *MEP2* in *gln3 Δ* and *ure2 Δ* mutants does not restore filamentation, indicating that Gln3 and Ure2 have additional targets that regulate dimorphism apart from Mep2 (Lorenz & Heitman, 1998). Consistent with this notion, *mep2 Δ* mutants of clinical *S. cerevisiae* isolates display equivalent virulence to wild type in a murine model of disseminated disease, while *gln3 Δ* mutants are slightly attenuated in vivo (Kingsbury *et al.*, 2006). This suggests that the ability to sense external ammonium is not essential for the survival of *S. cerevisiae* in vivo and that the *mep2 Δ* mutants may still have the ability to undergo pseudohyphal growth given the availability of other serum nitrogen sources such as amino acids and purines. Aside from ammonium, in the human blood glutamine, glutamate, asparagine, arginine, proline, uric acid, allantoin, urea and creatinine, among others, can all potentially serve as a nitrogen source (Table S2). Whether the attenuated virulence of the *gln3 Δ* mutants occurred through an inability to utilise a wide range of nitrogen sources or through a defective pseudohyphal developmental pathway remains open to speculation.

**Candida albicans**

The phylogenetically related *C. albicans* diverged from *S. cerevisiae* c. 200–800 million years ago, and two-thirds of the *C. albicans* 6500 genes have clear orthologues in the *S. cerevisiae* genome (Jones *et al.*, 2004). While this diploid commensal organism generally occupies the skin and alimentary canal of healthy mammals, it can also cause opportunistic infections in most tissues and organs of an immunocompromised host. During infection, *C. albicans* switches from the unicellular yeast form to multicellular, filamentous hyphal and pseudohyphal growth forms that serve as a means for tissue invasion (Lo *et al.*, 1997).

Neutral-to-alkaline extracellular pH and the presence of CO2 are critical determinants that autoinduce hyphal morphogenesis in *C. albicans* (Vylkova *et al.*, 2011). In an acidic, glucose-deprived environment (such as inside phagocytic cells), *C. albicans* utilises amino acids as a carbon source and excretes the amino nitrogen as ammonia, causing the surrounding pH to rise (Vylkova *et al.*, 2011). Mutations to *STP2* (encodes a transcription factor that regulates the expression of amino acid permease genes), *DUR1,2* (encodes urea amidolyase that catabolises urea to ammonia and CO2), and *ATO5* (encodes a putative ammonia exporter), either abolish or delay pH neutralisation (Vylkova *et al.*, 2011). Consistent with this notion, genes for arginine metabolism are induced following
internalisation by macrophages; CAR1-encoded arginase converts arginine to ornithine and urea for Dur1,2 to act upon (Lorenz et al., 2004). dur1,2Δ mutants are defective in germ tube formation in the presence of urea or arginine and are unable to pierce the macrophages cell membrane to facilitate escape following phagocytosis (Ghosh et al., 2009). Urea metabolism via the biotin-dependent Dur1,2 and dimorphic switching are therefore important for resistance against innate host immunity. In the human blood and cerebrospinal fluid, urea is present at very high concentrations (Table S2).

Starvation for amino acids or ammonium is also a potent morphogenetic signal that promotes switching from the budding yeast to filamentous hyphal form. In support of this observation, the regulator of the general amino acid starvation response, Gcn4, has been implicated in dimorphism (Tripathi et al., 2002). In addition, dimorphic switching is Mep2 ammonium permease-dependent during nitrogen-limiting conditions (Biswas & Morschhauser, 2005). So, unlike S. cerevisiae mep2Δ mutants that exhibit a filamentous growth defect during ammonium-limining conditions, the filamentation defect of C. albicans mep2Δ mutants is under general nitrogen limitation independent of nitrogen source (Lorenz & Heitman, 1998; Biswas & Morschhauser, 2005).

Similar to their counterparts in S. cerevisiae, both the GATA factors Gln3 and Gat1 activate the expression of MEP2 in C. albicans (Dabas & Morschhauser, 2007). While Gln3 is required for nitrogen limitation-induced filamentation, Gat1 is surprisingly dispensable for filamentation under both repressing and nonrepressing conditions (Limjindaporn et al., 2003; Dabas & Morschhauser, 2007). This conundrum is explained by the observation that a functional Mep2 is not required for filamentation in gat1Δ mutants, suggesting that the loss of GAT1 may lead to activation of other filamentous growth-inducing signalling pathways (Dabas & Morschhauser, 2007). gat1Δ mutants are avirulent in a murine model of disseminated candidiasis (Liao et al., 2008).

Additionally, while both Gln3 and Gat1 also regulate the expression of the proteolytically activated transcription factor-encoding gene STP1, Gat1 plays the predominant role (Dabas & Morschhauser, 2008). Stp1 mediates the induction of the major secreted aspartic protease isoenzyme SAP2 when proteins are the only nitrogen source available (Dabas & Morschhauser, 2008). Secretion of aspartic proteases is one of the earliest recognised virulence attributes of C. albicans as they directly modulate host immune response via their role in nutrient assimilation and degradation of extracellular matrix proteins and immunoglobulins (Naglik et al., 2004). Consequently, sap2Δ mutants also exhibit reduced virulence in various animal models of disseminated candidiasis (Hube et al., 1997).

**Aspergillus fumigatus**

Aspergillus fumigatus is an opportunistic pathogen that causes life-threatening invasive pulmonary infections in neutropenic and immunocompromised patients. This saprotrophic fungus is found ubiquitously in decaying soils and on organic debris, where it plays an important role in the recycling of carbon and nitrogen, which demands metabolic diversity (Latge, 1999). Like its well-studied sister species A. nidulans, A. fumigatus has the ability to utilise a wide array of nitrogen sources, and this nutritional versatility has led to its widespread distribution. Transmission of A. fumigatus occurs through the inhalation of germinated conidiospores that can lead to hyphal invasion of lung parenchymal tissue through the bronchioles, often resulting in severe asthma, sinusitis and even death.

Nitrogen starvation has been demonstrated to be a prominent host-imposed stress during early-stage A. fumigatus infection (McDonagh et al., 2008). Unlike C. albicans, whereby dimorphic transition from yeast to filamentous form is required for pathogenesis, after spore germination A. fumigatus exists only in its monomorphic mould state. Instead, proper regulation of pathways involved in growth control and nutrient sensing are the critical determinants that influence the ability of A. fumigatus to cause disease. The cross-pathway control system of amino acid biosynthesis is an example of a growth control pathway that is important for pulmonary aspergillosis. The bZIP transcription factor CpcA, an orthologue of C. albicans Gcn4, activates the expression of target genes encoding components of the amino acid biosynthetic pathways during amino acid starvation and other stresses (Rhodes & Brakhage, 2006). Although cpaAΔ mutants display wild-type growth on ammonium or nitrate as the sole nitrogen source, the mutants are unable to derepress the expression of genes associated with amino acid biosynthesis when starved for amino acids (Krappmann et al., 2004). Independent neutropenic murine models of pulmonary aspergillosis experiments have demonstrated that CpcA regulates pathogenesis (Krappmann et al., 2004).
Nutrient sensing-related pathways also contribute to A. fumigatus virulence. RhbA, a member of the family of Ras-related proteins, is implicated in sensing nitrogen availability (Panepinto et al., 2002). Transcription of rhbA occurs throughout the asexual developmental cycle, suggesting that RhbA functions in normal cellular processes instead of specialised developmental programmes; rhbA expression markedly increases in response to nitrogen starvation, however (Panepinto et al., 2002). rhbAA mutants exhibit wild-type growth on rich media or on defined medium supplemented with ammonium as the sole nitrogen source, but growth rates are impaired on secondary nitrogen sources such as proline, histidine and nitrate (Panepinto et al., 2003). A murine model of pulmonary aspergillosis indicates that the nutrient acquisition role of RhbA is required for pathogenesis (Panepinto et al., 2003).

Additionally, rhbAA mutants display enhanced sensitivity to the antibiotic drug rapamycin, suggesting that RhbA functions in the nutrient-sensing target of rapamycin (TOR) signalling pathway (Panepinto et al., 2003). The highly conserved TOR pathway acts through the GATA factors Gln3 and Gat1 in both S. cerevisiae and C. albicans to control growth in response to nutrient availability (Beck & Hall, 1999; Liao et al., 2008). Multiple lines of evidence suggest that mutants lacking the GATA factor known as AreA in A. fumigatus are also less virulent than wild type in murine models of invasive pulmonary aspergillosis (Hensel et al., 1998). According to Hensel et al., these observations are likely caused by the reduced competitive fitness of the areAA mutants given its inability to utilise a wide array of nitrogen sources.

**Coccidioides posadasii**

Filamentous, saprobic Coccidioides spp. are members of the Onygenales that are found in deserts and semi-arid soils (often several inches below the surface of the soil where nitrogen levels are higher) and have the ability to survive harsh environmental conditions such as alkaline soil with high salinity (Saubolle, 1996). Two species have been characterised based on molecular and biogeographical differences: Coccidioides immitis, which appears to be restricted to San Joaquin Valley of California, and C. posadasii, which is widespread throughout regions of endemcity in south-western USA and northern Mexico (Fisher et al., 2002). The multicellular hyphal form of this ascomycete can septate into single-celled arthroconidia and be inhaled, to cause potentially life-threatening pneumonia in both immunocompromised and immunocompetent individuals (Cole et al., 2006).

The host macrophages in lungs kill invading microorganisms largely through nitrosative and oxidative stresses. The production of toxic nitric oxide is mediated by the inducible nitric oxide synthase that requires arginine as a substrate. Like C. albicans, arginine metabolism by C. posadasii also serves as a means of innate immune evasion (Ghosh et al., 2009). One of the mechanisms employed by this respiratory pathogen to facilitate survival in the hostile host environment is to induce the production of macrophage cytosolic arginase I (Hung et al., 2007). Arginase I hydrolyses arginine to ornithine, a substrate for ornithine decarboxylase, a key enzyme in the polyamine biosynthetic pathway and a potential regulator of C. posadasii parasitic cell differentiation (Guevara-Olvera et al., 2000; Iniesta et al., 2002). Host-derived ornithine at the sites of fungal infection may enhance C. posadasii growth and proliferation through the provision of a pool of monoamines that the parasitic cells could utilise for the biosynthesis of glutamine, proline and polyamines (Iniesta et al., 2002). In the macrophage, arginase I competes with nitric oxide synthase for arginine, causing a reduction in the production of nitric oxide but an increase in ornithine and urea production, thereby promoting intracellular survival of C. posadasii (Moali et al., 1998). Treatment of C. posadasii-infected mice with the arginase physiological inhibitor, N-hydroxy-nor-L-arginine, results in augmented host survival, indicating that arginase activity facilitates disease development (Cole et al., 2006).

In C. albicans, arginine catabolism works in concert with Dur1,2-encoded urea amidolysis to promote hyphal switching that plays an important role in facilitating escape from host defences (Lorenz et al., 2004; Ghosh et al., 2009). Unlike hemiascomycetes that use Dur1,2 for urea utilisation, most other higher fungi including species of Coccidioides and Cryptococcus use the nickel-containing enzyme urease (Navarathna et al., 2010). Urease of C. posadasii catalyses the hydrolysis of both fungus- and host-derived urea to produce ammonia (Mirbod et al., 2002). Ammonia and urease released by C. posadasii during the endosporulation phase of the parasitic cycle creates an alkaline microenvironment that likely interferes with host defence function and contributes to exacerbated lung tissue damage (Cole, 1997; Mirbod-Donovan et al., 2006). Consistent with this notion, the expression of URE-encoded urease is upregulated during the parasitic cycle endosporulation stage of C. posadasii (Mirbod et al., 2002). Mice intranasally infected with ureA mutants exhibit a better-organised granulomatous response to infection and increase clearance of the pathogen, leading to better survival rates compared with mice inoculated with wild type (Mirbod-Donovan et al., 2006). Urease production is therefore required for the pathogenesis of C. posadasii parasitic cells.
Cryptococcus neoformans

Cryptococcosis is the leading invasive fungal infection in the world today, responsible for 1 million cases of cryptococcal meningitis per year. Over the past century, this opportunistic basidiomycete has risen from the status of medical curiosity to a common but life-threatening central nervous system pathogen of the immunocompromised population, particularly patients with AIDS (Casanovas & Perfect, 1998). The primary ecological niche of the ubiquitous C. neoformans is pigeon guano, and the majority of the nitrogen is present in the form of uric acid, with the remainder consisting primarily of xanthine, urea and creatinine (Staib et al., 1978). Normally isolated as a budding yeast, C. neoformans can also undergo a dimorphic transition to filamentous growth form via mating or monokaryotic fruiting that leads to the production of potentially infectious airborne basidiospores (Morrow & Fraser, 2009).

Progression through these sexual cycles is regulated by a specialised region of the genome that confers cell-type identity known as the mating-type locus, and environmental cues such as darkness, desiccation and nitrogen starvation (Fraser et al., 2004). During ammonium limitation, the high-affinity ammonium permease Amt2 is required for the induction of mating (Rutherford et al., 2008). Although both mating in C. neoformans and ammonium-dependent filamentous growth in S. cerevisiae and C. albicans appear superficially similar, the morphological changes associated with mating are distinct as it involves the formation of hyphae, basidia and spores (Morrow & Fraser, 2009). Like the S. cerevisiae ammonium permease Mep2, C. neoformansAmt2 is not required for virulence (Kingsbury et al., 2006; Rutherford et al., 2008). However, initiation of invasive growth in response to nitrogen limitation is dependent on Amt2 (Rutherford et al., 2008).

One particular nitrogen-scavenging enzyme in C. neoformans that has evoked as much interest as it has in C. posadasii is urease. Apart from converting the abundant urea to a usable nitrogen source, urease promotes yeast sequestration within the microcapillary beds of the brain during haematogenous dissemination, facilitating C. neoformans crossing the blood-brain barrier (Olszewski et al., 2004). Both murine intravenous and inhalation models of cryptococcosis revealed that mice infected with ure1A mutants lacking urease survived significantly longer than mice infected with wild type (Cox et al., 2000; Olszewski et al., 2004). However, direct co-inoculation of wild-type and ure1A mutants into the cerebrospinal fluid of corticosteroid-treated rabbits or into the brain of mice revealed no significant differences in

Table 1. Roles of a selected panel of fungal nitrogen regulatory proteins on virulence-associated phenotypes

<table>
<thead>
<tr>
<th>Gene</th>
<th>Protein</th>
<th>Mutant phenotype in mice</th>
<th>Other phenotypes in vitro</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>S.c. ME2</td>
<td>Ammonium permease</td>
<td>Wild type</td>
<td>Pseudohyphal growth defect</td>
<td>Lorenz &amp; Heitman (1998) and Kingsbury et al. (2006)</td>
</tr>
<tr>
<td>C.a. ME2</td>
<td>Ammonium permease</td>
<td>Unknown</td>
<td>Pseudohyphal growth defect</td>
<td>Biswas &amp; Morschhauser (2005)</td>
</tr>
<tr>
<td>C.n. AMT2</td>
<td>Ammonium permease</td>
<td>Wild type</td>
<td>Inability to initiate invasive growth form</td>
<td>Rutherford et al. (2008)</td>
</tr>
<tr>
<td>S.c. DUR1,2</td>
<td>Urea amidolase</td>
<td>Wild type</td>
<td>Inability to utilise urea as a nitrogen source</td>
<td>Kingsbury et al. (2006)</td>
</tr>
<tr>
<td>C.a. DUR1,2</td>
<td>Urea amidolase</td>
<td>Unknown</td>
<td>Inability to utilise urea as a nitrogen source. Germ tube formation defect</td>
<td>Ghosh et al. (2009) and Vylkova et al. (2011)</td>
</tr>
<tr>
<td>C.p. URE</td>
<td>Urease</td>
<td>Attenuated</td>
<td>Inability to utilise urea as a nitrogen source</td>
<td>Mirbod et al. (2002) and Mirbod-Donovan et al. (2006)</td>
</tr>
<tr>
<td>C.n. URE1</td>
<td>Urease</td>
<td>Attenuated</td>
<td>Inability to utilise urea as a nitrogen source</td>
<td>Cox et al. (2000) and Olszewski et al. (2004)</td>
</tr>
<tr>
<td>S.c. GLN3</td>
<td>GATA factor</td>
<td>Attenuated</td>
<td>Inability to utilise a wide array of nitrogen sources. Pseudohyphal growth defect</td>
<td>Lorenz &amp; Heitman (1998) and Kingsbury et al. (2006)</td>
</tr>
<tr>
<td>C.a. GAT1</td>
<td>GATA factor</td>
<td>Avirulent</td>
<td>Inability to utilise a wide array of nitrogen sources</td>
<td>Limjindaporn et al. (2003)</td>
</tr>
<tr>
<td>A.f. areA</td>
<td>GATA factor</td>
<td>Attenuated</td>
<td>Inability to utilise a wide array of nitrogen sources</td>
<td>Hensel et al. (1998)</td>
</tr>
</tbody>
</table>

S.c., Saccharomyces cerevisiae; C.a., Candida albicans; A.f., Aspergillus fumigatus; C.p., Coccioidoides posadasii; C.n., Cryptococcus neoformans.
colony counts when strains were recovered (Cox et al., 2000; Olszewski et al., 2004). Urease is therefore implicated in the dissemination process of pathogenesis, but the importance of this virulence factor appears infection site specific as its activity is not required for C. neoformans proliferation in the host brain or cerebrospinal fluid.

Instead, the enzyme laccase is crucial for C. neoformans survival in the brain (Nosanchuk et al., 2000). Both urease and laccase act synergistically to promote invasion of the host central nervous system. While urease facilitates invasion through brain microcapillaries, laccase enables yeast growth and proliferation within the brain parenchyma. Laccase catalyses the oxidation of catecholamines found in the central nervous system to produce the virulence factor melanin that protects against toxic free radicals generated by the host immune system (Nosanchuk et al., 2000). Interestingly, the nitrogen responsive GATA factor Gat1/Are1 negatively regulates LAC1-encoded laccase expression and consequently melanin production (Lee et al., 2011, 2012). Additionally, Gat1/Are1 impacts other virulence attributes including infectious basidiospore production, antiphagocytic capsule biosynthesis and febrile body temperature growth (Lee et al., 2011). These findings are consistent with classic studies demonstrating that nitrogen source utilisation affects cryptococcal virulence factor expression (Chaskes & Tyndall, 1975; Staib et al., 1978).

**Conclusions**

Pathogenic fungi are an increasing public health problem due to the growing immunocompromised population, particularly due to the advent of the AIDS pandemic. These fungi are found in diverse and often hostile conditions where they are constantly challenged by nutrient availability such as nitrogen limitation. The ability to survive, proliferate and colonise their respective niches is therefore dependent on well-coordinated regulation of nitrogen metabolism. While filamentous fungi have the ability to utilise a wide array of nitrogen sources; not surprisingly, GATA factor mutants of S. cerevisiae, C. albicans and A. fumigatus exhibit attenuated virulence. The other major nitrogen-associated mediator of fungal adaptation and pathogenesis, whose transcription is also dependent on GATA factors, is the virulence factor and catabolic enzyme urease that has been characterised in C. posadasii and C. neoformans. Activities of the common proteins among the fungal species described in this article are summarised in Table 1. The complex interplay between the host environment and the fungal pathogen is also a critical determinant; host immune cells generate highly toxic reactive nitrogen species against microbial invaders, but the nitrosative stress response of the pathogen in catabolising and detoxifying these chemical compounds enable its survival during infection. Clearly, it is becoming increasingly apparent that nitrogen regulation is a major factor modulating pathogenesis in fungi. An important area for future research is to dissect the nature and origin of intracellular signals that govern the metabolic processes involved in nitrogen acquisition, in the context of host-pathogen interactions.

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**References**


Gimeno CJ, Ljungdahl PO, Styles CA & Fink GR (1992) Molecular and phenotypic description of Coccidioides posadasii sp. nov., previously recognized as the non-California population of Coccidioides immitis. Mycologia 94: 73–84.


Navarathna DH, Das A, Morschhauser J, Nickerson KW & Roberts DD (2011) Dur3 is the major urea transporter in Candida albicans and is co-regulated with the urea amidolyase Dur1,2. Microbiology 157: 270–279.


Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. The presence or absence of a selected panel of nitrogen sources in various tissues of a healthy individual.

Table S2. The concentration (µM) of a selected panel of nitrogen sources in various biofluids of a healthy individual.