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Gene expression profiling of the nitrogen starvation stress response in the mycorrhizal ascomycete *Tuber borchii*

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Abstract

The focus of this work is on the nitrogen starvation stress responses operating in a plant symbiotic fungus. A cDNA array profiling analysis was conducted on N-limited mycelia of the mycorrhizal ascomycete *Tuber borchii*. Fifty-one unique transcripts, out of 2062 redundant arrayed cDNAs, were differentially expressed by at least 1.5-fold in response to N deprivation. Only two N assimilation components—a nitrate transporter and a high-affinity ammonium transporter—were found among differentially expressed genes. All the other N status responsive genes code for as yet unidentified hypothetical proteins or components not directly involved in N assimilation or metabolism, especially carbohydrate binding proteins and oligosaccharide as well as lipid modifying enzymes. A subset of cDNA array data were confirmed and extended by Northern blot analysis, which showed that most of the latter components respond not only to nitrogen, but also to carbon source depletion.
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1. Introduction

Nutrient, and especially nitrogen availability has a profound impact on the biology and environmental persistence of fungi (Lengeler et al., 2000; Lilleskov et al., 2002 and references therein). Two major, non mutually exclusive types of N starvation stress responses have emerged from a combination of targeted and untargeted gene expression studies carried out in yeast and in model multicellular fungi. The most general and straightforward of them relies on the compensatory upregulation of specific N transporters and assimilatory enzymes as a means to maintain a relatively constant intracellular N status in the face of variable and often harsh external environments (reviewed by Magasanik and Kaiser, 2002; Marzluf, 1997). The other is a more complex and functionally heterogeneous response (Gasch et al., 2000; Wu et al., 2004). It does not bear upon N acquisition directly (and often impinges on other nutrients as well) and involves changes in cell morphology and accumulation of outer surface modifying proteins. The paradigm of this 'indirect' response, which displays a high degree of organism idiosyncrasy and it is generally not so well understood, is pseudohypha formation following nitrogen (or carbon) source depletion in *Saccharomyces cerevisiae* (Cullen and Sprague, 2000; Lo and Dranginis, 1998; Madhani and Fink, 1998). Similar responses, many of which promiscuously induced by both N and C starvation, have been delineated

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in filamentous fungi such as the saprotroph *Trichoderma* (de las Mercedes Dana et al., 2001; Donzelli and Harman, 2001; Mach et al., 1999; Nakari-Setala et al., 1997) and the phytopathogens *Cladosporium fulvum* (Perez-Garcia et al., 2001; Segers et al., 1999; Van den Ackerveken et al., 1994) and *Magnaporthe grisea* (Soanes et al., 2002; Talbot et al., 1997). In these organisms, nutrient starvation is also one of the main abiotic stimuli promoting sexual differentiation and fruitbody formation (Brandrud and Timmermann, 1998; Kues, 2000; Uno and Ishikawa, 1974).

By comparison, relatively little is known about N starvation stress responses in ectomycorrhizal fungi. These organisms, which dramatically expand the ion-absorbing surfaces of their plant hosts through soil-penetrating mycelium, play a crucial ecological role in the forest ecosystems of boreal and temperate regions, especially during growth through N-depleted soil patches (Read and Perez-Moreno, 2003; Smith and Read, 1997). In addition, N shortage in the mycorrhizosfere is thought to favor the establishment of the ectomycorrhizal symbiosis (Bidartondo et al., 2001; Buscot et al., 2000; Lilleskov et al., 2002). This renewed perception of the ecological importance of ectomycorrhiza has revived efforts to elucidate the metabolic and molecular processes underlying nutrient uptake, assimilation, and starvation stress responses in plant symbiotic fungi (Javelle et al., 2003a, 2004; Nehls et al., 2001; Soragni et al., 2001 and references therein).

A number of genes coding for inorganic N transporters and N assimilation enzymes have been isolated from these organisms (Guescini et al., 2003; Jargeat et al., 2000, 2003; Javelle et al., 2001, 2003b; Juuti et al., 2004; Montanini et al., 2002, 2003; Vallorani et al., 2002). Most of them display the same kind of N status responsiveness previously documented in non-mycorrhizal fungi (Magasanik and Kaiser, 2002; Marzluf, 1997). The main exceptions are the lack of N-mediated repression for NADP-glutamate dehydrogenase in *Tuber borchii* (Montanini et al., 2003) and the nitrate-independent upregulation of nitrate assimilation components in both *Hebeloma cylindrosporum* (Jargeat et al., 2000, 2003) and *T. borchii* (Montanini et al., 2006). Additional genes coding for N metabolism components have been uncovered in the course of microarray studies aimed at exploring gene expression variations accompanying mycorrhiza (Morel et al., 2005; Podila et al., 2002; Wright et al., 2005) or fruitbody formation (Lacourt et al., 2002).

In contrast, only one mRNA not directly involved in N metabolism, yet strongly influenced by the N status, has been identified and functionally characterized so far in a mycorrhizal fungus (Soragni et al., 2001). It codes for a Ca^{2+}-dependent phospholipase A_2, named *T. borchii* Secreted Protein 1 (TbSP1). Although the specific functional role played by this enzyme in the context of the response to nutrient starvation is not yet understood, two features—namely, its strong upregulation following nutrient (either N or C) deprivation and its outer surface localization—point to it as a likely representative of the 'indirect' type of starvation stress responsive components previously described in other fungi. Additionally, the TbSP1 phospholipase has recently been found to be upregulated during mycorrhiza formation (Miozzi et al., 2005). What remains poorly known at present, is the variety, functionality, and nutritional status responsiveness of these components. It was thus of interest to conduct an extended (and largely untargeted) search for N starvation responsive genes in a mycorrhizal fungus such as the ascomycete *T. borchii*. To this end, cDNA arrays were used to monitor changes in transcript abundance in free-living mycelia subjected to well-defined N starvation conditions. This study allowed us to highlight the predominant upregulation of genes coding for components not directly involved in N acquisition or metabolism in response to N starvation and to identify new potential determinants of the changes that accompany the adaptation to nutrient-limited growth conditions in *T. borchii*.

Table 1
Arrayed cDNAs coding for proteins directly involved in N metabolism

Accession No.	Clone ID	Best BLASTX match[a]			Functional classification[c]
		Gene description	Accession No.	*E*-value	
CN487753	M08B06	Dihydrolipoamide dehydrogenase	EAL87307	2E-31	Nitrogen compound metabolism
CN487913	M11E02	2-Isopropylmalate synthase	CAE76195	5E-46	Nitrogen compound metabolism
CN487947	M11H11	Glutamate 5-kinase	EAA48823	2E-18	Nitrogen compound metabolism
CN487963	M12C07	Ornithine aminotransferase	EAL89873	3E-61	Nitrogen compound metabolism
CN487975	M12E03	Indoleamine 2,3-dioxygenase	AAW69319	6E-22	Nitrogen compound metabolism
CN488130	MR2M05G05	Xanthine dehydrogenase	EAL8966	6E-51	Nucleobase metabolism
CN488394	SA2H11	Allantoicase	CAA91956	2E-63	Nitrogen compound metabolism
BM266165	VA22	L-Amino acid oxidase	CAD21325	1E-24	Nitrogen compound metabolism
AF462031	TbAAT	Aspartate aminotransferase	EAL93260	1E-20	Nitrogen compound metabolism
AF462032	TbGOGAT	Glutamate synthase	AAL76245	2E-65	Nitrogen compound metabolism
AY050276	TbAMT1[b]	Ammonium transporter[b]	AAF80454	0	Cation transport
AF462037	TbGS[b]	Glutamine synthetase[b]	AAP23163	0	Nitrogen compound metabolism
AF309087	TbGDH[b]	Glutamate dehydrogenase[b]	AAG28788	6E-102	Nitrogen compound metabolism
AF462038	TbNrt2[b]	Nitrate transporter[b]	EAL91075	4E-150	Anion transport

[a] GenBank annotation of the best reciprocal hits retrieved from BLASTX analysis ($E \leqslant 10^{-5}$).
[b] Experimentally validated function: TbAMT1 (Montanini et al., 2002); TbGS (Montanini et al., 2003); TbGDH (Vallorani et al., 2002); TbNrt2 (Montanini et al., 2006).
[c] Functional classification according to Gene ontology, 'biological process' category (Ashburner et al., 2000).

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