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QID74 Cell wall protein of *Trichoderma harzianum* is involved in cell protection and adherence to hydrophobic surfaces

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Abstract

Trichoderma is widely used as biocontrol agent against phytopathogenic fungi, and as biofertilizer because of its ability to establish mycorriza-like association with plants. The key factor to the ecological success of this genus is the combination of very active mycoparasitic mechanisms plus effective defense strategies induced in plants. This work, different from most of the studies carried out that address the attacking mechanisms, focuses on elucidating how *Trichoderma* is able to tolerate hostile conditions. A gene from *Trichoderma harzianum* CECT 2413, *qid74*, was strongly expressed during starvation of carbon or nitrogen sources; it encoded a cell wall protein of 74 kDa that plays a significant role in mycelium protection. *qid74* was originally isolated and characterized, in a previous work, by a differential hybridization approach under simulated mycoparasitism conditions. Heterologous expression of Qid74 in *Saccharomyces cerevisiae* indicated that the protein, located in the cell wall, interfered with mating and sporulation but not with cell integrity. The *qid74* gene was disrupted by homologous recombination and it was overexpressed by isolating transformants selected for the *amdS* gene that carried several copies of *qid74* gene under the control of the *pki* promoter. Disruptants and transformants showed similar growth rate and viability when they were cultivated in different media, temperatures and osmolarities, or were subjected to different abiotic stress conditions. However, disruptants produced about 70% mass yield under any condition and were substantially more sensitive than the wild type to cell wall degradation by different lytic preparations. Transformants had similar mass yield and were more resistant to lytic enzymes but more sensitive to copper sulfate than the wild type. When experiments of adherence to hydrophobic surfaces were carried out, the disruptants had a reduced capacity to adhere, whereas that capacity in the overproducer transformants was slightly higher than that of the wild type. Results point to a significant role for Qid74 both in cell wall protection and adhesion to hydrophobic surfaces.

Keywords: Antagonism; Mycoparasitism; Fungal cell walls; Hydrolases; Cell integrity; Adherence; Fungal phytopathogens

1. Introduction

The fungal cell wall not only acts as a structural barrier protecting cells against hostile conditions but also plays a functional role in sensing environmental conditions and in cell–cell recognition (Kuhn et al., 1990; Georgopapadakou and Tkacz, 1995). Cell–cell interactions are essential to the vegetative growth and reproductive development of fungi, interactions varying, from fusion of cells in the same population, to interactions between fungal and foreign cells of a host organism (Kuhn et al., 1990; Osiewacz, 2002). Many pathogenic, symbiotic and parasitic fungi have surface molecules, that is, extensions of the walls that play a significant role in fungus-host interactions (Osiewacz, 2002; Harman et al., 2004; Djonovic et al., 2006; Viterbo and Chet, 2006; Sarrocco et al., 2006) and that are involved in the formation of specialised structures such as appressoria (Talbot et al., 1993; Tucker and Talbot, 2001; Harman et al., 2004).

Modification of the cell wall is an important mechanism by which fungi respond to stress conditions. Wall content is affected by oxygen, light, nitrogen deprivation, the presence

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of certain metal ions (Kuhn et al., 1990; Jung et al., 1998; Osiewacz, 2002) and, in pathogenic and parasitic fungi such as *Candida albicans* or *Pneumocystis carinii*, cell surface proteins are encoded by pH-regulated genes (Kottom et al., 2001; Sentandreu et al., 1998), pH being one of the main ambient traits affecting the activity of pathogenicity factors secreted by pathogens (Prusky and Yakoby, 2003).

Some conserved cell wall glycoproteins interact with glucan, chitin and structural mannoproteins and have a role in adherence (adhesins) (Sentandreu et al., 1994; Mrsa and Tanner, 1999). In addition, a large number of wall-bound enzymes and lectin-type proteins define surface properties such as cell hydrophobicity (Sentandreu et al., 1994; Wessels, 1994; Kieliszewski et al., 1994). Fungal pathogens, symbionts and parasites are likely to use a spectrum of adhesive, hydrophobic proteins, to interact with their hosts. Hydrophobins and lectins are involved in morphological events during ectomycorrhizal formation and plant-fungus interaction (Martín et al., 1997; Osiewacz, 2002; Lugones et al., 2004). Hydrophobins are essential in fungal morphogenesis (Wosten, 2001; Tucker and Talbot, 2001) and, in addition to forming hydrophobic wall coating, they play an important role in adherence of fungal hyphae to hydrophobic surfaces mediated by a non-specific binding (Wosten et al., 1994a,b). Hydrophobins are induced in cultures starved for nitrogen or carbon. They are capable of acting as phytotoxins, producing the same disease symptoms as the fungus itself (Takai, 1974; Stringer and Timberlake, 1993) or are involved in appressoria formation of pathogenic fungi and in attachment and plant disease development (Hwang and Kolattukudy, 1995). In *Trichoderma virens*, a hydrophobin-like elicitor of plant defence responses, Sm1, seems to play a role in the first stages of the *T. virens*-root interactions (Djonovic et al., 2006).

Repellents are fungal proteins with roles similar to those of hydrophobins. They were first reported in *Ustilago maydis* as abundant 8 kDa cell wall proteins, encoded by *rep1* gene (Wosten et al., 1996). Gene structure showed a secretion signal followed by 12 repeated sequences of between 37 and 55 amino acids, a consensus of 37 conserved aminoacids and 10 KEX2-like proteolytic cleavage sites, thus *rep1* encoded 12 peptides ranging in size from 35 to 53 amino acids.

Other cell wall proteins involved in adherence and cell surface hydrophobicity present repeated sequences that seem to arise by multiple duplications of a single genetic unit coding segment (Rey et al., 1998; Templeton et al., 2004). They have in common their ability to form extracellular protein-protein complexes, some of which maintain a cell barrier, whereas some conserved cysteine residues are involved in intermolecular disulfide bonds (Nielsen et al., 1991; Rey et al., 1998; Templeton et al., 2004). The cloning of a gene encoding a cellulose-binding protein from *Phytophthora nicotianae* (Gaulin et al., 2002), and of a gene encoding a protein secreted during attachment of spores of *Phytophtora*, have only recently given the first data on putative hyphal adhesines (Robold and Hardham, 2005). The latter contains 47 copies of the thrombospondine type 1 repeat, a motif found in adhesins of some parasites (Robold and Hardham, 2005).

Mycoparasites are also likely to use adhesive molecules to attach to their hosts. As in fungal phytopathogens, extracellular matrix material secreted by fungal conidia contains many different components, and this heterogeneity hampers the identification of fungal adhesins other than hydrophobins. Puyesky et al. (1999) reported on a multidomain conidiospore surface protein of *Trichoderma* and, although the presence of Arg-Gly-Asp (RGD) motifs suggested its involvement in cell interactions, its localization in the membrane wall interface argued against the protein to be present outside the wall. In addition, in the interaction between *T. harzianum* and soilborne plant pathogens such as *Rhizoctonia solani*, contact between the two fungi is mediated by an extracellular matrix followed by a series of degradation events in the host (Benhamou and Chet, 1993). This degradation process, mediated by the secretion of hydrolases by *Trichoderma* strains (Fravel, 2005; Montero et al., 2005; Sanz et al., 2005; Steyaert et al., 2004) raises the question of the way this fungus recognizes and adheres to the host and how it protects itself against its own enzymes.

In this study we present evidence about the role of a cell wall protein encoded by *qid74* gene from *Trichoderma*. The gene is induced by starvation and mycoparasite-like conditions and is conserved in all *Trichoderma* species tested (Rey et al., 1998). Qid74 protects the cells against lytic enzymes and enhances adherence to hydrophobic surfaces.

2. Materials and methods

2.1. Strains, media and growth conditions

Filamentous fungal strains *T. harzianum* Rifai CECT 2413, and the phytopathogens *R. solani* Kühn CECT 2815 AG4 (Boysen et al., 1996), *Botrytis cinerea* CECT 2100 and *Gibberella fujikuroi* CECT 2152 were obtained from Colección Española de Cultivos Tipo (CECT), Burjassot, Valencia, Spain (Belloch et al., 1998). All other *T. harzianum* strains used in this study derived from strain CECT 2413 and are named as T1 to Tn, when they are transformants that overexpress Qid74 and Δ1 to Δn (disruptants) when they are transformants with a disruption in the *qid74* gene and lack Qid74. *Saccharomyces cerevisiae* yeast strains GRF167 (MAT**a** *ura3 his3*), used for heterologous expression of *qid74* gene, and GWB-1 (MAT**α** *ura3 trp1*) used as a tester in conjugation, sporulation and tetrad analysis experiments, were kindly provided by Dr. A. Aguilera, Department of Genetics, University of Seville. The bacterial *Escherichia coli* strains NM522 (Stratagene, La Jolla, CA) and DH5α (Promega) were also used for the isolation of anti-Qid74 polyclonal antibodies. Media and procedures followed were as described (Sambrook and Russell, 2001).

Filamentous fungal strains were maintained on PPG or PDA (Limon et al., 2004). Salt minimal medium, MM (Penttila et al., 1987), supplemented with the indicated