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Review

Fungal wars: The underlying molecular repertoires of combating mycelia

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ABSTRACT

Non-self contact between fungi elicits strong morphological and biochemical reactions in the mycelia of interacting species. Although these reactions appear to be species- and interaction-specific, some responses such as pigmentation, increased secretion of phenol-oxidases, barrage formation and sealing of the mycelia front are common responses in most interactions. Hence, some species recruit similar molecular machineries in response to non-self. Increasing number of fully sequenced and annotated fungal genomes and advances in genome-wide and global proteome analytical tools now allow researchers to use techniques such as RNA sequencing, micro and macroarray analysis, 2-dimensional protein gel profiling, and differential display of mRNA to probe the underlying molecular mechanisms of combative mycelial interactions. This review provides an overview of the genes and proteins found to be differentially expressed in conflicting fungal mycelia by the use of 'omics' tools. Connections between observed gene and protein repertoires of competing mycelia and the attendant morphological and biochemical changes are presented.

1. Introduction

Fungi play a central role in the recycling of nutrients in nature (Hiscox and Boddy, 2017; van der Wal et al., 2013). Often, dead plant materials are simultaneously colonized by multiple species. As each species spreads in an attempt to gain control of colonized substrate, they come in contact with competing species. Depending on the arsenal and/or defense mechanisms available to each competitor, non-self mycelial contact is characterized by a range of biochemical and physiological reactions including pigmentation, formation of mycelial barrage to stave off opposing fungal mycelia; sealing of the mycelial front, and up-regulation of hydrophobic compounds (Rayner et al., 1994, 1995; Boddy, 2000; Peiris et al., 2008; Ujor et al., 2012a; Hiscox et al., 2015). These interactions are also characterized by the secretion of extracellular enzymes such as laccase, manganese peroxidase, lignin peroxidase, and chitinase (Boddy, 2000; Kubicek et al., 2001).

Antagonism between competing fungi may also occur at a distance, mediated by volatile organic compounds (VOCs) including alcohols, aldehydes, ketones, and aromatic compounds, which may possess antifungal properties (El Ariebi et al., 2016; Evans et al., 2008; Hynes et al., 2007; Humphries et al., 2002), as well as by non-volatile antifungal compounds (antibiosis) secreted by one or both interacting species (Waing et al., 2015). Broadly, interspecific mycelial interactions can result in replacement, where one species annexes the territory occupied by another, or deadlock where neither fungus gain the territory occupied by the other (Hiscox and Boddy, 2017; Boddy, 2000). Partial replacement is also reported where one species momentarily grows into the territory occupied by the other, but does not completely replace the opposition, and there is mutual replacement, which entails encroachment upon the opposing fungus' territory by each competing species (Boddy, 2000).

Extensive study of antagonistic mycelial interactions over the past four to five decades has led to the commercial use of aggressive species such as *Phlebiopsis gigantea* and *Trichoderma* species in the control of wood-rot and phytopathogenic fungi (Boddy, 2000; Kubicek et al., 2001; Adomas et al., 2006). More recently, studies

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of fungal interactions have revealed that *Fusarium verticillioides* has the potential to reduce the severity of corn smut caused by *Ustilago maydis* (Jonkers et al., 2012). However, the molecular mechanisms underlying characteristic responses during fungus–fungus interactions, vis-à-vis the genes and proteins that mediate these responses have only begun to emerge. Such knowledge is required to improve the robustness and persistence of biocontrol fungi in the field as well as to shed more light on the structure and dynamics of fungal communities, and how these interactions influence wood degradation and nutrient cycling (Boddy, 2000).

This is a rapidly evolving field of research due to the use of fungal-derived enzymes for accelerating lignocellulose hydrolysis to generate fermentable sugars in biofuel production, as well as other diverse applications relevant to bioremediation, food, paper, wine and textile industries (Hatakka, 2001; Hatvani et al., 2002; Binder and Raines, 2010). Early studies of combative interactions largely focused on the outcomes of interactions; namely, the patterns of pigmentation, enzyme activity profiling of the interaction interface, the metabolite repertoire and morphological changes associated with the combat zone. Recent advances in genomics and proteomics coupled with a marked increase in the availability of fully sequenced fungal genomes now make it possible for researchers to attempt to unravel the molecular mechanisms underlying the complex, biochemical and morphological reactions triggered by non-self contact between fungi. This has led to the identification of specific genes and proteins whose roles hitherto were poorly described or unknown in relation to non-self interactions between fungi. In this review, we summarize the cellular aspects of interspecific mycelial interactions between fungi with an emphasis on the genes and proteins whose differential expressions have highlighted likely response mechanisms (Table 1) recruited by various species during combat, and the occurrence of some of these mechanisms across different species.

1.1. Mycelial stabilization and cell wall fortification

Different fungi growing on the same substrate may interact from a distance by exchanging chemical cues, thereby resulting in mutual or selective inhibition without contact (Heilmann-Clausen and Boddy, 2005). In contrast, some species only respond to, or exhibit signs of response to the opposition after direct mycelial contact. In a combative scenario, modifications to the structure and strength of the targeted hyphae are warranted to withstand an invasive opponent. To stave off an aggressive competitor, most species form a dense mat of aerial mycelia (a barrage; Fig 1A) at the interaction interface (Rayner et al., 1994; Boddy, 2000). Interestingly, global mRNA profiling of non-self-interacting fungal mycelia showed increased expression of genes encoding hydrophobins. These are a group of small fungal proteins that have been implicated in hyphal aerial growth (Wösten et al., 1999; Torkkeli et al., 2002; Adomas et al., 2006). In fact, hydrophobins are involved in cell wall assembly, lowering surface tension, formation of amphipathic films, and assembly of surface layers in mycelia, which collectively enhance the formation of aerial hyphae (Wösten et al., 1999; Linder et al., 2002; Torkkeli et al., 2002; Adomas et al., 2006). In a similar study using cDNA macroarray, Adomas et al. (2006) reported varying expression levels for homologs of hydrophobins I, II, and III in *P. gigantea* and *Heterobasidion parviporum* at different stages of competitive interaction. Interestingly, hydrophobin I, which forms stable aggregates (Wösten et al., 1999) was strongly up-regulated within the confluence zone (barrage zone). Further, Jonkers et al. (2012) reported up-regulation of hydrophobin-encoding genes in the mycelia of *U. maydis* and *F. verticillioides* interacting with each other. It is likely that hydrophobins orchestrate barrage formation as they are upregulated in barrage-forming mycelia; most plausibly to seal off mycelial front against the approaching competitor (Fig 1A). In addition to sealing of mycelia front, more antagonistic species that metabolize competitor cell wall appear to recruit hydrophobins for attachment to host mycelia (Jonkers et al., 2012).

Hydrophobins are not the only biochemical players thought to participate in the sealing of the mycelial front and in barrage formation. Eyre et al. (2010) reported up-regulation of glycoside hydrolase, 1,3-beta glucan synthase, and α-1,2-mannosyltransferase genes in *Trametes versicolor*, while Ujor et al. (2012b) detected an increase in the amount of a glycosyltransferase in *Schizophyllum commune* during various interaction pairings. In addition to their roles in carbohydrate metabolism, glycoside hydrolase, 1,3-beta glucan synthase, and α-1,2-mannosyltransferase have been implicated in the strengthening and improvement of the plasticity of fungal cell wall, as well as in hyphal branching (Yuan et al., 2008; Latgé, 2007; Häusler et al., 1992). Similarly, glycosyltransferases are involved in the construction and polymerization of cell wall components in both prokaryotes and eukaryotes (Lim and Bowles, 2004; Hashimoto et al., 2009). Increases in the levels of these enzymes during combative interactions may indicate an attempt to fortify the mycelia following non-self contact. Such mycelial fortification would not only prevent the progress of the competitor (in the case of barrage formation), but also attempt to limit the passage of toxic metabolites often associated with combative mycelial interactions via the cell wall barrier.

Barrage formation and modification of cell wall plasticity serve as transient resistance when faced with an aggressive cell wall-lysing fungus such as a *Trichoderma* species (Fig 1). Once the cell wall barrier is breached (Fig 1D and E) it is critical to protect the cell membrane and cytosolic components. This entails recruitment of

Table 1
Mechanisms of attack and defense recruited by fungal mycelia interacting with non-self.

Mechanism	Fungal species	References
Enhanced nutrient uptake and metabolism	*P. gigantea, H. parviporum, T. versicolor, U. maydis, T. viride, F. verticillioides*	Adomas et al., 2006; Jonkers et al., 2012; Eyre et al., 2010; Ujor et al., 2012a
Hydrolysis of competitor cell wall	*Trichoderma species, F. verticillioides, P. coccineus*	Kubicek et al., 2001; Dana et al., 2001; Boddy, 2000; Ujor et al., 2012a; Ujor, 2010; Jonkers et al., 2012
Protein stabilization and recycling	*P. gigantea, S. commune, T. versicolor, P. coccineus*	Adomas et al., 2006; Ujor et al., 2012b; Arfi et al., 2013; Eyre et al., 2010
ROS production & anti-oxidation	*P. gigantea, U. maydis, F. verticillioides, T. versicolor, H. annosum*	Iakovlev et al., 2004; Eyre et al., 2010; Adomas et al., 2006; Jonkers et al., 2012
Detoxification of toxic metabolites	*P. coccineus, T. versicolor, F. verticillioides, S. commune*	Arfi et al., 2013; Eyre et al., 2010; Jonkers et al., 2012; Ujor et al., 2012b; Ujor, 2010
Sealing of mycelial front, attachment, and septal plugging	*P. gigantea, H. parviporum, U. maydis, S. commune, F. verticillioides, U. maydis*	Adomas et al., 2006; Jonkers et al., 2012; Eyre et al., 2010; Ujor et al., 2012b; Ujor, 2010
Induction of secondary metabolism	*U. maydis, S. commune, F. verticillioides, H. annosum*	Jonkers et al., 2012; Eyre et al., 2010; Ujor et al., 2012b; Ujor, 2010

ROS – reactive oxygen species.

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