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Interaction of entomopathogenic fungi with the host immune system

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A B S T R A C T

Entomopathogenic fungi can invade wide range of insect hosts in the natural world and have been used as environmentally friendly alternatives to chemical insecticides for pest control. Studies of host-pathogen interactions provide valuable insights into the coevolutionay arms race between fungal pathogens and their hosts. Entomopathogenic fungi have evolved a series of sophisticated strategies to counter insect immune defenses. In response to fungal infection, insect hosts rely on behavior avoidance, physical barrier and innate immune defenses in the fight against invading pathogens. The insect cuticle acts as the first physical barrier against pathogens. It is an inhospitable physiological environment that contains chemicals (e.g., antimicrobial peptides and reactive oxygen species), which inhibit fungal growth. In addition, innate immune responses, including cellular immunity and humoral immunity, play critical roles in preventing fungal infection. In this review, we outline the current state of our knowledge of insect defenses to fungal infection and discuss the strategies by which entomopathogenic fungi counter the host immune system. Increased knowledge regarding the molecular interactions between entomopathogenic fungi and the insect host could provide new strategies for pest management.

1. Introduction

Entomopathogenic fungi cause many insect diseases and play important roles in regulating insect populations in nature (Hajek, 1997; Wang et al., 2011b). These fungi have many advantages as eco-friendly alternatives to chemical insecticide (Chen et al., 2017; Hajek, 1997). Those belonging to the genera *Beauveria* and *Metarhizium* have been widely used for the control of various agricultural insect pests and vectors of human diseases (Blanford et al., 2005; Farenhorst et al., 2009; Knols et al., 2010).

To combat infection, insects have evolved various defense systems. Cuticular integument provides the first and most effective physical barrier to prevent the entrance of pathogens into the body (Leger et al., 1991). Furthermore, insects employ both cellular and humoral immune defense mechanisms to fight fungal infections (Gottar et al., 2006). Cellular immunity is mainly mediated by hemocytes, which circulate in the hemolymph, and involves coagulation, phagocytosis, and encapsulation (Valanne et al., 2011). The humoral defense responses rely on specific signaling pathways. Among them, the Toll signaling pathway is significantly activated in response to fungal infection and eventually leads to production of anti-fungal peptides like drosomycin and metchnikowin in *Drosophila* (Lemaitre and Hoffmann, 2007). During long-term coevolutionary arms race, insect pathogenic fungi have developed multiple strategies that allow the fungus to overcome or evade the immune defense responses of the host.

Here we provide an overview of entomopathogenic fungi invasive and developmental processes in insect hosts and present a review of behavioral immunity in insects. We summarize studies that characterize signaling pathways or mechanisms of innate immunity of insects in response to infection. We also emphasize the current state of knowledge regarding fungal strategies that counter the host immune response. Finally, this review discusses the coevolutionary arms race between entomopathogens and insect host immune systems.

2. Invasion of entomopathogenic fungi and interactions on the surface

Entomopathogenic fungi typically infect their host insects by direct penetration of the external cuticle (Vega et al., 2012). The first step is adhesion of fungal conidia to the host surface. This occurs with the aid of mucilage and adhesive proteins (Wang and St Leger, 2007). Under appropriate conditions and given adequate

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nutrient availability, fungal spores can germinate on the insect cuticle and form a specialized structure called appressorium (Wang and Wang, 2017), then breach the insect cuticle via cuticle-degrading enzymes (Beys da Silva et al., 2010). The fungi then enter the insect hemocoel, where the fungal cells absorb nutrients, produce toxins, destroy host cells, and eventually kill the insect. Next, transmission is achieved by sporulation from the cadaver (Anderson et al., 2011; Mishra et al., 2015).

The insect cuticle is a complex multilayered structure composed of macromolecules such as tanned proteins and chitin that provides a series of challenges to the entry of pathogens (Leger et al., 1991). As the first physical barrier to pathogen infection, physiological environment of the cuticle is inhospitable for pathogens: It has low water activity, few nutrients, and contains antimicrobial compounds. Entomopathogenic fungi can actively penetrate the host cuticle by physical and enzymatic mechanisms.

The insect cuticle is hydrophobin, and some chemicals produced by host itself or the resident microbial community prevent fungal conidia from adhesion and germination (Greenfield et al., 2014; Ortiz-Urquiza and Keyhani, 2013; Toledo et al., 2011). For example, *Bacillus pumilus* on the surface of *Delphacodes kuscheli* and *Dalbulus maidis* cuticles inhibit *B. bassiana* conidial germination (Toledo et al., 2011). In addition, some social insects use microbicidal secretions to avoid fungal infection and disease spread. For example, ants secrete antimicrobials in response to fungal infection (Fernandez-Marin et al., 2006). *Tribolium castaneum* is resistant to *B. bassiana* infection by secreting defensive compounds called benzoquinones (Pedrini et al., 2015). The insect cuticle thus plays an important role in defense against fungi.

On the pathogen side of the arms race, fungi have evolved specific mechanisms that circumvent these defenses. Successful adhesion and fast germination of fungi spores on the insect cuticle surface are important attributes of virulent fungal strains. Fungal blastospores can produce mucilage that enables their adhesion on the insect cuticle, and it appears to provide a relatively stable underwater condition for spore germination. Entomopathogenic fungi are known to possess hydrophobin (*hyd*) or adhesion (*mad1*and *mad2*) genes, some of which are responsible for hydrophobicity, adhesion and virulence of strains (Holder et al., 2007; Sevim et al., 2012; Wang and St Leger, 2007; Zhang et al., 2011). In addition, other cell wall proteins play critical roles in adhesion and stress management. Examples are the non-hydrophobic cell wall proteins CWP10 and CP15, which are involved in the resistance to thermal and oxidative stresses (Li et al., 2010; Ying and Feng, 2011).

For entomopathogenic fungi, crossing the cuticle protein-chitin barrier is critical for infection. Entomopathogenic fungi produce many enzymes, including proteases, chitinases, lipases, esterase, phospholipase C, and catalase (Beys da Silva et al., 2010; Santi et al., 2010; Wang et al., 2009, 2011a; Wei et al., 2017), which are virulence determinants and necessary for penetration of the insect cuticle (Nunes et al., 2010; St Leger et al., 1998). Proteases, such as a virulence factor Pr1 (Shah et al., 2005), can enhance fungal spore germination and, together with other enzymes (e.g., Pr2 and chitinases) degrade the cuticle to enable the fungus to gain access to the hemocoel (Ortiz-Urquiza and Keyhani, 2013; Santi et al., 2010; Small and Bidochka, 2005). Comparative genomics studies show that entomopathogenic fungi have more genes encoding proteases and chitinases compared to plant pathogens (Gao et al., 2011; Hu et al., 2014; Xiao et al., 2012; Xiaoi et al., 2013; Zheng et al., 2011). This is likely an adaptation to the amount of chitin in the insect cuticle, and the expansion of proteases might reflect the insect host range of entomopathogenic fungi. In addition, internal lipids of the insect cuticle can also alternatively act as antifungal barriers. Thus, lipid assimilation can be considered as forming a coevolutionary web between insect hosts and entomogenous fungi (Keyhani, 2017).

Insect hosts exhibit a vast array of responses to counter entomopathogenic fungi during penetration (Fig. 1). In a bid to reduce hemolymph leakage during fungal penetration process, the insect hemostatic response is activated (Vilmos and Kurucz, 1998). Insect hemostasis involves clotting proteins such as lipophorins, vitellogenin-like proteins, and calcium-dependent transglutaminases containing a cysteine-rich domain homologous to the von Willebrand factor of mammals (Vilmos and Kurucz, 1998). In *Drosophila*, transglutaminase anchors the invading microbes to microclots to allow killing in the hemolymph by antimicrobial peptides (AMPs) and hemocytes (Wang et al., 2010). In addition, ecdysis can greatly improve insect larval survival when fungi have not reached the underling new cuticle; in this case, the pathogens are shed with the old cuticle (Vestergaard et al., 1995).

In addition to hemostasis and ecdysis, the cuticle also contributes to insect host immunity (Dubovskiy et al., 2013a). The epidermis initiates the integumentary defense by protease inhibitors and melanin synthesized by epidermal cells. An array of protease inhibitors are expressed by insects. One example is insect metalloprotease inhibitor (IMPI) that is involved in resistance to the entomopathogen-specific metalloproteases (Qazi and Khachatourians, 2007; Vilcinskas, 2010). During fungal penetration of the cuticle, the prophenoloxidase (proPO) cascade in epidermal cells is activated to synthesize melanin. Melanin and its precursors play important roles in protecting the insect cuticle against fungi invasion, not only limiting the growth of certain fungi but also preventing synthesis of cuticle-degrading enzymes (Söderhäll and Ajaxon, 1982; Yassine et al., 2012; Zhang et al., 2017). Moreover, it was recently found that prophenoloxidase activation is required for survival to infection by fungi and Gram-positive bacteria in *Drosophila* (Binggeli et al., 2014). AMPs are also detected in the integument (Brey et al., 1993). Hemocytes can penetrate the basement membrane and reach the fungi penetration sites to release AMPs (Gunnarsson, 1988). Although the extent to which AMPs in most insects contribute to defense against entomopathogenic fungi remains unclear, recent studies showed that *Bombyx mori* cecropin A and gloverin2 have high antifungal activity against entomopathogenic fungus *Beauveria bassiana* both *in vitro* and *in vivo* (Lu et al., 2016, 2017). The AMPs are likely involved in the early recognition and destruction of fungal structures in the external cuticle (Butt et al., 2016).

Once entomopathogenic fungi successfully gain entry into the insect hemolymph, they propagate as yeast-like blastospores or hyphal bodies. Then blastospores rapidly colonize the hemocoel, where they encounter host cellular and humoral defenses. The invading pathogen is recognized via pattern recognition receptors (PRRs) including peptidoglycan recognition proteins (PGRPs), Gram-negative-binding proteins (GNBPs), β-glucan-binding proteins (βGRPs), C-type lectins and others (Stokes et al., 2015). Carbohydrate heterogeneity at the fungal surface plays a critical role in nonself detection by insect hosts (Wanchoo et al., 2009). Compared with fungal conidia or hyphae, hyphal bodies appear to possess fewer carbohydrate epitopes, which allow them to avoid recognition by the host immune system in the hemocoel (Pendland and Boucias, 1993; Wanchoo et al., 2009). In addition, fungal protease PR1 could be detected and lead to cleavage of the insect host Persephone protease and then activates the serine protease cascade acting upstream of the Toll pathway (Ligoxygakis et al., 2002; Gottar et al., 2006).

3. Behavioral and social immunity to fungal infection

Entomopathogenic fungi have a dramatic impact on their insect hosts, and the survival of insects depends on their defense