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# THE ROLE OF HYDROLYTIC ENZYMES PRODUCED BY ENTOMOPATHOGENIC FUNGI IN PATHOGENESIS OF INSECTS MINI REVIEW

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**Abstract:** Entomopathogenic fungi are a major component of integrated pest management techniques as biological control agents against insect pests and other arthropods in horticulture, forestry and agriculture. Fungal virulence of different entomopathogenic fungi, like *Beauveria bassiana*, *B. brogniarti*, *Metarhizium anisopliae, Isaria fumosoroseus* and *Lecanicilium lecanii*, has been mostly associated with cuticle-degrading enzymes that can be regulated depending on nutrient conditions. These enzymes are pointed out as important in the infection process, since they hydrolyze polymer protein, chitin and lipid complexes, the major components of the insect's cuticle. Establishing a correlation between the production of these enzymes and the virulence and also understanding of their action mechanism could be useful for producing more effective and safer environmental micoinsecticides.The purpose of this study is to synthesize existing data on the enzymatic complex produced by entomopathogenic fungi, its mechanism of action and its role in microbiological control of insects.

***Key words****: chitinases, proteases, lipases, cuticle*

## INTRODUCTION

Chemical insecticides are usually applied to control insects, but high tolerance to most insecticides and associated environmental problems have limited their use. In recent decades, sustained efforts for development of alternative non-chemical strategies have been made. Entomopathogenic fungi are well-known biological control agents of insect pests that have broadly replaced the chemicals used in biopesticides for agricultural purposes (Charnley & Collins, 2007; Matias-Montesinos et al., 2011; Mondal et al., 2016). Entomopathogenic fungi infect the insect through the cuticle, penetration of this is the result of combined action of mechanical force and the action of enzymes secreted by the fungus (St Leger et al., 1991; Sanchez-Perez et al., 2014). Entomopathogenic fungi produce a variety of destructive enzymes and metabolites which act as toxins, playing an important role in the infectious process and provoking a series of symptoms in the insect. Extracellular enzymes, including proteases, chitinases, and lipases are shown to be important pathogenicity/virulence factors that can degrade the main chemical constituents of the insect cuticle but also they can also be important during adhesion of conidia to the host cuticle and during germination and the colonization process after penetration (St. Leger et al., 1986a; St. Leger et al., 1986b; Charnley, 2003; Samuels et al., 2011).

The purpose of this brief review is to present the enzymes systems synthetized by the entomopathogenic fungi in the infective process and discuss the potential role of those enzymes as control agents of insects.

**Role of extracellular enzyme produced by enthomopathogenic fungi in insect pathogenesis**

Fungal pathogenesis represents a complex of the biochemical, physiological, and genetic processes during insect infection and the occurrence of the disease. Virulence is the ability of an entomopathogenic fungus to cause death and can be defined as a process involved in insect death during pathogenesis (Bidochka & Khachatourians, 1990; Charnley & St. Leger, 1991; Mondal et al., 2016).

The infection process of entomopathogenic fungi on insects comprises several stage the most important being penetration of the cuticule utilizing enzymatic and/or physical mechanisms (Charnley & Collins, 2007; Samuels et al., 2011; Mondal et al., 2016). The insect cuticle is a highly heterogeneous structure that can vary greatly in composition even during the various life-stages of a particular insect (figure 1) (Ortiz-Urquiza & Keyhani, 2013). The insect cuticle is composed of wax, protein, chitin associated with lipids and phenolic compounds, these representing a significant barrier to the invading fungus. The entomopathogenic fungus can produce diverse enzymes as virulence factors in response to different insects. Several studies have carried out the biochemical characterization of cuticle degrading enzymes such as chitinase, protease, and lipase, the key enzymes involved in the process of pathogenesis, has been carried out to understand the host-pathogen interaction (El-Sayed et al., 1989; Charnley & St. Leger, 1991; Cheong et al., 2016). Comparisons between isolates for pathogenicity and production of enzymes may only reveal the great variability within a species for numerous factors, many of which may influence but be unrelated to cuticle-degrading enzyme activity.

The production of cuticle-degrading enzymes has been proposed as an important attribute determining the virulence of the entomopathogenic fungi toward their hosts. St. Leger and co-workers, (1986a and 1986b) have shown that entomopathogenic fungi produce a wide range of cuticle degrading enzymes; they supported that although proteases initiate degradation, these enzymes act synergistically with chitinases in the solubilization of the insect cuticle. Studies of Gupta et al. (1994) supports the notion that cuticle degrading enzymes may determine not only specific virulence parameters, but also host specificity.

Most studies were focused on cuticle-degrading enzymes produced by entomopathogenic fungi and on extracellular activities of them (St. Leger et al., 1986a; St. Leger et al., 1986b; Charnley & St. Leger, 1991; Bidocka et al., 1993; Charnley, 2003; Ali et al., 2009; Mondal et al., 2016). Experiments of Chui-Chai et al. (2012) showed higher insecticidal efficacy and hydrolytic enzyme activities of *Metarhizium* isolates compared with *Beauveria* isolates. Therefore, they suggested that the insecticidal effects of entomopathogenic fungi could be directly linked with the activity of cuticle hydrolytic enzymes. Enzyme secretion by entomopathogenic fungi may be involved in the degradation of cuticular polymers during pathogenesis, assisting in the penetration of the insect exoskeleton and providing nutrients for fungal growth. These enzymes can act synergistically, helping fungi to control insect pests and pathogens that attack productive crops, and offer potential economic benefit to agribusiness.

Establishing a relationship between the production of hydrolytic enzymes and the virulence of entomopathogenic fungi may be useful in developing of screening methods for identifying new isolates with increased virulence and also for the development of bioproducts based on them (Matias-Montesinos et al., 2011).

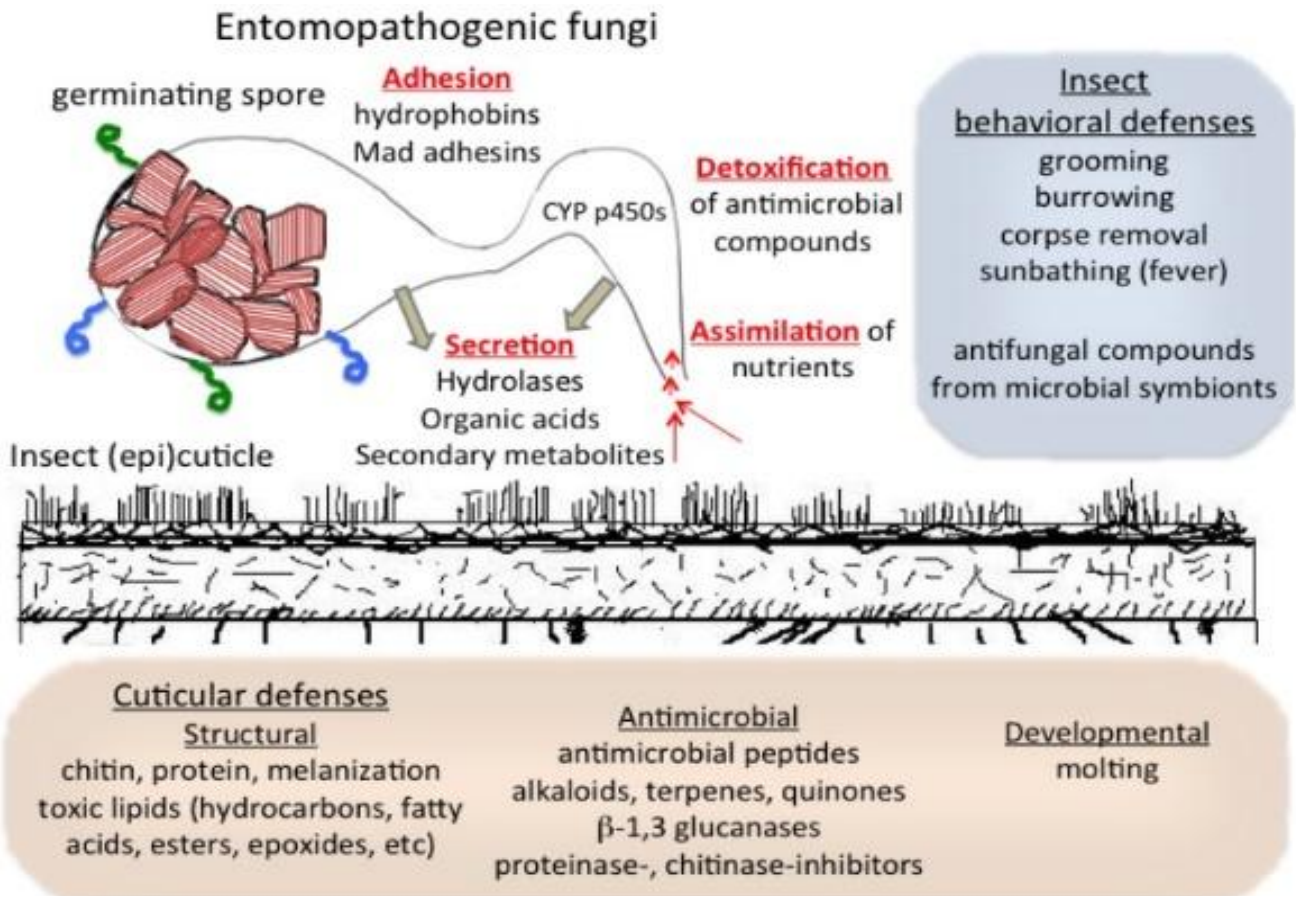


**Figure1**. Overview of surface interactions between entomopathogenic fungi and the insect cuticle and host behaviors (according to Ortiz-Urquiza & Keyhani, 2013)

**Proteases secreted by enthomopathogenic fungi**

Because proteins constitute the majority of insects' cuticle (about 70%), proteases (endopeptidase, aminopeptidase, carboxypeptidase) attack insect cuticle before chitinases because this mask cuticular chitin microfibers, followed by lipases. Entomopathogemc fungi produce a variety of endo- and exo-acting proteolytic enzymes in culture. Many of the protease of enthomopathogens are classified as collagenases or chymoelastases that show strong homology with the subtilisin family of proteases (St. Leger et al., 1988; Chrzanowska & Kolaczkowska, 1998) A different assays have been used to detect protease production by entomopathogens in culture (St. Leger et al., 1987a) and also purification of pathogen proteases by isoelectric focusing and affinity chromatography (St. Leger *et al.*, l987a) was achieved. The high levels of peptidase activity detected in the culture filtrates of *Metarhizium anisopliae* (strain MEl), suggested a key role for this type of enzyme in cuticle penetration (St. Leger *et al.*, 1987b). Subtilisin like serine protease (designated Pr1) produced by *M.anisopliae*, is adapted to extensively degrade insect cuticular protein (St. Leger et al., 1987) and its determinant role in host invasion has been clearly demonstrated by St. Leger et al. (1988). Subsequent, Donatti et al., (2008) examined for the first time the production of subtilisin-like activity (Pr1) and trypsin-like activity (Pr2) proteases by *Beauveria bassiana* in the presence of *Rhammatocerus scistocercoides* cuticle. Those authors described that *B. bassiana* produced proteases (Pr1 and Pr2) in all media tested but the amount of secreted

proteases varied. They supported that highest levels of both protease types were found in culture supernatants from grasshopper cuticle.

Studies of Bidochka and Khachatourianis (1990) confirmed the virulence of *B. bassiana* toward *Melanoplus sanguinipes* under laboratory conditions. Furthermore, their study identified the production of extracellular protease by *B.bassiana* as a virulence factor in pathogenesis of *M. sanguinipes*. Also**,** high level of proteases produced by *B.bassiana* has been shown to be directly related to early onset of mortality in the larvae of the wax moth *Galleria mellonella* (Lepidoptera: Pyralidae) (Gupta et al., 1994).

Zare et al. (2014) founded significant differences in proteolytic activities of the *B.bassiana* isolates studied, differences between them being in accordance with the changes in their virulence, such that the higher the proteolytic activity of a given isolate, the higher the virulence of that isolate. However, other studies suggest that the enzymes produced by *B.bassiana* were expressed differently according to the type of insect cuticle and there was not clear relationship between the protease activity and virulence of this entomopathogenic fungi (Dias et al., 2008).

**Chitinases secreted by enthomopathogenic fungi**

The importance of chitinases produced by enthomophagous fungi has been highlighted through experiments with different mutants deficiente in chitinase production, from *Beauveria brongniartii*, and *M. anisopliae*, exhibited reduced virulence towards *Melolontha melolonta*, and *Dysdercus peruvianus*, respectively (Boldo et al. 2009; Matias- Montesinos et al., 2011). In their studies El-Sayed et al. (1989) reported that the greater virulence of *Nomuraea rileyi* strains on *Trichoplusia ni* larvae was associated with high levels of exochitinase and total chitinase. Additionally, studies of Matias-Montesinos et al. (2011) clearly indicate that higher virulence of the *B.bassiana* mutant 881.2 was associated with increased production of chitinase compared to wild type. Also, Kim et al., 2010 found that high levels of exochitinases in *B. bassiana* SFB-205 are associated with an increased virulence towards the aphid *Aphis gossypii*. It has been observed that N-acetyl-D-glucosaminidase, endochitinase, and exochitinase are extensively produced by *B. bassiana* and *M. anisopliae* and this was positively correlated with increased virulence against the insects (Bidochka et al., 1993; Pinto et al.1997; Fang et al., 2007).

The role of chitin metabolizing enzymes like chitin deacetylase and chitosanase of *M. anisopliae* in initiating the process of fungus-insect interaction has been evaluated for the first time by the Nahar (2004). The extracellular constitutive production of chitin deacetylase not only helps in softening the insect cuticle but also elicits defense mechanism on part of the fungus by converting its cell wall chitin into chitosan so as to evade the attack from insect chitinases.

Fang et al. (2005) proved that an overexpression of a chitinase gene (Bbchit1) enhanced the virulence of *B. bassiana* to aphids *Myzus persicae*, compared with a wild-type strain. In order to enhance the potency of *B. bassiana* further, Fang et al. (2009) used mixture of the *B. bassiana* Pr1A homolog (CDEP1) and Bbchit1 for degraded insect cuticle in vitro which proved more effective than either CDEP1 or Bbchit1 alone.

Dhawan and Joshi (2017) reported that among four isolates of *B.bassiana* studied, the highest mean chitinase activity was recorded in *B. bassiana* MTCC 4495, which showed maximum mortality of third instar larvae of *Pieris brassicae.* Similar observations were recorded by Pelizza et al. (2012) that reported that *B. bassiana* isolates with highest levels of chitinase activity was more pathogenic against *Tropida criscollaris*. On the other hand, Silva et al. (2005) tested the larvicidal effect of *M. anisopliae* isolates to *Aedes aegypti* showed that

it could not be detected none relationship between enzyme levels and insecticidal activity suggesting that other factors may be involved in the process.

**Lipases secreted by enthomopathogenic fungi**

Lipases are responsible for the hydrolysis of ester bonds of lipoproteins, fats and waxes found at the interior part of the insect integument (Ali et al., 2009; Mondal et al., 2016). The importance of lipases in the tegument penetration and breaking down process and defense mechanism in the insect has already been demonstrated (Silva et al., 2010).

Lipase production by microorganisms varies not only by the lipid source but also by its concentration. Thus, experimental studies of Silva et al. (2005) supported that the best lipid source that induced lipase production in *M. anisopliae* were rice oil, soybean oil, olive oil, sunflower oil, sesame oil and hydrogenated soybean fat. In vitro, lipase production was induced by triglyceride substrates and was not inhibited by fatty acids present in the culture medium. Hegedus and Khachatourians (1988) showed that *B. bassiana* cultured *in vitro* produced lipase when grown on a yeast extract-peptone-dextrose broth (YPD) medium. They conclude that the addition of fatty acids, such as, myristic, palmitic, stearic, oleic, linoleic and arachidic acids, inhibited both growth and lipase production but addition of olive oil induce lipase.

Dhawan and Joshi (2017) showed that the *B. bassiana* MTCC 4495 strain were more virulent to third instar larvae of *P. brassicae* exhibited the highest levels of lypolitic activity.

Silva et al. (2010) also reported that the lipase secreted by entomopathogenic fungi was involved in the initial stages of the adhesion and penetration of insects. In addition, extracellular lipase activity was found to be more as compared to protease and chitinase; hence, lipase may be considered as an important enzyme in metabolic activities of *B. bassiana*. According to Feng et al. (1998) lipase production by *B. bassiana* had little correlation to either the mortalities *Melanoplus sanguinipes*. In *B. bassiana* the lipolytic activity was proposed as a 'virulence index', contributing to the evolution of host-parasite relationship, contributing to the selection for efficient isolates in order to adapt to the insect diversity (Kaur & Padjama, 2009)

## CONCLUSIONS

The present study provides information about diversity of extracellular enzymes produced by entomopathogenic fungi. These varied from isolate to isolate, hypothesizing that the enzyme production depends on the type of host and its habitats.

Based on the data and information presented above novel biocontrol strategies can be employed by using of the cuticle degrading enzyme complex of entomopathogenic fungi for the effective control of pests in agriculture.

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