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Journey of enzymes in entomopathogenic fungi

Subhoshmita Mondal [a, b, *], Sibashish Baksi [b], Andras Koris [a], Gyula Vatai [a]

[a] *Department of Food Engineering, Faculty of Food Science, Szent Istvan University, Gödöllő, Páter Károly u. 1, 2100, Hungary*
[b] *Department of Chemical Engineering, Jadavpur University, Kolkata, 700032, India*

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ABSTRACT

Entomopathogenic fungi are well-known biological control agents of insects that have broadly replaced the chemicals used in biopesticides for agricultural purposes. The pathogenicity of entomopathogenic fungi depends on the ability of its enzymatic equipment, consisting of lipases, proteases and chitinases, which degrade the insect's integument. Additionally, the researchers studied the content of β-galactosidase, -glutaminase, and catalase within entomopathogenic fungi. With highly focused investigations on the use of enzymes for green technology, the group of entomopathogens are slowly gaining applications in these areas, even as phytopathogenic fungi (disease originator). This brief review will serve as a reference of the enzymes derived from entomopathogenic fungi and of their current and potential applications.

1. Introduction

The footprint of enzyme-mediated processes has been observed even in ancient civilization. Today, of the approximately 4000 known enzymes, 200 enzymes are used commercially. Until the 1960s, total annual sales of enzymes were only several million dollars, but since then, the market has grown spectacularly [1,2]. Today, an increasing number of enzymes are produced affordably as a result of a better understanding of production biochemistry, bioprocess technologies, and protein engineering. Depending on environmental factors such as pH and temperature, one particular enzyme can catalyse different transformations, and as a result, the commercial use of enzymes has continued to increase. Twelve major producers along with 400 minor suppliers fulfil the industrial enzyme demand globally, but not many investigations have been proposed on the development of robust lipase bioreactor systems for commercial use [3]. Enzymes of fungal origin are more preferable due to the easy removal of cells during downstream processing. In recent years, microorganisms have been used as potential sources of industrially relevant enzymes, accelerating the interest in the study of extracellular enzymatic activity in several microorganisms [4]. Many investigations and studies have been focused on the discovery and characterization of novel and naturally occurring enzymes from sources that have been overlooked [5]. Searching for novel enzymes has renewed the interest in enzyme profiles of fungal strains following isolation from diverse and hitherto unexplored habitats. The entomopathogenic fungi are considerably vary in terms of their parasitic activity and virulence. The ability of a microorganism to invade into a host is called its degree of pathogenicity, and the ability to kill its host in controlled conditions is known as virulence. Evolution has the greatest impact on the host–pathogen relation. On the other hand, insects develop different strategies through the course of evolution for defending themselves from pathogens [5]. The fungi genera have showed evolutionary adaptations that allow them to invade through the immune system of insects to complete the infection process [6]. Recently new data have been revealed about genetic evolution that is responsible for the development of host resistance or pathogen virulence (shown in Fig. 1). Apparently, entomopathogenic fungi double their genes following replication; one copy of the replicated genes keeps the original properties, while the other copies get evolutionarily modified in respect to amino acids, stimulating a functional divergence. These important adaptations carry forward its evolution and persistence within the ecosystem, where the pathogen-host interaction occurs [7].

* Corresponding author. Department of Chemical Engineering, Jadavpur University, Kolkata, 700032, India.
E-mail address: subhoshmita@gmail.com (S. Mondal).

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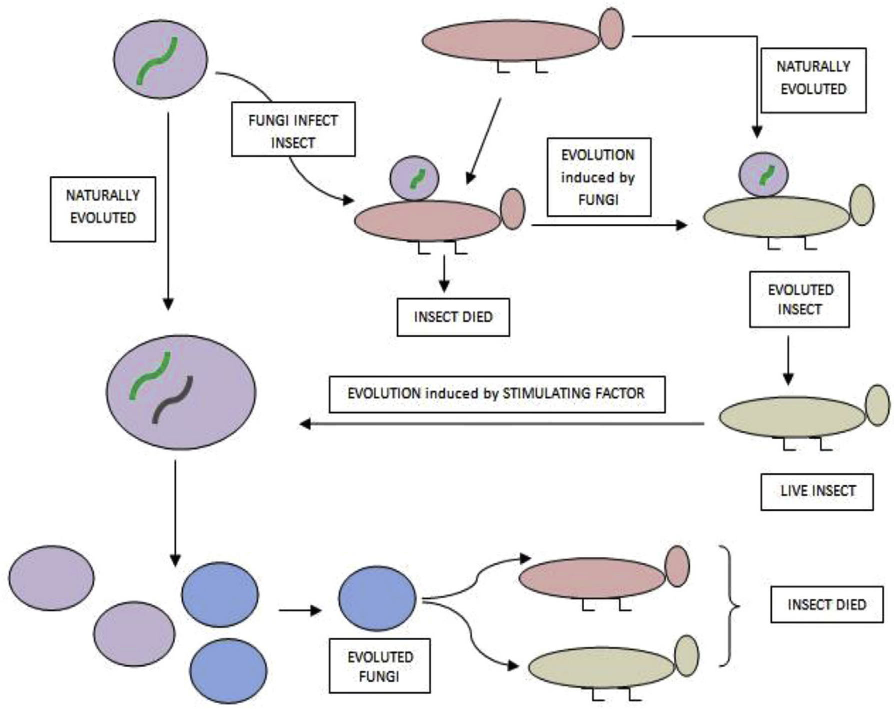

Fig. 1. Evolution of resistant fungi.

The present review discusses the evolutionary journey of many entomopathogenic microorganisms with variant enzymatic activity of different enzymes.

2. Occurrence and biology of entomopathogenic fungi

In broad terms, entomopathogenic fungi have life cycles that organise and adapt with insect-host stages and varying environmental conditions. Species and sometimes isolates within a species have been seen to behave differently depending on insect-host range, infection levels, germination rates and optimum temperature [8–10]. Members from the family of entomopathogenic fungi are generally considered to be opportunists that infect many species, including a range of insects, and host death is due to toxin production, which initiates defence responses [10,11]. Infection does not get in the way of the insect feeding system and movement, but conidia penetrate through a cavity in the abdomen of infected insects over a long period of time prior to death [8]. The families of Entomophthorales have biotrophic relationships with host insects following little or no saprophytism [11–14].

Entomopathogenic fungi are found amongst the families of Zygomycota and Ascomycota and in the class of Hyphomycetes in Deuteromycota [11], as well as in the families of Chytridiomycota and Oomycota. It is important to cite that fungal infections occur in other arthropods as well as insects and/or species that are not pests for cultivated crops. The asexually produced fungal spores or conidia that are largely responsible for infection are dispersed throughout the environment in which the insect hosts are present. When conidia get attached to the cuticle of a suitable host, they germinate following host recognition and activation of enzymatic reactions of the host as well as those of the fungal parasite [11]. The invasion of the insect body and circulatory system (hemolymph) starts once the fungus has penetrated the cuticle of the external insect skeleton. Structures and steps for the invasion of insect tissues include the formation of germ tubes, appresoria and penetration pegs [11]. The entomophthoralean fungi, with chitinuous walls resembling hyphal bodies, spread throughout the insect by penetration and utilization of host nutrients, which causes death of the host by physiological starvation 3–7 days after infection. Some entomophthoralean species initially form round-shaped protoplasts that either lack sugar residuesin the outer cell layers or veil their presence to evade recognition by insect haemocytes [8,11,15]. However, after the death of the insect host, the fungus has been found to appear from the dead host, and sporulation occurs outside the insect skeleton. Sporulation can occur internally when ambient humidity hinders external sporulation. *Metarhizium anisopliae* were seen to sporulate on the internal surfaces of the dried out hosts. The attachment structures signify that fungi continue to stay in the new hosts for subsequent transmission. Entomopathogenic fungi are found naturally; they can also be taken from the external environment and can be cultured in the laboratory. For example, the fungus *Beauveria bassiana* has been reported to exist naturally in more than 700 species of hosts [16]. However, it is quiet fortunate that *B. bassiana* is the most common natural host for almost all major insect taxa found in generally temperate regions. The fungal life cycle is defined by the infection of hosts, where the fungi build up a large population after producing huge numbers of conidia.

Entomopathogenic fungi play an important role in natural biological control agents for many insects and other arthropods and frequently behave as epizootics that significantly decrease host populations [17–20]. The significance of fungi in regulating the insect population was noticed early in recorded history by the ancient Chinese [10]. This was due to the existence of many natural epizootics and their distinct symptoms related to fungus-induced mortality [20,21]. Approximately 750 species of entomopathogenic fungi are known; among them, 85 genera have been found all through the classes of fungi [10,20,22,23]. The most important difference was observed in the method of infection; most entomopathogens infect hosts through their gut (shown in Fig. 2).

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