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REVIEW ARTICLE

Molecular interactions and genetic improvements of fungal entomopathogens

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Abstract

In the natural world, entomopathogenic fungi are crucial for maintaining the population balances of agricultural pests and disease-carrying organisms. It is impossible to fully address the limitations of mycoinsecticides for field pest management by enhancing a single fungal biocontrol property. Thus, it is desirable to use genetic engineering to increase the biocontrol capability of entomopathogenic fungi in numerous ways. By breaching the host's cuticle, they have the capability to propagate. When coupled with attempts to boost fungal virulence and stress resilience through genetic modifications, comprehending entomopathogenic fungi could improve the economic effectiveness of employing mycoinsecticides for pest control in agricultural environments. Additional research is necessary to elucidate the gene-for-gene connections in fungus-insect interaction models, given the advancing knowledge of fungal diseases in plants and humans.

Keywords

Entomopathogenic fungus; virulence; genetic management; biopesticides

Introduction

Entomopathogenic fungus has been identified as a potentially effective biocontrol substitute for chemical pesticides. There are now 10 entomopathogenic fungal species in development for pest control out of the approximately 700 known species (1). Entomopathogenic fungi, which kill insects, have ancestral ties with plant endophytes. Through evolution, they have developed unique mechanisms and biomolecules that distinguish them from other fungi, allowing them to adapt and thrive in their specialized ecological niche (2).

Entomopathogenic fungi can range from obligatory parasites to opportunistic infections that survive without live hosts. Numerous entomopathogenic fungi, particularly those belonging to the Entomophthorales order, are accountable for generating epizootics that effectively control the populations of insect pests (3). Today, a spectrum of entomopathogens are used to reduce pest and vector insects of veterinary and medical value as well as to manage invertebrate pests in glasshouses, row crops, orchards, ornamentals, turf and grass, stored goods and forestry. Oftentimes, epizootics resulting from naturally occurring fungal and viral infections induce dramatic declines in insect pest populations (4).

Recent advances in molecular biology techniques have made it possible to identify and thoroughly analyze the genes involved in the interactions between the pathogenic fungus and its host (5). Genes involved in signaling events, which accompany and regulate every stage of the infection and colonization processes are the subject of current research. Because they are believed to be predominantly involved in this necrotrophic stage of disease, efforts to understand the molecular characteristics of fungal virulence have largely concentrated on the broad array of enzymes they produce during penetration and colonization (6). Infections can gain a notable selection advantage by developing enzymes that compromise the host's physiological integrity (7).

Entomopathogenic fungi infect insect pests by penetrating their cuticle and producing enzymes like chitinases, proteases, lipases and others that break them down (8). The conidium or spore is the infectious propagule for most entomopathogens. It sticks to the host surface and germinates before breaking through the insect's exoskeleton into its hemolymph. This process allows the pathogen to infect and ultimately kill the insect host (9) (Fig. 1).

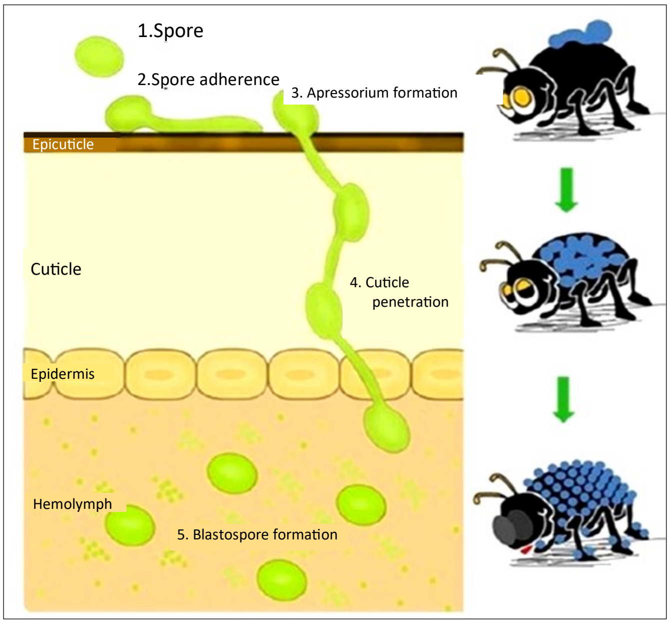

Fig. 1. Role of entomopathogenic fungi affecting the insect pest.

Fungal biocontrol agents are less popular because of their lower virulence, reduced capacity to withstand abiotic stress and ability to remain in the field, all of which are supported by the use of chemical pesticides. Increased use of chemical pesticides caused insects to become resistant and had unpredictable effects on non-target organisms (10). On the other hand, biocontrol agents show further benefits by postponing the emergence of resistance. In order to increase the virulence of entomopathogenic fungi and consequently, agricultural output, it is necessary to address the combinatorial effect of multifunctional proteins (11). Therefore, the purpose of this review is to assess how effective entomopathogenic fungi are against the particular pests that they target. We highlight recent molecular and genetic biology developments in this review (12).

Food security challenges associated with agricultural pest

According to the Food and Agricultural Organization of the United Nations, there will be a 70 % rise in global food consumption by 2050 and in order to sustainably feed this population, food production must double. A future free from hunger and malnutrition will require revolutionary reforms in agriculture and food systems. Several variables, including deteriorating soil health, temperature, precipitation and appropriate crop models, impact agricultural production (13). Unexpected outbreaks of disease and pests could limit growth and output. We can only generate two-thirds of the food grains that are lost due to pests and diseases; the remaining one-third is lost due to new agricultural crop protection techniques (14). Foods that have been stored are damaged by insects, accounting for 30 % of post-harvest losses worth over 100 billion US dollars. Over 10000 insect species damage food crops, causing a 13.6 % global yearly loss. Additionally, mosquitoes spreading malaria claim the lives of over 3 million people annually (15). Tobacco whitefly (*Bemisia tabaci*), cotton aphid (*Aphis gossypii*), diamondback moth (*Plutella xylostella*) and cotton bollworm (*Helicoverpa armigera*) are some of the most prevalent pests worldwide and infesting a wide range of crops in 135 countries. In India alone, there are 1063 pest species causing devastating effects on plants (16).

Global and Indian scenario of insect pest control

Fifty percent of Indians make their primary living from agriculture. The Indian community's main concerns regarding sustainability are undoubtedly related to land resources, population growth, agricultural loss caused by pests and post-harvest losses. Pesticides are widely used to prevent insect pests from harming agricultural crops, which helps feed the world's expanding population and is crucial in avoiding the spread of malaria and other diseases carried by insects, which kill millions of people (17). When there is a high prevalence of different pests, many synthetic pesticides are used.

Pesticides are used globally, with an estimated 4.6 million tonnes being used for vector-borne disease management and crop protection (18). Over one-third of the worldwide pesticide business comprises neonicotinoids and fipronil systemic neurotoxic insecticides, which are used to control insect pest damage.

Most insecticides target specific receptors in insects, such as the GABA-gated chloride receptor, acetylcholinesterase receptor, voltage-gated sodium and chloride channels (VGCC) and the mitochondrial complex II electron transport system. Insecticides also disrupt hormones and affect the feeding habits of insects (19). These methods are used worldwide to control insect populations efficiently.

Fungi as bio-control agents

Recent advances in molecular biology techniques have made it possible to identify and thoroughly analyze the genes involved in the interactions between pathogenic fungus and their host (20). Genes involved in signaling events, which accompany and regulate every stage of the infection and colonization processes are the subject of current research. The focus on unraveling the molecular traits of fungal virulence has primarily centered on the extensive range of enzymes they generate during penetration and colonization, as they are thought to play a significant role in the necrotrophic stage of disease (20).

Entomopathogenic fungi like *Beauveria bassiana* (Balsamo) Vuillemin, *Isaria fumosorosea* (wize) Brown and Smith, *Lecanicillium lecanii* (Zimmermann) (formerly *Verticillium lecanii*) and *Metarhizium anisopliae* (Metschnikoff) Sorokin are natural biopesticides that are environmentally safe and effective in controlling crop pests and disease-carrying insects. These fungi target a wide range of insect orders, including Coleoptera and have shown promise in replacing traditional chemical pesticides with a more sustainable and eco-friendly alternative (21).

Strategies to improve virulence of entomopathogenic fungi

Enzymes of entomopathogenic fungi

Enzymes coupled to cell walls or extracellular entities may be responsible for the enzymatic breakdown of the host cuticle (Table 1). Before 1986, extracellular enzymes that correlate to the primary chemical components of insect cuticle protein, chitin and lipids had been identified (22). However, the fungal mycelia may secrete hydrolytic enzymes like lipase, protease and chitinase which allow it to pass right through these barriers. Depending on the integument's characteristics, the secretion will vary. One of the hydrolytic enzymes of entomopathogenic fungi (EPF) that is in charge of secretions is protease, which is thought to be an element of the virulence of EPF (23).

Table 1. List of entomopathogenic fungi with corresponding enzymes

Enzymes	Entomopathogenic fungi	References
Lipase	*Fusarium oxysporum, Metarhizium anisopliae, Aspergillus flavus, Beauveria bassiana*	(53, 54)
Protease	*Metarhizium anisopliae, Beauveria bassiana, Verticilium lecanii, Paecilomyces fumsoroseus, Isaria fumsoroseus, Tolypocladium niveum*	(55)
Chitinase	*Trichoderma atroviride, Trichoderma harzianum, Tichoderma virens, Metarhizium anisopliae, Beauveria bassiana, Nomurae rileyi, Aschersonia aleyrodis, Verticillium lecanii, Isaria fmosorosea*	(56-58)
β-galactosidase	*Aspergillus* spp.*, Aspergillus foelidis, Beauveria bassiana, Aspergillus fonsecaeus, Aspergillus oryzae, Auerobasidium pullulans, Curvularia inequalis, Fusarium monilliforme, Metarhizium anisopliae, Metarhizium robertsii*	(59)
Catalase	*Lecanicillium muscarium, Fusarium oxysporum, Verticillum dahlia, Aspergillus phoenicis*	(60)
L-glutaminase	*Beauveria bassiana, Trichoderma koningii, Aspergillus flavus, Acremonium forcatum, Aspergillus wentii* MTCC 1901*, Trichoderma harzianum*	(49)

The disintegration of the host cuticle is caused by their ability to release special extracellular serine proteases, including families of exo-acting peptidases, subtilisin-like proteases and trypsin-like proteases (24) (Fig. 2). The primary hydrolytic secretion is Pr1 and the EPF's ability to cause disease is determined by the amount of active Pr1 secretion. Moreover, chitinase catalyzes the hydrolysis of chitin (polymer of N-acetyl-D-glucosamine), a crucial structural element of insect cuticles (25). Additionally, it was proposed that lipases were also involved in the hydrolysis of fats, the wax layer of insect integuments and the ester bonds of lipoenzymes. Similar to the other 2 hydrolytic enzymes, lipase is crucial for breaking down lipids in the epicuticle, the first line of defense against microorganisms that pose a threat to arthropods (26).

Molecular approaches in entomopathogenic fungi research

An investigation of the existence and mechanism of recombination in *B. bassiana* and *Nomuraea rileyi* was done by previous researchers have been stated below *B. bassiana* and *N. rileyi* are anamorphs with no gregarious sex. However, molecular genetic characterization of populations of these and other mitosporic entomopathogenic fungi has shown larger amounts of gene flow than might be expected for asexual organisms (27). In a model-based investigation on the potential effects of a lack of recombination using *B. bassiana* and *M. anisopliae*, the results revealed an intriguing possibility. It was theoretically demonstrated that mitosporic entomopathogenic fungi could reproduce exclusively through clonal lineages without any significant impact on the fitness of the populations due to the absence of the critical Muller's Ratchet effect (24).

Asexual organisms are, however, believed to be evolutionary scandals and at best, extremely rare. Most asexual organisms examined show evidence of recombination in their population genetic structure (27). Entomopathogenic fungal populations, including species like *B. bassiana* and *N. rileyi* have opportunities for genetic exchange through protoplast fusion in the hemolymph of their insect hosts (28). While genetic recombination has been observed between isolates of *M. anisopliae* co-infecting an insect (29), these events are not commonly observed. More evidence is needed to understand the frequency distribution of genotypes rather than relying on direct observations or assumptions of clonal reproduction (30).

This can be attained from molecular genetic analysis of populations. Through these methods, the prevalence of recombination has been ascertained in *B. bassiana* and *M. anisopliae* (31). In *B. bassiana*, recombination was observed between isolates sharing the same habitat when they were genetically closely related (belonging to the same genetic group); genetically dissimilar isolates (of

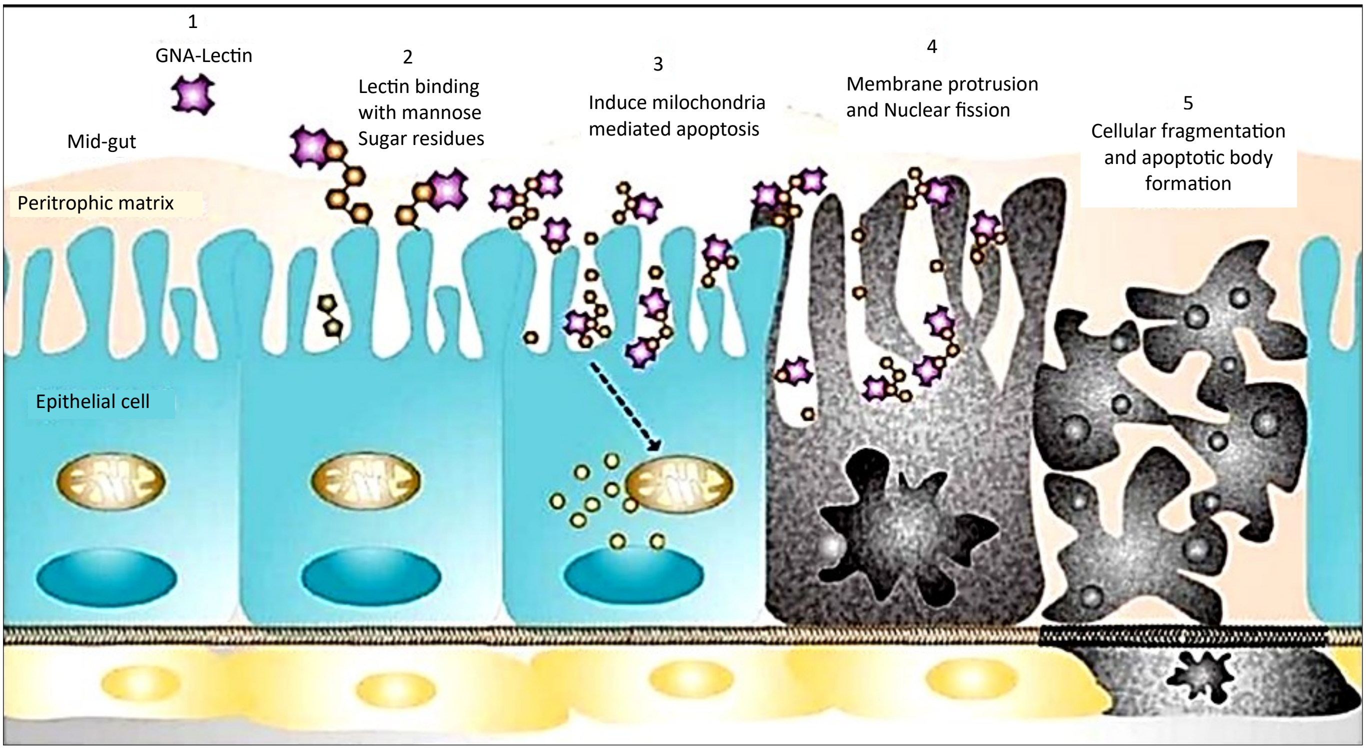

Fig. 2. Enzymatic interactions of entomopathogenic fungi.

different genetic groups), however, in the same habitat showed no recombination (32). While a population genetic approach to predict recombination has been possible with most asexual organisms, this approach does not indicate the mechanism by which it is achieved (33). The molecular genetic tests available to confirm the prevalence of recombination in a population cannot tell how recombination occurs, how often it occurs or when it occurs. Answering these questions is the next challenge in understanding the population biology of mitosporic fungi (34).

The parasexual cycle has been observed in the lab for various fungi, including *B. bassiana*, but has not been observed in nature yet (35). Demonstration of recombination in the genome of mitochondria - the cytoplasmic cell organelles, can be evidence of a mix of cytoplasm and therefore, the operation of the para-sexual cycle. In fungi, there is plentiful evidence of genetic recombination between mitochondrial DNAs originating from distinct lineages, observed in both artificial and natural environments with established heteroplasmy (36). SSCP analysis was done to investigate the prevalence and mechanism of recombination in *B. bassiana* and *N. rileyi*. Mitochondria genes, along with a nuclear gene were analyzed. The signature of the para-sexual cycle may be evident in the mitochondrial genome. Besides, alternate recombination mechanisms like gene conversion have often been reported in mitochondrial genomes in fungi (37). Research on the 15 variations within the rDNA-ITS region of 48 isolates of *I. fumosorosea* from various geographical and biological sources identified 3 distinct groups within the *I. fumosorosea* complex (38). These groups, isolated exclusively from *B. tabaci*/ *B. argentifolii*, displayed a significant level of polymorphism. The findings were further supported by the use of nine microsatellite markers obtained from *I. fumosorosea* (39).

A group of researchers examined the New Zealand *Pinus radiata* trees, along with other exotic and native trees, to find *B. bassiana* (40). They found 21 fungal isolates that resembling *Beauveria* sp. from 167 trees. These isolates were identified as *B. bassiana* Clade A through DNA sequence analysis of the EF1-α locus. Different *B. bassiana* isolates from various locations were analyzed using Polymerase Chain Reaction (PCR) based Random Amplified Polymorphic DNA (RAPD) markers (41). Researchers discovered and studied *P. reniformis* in a long-horned grasshopper in Sulawesia, Indonesia. They used morphological characteristics, sequencing of the ITS1-5.8S - ITS2 region, D1/D2 regions of 28S rDNA and a portion of the tubulin gene for their analysis (42).

A recent study suggested that conducting a thorough genetic analysis of asexual entomopathogenic fungi populations worldwide could provide insights into allopatric speciation (43). A study on nucleotide sequence variation in 6 different loci revealed hidden speciation in *M. anisopliae* (44). In a study on *B. bassiana*, researchers analysed sequences of the ITS (Internal Transcribed Spacer) of the nuclear rRNA gene and the EF1a gene (elongation factor 1). They found that a comprehensive analysis of globally distributed species complexes, along with molecular phylogenetic analysis, is an effective approach to assess species diversity. This is an essential first step in understanding the evolutionary history and historical ecology of this species (45). While *N. rileyi* has not been identified as a species complex in existing literature, a study using SSCP analysis of allelic forms of the J-tubulin gene in an epizootic population of *N. rileyi* revealed deep phylogenetic lineages. To further investigate the species structure in *B. bassiana* and *N. rileyi*, researchers examined samples from these 2 species worldwide (46).

Several studies have explored the phylogenetic placement of *B. bassiana* in relation to sexually reproducing fungi with established taxonomic status. The conclusion drawn was that *B. bassiana* exhibited a closer relationship to the Euriotalean Ascomycete, *Aspergillus nidulans*, than to Sordialean members like *Podospora anserine* and *Neurospora crassa*. Another study, using the complete sequence of the small subunit rRNA gene of mitochondrial ribosomes, suggested a closer affiliation of *B. bassiana* to pyrenomycete than to plectomycete ascomycetous fungi (47).

Research has identified the stage (teleomorph) of *B. bassiana* as *Cordyceps bassiana* within the order Hypocreales of Ascomycotina (48). Molecular phylogenetic analyses using sequences of the internal transcribed spacer (ITS) region and the gene for elongation factor 1-alpha (EF1-a) have confirmed the relationship between *B. bassiana* and its teleomorph species, *Cordyceps bassiana*/ staphylinidaecola and *Cordyceps scardbaeicola* (49). This research recommends the taxonomic placement of *B. bassiana* in Hypocreales within the family Clavicipitaceae, under the subfamily Cordycipitoideae.

Phylogenetic relatedness among some asexual entomopathogenic fungi, with a focus on the genus *Nomuraea* (50). Utilizing sequences of 5.8S, 28S rDNA and ITS, the researchers found that *Nomuraea anemonoides*, *N. rileyi* and *N. atypicola* could be well distinguished from each other. Isolates of *Nomuraea rileyi* were discovered to have a closer relationship with *B. bassiana*, *M. anisopliae* and *Metarhizium flavoviride* than with *Nomuraea anemonoides* and *N. atypicola*. Phylogenetic relationship between the 3 *Nomuraea* species using partial β-tubulin gene sequence analysis, finding that *N. anemonoides* showed a close relationship with the *Nomuraea rileyi* - *Epichloe typhina* cluster, while *N. atypicola* diverged from this clade (51). Notably, there has been no comprehensive study to date investigating the interrelationships among economically important asexual fungi with entomopathogenic habits and their taxonomic affiliations (52).

Conclusion and future prospects

The goal of biochemical and molecular studies on host-pathogen interactions is to manipulate particular fungal processes by identifying the characteristics that lead to enhanced pathogenicity. Studies conducted at the organism level involve examining the growth and behavior of different stages of both the pathogen and the host, often in relation to changes in the surrounding environment. Beneficial in advancing our knowledge of how to employ fungi to manage insects. Numerous and varied entomopathogenic fungal species have a major effect on host populations. Regretfully, not many fungal-insect systems have undergone extensive research. However, research so far has generated hypotheses to test in different host-pathogen systems and offers frameworks for additional study. It goes without saying that not everything that is discovered about a few model systems will be applicable to other systems due to the wide variety of fungi and the many ways in which they have become successful pathogens. Nevertheless, we are building a body of information that will allow us to compare systems and identify patterns in pathogenicity.

Authors' contributions

SP collected the data and drafted the manuscript. VR & PS revised the manuscript. The authors read and approved the final manuscript. All authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interests to declare.

Ethical issues: None.

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