



Advances in fundamental and applied studies in China of fungal biocontrol agents for use against arthropod pests



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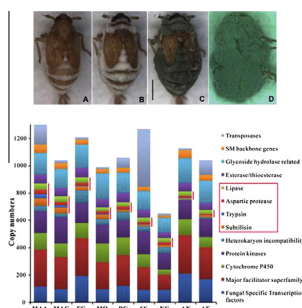
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HIGHLIGHTS

- Biocontrol potential of entomopathogenic fungi depends on fungal virulence and field adaptation.
- Genetic improvement of fungal biocontrol potential needs new knowledge in genomics and molecular biology.
- Genomic and molecular studies in China speed up for creating new-generation mycoinsecticides.

GRAPHICAL ABSTRACT



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ABSTRACT

Entomopathogenic fungi, such as *Beauveria bassiana* and *Metarhizium anisopliae*, are environmentally friendly biocontrol agents (BCAs) against various arthropod pests. We provide an overview to the past-decade advances in fungal BCA research and application in China. Since 1960s, fungal BCAs have been mass-produced for application and at present, thousands of tons of their formulations are annually applied to control forest, agricultural, greenhouse and grassland insect pests throughout the country. Apart from technical advances in mass production, formulation and application of fungal BCAs, basic studies on the genomics, molecular biology, genetic engineering and population genetics of fungal entomopathogens have rapidly progressed in the past few years in China. The completed genomic studies of *M. anisopliae*, *Metarhizium acridum*, *B. bassiana* and *Cordyceps militaris* provide profound insights into crucial gene functions, fungal pathogenesis, host–pathogen interactions and mechanisms involved in fungal sexuality. New knowledge gained from the basic studies has been applied to improve fungal virulence and stress tolerance for developing more efficacious and field-persistent mycoinsecticides by means of microbial biotechnology, such as genetic engineering. To alleviate environmental safety concerns, more efforts are needed to generate new data not only on the effects of engineered BCAs on target and non-target arthropods but also on their potential effects on gene flow and genetic recombination before field release.

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1. Introduction

There are approximately 1000 known species of entomopathogenic fungi, many of which are the ascomycetes against most, if not all, orthopteran, homopteran, lepidopteran, coleopteran, dipteran and acrina pests (Vega et al., 2012). Fungi can infect insects by direct penetration of host cuticle. Thus, unlike bacteria and viruses,

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they enable to kill not only chewing pests but also sucking pests, such as aphids and mosquitoes, with no requirement of ingestion by the hosts. Several entomopathogenic fungi, including *Metarhizium anisopliae*, *Metarhizium acridum*, *Beauveria bassiana*, *Beauveria brongniartii* and *Isaria fumosorosea* (formerly *Paecilomyces fumosoroseus*), are considered as environmentally friendly biocontrol agents (BCAs) and have been developed into a long list of mycoinsecticides and mycoacaricides (de Faria and Wraight, 2007). Such fungi are readily mass-produced and can be applied in an inundative manner rather than in classical biological control. Due to the lack of market surveys for the BCA products made by small regional companies (Feng, 2003), none of the listed products registered in many countries was from China (Li, 2007; Wang and Li, 2010). However, in terms of annual application scale, application of fungal BCAs in China, for example, the use of *B. bassiana* against pine caterpillars and the Asian corn borer, has achieved great successes (Li et al., 2010a,b). More importantly, Chinese scientists have made great progress in basic research of fungal BCAs in the recent years, including genomics and molecular biology, genetic engineering, molecular ecology, and population genetics.

2. Genomics and molecular biology

Until recently, genomic studies of fungal BCAs were far behind those done for other eukaryotes, such as model and phytopathogenic fungi. By collaborating with international colleagues, Chinese scientists initiated projects for sequencing the genomes of *B. bassiana*, *M. anisopliae*, *M. acridum*, *Cordyceps militaris* and *Cordyceps sinensis* a few years ago (An et al., 2010). A comparative genomic analysis of the broad-spectrum *M. anisopliae* and the locust-specific *M. acridum* has demonstrated that *Metarhizium* lineage may have evolved from plant endophytes or pathogens ca. 34–40 million years (MY) ago (Gao et al., 2011). The sexual stages of *Metarhizium* spp. and *Beauveria* spp. were considered to be *Cordyceps* spp. (Sung et al., 2007). However, phylogenomics analysis including the use of the genomic databases of *B. bassiana* and *C. militaris* indicated that fungal entomopathogenicity evolved around the Triassic-Jurassic boundary (200 MY) and that the *Cordyceps* lineage appeared on the earth 130 MY earlier than the *Metarhizium* lineage (Zheng et al., 2011; Xiao et al., 2012). Around one third of *Metarhizium* genes are putatively involved in fungus-insect interactions. In

comparison with plant pathogens and saprophytes, *Metarhizium* spp. possess the well expanded protein families of proteases and lipases (Fig. 1) and a larger number of secreted proteins, particularly proteases, suggesting a greater complexity and subtlety in the interactions between the insect pathogens and their hosts or environments (Gao et al., 2011). Developmental transcriptome analysis of *C. militaris* indicated that the mitogen-activated protein kinase (MAPK) pathway instead of the cAMP-dependent kinase A pathway regulates fruiting-body development (Zheng et al., 2011). Compared to the specialist *M. acridum*, the generalist *M. anisopliae* has acquired many additional proteins in the families of transmembrane receptors, lipases, chitinases, transporters, detoxification enzymes and secondary metabolites that function to cope with diverse insect hosts in various environments. In the high throughput transcriptomics analysis of both fungi, the genes and pathways involved in host recognition, appressorial differentiation and infectivity were shown to be differentially expressed during early infection, an indication for the effectors of fungal host specificity (Gao et al., 2011). The sexual stage of *B. bassiana* has been identified as *Cordyceps bassiana* (Li et al., 2001). However, unlike *C. militaris* being able to readily perform a sexual life cycle (Zheng et al., 2011), the sexual stage of *B. bassiana* is hardly induced under controlled conditions or observed in the field. Perhaps for this reason, comparative analyses of genomic structure revealed that the overall syntenic relationship could not be evident between *C. militaris* and *B. bassiana* (Xiao et al., 2012) but between *M. robertsii* and *M. acridum* (Gao et al., 2011). Future ongoing studies on the genomics and comparative genomics of more fungal BCAs will help to better understand the evolution and speciation of the fungal entomopathogens as well as the evolution of entomopathogenicity in fungal tree of life.

Before the acquisition of the fungal genome data, Chinese scientists had made their efforts or joint-efforts by collaborating with international colleagues to functionally characterize various fungal genes for understanding the pathogenicity, cell differentiation and stress responses of different insect pathogens (Table 1). Of those, identified adhesins (Mad1 and Mad2) enabled *M. anisopliae* conidia to attach to insect cuticle and plant surface, respectively, and their inactivation significantly reduced the fungal virulence (Wang and St. Leger, 2007a). Hydrophobins in *B. bassiana* and cell wall proteins, such as Cwp10, in *M. anisopliae* was also found to mediate conidial adhesion to insects (Li et al., 2010a,b; Zhang et al.,

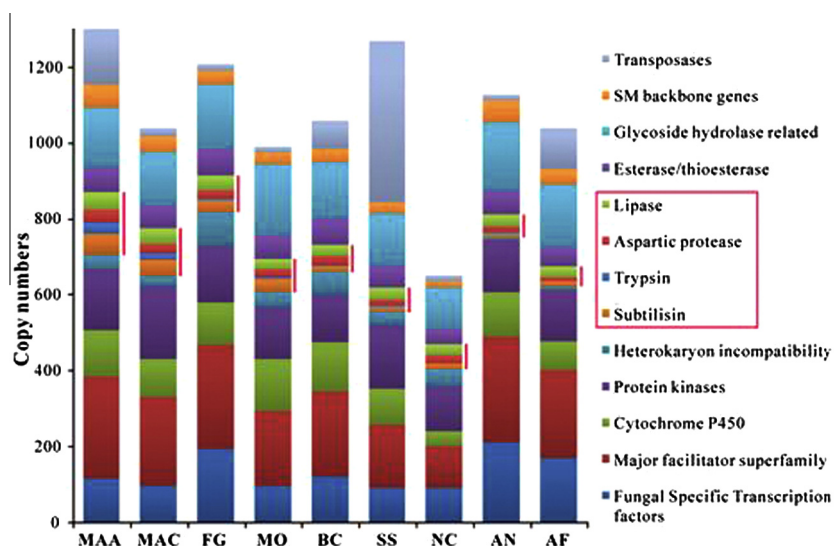


Fig. 1. Comparative genomic analysis of protein family size between *Metarhizium* spp. and other fungi (MAA: *Metarhizium robertsii*; MAC: *M. acridum*; FG: *Fusarium graminearum*; MO: *Magnaporthe oryzae*; BC: *Botrytis cinerea*; SS: *Sclerotinia sclerotiorum*; NC: *Neurospora crassa*; AN: *Aspergillus nidulans*; AF: *A. fumigatus*). The scale bars show the sizes of framed protein families.

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