Chapter 10
Beauveria bassiana: Biocontrol Beyond Lepidopteran Pests

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10.1 Introduction

Beauveria bassiana, the anamorph stage of *Cordyceps bassiana*, is a facultative cosmopolitan entomopathogen with an extremely broad host range. First discovered by Agostino Bassi de Lodi (Keswani et al. 2013) in larval silkworms, the fungus grows as a white (hyaline) mold producing single-celled, haploid, and hydrophobic conidia. RNA-sequence transcriptomic studies have revealed the startling ability of this fungus to adapt to varied environmental niches including survival and interactions outside the insect host (Xiao et al. 2012). Thus, the ecological habitat of this entomopathogen extends from the simple insect–host interaction to a broader perspective including plant rhizosphere with a well-equipped growth-promotion attribute (Bruck 2010). A diverse array of plant groups, including the agronomic, medicinal, and cash crops, serve as a host for the endophytic form of this fungus (Ownley et al. 2008; Gurulingappa et al. 2010).

Microbial control of plant pathogens and insect pests not only reduces the dependence on chemical pesticides but also increases sustainability of agriculture. Although the number of registered microbial products has increased in recent years, many potential biopesticides either have not been developed for

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Table 10.1 Various host ranges of *B. bassiana*

Fungi	Bacteria	Pests
Rhizoctonia solani	*Xanthomonas axonopodis* pv. *malvacearum*	(**Coleoptera order**) *Lathrobium brunnipes*, *Calvia quattuordecimguttata*, *Phytodecta olivacea*, *Otiorhynchus sulcatus*, *Sitona lineatus*, *S. sulcifrons*, *S. macularius*, *S. hispidulus*, *Anthonomus pomorum*, *Hylastes ater*
Pythium ultimum, *P. debaryanum*, *P. myriotylum*	*Clostridium perfringens*	(**Hymenoptera order**) *Ichneumonidae*, *Lasius fuliginosus*, *Vespula* spp., *Bombus pratorum*
Sclerotinia sclerotiorum	*Listeria monocytogenes*	(**Heteroptera order**) *Picromerus bidens*, *Anthocoris nemorum*
Alternaria solani, *A. tenuis*	*Yersinia enterocolitica*	(**Homoptera order**) *Eulecanium* spp.
Colletotrichum gloeosporioides	*Salmonella enterica*	(**Diptera order**) *Leria serrata*
Fusarium oxysporum, *F. moniliforme*, *F. graminearum*, *F. avenaceum*	*Shigella dysenteriae*	(**Lepidoptera order**) *Hepialus* spp., *Hypocrita jacobaea*, *Cydia nigricana*
Aspergillus niger, *A. parasiticus*	*Staphylococcus aureus*	
Septoria nodorum	*Enterococcus faecium*	

commercial use or have had limited success due to their pathogen or pest specificity. Moreover, its inconsistent performance across environments, or a lack of understanding of the mechanism(s) of biocontrol, results into ineffective use. Hypocrean fungi, such as *B. bassiana*, offer a quick respite to this problem as they become established as epiphytes and endophytes, thereby enhancing the induced resistance of the plant or direct disease suppression by antibiosis, competition, or mycoparasitism (Ownley et al. 2010). The detailed host range of *B. bassiana* has been shown in Table 10.1.

B. bassiana has been included in a schedule as an amendment in the Insecticide Act, 1968, for commercial production as biopesticide and published in the Gazette of India dated 26th March 1999 (Keswani et al. 2013). However, its availability is still limited to some selected states in our country (Singh 2013). The present chapter focuses on the crucial attributes of *B. bassiana* that are responsible for its biocontrol activity. In addition, certain strain improvement techniques have also been taken under consideration that would augment the in situ action of the fungus. Besides, the heavy-metal remediation by *B. bassiana* has also been discussed.

10.1.1 Entomopathogenic vs. Endophytic Nature of *B. bassiana*

B. bassiana is the most appreciated endophytic fungal entomopathogen to date, with a widespread commercial availability as a potent mycopesticide. The fungus establishes itself as an endophyte either naturally, e.g., by stomatal penetration, or with the aid of inoculation methods such as soil drenches, seed coatings and immersions, radicle dressings, root and rhizome immersions, stem injection, and foliar and flower sprays. Hence, it is widely acknowledged as a success in a variety of plants such as grasses; agricultural crops, viz., tomato, cotton, corn, and potato; the medicinal group including opium poppy, cocoa, and coffee; and trees such as *Carpinus caroliniana* and western white pine (Vega et al. 2008; Ownley et al. 2008). The most preferred protocol for endophytic colonization of *B. bassiana* includes the use of formulations augmented with solid inert carriers or diluents such as diatomaceous earth, talc, clay, vermiculite, corn cob grits, and alginate gels (Wagner and Lewis 2000; Parsa et al. 2013). Irrespective of the multifarious inoculation techniques, the extent of colonization depends on the type of plant part evaluated, the inoculation method used, and the initial spore density on the plant tissue (Posada et al. 2007; Ownley et al. 2008). While leaves respond best to foliar sprays, roots colonize only to drench inoculation with stems responding equally to both forms. A plausible yet untested hypothesis suggests that the extent of endophytic colonization correlates positively with the extent of endophyte-mediated resistance by the host.

The insecticidal attribute of *B. bassiana* recommends its use in biopesticide industry with particular reference to the malaria-causing mosquito vector. The spores of *B. bassiana* have a high affinity for the female *Anopheles* mosquito. However, for proper infestation, spore sufficiency and direct contact with the insect is required so that the fungus can efficiently penetrate and germinate the insect cuticle. Female *Anopheles stephensi*, the causal agent of human malaria in Asia, relishes on the dead and dying caterpillars heavily infested with *B. bassiana*, thereby letting themselves be infested in turn with this fungus. George et al. (2013) premiered a caterpillar sans innovative technique of using oil-formulated dried spores sprayed on a cloth that resulted in the fungal infestation efficiency of 95 % in the mosquitoes. However, the utility of *B. bassiana* larvicide can be good when we have regulated resistance in bacterial *Bacillus sphaericus* and chemical larvicides.

10.2 Formulation Types of *B. bassiana*

Novel isolates of *B. bassiana* have been recommended for developing an efficient bioformulation of biopesticide and mycopesticide. Primarily, three basic types of formulations have been proposed for *B. bassiana*.

10.2.1 Conidia Mixed with Metabolites

Eyal (1993) developed a novel formulation using a saprophytic isolate producing oosporin in high yield and evaluated its efficacy as biopesticide. This bioformulation comprising of the fungal conidia (the biologically active form of the fungus) treated with oosporin (produced by submerged culture) provides an effective pest control measure particularly against Aphididae, Delphacidae, Cicadellidae, Cercopidae, Aleyrodidae, Coccidae, Coleoptera, and Lepidoptera. Moreover, it also showed potent application against mealybugs, spider mites, and other foliar insects. The mode of application involves prilling of the mycelia as it assures the retention of the biological activity of the product until application. At the time of application, the dried prill is reactivated post-wetting and further used for treating soil, seed, root, or plant (Eyal et al. 1994). In addition, the amendment with food attractants or vegetable oils rich in oleic, linoleic, and linolenic acids in bioformulation has proved a robust application in stimulation of necrophagy (Jackson et al. 2010).

10.2.2 Extracted Protein

An alternative mode of application suggests the use of finely emulated beauverial protein extract, weighing about 5 kDa, either as granule, wettable powder, or dust combined with inert materials, such as inorganic or botanical, or in liquid form such as aerosol, foam, gel, suspension, or emulsifiable concentrates. The suggested protein content in the bioformulation ranges from 1 to 95 % of the total weight of the pesticide in dry form, while the liquid formulation consists of 1–60 % of the total weight of solids in liquid phase (Leckie and Stewart 2006).

10.2.3 Endophytic Beauveria

Another novel isolate displaying endophytic colonization, reported by Vidal and Tefera (2011), is much cashed upon for commercialization purpose. The said isolate not only enjoys a broad prey range, including root weevils, wireworms, maggots, bugs, aphids, beetles, soil grubs, root maggots, termites, and ants, but also inhabits a diverse range of hosts, such as the vegetable crops and the cash crops. The added advantage of using endophytic isolates is that they posses tolerance against environmental stresses, such as UV, high temperature, rainfall, etc. The fungal strain grows along with the host, and its additional entomopathogenic quality renders a substantial lifelong protection to the host plants, particularly to agriculture crops. The mode of incorporation involves dispersion, spray, gel, emulsion, layer,

cream, coating, dip, encapsulation, or granule. Moreover, they may also be incorporated as conventional microparticles or microcapsules.

10.3 Bioremediation of Heavy Metals: Potential Capacity of *B. bassiana* in the Biosorption of Heavy Metals

Heavy metals render a cumulative harm to the living system by denaturation of enzymes and proteins, production of ROS, displacement of essential metal ions from biomolecules leading to conformational changes, and damage of membrane integrity. Fungi are comparatively more tolerant to heavy-metal stress and thrive well in the acidified environment owing to their adaptation of several strategies of rendering the metal ions into innocuous forms. Hence, their use in bioremediation of industrial effluents and wastewaters containing heavy metals has been reported globally (Rajapaksha et al. 2004). The main principle for bioremediation is biosorption that primarily utilizes amino, carboxyl, hydroxyl, and carbonyl groups of the cell wall for metal binding as elucidated by FTIR analysis. A plethora of fungal species, such as *Aspergillus niger*, *Mucor rouxii*, *Rhizopus* spp., etc., have been reported to be utilized in heavy-metal remediation. Besides this, *B. bassiana* is reported to efficiently adsorb Cd and Pb from aqueous metal solutions. Physicochemical factors like solution pH and contact time at room temperature positively affected the rate of metal biosorption (Tomko et al. 2006).

Another strategy of heavy-metal uptake by fungal species involves immobilization followed by precipitation. Fungal metal leaching is promoted by proton efflux or metabolites with chelating properties. Metal immobilization can also occur through other processes of reductive metal precipitation such as synthesis of metallic nanoparticles that suggests its novel applications in the corrosion control of metal and stone artifacts. *B. bassiana* is reported to be endowed with the ability of heavy-metal chelation using oxalates as organic chelators. The beauverial oxalate reportedly chelates a variety of metals, involving iron (iron oxalates were directly sequestered on the fungal hyphae), copper (formation of characteristic "Liesegang rings" due to simultaneous diffusion and precipitation of copper oxalates), and silver (coprecipitation of copper and silver oxalates) (Joseph et al. 2012).

10.4 Secondary Metabolites of *B. bassiana*: A Boon in Disguise

B. bassiana produces a plethora of secondary metabolites having multifarious roles not only in pest control but in other human benefits too (Fig. 10.1). Some of the essential secondary metabolites of this fungus have been discussed below.

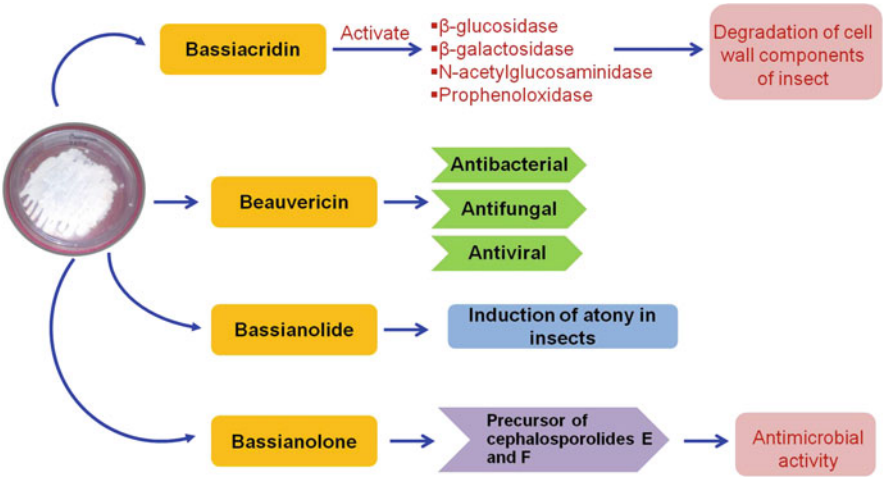

Fig. 10.1 Multifarious roles of various secondary metabolites of *B. bassiana*

10.4.1 Bassiacridin

B. bassiana is reported to secrete a protein, bassiacridin, exhibiting toxicity for locusts and comprising of about 0.1–0.3 % of the total protein content of the crude extract. The protein exhibits a monomeric structure with a molecular weight of 60 kDa and an isoelectric point of 9.5. It shows an amino acid sequence homology to the yeast chitin-binding protein, but its chitin-binding properties have not yet been determined. Being neutral for ion exchangers, bassiacridin actively participates in β-glucosidase, β-galactosidase, and *N*-acetylglucosaminidase activities. Fourth-instar nymphs of *Locusta migratoria* injected with the protein even at low dosage expressed a mortality rate of about 50 %. The root cause of mortality as revealed by the ultrastructural studies showed melanin pigmentation in the trachea, air sacs and a formation of melanized nodules on the fat bodies leading to an alteration of the epithelial cell structure of the trachea, air-bags, and integuments, thereby turning them necrotic. However, these structural changes were not reported to be caused by hemolymph pigmentation with the simultaneous production of toxic quinones. The pigmentation activity was carried out by the enzyme prophenoloxidase, activated by bassiacridin. Being novel, research needs to be targeted on the understanding of the mechanism of interaction of bassiacridin with host cells and its impact on the environment so as to include the protein as an active ingredient of biopesticides (Quesada-Moraga and Vey 2004).

10.4.2 Beauvericin

First discovered by Hamill et al. (1969), beauvericin is a cyclo-oligomeric hexadepsipeptidic ionophore produced by *B. bassiana* with a widely acknowledged insecticidal activity. The peptide binds to monovalent cations and facilitates their transport across the membrane, thereby uncoupling oxidative phosphorylation (Xu et al. 2008). Irrespective of its strong insecticidal potential, the preferential use of a potent beauvericin-producing strain as a commercial insecticide is far more advantageous than the pure compound primarily because its biosynthesis coincides with infection. During the pathogenesis phase, the developing hyphae of *B. bassiana* release extracellular hydrolytic enzymes aiding the fungal penetration of insect integument (Fan et al. 2007) and virulence factors that disables and crumbles the host immune system leading to its death. The activity of pure beauvericin in entomopathogenesis is still controversial as it was reported to be well tolerated by *Helicoverpa zea*.

Besides its role as an active insecticide, beauvericin displays a wide range of in vitro biological activities, such as antibiotic, antiviral, and cytotoxic, and an augmented antifungal activity when annexed with other antifungal agents in consortia, as mentioned below.

10.4.2.1 Antitumor Activity

The cytotoxicity of beauvericin to a human leukemia cell has been frequently reported. The peptide activates a calcium-sensitive cell apoptotic pathway. Beauvericin induces Ca^{2+} ion transport from the extracellular environment to the cytosol resulting in an augmentation of cytosolic Ca^{2+} concentration, thereby triggering the onset of an "unknown signal system" with the subsequent release of cyt c from mitochondria. The cyt c further activates the caspase inducing apoptosis (Wang and Xu 2012). Beauvericin also inhibits directional cell motility (haptotaxis) of cancer cells at sub-cytotoxic concentrations (Zhan et al. 2007)

10.4.2.2 Antibacterial Activity

Beauvericin displays a strong antibacterial activity against human, animal, and plant pathogenic bacteria with no discrimination between Gram-positive and Gram-negative bacteria. The target of action of beauvericin, contrary to other fungal antibiotics, does not involve the peptidoglycan cell wall biosynthesis, but the cell organelles and enzyme system of the bacteria. Based on its broad-spectrum antibacterial activity, beauvericin could be used to figure out the solution to drug resistance, deadly bacterial infections, and nonfood crop disease (Wang et al. 2012).

10.4.2.3 Antifungal Activity

Beauvericin, being a fungal metabolite, displays no activity as an antifungal agent. However, when used in combination with another compound, a whole new way of development and utilization of the biological activity of beauvericin is unraveled, e.g., beauvericin with ketoconazole in combination acts against *Candida parapsilosis*, one of the major culprits of neonatal mortality, while both the compounds, when applied singly, have little or no effect on this deadly fungus (Zhang et al. 2007).

10.4.2.4 Antiviral Activity

The antiviral activity of beauvericin has also been detected. Shin et al. (2009) proposed beauvericin as the most effective inhibitor of cyclic hexadepsipeptides that inhibit HIV-1 integrase. However, an effective focus on the antiviral potential of beauvericin is required for the exploration of its activity against the more fatal and epidemic disease-causing viruses, such as HBV, SARS, H1N1, and AIV (Wang et al. 2012).

10.4.3 Bassianolide

Another secondary metabolite of *B. bassiana* with insecticidal property is bassianolide, a cyclo-oligomer depsipeptide (Xu et al. 2009). Comparative sequence analysis of bassianolide synthetase (BbBSLS) suggested the presence of catalytic domains intermediate from D-2-hydroxyisovalerate and L-leucine, responsible for the iterative synthesis of dipeptidol monomer, D-hydroxyisovalerate (D-Hiv) *N*-methyl-L-leucine (*N*-Me-Leu) bound to the enzyme. Post synthesis, these domains catalyze the condensation of the monomers to form the octadepsipeptide with a 24-membered macrolactone ring. This structure further isomerizes to the final cyclic tetrameric ester form (Suzuki et al. 1977). Comparative in vitro infection studies against selected insect host establish bassianolide as a highly significant virulence factor of *B. bassiana*, inducing atony to the larvae of *H. zea* and toxicity to silkworm larvae. Knockout mutants with a targeted disruption of *bbBsls* gene were unable to produce bassianolide and showed a drastic reduction in virulence against the model insects *Galleria mellonella* and *H. zea*. However, *B. bassiana* orchestrates a definite strategy for carrying out the killing operation of insect larvae in which the metabolite production coincides with the host infection. Thus, bassianolide represents a bona fide virulence factor of the entomopathogen significantly contributing to the commercial biological insecticide preparation containing the spores of the fungus. Besides being a potent insecticide, purified bassianolide inhibits the acetylcholine-induced smooth muscle contraction, as well

as displays in vitro cytotoxic, moderate antiplasmodial and antimycobacterial activities (Jirakkakul et al. 2008).

10.4.4 Bassianolone

Ackland et al. (1985) isolated two rare metabolites, cephalosporolides E and F, as a constituent of the fermentation products of the fungus, *Cephalosporium aphidicola*, growing under sulfur-limiting conditions. Soon after this serendipitous discovery, the same process could not be mimicked in laboratory conditions nor could these metabolites be isolated from elsewhere in nature. However, Oller-López et al. (2005) unexpectedly found cephalosporolides E and F together with bassianolone, an antimicrobial precursor of cephalosporolides E and F from *B. bassiana* growing under low-nitrogen conditions. Though the in vitro antimicrobial activity of cephalosporolides E and F was completely negative, bassianolone contradicted them by displaying a high antimicrobial activity with a complete inhibition of the growth of *Staphylococcus aureus* and *Candida albicans*.

10.5 Internal Machinery that Renders the Fungal Bioformulation a Success

10.5.1 Superoxide Dismutase

The basic criteria that determine the credibility of success of a particular fungal formulation are its virulence and its tolerance to environmental stresses that drastically affect the field efficacy and persistence of fungal sprays. Stress, in any form, be it UV, heat, or drought, induces the production of reactive oxygen species (ROS) which damages the essential biomolecules, like DNA, proteins, and lipids. Fungal enzymes, particularly superoxide dismutases (SODs) capable of scavenging the ROS, establish their virulence and field persistence. SODs are superoxide species scavenging metalloproteins that offer the first line of defense against superoxide damage. The SODs function in cell response, cell differentiation and infection yielded the benefit of mycelial growth, ordered conidiation rhythm, and increased sporulation potential and timely conidial germination in fungi. Fungal SODs are Cu/Zn factored and existent mainly in cytosol or mitochondria (Mn). Mn-SOD has proven to participate in the survival and resistance of aerobic fungi to multiple stresses. A new Mn-SOD has been identified from *B. bassiana* that offers multifaceted attributes to the entomopathogen, including amplification of the SOD activity by 4–10-fold, virulence to *Spodoptera litura*, and tolerance to chemical oxidation and UV-B irradiation (Xie et al. 2010).

Two isomeric forms of BbSOD, BbSOD2 and BbSOD3, characterized as cytosolic and mitochondrial isoenzymes, dominated the total SOD activity in *B. bassiana* under normal growth conditions. The biocontrol and stress retention potential of the isoenzymes was determined by constructing knockout (DBbSOD2 and DBbSOD3) and RNAi mutants with a 91–97 % suppression of the BbSOD2 and BbSOD3 transcripts. The constructs were used in conjunction with DBbSOD2/BbSOD2, DBbSOD3/BbSOD3, and wild type. Both the mutant types displayed marked phenotypic alterations, such as delayed sporulation, reduced conidial yield, and impaired conidial quality, but little change in colony morphology. The mycelia and conidia expressed greater sensitivity to menadione- or H_2O_2-induced oxidative stress but little or no response to hyperosmolarity or elevated temperature, in contrast to the yeast mitochondrial Mn-SOD which protected the yeast cells from osmotic, oxidative, and thermal stress. While disruption of either of the genes resulted in reduced conidial yield and delayed germination, the double-gene-silenced mutants expressed defective sporulation, increased sensitivity to menadione and H_2O_2, a longer delay in conidial germination, and a decreased virulence, due to a greater loss of the antioxidative capability leading to an increased level of intracellular superoxide species. This fact suggests the additive influence exertion of the two Mn-SODs mediating fungal development, antioxidative capacity, UV resistance, and virulence. However, the increased tolerance of mycelia and conidia of DBbSOD2 to the two oxidants than DBbSOD3 suggested the greater contribution of the mitochondrial BbSOD3 to the fungal antioxidative capability.

10.5.2 Hydrophobins

The entomopathogenic *B. bassiana*, existing both as a saprobe and endophyte, comprises of several infectious propagules, such as conidia (aerial and submerged), in vitro unicellular blastospores, and in vivo insect hemolymph derived cells, hyphal bodies. The process of pathogenesis commences with the adhesive interaction between the conidial surface and the insect epicuticle by nonspecific hydrophobic interactions, mediated by the spore-coat hydrophobins. *B. bassiana* comprises two genes, *hyd1* and *hyd2*, termed as the hydrophobin genes, responsible for cell-surface hydrophobicity, adhesion, virulence, spore thermotolerance, and formation of the rodlet layer, a protective spore-coat structure. Bioinformatic and phylogenetic studies classified both the proteins as class I hydrophobins with no primary sequence homology between them. N-terminal amino acid sequencing of a rodlet layer protein suggested Hyd2 as a spore-coat component. Deactivation of *hyd1*, i.e., *dhyd1*, displayed characteristic phenotypic alterations such as apparently "bald" conidia with modified surface fascicles or bundles. The spores were hydrophobic with different surface carbohydrate epitopes and β-1,3 glucan distribution. Further, their mode of dispersal was sans water, and their virulence was also lowered, but adhesion was not affected. On the contrary, adhesion was lowered in *Dhyd2*, but the mutants were unaffected in virulence. The double-gene-silenced

mutants expressed a lack of bundles and rodlets and a drastically lower surface hydrophobicity, cell attachment, and virulence (Ying and Feng 2004; Zhang et al. 2011).

Apart from being a spore surface component, hydrophobins play a characteristic role in the physiology of the organism. The hydrophobin acts as a regulator that senses the environmental conditions, and if found suitable, the spore is allowed to germinate. Thus, at an elevated temperature, hyphal growth would be compromised, and conidial germination inhibition would be adaptive. The above explanation was derived from a distinctly impaired adhesion phenotype in single- and double-gene knockout mutants. *Dhyd1* mutant displayed a drastic reduction in virulence when topically assayed against *G. mellonella* larvae as compared to *Dhyd2* mutants though the latter one showed a prominent reduction in adhesion. The intrigue could be explained by the probable clumping of the *Dhyd1* conidia resulting in a decreased germination and virulence. In conclusion, *hyd1* and *hyd2* encode class I hydrophobins that are essential for spore-coat rodlet layer and fascicle formation. Besides, *Hyd1* forms the rodlet with *Hyd2* acting as an organizing partner (Zhang et al. 2011).

10.5.3 Primary Roles of Two Dehydrogenases in the Mannitol Metabolism and Multistress Tolerance of B. bassiana

Mannitol metabolism in *B. bassiana* corresponds with multistress tolerance and virulence. This attribute is of prime importance where a constant challenge from the various stress factors, such as UV and high temperature, is faced and, particularly in the case of the fungal bioformulation, exposed to the environment. *B. bassiana* hosts two enzymes, namely, mannitol-1-phosphate dehydrogenase (MPD) for reducing fructose-6-phosphate to mannitol-1-phosphate and mannitol dehydrogenase (MTD) for oxidizing mannitol to fructose. Intracellular mannitol accumulation relates to the increased conidial tolerance to various stresses. This fact was evidenced in single knockout mutants DBbMPD and DBbMTD where a drastic abatement in the mannitol content resulted in a steep decline in conidial tolerance to the various stresses as well as virulence ability as observed against *S. litura* larvae. Also, mannitol-supplemented nitrate-based minimal medium suppressed the colony growth and conidial germination of DBbMTD at a larger level as compared to DBbMPD (Wang et al. 2012). However, mannitol content decline was supported by trehalose accumulation. Trehalose accumulation is regulated by the enzyme trehalase. Trehalose acts as a carbohydrate store and offers a temporary respite during the stress conditions (Liu et al. 2009). However, owing to its compartmentalization, trehalose cannot replace mannitol to increase conidial thermotolerance. Conclusively, BbMPD and BbMTD represent the flag bearers of mannitol

metabolism in *B. bassiana* contributing to conidial thermotolerance, UV-B resistance, and virulence.

10.5.4 MAP Kinase Activity

MAP kinases are widely described in eukaryotes, including fungi, and are known to play essential roles in the transduction of extracellular environmental signals, regulating development and differentiation process. Filamentous fungi harbor basically three classes of MAP kinases, Fus3/Kss1, Hog 1, and Slt2, with orthologs of each of these kinases being present in *B. bassiana*. Fus3/Kss1 MAPK deals with the regulation of infection-related development or process leading to the penetration of host tissues. The Hog1 MAPK is known to be involved in the mediation of virulence and responses against various environmental stresses (osmotic, oxidative, and thermal) and fungicides. Slt2 MAPK is likely to be crucial for the fungal survival in the environment. Bbslt2 controls growth, conidiation, cell wall integrity, response to oxidative and heat stress, heterokaryon formation, secondary metabolic pathways, and virulence in the entomopathogenic fungus. The above attributes were antipodally expressed in the knockout mutant of DBbslt2, as reduction in conidial production and viability, temperature-dependent chitin accumulation but with a simultaneous chitinal sensitivity to Congo red and fungal cell wall-degrading enzymes, and decreased conidial and hyphal hydrophobicity without alteration in the hydrophobin-encoding genes, hyd 1 and hyd 2. Besides, the cell-surface carbohydrate epitopes also expressed an alteration with a change in the content of acid-soluble, alkali-insoluble, and β-glucans as well as an attenuation in virulence as observed on topical and intra-hemocoel application. The content of trehalose was also reported to be elevated in the mutated strains as a stabilizing factor during stress conditions (Zhang et al. 2009; Luo et al. 2012).

10.5.5 Role of G-Protein Signaling (BbRGS1) in Conidiation and Conidial Thermotolerance of *B. bassiana*

The chief virulence attribute of the entomopathogenic fungus involves conidial germination leading to pathogenesis and disease transmission. As in other fungi, conidiation and germination in *B. bassiana* is regulated by regulatory G-protein signaling RGS protein encoded by *Bbrgs1*. RGS functions in a G-protein-mediated signaling pathway that responds to environmental signals by regulating the switches between vegetative growth and conidiation. Activation of the gene results in the initiation of vegetative growth and termination in conidiation, while gene repression accelerates conidiation. However, the knockout mutant exhibiting the

complete loss of *Bbrgs1* gene only reduced conidial production in contrast to other fungi suggesting an alternative mechanism or other RGS proteins in addition to BbRGS1 controlling conidiation in *B. bassiana* (Fang et al. 2008). Apart from conidiation, *Bbrgs1* is actively involved in toxin synthesis, pigment production, stress tolerance, and thermotolerance. The ability to control conidiation and thermotolerance in insect pathogenic fungus as well as the ability to control the dissemination of genetically modified strains in field application has an important implication for the mass production of this biocontrol agent.

10.6 Strain Improvement in *B. bassiana*

The entomopathogenic ability of *B. bassiana* has been extensively studied and advocated in insect pest management (Roberts and St Leger 2004; Wang et al. 2004; Thomas and Read 2007). However, a major hindrance toward its commercialization and application is its slower action compared to chemical insecticides, which enables the infected insects to cause a serious damage to crops until controlled (St Leger et al. 1996). To overcome such obstacles, there have been constant efforts for the improvement of the desired strains which render their quick action and high reproducibility. Various approaches have been employed to develop enhanced *B. bassiana* strains, including the recovery of mutants after UV-light irradiation (Hegedus and Khachatourians 1995; Meirelles et al. 1997). Other reported methods involve genetic recombination (Viaud et al. 1998) and genetic constructs (Fan et al. 2007; Fang et al. 2005; Joshi et al. 1995; Sandhu et al. 2001).

Genetic engineering, involving the identification and manipulation of the virulent genes, has elevated the insecticidal efficacy and biocontrol potential of the fungus. The expression of a neurotoxin AAIT from the scorpion *Androctonus australis* and an insect cuticle-degrading protease PR1A from *Metarhizium anisopliae* has permitted a high efficiency of *B. bassiana*. When assayed against the larvae of Masson's pine caterpillar *Dendrolimus punctatus* and the wax moth *G. mellonella*, engineered strains required less spores to kill 50 % of pine caterpillars (LD_{50}) (Lu et al. 2008).

Insect cuticle is mainly composed of chitin, embedded with proteins and acts as a primary barrier against pathogen attack. *B. bassiana* produces chitinases and proteases to disintegrate insect cuticle. Two chitinases (*Bbchit1* and *Bbchit2*) have been reported in *B. bassiana* lacking a chitin-binding domain. However, hybrid chitinases were developed in which *Bbchit1* was fused to chitin-binding domains derived from plant, bacterial, or insect sources. The hybrid chitinase gene was transformed in *B. bassiana*, and the transformed strains confirmed higher levels of virulence resulting in 23 % less time to kill the targeted insects (Fang et al. 2005).

Protoplast fusion may provide as a striking method for genetic improvement of biocontrol efficacy of *B. bassiana*. Paris (1977) first described the construction of parasexual heterokaryons through a protoplast fusion between two strains of

Beauveria tenella. Till date, many intraspecific and interspecific fusions have been reported in genus *Beauveria*. The fusants obtained through protoplast fusion of *B. bassiana* with *B. sulfurescens* have been reported to possess enhanced antagonistic activity against Colorado potato beetle (*Leptinotarsa decemlineata*) and European corn borer (*Ostrinia nubilalis*) (Couteaudier et al. 1996).

T-DNA insertion mutagenesis is being used to identify and isolate the genes governing thermotolerance and osmotolerance. A pool of T-DNA inserts of *B. bassiana* have been constructed for detection of mutants deficient in thermotolerance and osmotolerance ability. Five mutants were reported which posses high conidial yield, virulence, and resistance to adverse conditions (Luo et al. 2009).

10.7 Conclusion

B. bassiana is a profusely growing saprophytic as well as endophytic fungus. However, many of its potentials remain unrealized till date. This chapter was a small attempt to enhance the knowledge of some of the beneficial attributes of *B. bassiana*. The bioformulations of this fungus will not only be realized in pathogen control but will also augment the remediation of heavy metals. These attributes of *B. bassiana* are in perfect synchronization with the environment and when optimized will lead to surplus production, thereby reducing its price and making the formulations readily available.

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