Perspectives of Microbial Metabolites as Pesticides in Agricultural Pest Management

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Abstract

In the present-day agriculture, crop protection has become an inevitable event to sustain production. Chemical pesticides are considered to be an excellent strategy to any given pest problem, but overreliance on them raised different

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environmental concerns besides being ineffective due to resistance development. At this juncture, microbial pesticides had emerged as an alternative strategy due to high target specificity and ecological safety. Although a variety of microbes (bacteria, fungi, and nematodes) are commercially available and in the process of development as well, the actual pathogenicity and host killing are achieved by the metabolites they produce. So, it is obvious that the selection of a strain of any given microbes for pest management is a function of pesticidal metabolites it produces and their bioactivity against target pest. With the advances in applied microbiology and genetic engineering, isolation and characterization of bioactive genes and their products of microbial origin had become one of the fast-growing wing of pesticide chemistry. These efforts lead to commercialization of avermectins and spinosad, the biopesticides with metabolites of microbial origin as active ingredients with wider application in pest management. This chapter includes pesticidal (insecticidal, antifungal, antibacterial, and nematicidal) activities (target pests, modes of action, chemical structures, etc.) of different metabolites produced by diverse pathogenic microorganisms of agricultural importance. The molecular modifications for improving bioactivity, biotechnological approaches, and commercial implications of these microbial origin metabolites are also discussed in view of the existing literature.

Keywords

Secondary metabolites · Microbes · Biopesticides · Insecticidal · Antifungal · Nematicidal · Formulations · Genetic improvements

Abbreviations

ATP	Adenosine triphosphate
Bt	*Bacillus thuringiensis*
CAGR	Compound annual growth rate
Cry	Crystal
EPB	Entomopathogenic bacteria
EPF	Entomopathogenic fungi
EPN	Entomopathogenic nematodes
GABA	Gamma-aminobutyric acid
GlcNAc	*N*-Acetylglucosamine
HSP	Host-specific phytotoxins
kDa	Kilodaltons
Mcf	Makes caterpillars floppy
NHSP	Non-host-specific phytotoxins
ORF	Open reading frame
Pir	*Photorhabdus* insect related
RNA	Ribonucleic acid
Tc	Toxin complex
VIP	Vegetative insecticidal proteins

1 Introduction

Microbial diversity is one of the rich resources for a variety of products and processes having vast applications in industrial, pharmaceutical, and agricultural sectors. In particular, predominant use of microbials in agriculture is targeted against insect pests and diseases as biocontrol agents. Although the microbial biocontrol of pests is reported during the mid-1990s, their action and potential are over-masked by the chemical pesticides. After "silent spring" scientists and society realized that the chemical intensive pest management is lethal to the environment and did not support safe food security for growing population. So, the recent approaches of sustainable agriculture reoriented the therapeutic pesticidal control toward preventive pest management practices with different economical, ecological, and human concerns.

The pest problems in present-day intensive agriculture make the plant protection an inevitable event. Besides, development of pesticide resistance aggravated the pest problems, and the global trade increased the problems of nonnative invasive pest species. At this juncture, restricting the use of chemical pesticides and ecofriendly protection of crop plants is possible with realizing the importance of microbial pathogens or microorganism and their products. Probably, the basic reasons behind the poor adoption of microbial pesticides by farming community are unavailability of quality commercial products, poor visualization of action under field conditions, inconsistent performance, short shelf life, lack of awareness, etc. [1]. Laborious processes involved in isolation, identification, suitable formulation, and ecotoxicity establishment of microbial pathogen are some of the backstopping issues of scientific community [2]. However, with the advent of different molecular tools, identification and characterization of microbial pathogens became easy, and the biological control intensified with microbial pathogens became reality. At present, microbial pesticides are considered as imperative alternatives to chemical pesticides with high host specificity, biodegradability, and environmental safety.

In 2013, the global biopesticide market was estimated to be approximately $3 billion which accounts to 5% of total pesticide market. This is expected to grow to more than $4.5 billion by 2023 [3], among which microbial products are the fastest-growing segment [4]. Over the years, strains of *Bacillus thuringiensis* occupied prime position in biopesticide market followed by entomopathogenic fungi (*Beauveria bassiana*, *B. brongniartii*, *Metarhizium anisopliae*, *Lecanicillium lecanii*, and *Hirsutella thompsonii*) targeting wide range of arthropod pests. Different strains of *Bacillus*, *Pseudomonas*, and *Trichoderma* are being applied against a variety of plant pathogens [5]. In the United States, 356 biopesticides are registered with a total of 57 species of microbes [1]. Whereas in developing country like India, 970 formulations with 15 species are registered by 2017 [6]. Majority of these products contain the fermented cultures of species or strain of microbial agent, and some contain their by-products or synthetic chemical analogues as active ingredient.

The toxicity or pathogenicity of any given microbial biocontrol agent against target pest is manifested by microbial origin metabolites. These metabolites either

have direct toxicity to invading cells or weaken the system there by facilitating the microbial invasion. This pesticidal activity has received greater attention in recent years due to their versatile structures and novel modes of action with fascinating target sites. The discovery of avermectins and spinosyns proved that microbial metabolites are interesting targets for identification of environmentally safe, biodegradable, target-specific, and effective pesticidal compounds [7]. There are over 23,000 known secondary metabolites [8] including fungicides (blasticidin S, polyoxin, kasugamycin, validamycin, mildiomycin, etc.), insecticides (avermectin, milbemycin, bialaphos, etc.), and miticides (tetranactin) with excellent activity against target pests (structures of some prominent pesticidal metabolites are detailed in Figs 1 and 2) indicating many other pesticidal metabolites to be uncovered [7]. However, the discovery of new metabolites is a function of keen interest for novel pesticides which depends on improvements in screening technologies, exploration in novel ecological niches, applications of genetic techniques, progress in biochemistry of pesticide sciences, and ultimately the synthetic chemistry with commercial product facet.

There is an uncountable list of pesticidal microbes and metabolites. *Streptomyces* species have a special mention in production of pesticidal secondary metabolites. In the late 1990s, about 60% of insecticidal and herbicidal compounds reported are of *Streptomyces* origin [7]. Similarly, the metabolites of *Bacillus thuringiensis* (Cry, VIP proteins, and chitinases) are also considered to be an ever-growing list of insecticidal, nematicidal, and antifungal pesticides [9, 10]. In recent past, metabolites of entomopathogenic fungi and other novel groups of microbes have gained importance due to great biodiversity and possible identification of competent pesticides [11]. Besides, the advancements in cost-effective high-throughput whole genome sequencing techniques also facilitated the exact identification of pathogenic metabolites and prediction of modes of action. In this chapter, we chiefly focused on details of important pesticidal toxins reported against economically important agricultural pests that are derived from microorganisms PN). Further improvements and applications of these metabolites as pesticides are also discussed in view of the existing literature for successful pest management strategies.

2 Secondary Metabolites of Microbial Origin with Insecticidal Properties

2.1 Insecticidal Metabolites of EPB Origin

2.1.1 Cry Toxins

Cry toxins are the most prominent and commercially used insecticidal proteins against a wide range of insect pests and nematodes also. They are constitutively expressed as water-soluble parasporal crystals during sporulation of *Bacillus thuringiensis* (Bt), a soil bacterium with more than a century of history in agricultural pest management and nearly two decades of viable application in production of pest-resistant transgenic crops [12]. Studies also reported the production of Cry toxins

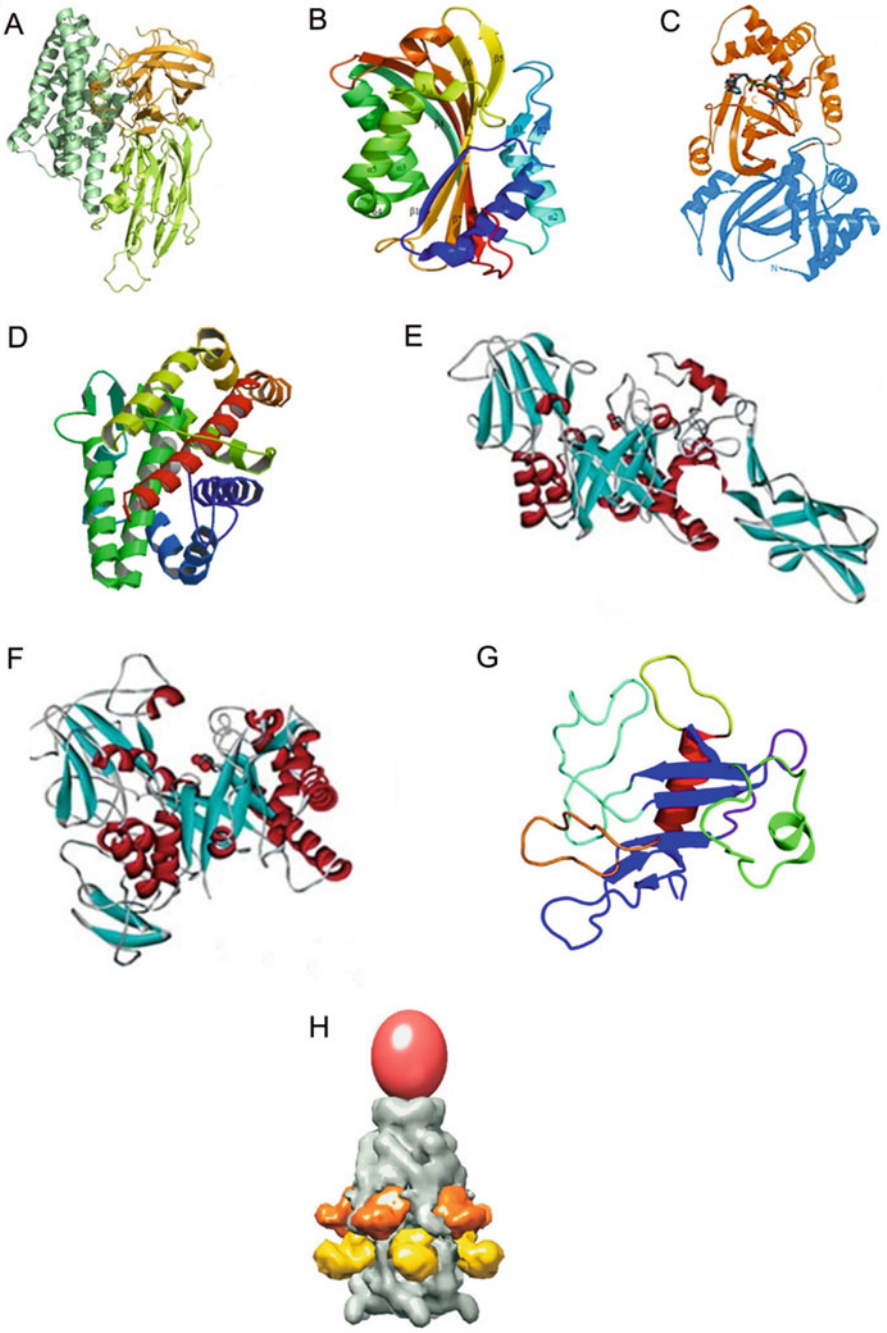

Fig. 1 3D structures of (**A**) an activated cry toxin, (**B**) Cyt toxin (adopted from Xu et al. [107]), (**C**) Vip2 toxin (adopted from Chakroun et al. [19]), (**D**) phospholipase C (adopted from Hough et

in other bacteria like *Bacillus popilliae* and *Clostridium bifermentans* [13]. Single gene-derived toxins, target specificity, risk-free against humans, nontargets and beneficials, biodegradability, etc. are the major characteristic features governing its wide usage.

Based on the primary structure (amino acid sequence), Cry toxins are classified into 67 families (Cry1 to Cry67) with more than 500 genes [14]. Structurally they are a three domain components and, after conformational alterations, interact with several pest-specific midgut proteins (cadherin, aminopeptidase-*N*, and alkaline phosphatase in lepidopteran, dipteran, and coleopteran insects, respectively) of susceptible insects [15] with sequential formation of pre-pore oligomers, membrane insertion, and pore in plasma membrane of midgut epithelial cells resulting in osmotic imbalance [13, 16]. These disruptions in midgut cells lead to immediate cessation of feeding and ultimately death (reviewed by Bravo et al. [16]) suggesting a conserved bio-toxicity. In recent past, a different mechanism of Cry toxin action by necrotic death of target cells due to disturbances in intracellular signaling is also proposed [9].

2.1.2 Cytolysins

They are reported from *B. thuringiensis* extracellular proteins produced during vegetative growth of the bacterium. Cytolysins cause cell lysis and can synergize Cry and other insecticidal proteins [17]. They chiefly interact with phospholipid receptors or phosphatidylethanolamine of cell membrane either specifically or non-specifically in a detergent-like manner, where the structural hydrophobic patches bind with amphipathic phospholipids leading to pore formation as that of Cry toxins and subsequently leading to cell death by a process called colloidal osmotic lysis [18].

2.1.3 Vegetative Insecticidal Proteins

Studies on the cultural supernatant proteins of *B. thuringiensis* and *B. cereus* led to identification of insecticidal protein called vegetative insecticidal proteins (VIPs) that are produced during vegetative growth stage of bacteria. They are completely unrelated insecticidal toxins and share no homology with Cry proteins. Till date, four families of VIP genes are identified, viz., Vip1, Vip2 specific to coleoptera and hemiptera, VIP3 specific to lepidopteran pests, and VIP4 with unknown toxicity [19]. Structurally VIP1 and VIP2 toxins contain N-terminal signal sequence, while VIP3 lacks it. Although individually toxic, in some instances, both VIP1 and VIP2 are located in single operon and are required together for bioactivity against some insects thus are considered as binary toxins [20]. As a binary toxin, VIP1 component binds with specific receptors and forms an oligomer that allows translocation of the

Fig. 1 (continued) al. [108]), (**E**) ChiA from *Serritia marcescens*, (**F**) ChiB from *Serritia marcescens* (adopted from Horn et al. [109]), (**G**) hirsutellin (adopted from Olombrada et al. [51]), (**H**) *Yersinia entomophaga* toxin complex. (Adopted from Landsberg et al. [110])

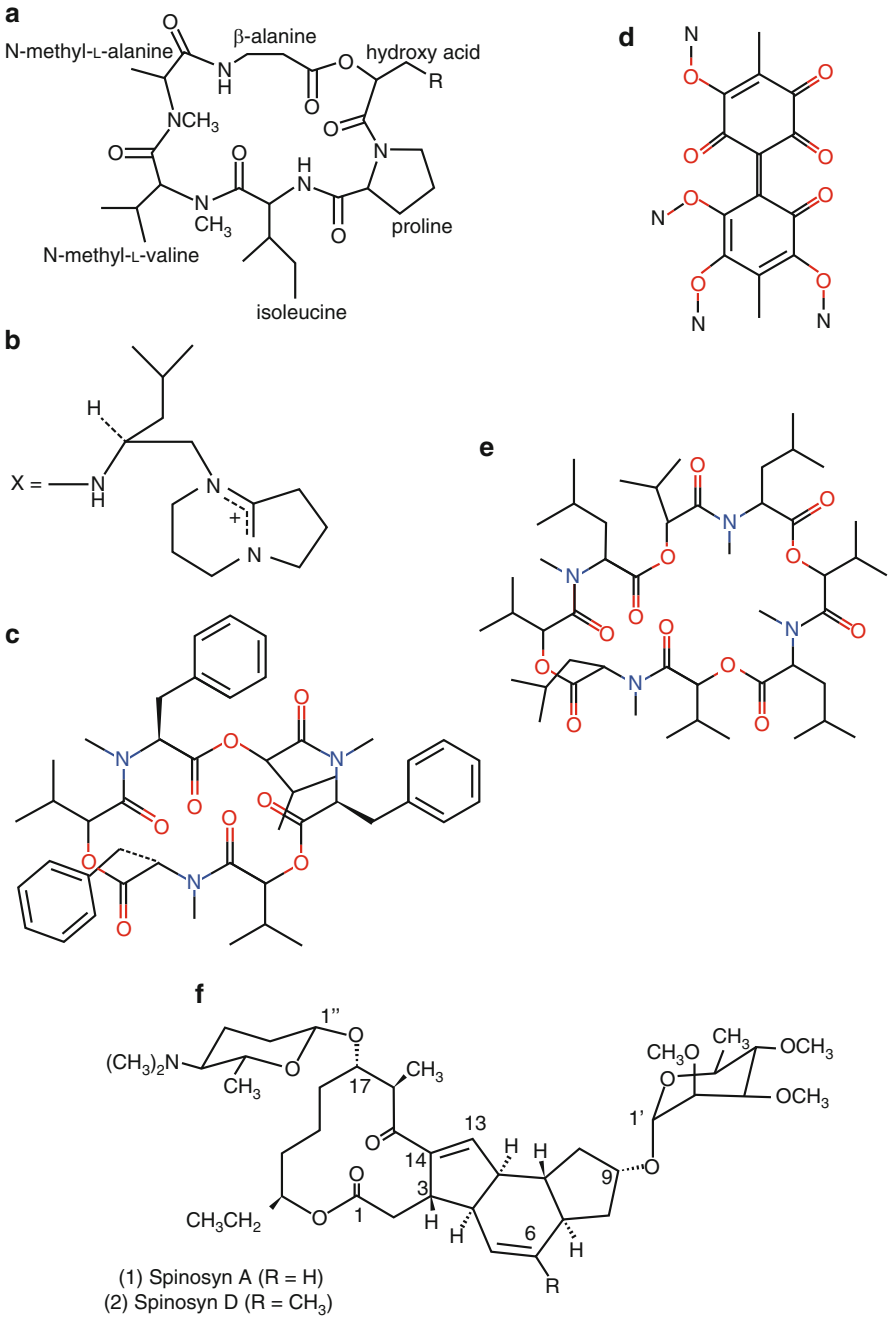

Fig. 2 (continued)

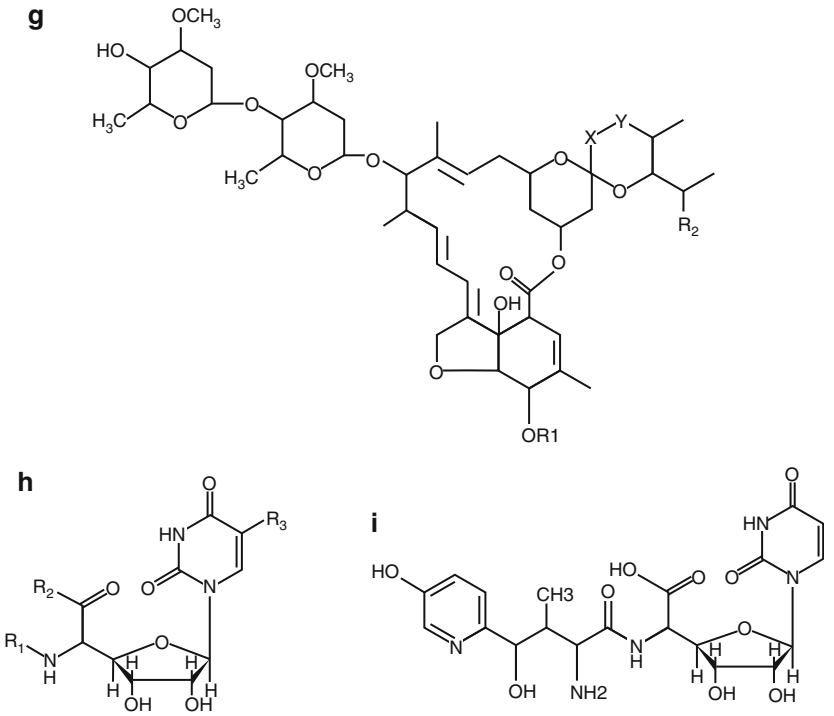

Fig. 2 Chemical structures of **(a)** destruxins (adopted from Donzelli et al. [111]), (**b**) efrapeptins (adopted from Krasnoff and Gupta [43]), (**c**) beauvericin, (**d**) oosporein, (**e**) bassianolide (adopted from Ortiz-Urquiza and Keyhani [112]), (**f**) spinosyns (adopted from Kirst [113]), (**g**) avermectins (adopted from Qiu et al. [114]), (**h**) polyoxins, (**i**) nikkomycin. (Adopted from Li et al. [115])

enzyme domain, the VIP2 component which acts as ADP-ribosyltransferase against actin, thereby preventing formation of microfilaments. The proteolytically activated Vip3 proteins upon receptor binding in midgut epithelial cells of susceptible insects cause apoptotic cell death. Although it shares similar mode of action as that of Cry toxins, binding sites are unique. Due to this differential binding sites, both the toxin genes can be used in gene pyramiding for increased target pest spectrum and delayed resistance. Interestingly, in case of insensitive insects, VIP3 proteins didn't bind with epithelial cells [21] giving its target specificity.

2.1.4 Thuringiensin

It is also known as β-exotoxin and is a thermostable metabolite of oligosaccharide nature with insecticidal activity against insect pests of Diptera, Lepidoptera, Coleoptera, Orthoptera, Hymenoptera, and Isoptera and even some nematode pests. The insecticidal activity is manifested by interfering the RNA polymerase activity by competing with ATP binding sites. The toxicity of thuringiensis is visualized only at the time of moulting and pupation [22].

2.1.5 Phospholipase C

It is a heterogenous group of esterase which is usually associated as surface protein and sometimes secreted into medium. Although produced by a large variety of Gram-positive and Gram-negative bacteria, phospholipase C is directly involved in pathogenicity of pesticidal *B. cereus* strains against coleopteran pests. These enzymes are involved in hydrolysis of glycerophospholipids which directly influence membrane dynamics and cellular signaling in particular. It is important to note that phopholipases from other than *B. cereus* showed human toxicity [23].

2.1.6 Other Metabolites from EPB in Support of Pathogenicity

In addition to Cry and VIP toxins, recent discoveries of novel insecticidal metabolites from *B. thuringiensis* like secretary insecticidal proteins, thuringiensin with oligosaccharide nature, insecticidal lipoproteins and PS201T6 strain toxicity against some hymenopteran pests (reviewed in Mnif and Ghribi [10]), and antimicrobial secondary metabolites like zwittermicin, thuricin, kurstakins, etc. show the bacterium is an eternal source of pesticidal toxins. Some unfamous EPBs like *Yersinia entomophaga*, *Chromobacterium subtsugae*, *Brevibacterium frigoritolerans*, *Pseudomonas entomophila*, etc. may also harbor novel range of pesticidal compounds.

2.2 Insecticidal Metabolites of EPN Origin

2.2.1 Toxin Complex (Tc) Proteins

The toxin complex (Tc) proteins are multiple-subunit, high molecular weight (more than 100 kDa) insecticidal toxins identified in both Gram-negative and Gram-positive bacteria [24]. They were initially identified from supernatant protein of a Gram-negative bacterium *Photorhabdus luminescens* strain W14 [25] and *Xenorhabdus nematophila* [26] the symbionts of entomopathogenic nematodes. Although the individual subunits exert toxicity, their complex showed multiple pathogenicity against variety of insect pests and so potentiates each other, an evolutionary adaptation to invade different hosts. Recent studies also reported existence of Tc complexes in some other entomopathogenic bacteria like *Serratia entomophila* [24], the causal agent of "amber" disease in New Zealand grass-grub [27]. The Tc complexes are highly conserved with respect to amino acid sequences as they are encoded by multiple copies of single Tc loci which have different open reading frames [25, 28].

The insecticidal potential of these toxins include both coleopteran and lepidopteran pests. Oral toxicity is most prominent and reported to cause progressive deformations and deteriorations in midgut epithelial cells [29]. Pathogenic *Pseudomonas* species are also reported to be efficient producers of Tcs [30, 31] and induce apoptic cell death in host through antioxidative stress and activity against macrophagosis. So, Tcs can be used as alternatives to *B. thuringiensis* Cry toxins in transgenic production [28, 32] with an additional advantage of multiple and diversified pest resistance. Additionally, cross-potentiation between different toxin complexes of varied origin is also reported which helps in the production of "stacked"

transgenic plants. However, Tcs consist of number of protein subunits which might be difficult to express together transgenically to realize full potential.

2.2.2 *Photorhabdus* Insect-Related (Pir) Binary Toxins

This is a two-component insecticidal toxin derived from *PirAB* gene with two genetic loci from *P. luminescens* and *P. asymbiotica* [33]. They are orally active against *Plutella xylostella* and different mosquito larvae. The mode of action is similar to Cry toxin pore formation and leptinotarsin as well which has amino acid similarity with juvenile hormone esterase-like protein. This also suggests neurotoxicity by promoting Ca^{2+} influx and release of neurotransmitters from presynaptic nerve.

2.2.3 Makes Caterpillars Floppy Toxins

These toxin genes (Mcf) are identified during screening of a cosmid library of *P. luminescens* strain W14 as a single 8.8 kb open reading frame (ORF). Injection of transformed *E. coli* with these toxin resulted in loss of turgor pressure in larvae of *Manduca sexta* leading to death thus the name Mcf [34]. Another ORF of Mcf gene with similar symptomology is also identified during further screening of same cosmid library with differences in N-terminal region. Further progress in genome sequencing also showed the presence of Mcf genes in *P. fluorescens*, *Providencia* sp., and *Vibrio* spp [32]. These toxins cause apoptosis in insect midgut epithelial cells and hemocytes which may cause disturbance in osmoregulation leading to typical floppy phenology.

2.2.4 Other Metabolites from EPN in Support of Pathogenicity

The insect immune system directly responds to any foreign entities by various humoral and cellular responses. Other than direct toxic metabolites, evasion of these responses of host immune system is a challenge to invade host for which a variety of antimicrobials such as proteases, lysozyme, cecropins, hemolysins, etc. are reported [35, 36–38]. In addition, an indirect pathway of inhibiting melanization, phagocytosis, and nodule formation (usually associated with cellular response of immune system) through effecting phenoloxidase cascade is also a response against cellular immunity. *Photorhabditus* has a dedicated type III secretion system for these activities, whereas *Xenorhabdus* species had several cytotoxic strategies. Indeed, the whole genome sequence of *P. luminescens* strain TT01 revealed that it encodes a huge number of insecticidal genes than any other bacteria known [38], indicating an extensive resourceful nature of these symbiotic bacteria for pesticidal toxins.

2.3 Insecticidal Metabolites of EPF Origin

2.3.1 Destruxins

They were first discovered in *Metarhizium anisopliae* later reported in majority of entomopathogenic fungi, viz., *Aschersonia* sp., *Nigrosabulum globosum*, and *Beauveria feline*, as well as some plant pathogenic fungi [39, 40]. They are classified

into families, destruxins A, B, C, D, and E, which occur as isomers or congeners with a basic structural backbone of five amino acids and an α-hydroxyl acid. They are structurally cyclic depsipeptides with insecticidal, antiviral, and phytotoxic properties. Till date, a total of 39 destruxins were identified mostly from *M. anisopliae* [41]. Some natural pathogenic analogues like roseotoxin and bursephalocids (A and B) are also identified from different sources [39].

Lepidopteran insects are reported to be highly susceptible to destruxins with typical tetanus followed by flaccid paralysis upon injection. They are mainly responsible in weakening the host immune defense and damaging muscular and digestive systems [42]. They are also known to inhibit nucleic acid and protein synthesis [23, 43]. Upon feeding they are reported to cause growth reduction and influence pupal weight and adult emergence. However, the contact toxicity of destruxins is controversial, and mode of action is still unclear. It is also important to note that destruxins E have systemic toxicity against aphid pests like *Brevicoryne brassicae* and *Myzus persicae*.

2.3.2 Efrapeptins

These are a linear peptide molecules with 15 amino acids isolated from an entomopathogenic fungus, *Tolypocladium* sp. [43], a soil hyphomycetes. They are reported to be inhibitors of intracellular protein transport system and ATPase of mitochondria [44] by acting as catalytic site competitive inhibitors with insecticidal and miticidal properties and limited antimicrobial activity. Low doses results in antifeedant and growth inhibitory activities.

2.3.3 Oosporein

Oosporeins are chiefly produced by *Beauveria* sp. and are known to be produced during infection process on cuticle [45]. They are red pigmented dibenzoquinone antimicrobial substances against Gram-positive bacteria. They result in malfunctioning of different enzymes by disorienting tertiary structures through SH group redox reaction of amino acids and also showed inhibitory effect on ATPases [46].

2.3.4 Beauvericin

Beauvericins are isolated from *Beauveria* and *Paecilomyces* species. They structurally represent hexadepsipeptide with cyclic repeats of phenylalanine and hydroisovaleric acid and are reported to be similar to membrane damaging antibiotic, enniatin [47], with adequate antibacterial property. They are cationophoric and usually increase the permeability of cell membranes by forming Na^+ and K^+ complexes. In addition, Ojcious et al. [48] reported beauvericins can act as cholesterol acyltransferase inhibitors and are also capable of fragmenting DNA.

2.3.5 Bassianolide

They are cyclo-octadepsipeptide of four molecules each of L-*N*-methyl leucine and D-α-hydroxyisovaleric acid produced by *B. bassiana* and act like ionophore antibiotic with differential reaction to cations. At high doses bassianolides are lethal, but at low doses they simply caused atonic symptoms [49].

2.3.6 Bassiacridin

It is a monomeric 60 kDa protein fraction isolated from a locust infesting strain of *B. bassiana* with β-glucosidase, β-galactosidase, and *N*-acetylglucosaminidase activities. The toxicity of bassiacridin is distinct from other *Beauveria*-originated macromolecular toxins, and at cellular level, it causes melanized spots and structural deformities on tracheal system.

2.3.7 Hirsutellin

They are non-glycosylated and thermostable proteins produced by *Hirsutella thompsonii* by a unique gene. They showed both ingestion and injection toxicity against variety of aphids, mites, and fruit flies. In some instances contact toxicity is also observed. They are cytolytic and inhibit protein synthesis by specific cleavage of rRNA and so-called ribotoxins [50, 51].

2.3.8 Organic Acids

Different EPF origin organic acids like oxlic, kojic, cylcopyazonic, fusaric, 4-hydroxymethylazoxybenzene-4-carboxylic acids, etc. have been reported to be toxic to various lepidopteran and dipteran pests. They are important in solubilizing specific cuticular proteins and can synergize proteases and chitinases.

2.3.9 Other Metabolites from EPF in Support of Pathogenicity

Some genes and their products are specifically designed to cater specific needs of EPF. For example, adhesins in *M. anisopliae* (Mad1 and Mad2) are involved in holding of spores to insect cuticle and plant cells; immune evasion genes (Mcl1) are involved in weakening the host immune system, etc. [52]. Viridoxins are nonprotein metabolites identified in *M. anisopliae* var. *flavoviride* having insecticidal activity against *Leptinotarsa decemlineata* [53]. In majority of cases and especially in species-specific pathogens, such compounds are interaction specific. In addition, some unfamous EPF species like *Agerata*, *Sphaerostilbe*, *Podonectria*, *Myriangium*, *Aschersonia*, etc. and nematicidal fungi like *Purpureocillium lilacinum* and *Pochonia chlamydosporia* are still needed to be explored for their novel pesticidal metabolites.

2.4 Insecticidal Metabolites of Actinomycetes Origin

2.4.1 Spinosyns

They are derived from actinomycetes, *Saccharopolyspora spinosa*, with two major families, viz., A and D, that are most active and unique insecticidal compounds with specific activity against Lepidoptera, Diptera, Thysanoptera, and some species of Coleoptera and Orthoptera [54]. Structurally spinosyns are macrolides containing a backbone of 21-carbon tetracyclic lactone attached with two deoxysugars (an amino sugar, tri-*O*-methylated rhamnose, and a neutral sugar, forosamine) that are essential for insecticidal activity. Except for rhamnose, a cluster of biosynthetic and bioconversion genes for spinosyn components spans 74 kb of the *S. spinosa* genome

that includes five genes for type I polyketide synthase and 14 genes for sugar biosynthesis and their attachment to the polyketide [55].

Spinosad exhibits rapid contact and ingestion toxicity. Like organophosphates and carbamates pesticides, it shows excitation of the nervous system, involuntary muscle contractions, tremors, and paralysis which are unusual for a biological product. All these effects are consistently expressed through activation of nicotinic acetylcholine receptor and GABA receptors which are unique to spinosad. The site of action of spinosad is also different from the other nerve acting neonicotinoids and avermectins with no known cross resistance. Low toxicity to nontarget organisms including humans and relatively fast degradation by photolysis make it a relatively safe insecticide [54].

2.4.2 Avermectins

Avermectins (abamectin, ivermectin, and emamectin benzoate) are a novel class of macrocyclic lactones produced by soil actinomycetes, *Streptomyces avermitilis* [56]. Although highly toxic to bees and fish, rapid photodegradation and no apparent bioaccumulation result in environmental acceptance of avermectins. They showed bioactivity against around 84 species of insect pests belonging to ten insect orders and also have nematicidal and acaricidal activity. However, its worldwide commercial use is as acaricide against variety of mite species (Tarsonemidae, Tetranychidae, and Eriophyidae) associated with horticultural, food, commercial crops, and even livestock.

At cellular level, avermectins affect neural and neuromuscular transmissions in central nervous system by disrupting the receptors for γ-aminobutyric acid and glutamic acid (GABA-gated chloride channels) resulting in chloride ion influx at neuromuscular junction. Disturbances in water balance, moulting, metamorphosis, reproductive developments, etc. are the major symptoms associated with poisoning by avermectins. Most importantly, they also have translaminar activity which provides prolonged residual pest management.

2.4.3 Polyoxins and Nikkomycins

These are a group of peptidyl nucleoside antibiotics produced by *Streptomyces* species. They inhibit the enzyme chitin synthetase by acting as competitive inhibitors thereby inhibiting chitin formation. Thus both polyoxins and nikkomycins act as insect growth regulators and have potential in controlling of various insect, mite, and fungal pests. Due to the absence of chitin in higher organisms and humans, these compounds show substantial target specificity. Structurally they are pyrimidine rings with attached dipeptide uridyl-ribose moiety and are produced in complex pathway [23].

2.4.4 Chitinases

Chitinases (EC 3.2.1.14) are the catalytic enzymes belonging to glycoside hydrolases involved in degradation of chitin, the second most abundant natural homopolymer after cellulose. They hydrolyze the β-1,4-linkage between the monomeric units of chitin chains, *N*-acetyl-D-glucosamine. These enzymes are produced by a variety

of organisms including bacteria, fungi, viruses, insects, plants, and even humans [57] either constitutively or inductively, mostly the latter by substrate. However, the release of free *N*-acetyl glucosamine from chitin chains is undertaken by a complex combination of enzymes, i.e., chitototetriose, chitotriose, and diacetylochitobiose and chitobiases and β-*N*-acetylglucosaminidases. Based on the cleavage site, these enzymes were categorized as exochitinases (cleaves chitin chains from reducing or nonreducing end of the chitin chain) and endochitinases (cleaves chitin chains at random locations) [58]. Based on amino acid similarity, chitinases are classified into three different families, viz., families 18, 19, and 20. Majority of family 18 chitinases are produced by microorganisms like bacteria, fungi, viruses, and some insects and plants. Family 19 chitinases are especially plant derived and some from bacterial like *Streptomyces griseus* [59]. Family 20 are the newly identified chitinases from *Vibrio harveyi*, *Dictyostelium discoideum*, and humans.

In any given insect pest, chitin is the major structural component of vital organs (exoskeleton, appendages, peritrophic membrane, etc.), and in the case of PPF, mycelia are made up of chitin, so chitin metabolism is one of the essential biological activity. Many studies reported that an extraneous application of microbial-derived chitinases results in damage to midgut peritrophic membrane and epithelial cells [60] effecting feeding, digestion, nutrient utilization, and ultimately growth [61]. Although no direct toxicity is observed, antifeeding effects, growth reduction, and developmental deformities are prominent against a variety of insect pests [62–67]. It is important to note that chitinases are produced by a variety of entomopathogens as a part of pathogenicity and in some cases chitinolytic strains exhibited greater toxicity over non-chitinolytic strains [60]. Studies also reported that they can be considered as biological synergists for toxicity with a variety of chemical and biological insecticides (reviewed by Subbanna et al. [61]).

2.4.5 Other Pesticidal Metabolites

Newly identified heat-stable low molecular weight proteins, Cry protein homologues binary toxins [62, 63], species-specific toxins like *Photorhabdus* virulence cassettes [27], variety of enzymes (collagenases, proteinase, proteases), and proteinaceous metabolites (surface proteins, GlcNAc-binding protein, antigen proteins, bacillolysins) are also reported to have either direct insecticidal activity or assist in pathogenicity of respective bioagents [62, 63]. One day all these compounds may have their say in pest management.

3 Secondary Metabolites of Microbial Origin with Antimicrobial Properties

3.1 Antifungal Metabolites Produced by Fungi

Secondary metabolites are small organic compounds (molecular masses generally less than 3000 Da); secondary metabolites are interesting for various reasons, e.g., their structural diversity and their potential as drug candidates or as natural

Table 1 Microbial metabolites produced by different microorganisms with fungicidal and antibiotic action against different diseases

Pesticidal metabolite	Origin microorganism	Target pest	References
Macrolactin A	*Bacillus* sp. *sunhua*	Potato scab pathogen (*Streptomyces scabies*)	Han et al. [74]
Syringomycin E	*Pseudomonas syringae*	Citrus green mold (*Penicillium digitatum*)	Bull et al. [75]
Blasticidin S	*Streptomyces griseochromogenes*	Rice blast caused by *Pyricularia oryzae*	Fukunaga [116]
Kasugamycin	*Streptomyces kasugaensis*	Rice blast (*Pyricularia oryzae)*, leaf spot in sugar beet and celery (*Cercospora* sp.), and scab in pears and apples (*Venturia* sp.)	Umezawa et al. [117]
Cryptocin	*Cryptosporiopsis quercina*	Rice blast (*Pyricularia oryzae)*	Strobel et al. [118]
Cytochalsins	*Phomopsis* sp.	*Sclerotinia sclerotiorum*, *Fusarium oxysporum*, and *Botrytis cinerea*	Fu et al. [119]
Colletotric acid	*Colletotrichum gloesporioides*	Brown spot of rice (*Helminthosporium sativum*)	Zou et al. [120]
Rufuslactone	*Lactarius rufus*	*Alternaria brassicae*, *Botrytis cinerea*	Luo et al. [121]
Oxytetracycline	*Streptomyces rimosus*	Fire blight of apple (*Erwinia amylovora*)	Finlay et al. [122]
Streptomycin	*Streptomyces griseus*	*Xanthomonas oryzae*, *X. citri*, *Pseudomonas tabaci*	Saxena [8]

pesticides. Secondary metabolites are low molecular weight compounds and well known for their ability to restrict the growth of other microorganisms. Microbes are ubiquitous and display various interactions with other living organisms mediated by a myriad of chemical interactions that exhibit diverse biological activities. There are over 23,000 known secondary metabolites, of which 42% are produced by different fungi, 42% by actinomycetes, and 16% by other bacterial species. (Details of some prominent ones are given in Table 1.) A wide range of antimicrobial compounds have been isolated from microbes and developed into drugs [8] like streptomycin (*Streptomyces griseus*), penicillin (*Penicillium chrysogenum*), and bacitracin (*Bacillus subtilis*). The ascomycetes filamentous fungus *Aspergillus fumigates* secretes more than 226 secondary metabolites including commonly studied polyketides, such as cyclic peptides, alkaloids, and sesquiterpenoids [64, 65]. Members of another class of secondary metabolites produced by *A. fumigatus*, termed the epipolythiodioxopiperazines (ETPs), are characterized by an internal disulfide bridge across a diketopiperazine ring, where the first and best characterized member being gliotoxin [66, 67]. Among different *Aspergillus* species, only those associated with aspergillosis, such as *A. fumigatus*, *A. terreus*, *A. flavus*, and *A. niger*, produce gliotoxin [68, 69].

A phytotoxin is a microbial metabolite excreted (exotoxin) or released by lysed cells (endotoxin), which, in very low concentration, is directly toxic to cells of the susceptible host. Plant-pathogenic fungi mediate their pathogenesis by virtue of biochemicals which overcome the defense mechanisms of plants and induce wilting, suppression of growth, chlorosis, necrosis, and leaf spots. The partial success of fungal biological control agents is attributed to the production of phytotoxins. These have been categorized as host-specific phytotoxins (HSPs) and non-host-specific phytotoxins (NHSPs). HSPs are active toward the plants which are host of the toxin-producing fungus and essential determinant for pathogenicity [70], while NHSPs are not primary determinants of pathogenicity and may contribute to the virulence of the fungus. The fungi which produce HSPs are of the genera *Alternaria*, *Cochliobolus*, *Leptosphaeria*, *Venturia*, *Ascochyta*, and *Pyrenophora*. AK-toxin and AM-toxin host-specific phytotoxins produced by *Alternaria kikuchiana* and *Alternaria mali* are the causative agents of black spot disease and necrotic spots on leaves of pears and apples, respectively [71]. Nonselective phytotoxins include tentoxin, a cyclic tetrapeptide produced by *Alternaria alternata*.

3.2 Antibacterial Metabolites Produced by Bacteria

Many bacteria produce antimicrobial substances such as non-ribosomally synthesized antibiotics and ribosomally synthesized proteinaceous compounds referred to as bacteriocins. Bacteriocins most often act on closely related species only and are thus of interest for application as targeted narrow-spectrum antimicrobials with few side effects. Bacteriocins that exert their antimicrobial action by self-assembling into cytotoxic phage tail-like fibers have also been observed in Gram-negative plant pathogens [72]. Bacteriocins are classified as protein bacteriocins and colicin/S-type pyocins produced by *Pseudomonas syringae* pv. *syringae* [73] effective against other *Pseudomonas* sp. Other bacteriocins include peptide bacteriocins; trifolitoxins are peptide bacteriocins produced by Gram-negative species such as *Agrobacterium tumefaciens* and *Rhizobium leguminosarum*. Macrolactin A, macrolactin A (IV), and iturin A produced by *Bacillus* sp. *sunhua* inhibited the potato scab pathogen *Streptomyces scabies* and are also fungicidal to *Fusarium oxysporum* causing dry rot disease [74]. Similarly, s Syringomycin E from *Pseudomonas syringae* ESC 10/11 controls the citrus green mold, *Penicillium digitatum* [75].

3.3 Nematicidal Metabolites Produced by Fungi

Fungi also parasitize the nematodes directly or indirectly and play major role in their biological control. Thus secondary metabolites produced by nematode-predating fungi may be exploited to develop biorational nematicides. Omphalotin A, cyclic dodecapeptide produced by *Omphalotus olearius*, is known to produce ivermectin with high selectivity [76, 77]. *Caryospora callicarpa* produces caryospomycins A, B, and C with potential nematicidal activity to pinewood nematode *Bursaphelenchus xylophilus* [78]. *Paecilomyces* sp. produces a unique nematicidal compound 4-(4′-

carboxy-2′-ethyl-hydroxypentyl)-5,6,-dihydro-6-methylcyclobuta[b]-pyridine-3,6-dicarboxylic acid which is effective against root-knot nematode, *Meloidogyne incognita* [79].

4 Genetic Improvements in Pesticidal Metabolites

Genetic improvement in pesticidal microbes is by manipulating and improving the strains for enhanced metabolite production and also for improving the efficacy of the metabolites and also for the exclusion of unwanted cometabolites. Microbial strain improvement can be done by classical genetic methods (including genetic recombination) and by molecular genetic methods [80]. Classical genetic methods for improvement in microbial metabolites rely mostly on mutation (both using physical and chemical mutagens) followed by rational screening. Rational screening is made for a particular characteristic which is different from that of final interest but easier to detect. Microorganisms possess regulatory mechanisms that regulate metabolite production and thus prevent overproduction. So the mutants are to be selected for over production of desired metabolites. Genetic recombination methods for improvement are by sexual or parasexual cross in fungi and conjugation in actinomycetes and protoplast fusion in both [81].

Molecular methods of genetic improvements require biochemical and molecular genetic tools apart from knowledge on the biosynthetic pathway and effective transformation protocols [80]. There are many methods used for molecular genetic improvement for secondary metabolite production. It is reported that the genes responsible for metabolite biosynthesis are found in clusters in the organisms and which are amplified for higher copies. Molecular improvements can also be made by targeted duplication or amplification of secondary metabolite production genes and amplification of whole pathway. The negative process of inactivating the competing pathways, silencing the regulatory genes, etc. can be also used in the genetic improvement process. Genetic improvements in different Cry toxins are discussed by different authors by using variety of molecular techniques [12, 15, 16, 82].

4.1 Genetic Improvement in Cytolysins

The *Enterococcus faecalis* cytolysin is related to antibacterial peptides termed lantibiotics which can be engineered to desired levels of cytotoxicity [83]. The lantibiotics are ribosomally synthesized, posttranslationally modified peptide containing unusual amino acids, such as dehydrated and lanthionine residues [84].

4.2 Genetic Improvement in Vegetative Insecticidal Proteins

Genetic improvement of vegetative insecticidal proteins aims in broadening the target pest spectrum along with higher toxicity to the pests. The gene vip3Aa7, its native promoter and cry3A promoter, was subcloned into *B. thuringiensis*

acrystalliferous BMB171 to generate BMB8901 and BMBvip, respectively, and the latter produced 3.2-fold Vip3Aa7 protein. Therefore, the vip3Aa7 gene under the control of cry3A promoter was transformed into strain YBT152, which was tenfold more toxic to *Spodoptera exigua* without meddling the toxicity of against *Helicoverpa armigera*. This will widen the spectrum of effect of *B. thuringiensis* against *S. exigua* apart from *H. armigera* and *P. xylostella* [85]. The vip3A(a) gene product has already demonstrated its activity against *Agrotis ipsilon*, *Spodoptera frugiperda*, *Spodoptera exigua*, *Heliothis virescens*, and *Helicoverpa zea* [21]. The deduced amino acid sequence of the vip3Aa14 gene from *B. thuringiensis tolworthi* was reported effective against *S. litura* and *P. xylostella* [86]. In a study, two genes encoding the corresponding proteins of the binary toxin, designated as vip2Ae and vip1Ae, were cloned, sequenced, and expressed in *E. coli.* Bioassays on aphids with the recombinant proteins confirmed toxicity of both the toxins in combination. The use of this gene for developing transgenic crop plants against sap-sucking insect pests is warranted [87]. Genes encoding inhibitors of insect proteases and vegetative insecticidal proteins (VIP) were considered for introduction into transgenic tomatoes in conjunction with cry1Ac gene [88].

4.3 Genetic Improvement in Chitinases

Entomopathogens can produce a series of chitinases which cause pathogenicity to insects and also fungi by degrading the cuticle/cell wall. Genetic improvement in chitinases aims in enhanced production of chitinase and its efficacy against target pests, e.g., overproduction of Bbchit1 by molecular means enhanced the virulence of *B. bassiana* on aphids. Transgenic rice plants with rice chitinase *chi11* gene were reportedly conferring resistance to sheath blight [89].

4.4 Genetic Improvement in Avermectins

Avermectins possessed activity against nemathelminthes and arthropods. Ivermectins and selamectins are semisynthetic derivative of avermectin. Ivermectins have effect against human onchocerciasis and strongyloidiasis, whereas selamectin is effective against heartworms and fleas. Genetic improvement in avermectin producing *S. avermitilis* has aimed in higher production of avermectins especially by cloning multiple copies of genes and eliminating unwanted cometabolites. *Streptomyces avermitilis* has gene *afsR2*, and incorporation of multiple copies of *afsR2* from *S. lividans* into *S. avermitilis* increased avermectin production by 2.3-fold [90]. Novel erythromycins were produced using *Saccharopolyspora erythraea* with the loading domain of the erythromycin PKS replaced by *Streptomyces avermitilis*, producer of avermectin [91]. Another regulatory gene of *S. avermitilis* which stimulates actinorhodin and undecylprodigiosin has also found stimulating avermectin production in *S. avermitilis* by 2.5-fold [92]. A troublesome toxic oligomycin in *S. avermitilis* was eliminated by transposon mutagenesis [93].

Ivermectins are synthesized by hydrogenation of avermectins B1a and B1b in the presence of catalyst rhodium chloride [94]. It was demonstrated that the ivermectins can be directly produced by replacing PKS genes of *S. avermitilis* with that of *S. venezuelae* by genetic engineering [95].

4.5 Genetic Improvement in Spinosyns

Saccharopolyspora spinosa produced secondary metabolite, spinosad which is widely used as insecticide with exceptional nontarget safety. Improved strains of *S. spinosa* were obtained by four rounds of genome shuffling of ten strains using nitrosoguanidine and UV irradiation [96]. Most of the genes involved in the biosynthesis of spinosyn are found in a contiguous 74 kb region of *S. spinosa* genome [55]. Gene duplication was also been done to increase the spinosyn yield significantly [97] which is about 288 times higher than the parent [98]. Metabolic engineering of the spinosyn gene cluster yielded 21-cyclobutyl-spinosyn A and 21-cyclobutyl-spinosyn D, which have enhanced insecticidal action against cotton aphid and tobacco budworm than that of spinosyns A and D [99].

5 Biotechnological and Commercial Implications of Pesticidal Metabolites of Microbial Origin

Plant genetic engineering paves a way for insect-resistant plants by insertion and expression of entomopathogenic proteins in the plants itself. The most successful and widely used biotechnological approach for pest management is the transgenic plants expressing insecticidal Cry proteins derived from *Bacillus thuringiensis*. The crystal (Cry) and cytolitic (Cyt) proteins of Bt are reported to be active against insects of different orders, Lepidoptera, Coleoptera, and Diptera, and also against other invertebrates such as nematodes. *Bacillus thuringiensis* produces insecticidal crystalline inclusions which are made of protoxins while sporulation. Genetic engineering helps in the transfer and expression of *B. thuringiensis* genes in the plants to protect them from any kind of target insect infestation. Since the inception of transfer of Bt genes in tobacco and tomato during 1987, it is been transferred into cotton, rice, maize, etc. with Lepidoptera as the main targets. Later, desired genes were synthesized partially or totally, in which the nucleotide sequence was modified with no alteration in amino acid sequence. Transgenic tobacco plants expressing *B. thuringiensis* toxins were experimented in the fields. In 1995, the first transgenic plant of corn (Maximizer™) with CryIA(b) toxin, cotton (Bollgard™) with CryIA(c) toxin, and potato (Newleaf™) with CryIIIA toxin was approved for commercial purpose [100]. Vegetative insecticidal protein (VIP) from Bt was also put into the genetically engineered plants to confer broader resistance against insect pests, viz., rice plants [82], thus serving as a potential alternative to Cry proteins. Corn plants with insecticidal protein isolated from *Pseudomonas chlororaphis* were found

effective against western corn rootworm which are reported resistant to cry toxins [101].

Development of insecticide resistance and public awareness are the major concerns about the continuation of traditional chemical pesticides. This necessitates the use of novel pesticidal compounds of which tackle both these issues. The commercial formulations of microbial origin metabolites, avermectins [56], and spinosad [54] have been established as potential protective pesticides with diverse group of target pests. Both these pesticides are recommended by the World Health Organization as safe and even recommended for pest management in organic agriculture. The commercial formulations of other actinomycete metabolites like milbemycin (Matsuguard, Koromite, Milbeknock, etc.) and polynactins are available in market as potential miticides [102]. This alleviated the interests on screening of pesticidal metabolites from microorganisms. Besides, due to their novel modes of action, they are recommended against resistant populations also [54]. This story comprehends the potential of pesticidal metabolites of microbial origin, and their putative chemical structures can be developed into a biorational pesticide to complement and even substitute the conventional chemical pesticides [8]. In addition, the novel site of action may become a target for further improvements in traditional pesticide developments.

Being living agents, microbials may alter native microflora. Sometimes, complete replacement of native pathogenic species may be possible. But these issues may not arise with the metabolites as pesticides, as they are the inert chemical compounds with respect to nontarget species including microflora. Due to their high target specificity and biodegradability, no bioaccumulation is possible.

6 Future Prospects and Conclusions

The positive public perception and acceptance of microbial pesticides due to their safety to nontargets made them an ecofriendly and sustainable strategy against a variety of agricultural pest problems. In the United States, the growth rate of commercial biopesticides is projected to be 17% of CAGR from 2016 to 2022 as against 3% CAGR of conventional chemical pesticides [103]. As a whole, the bacterial biopesticides claim major share (74%) followed by fungal (10%) and viral (5%) pesticides [104]. Although the bacterial pesticides contribute major share, majority of the products are based on *B. thuringiensis* only. In addition to these commercialized products, there exists a vast literature reporting new species of entomopathogens and strains of existing species with improved bioactivity. We suppose that unavailability of commercial products of these isolates might be due to lack of environmental competency data, problems in mass multiplication and formulations, etc. In addition, a true transformational technology interventions are not yet achieved to realize the full potential of biopesticides and the associated bioactive metabolites [105]. These problems can be conquered by utilization of pesticidal secondary metabolites either directly or by their chemical analogues which are easy to handle. These molecules have been evolutionarily selected over

millennia to enable microbes to interact with their environments. Besides, these compounds might have good environmental fitness due to their immediate pesticidal activity and ready biodegradability. However, the identification, characterization, further improvements, formulation, pesticidal evaluation, and safety analysis of these secondary metabolites from diverse entomopathogens require an interdisciplinary approach involving pest scientists, biotechnologist, organic chemist, environmental scientist, etc. In view of the importance of pesticidal microbes and metabolites, a centralized facility might tackle all these issues and facilitate the availability of microbial biopesticides to cater the needs of diverse pest problems.

A survey of recent literature revealed identification and characterization of many new bioactive genes and metabolites. But majority of the studies failed to understand the actual biophysical and biochemical changes they incur in host tissues. Understanding these induced changes and pathogenicity mechanisms may help in the interpretation of novel target sites for pesticidal activity. Some metabolites of pesticidal pathogens, although not directly involved in pathogenicity, synergize the pesticidal activity. For example, chitinase-producing strains of *B. thuringiensis* have greater toxicity than nonproducers [61]. Identification of these compounds may open new avenues in bio-synergism with greater efficacy. Similarly, adhesion, penetration, and nutrient uptake by contact pathogens are some of the poorly understood issues which may yield bioactive enzymes. Above all, patenting of novel pesticidal toxins and secrecy of industry have made some potent pesticidal metabolites unavailable and under progressed.

Omura [106] edited a book that describes different strategies and methodologies for screening of bioactive microbial metabolites after which many molecular and biological screening techniques came into picture. In general, majority of the pesticidal metabolites identified are based on conventional, biological, and sometimes chemical screening of pesticidal activity [7]. Differences in screening procedures may overlook some important metabolites, as production is a function of existing nutrient environment. In general, majority of secondary metabolites are repressed during logarithmic growth and is depressed during the suboptimal or stationary growth phases. Sometimes they are inducible as well. In such situations, generalized screening procedures may not portray all the capable bioactive compounds of a given bioagent. So a set of diversified screening techniques may be adopted to understand the complete set of metabolites involved in pathogenicity and mortality.

Besides pathogenic microorganisms, different pest-associated microorganisms like gut symbionts are in direct relation with host biology and physiology. Any induced disturbances to this relationship are lethal to the pest. Similarly, plant growth-promoting endophytes influence the pest invasion and biology by different classes of secondary metabolites (aliphatic compounds, peptides, phenylpropanoids, alkaloids, polyketides, and terpenoids). Understanding the biology, genetics, and biochemistry of these compounds may open novel possibility of pest management. Some of the microbial metabolites are claimed as "biostimulants" which induce or aggravate plant defense, thereby offering protection against diverse pest species. This multifaceted pest management strategy may also contribute to consistent production systems.

Insecticide resistance development, withdrawal or de-registration of many synthetic pesticides, public awareness about environmental issues, etc. direct crop protection toward sensible pest management practices. In view of this, interest and importance of pesticidal metabolites of microorganism are growing significantly. In general, the present-day pesticide chemistry is progressed by unexpected discoveries but it now needs an enhanced interest. Increasing number of publications on genome-wide analysis of microbial pesticides may answer the long-standing questions about pathogenesis besides revealing metabolic complexes involved. So a concrete knowledge of organic chemistry and basic sciences coupled with interdisciplinary approach on pesticidal metabolites of microbial origin with commercial product facet may overcome issues related with good quality market products of biopesticides.

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