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Feature Article

Mini-Review on Microbial Pesticide Research for Crop Protection Assisted by Generative AI

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Abstract

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Research to develop safer pest control technologies is critical for modern agricultural crop protection. This need is heightened by public concern with drawbacks associated with the intense use of synthetic chemical pesticides resulting in human and non-target organism toxicity and pests resistant to agrochemical treatments. In contrast, microbial pesticides are environmentally friendly, and their use presents low risk for the development of resistance in target pest populations. Thousands of research publications on microbial pesticides accumulated since the 1950s and available in PDF (electronic format) could be analyzed to identify research areas to innovate microbial pesticides for sustainable pest control. However, searching through a large volume of research manuscripts is challenging for researchers due to limitations for exhaustive data mining and potential inaccuracies extracting massive science data. The use of Generative Artificial Intelligence (GenAI) tools could assist in reviews by facilitating the identification of publications across scientific disciplines with information that scientists can use to accelerate microbial pesticide research and innovation. This study employed a GenAI tool to assist reviewing 750 PDF publications on microbial pesticides collected from public databases. Summaries of relevant papers identified rapidly were queried by the co-authors with expertise in plant pathology and entomology to prepare a manuscript. Actual intelligence was practiced further by subjecting the manuscript to further evaluation and revision incorporating the co-authors' expertise emphasizing genomic research. Advantages and disadvantages of using GenAI technology in making literature reviews are discussed herein. This mini-review highlights the use of microbial pesticides in agriculture to provide long-term pest management options and documents how GenAI can assist actual intelligence to ideate agricultural research for sustainable crop protection.

Key words: Microbial pesticide, Generative artificial intelligence (GenAI), PDF publications, Bacterial biopesticides, Fungal biopesticides, Genomic.

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INTRODUCTION

Pest management is crucial for producing crops to feed the world. Several species of invertebrate animals, including insects, mites, and nematodes, and microbes including fungi, bacteria, and viruses are considered pests because they damage and reduce the yields of crops. Other than viruses, crop pests are commonly controlled by chemical pesticides produced synthetically, or by biological pesticides also known as biopesticides. Chemical pesticides have been used intensely since the last century, which selects crop pest populations resistant to them. Additionally, the public is concerned with the pervasive use of chemical pesticides that can result in toxicity to human and non-target organisms, and pest resistance that render the pesticides ineffective. Thus, interest in research and development to use biopesticides as alternatives to chemical pesticides keeps growing (Thomas 1999; Ruiu 2018; van Lenteren *et al*. 2018; Keswani *et al*. 2019; Rani *et al*. 2021; da Silva Folli-Pereira *et al*. 2022; Elnahal *et al*. 2022; Mishra & Patni 2022; Thakur 2022; Ayilara *et al*. 2023; Aioub *et al*. 2024; Tadesse-Mawcha *et al*. 2024). There are three general types of biopesticides: (1) microbial biopesticides, consisting of microorganisms such as bacteria, fungi, viruses, and protozoa; (2) biochemical biopesticides or biorationals, derived from natural substances such as plant extracts and growth regulators; and (3) plant incorporated protectants, which are derived from genetically modified plants incorporated with genetic material that shows pesticide activity (EPA 2025) (https://www.epa.gov/ingredients-used-pesticide-products/what-are-biopesticides#classes).

Our program involves research and development of effective microbial pesticides including the discovery and characterization of bacteria and fungi that can be used as biopesticides for important crops. Microbial biopesticide research is documented in public domains and disseminated electronically in portable document format (PDF) files. Searches of scientific literature using PDFs could identify research areas to innovate microbial pesticides. However, searching through a large volume of research manuscripts is challenging for researchers due to limitations for exhaustive data mining and potential inaccuracies extracting massive science data. For example, a literature search we conducted using “microbial biopesticides” yielded a total of 5,045 entries in PubMed database of National Center for Biotechnology Information (NCBI) as of March 2025 (Fig. 1). And more specific searches using “bacterial biopesticides” yielded 8,660; “fungal biopesticides” yielded 11,190; “viral biopesticides yielded 227; and “nematode biopesticides” yielded 1,111 (Fig. 1). Interestingly, using “microbial” in the combined keyword yielded fewer entries than when using “bacterial” and “fungal” with “biopesticides”. This was likely due to the uncommon use of the term “microbial biopesticides” as a keyword in published literature, which reflected complexity that complicates comprehensive literature searches.

Searching through and summarizing a large volume of research papers is time-consuming and prone to human error that may result in synthesizing inaccurate information due to a large volume of data processing. Recent developments in artificial intelligence (AI) produced tools to automate electronic document reviewing processes (van Dijk *et al*. 2023; Fabiano *et al*. 2024; Ge *et al*. 2024). Large Language Models (LLMs) are a type of AI tool allowing the application of Generative Artificial Intelligence (GenAI) to assist literature reviews. Some LLMs may be accessed through subscription-serviced vendors such as OpenAI, Google, and Anthropic. However, there are a few that are available through publicly available resources such as huggingface.co and ollama.ai. Offering such as ollama.ai may be used offline if data security is a concern. Additionally, current public browsers such as Google Chrome and Microsoft Edge are equipped with AI functions that significantly improve the efficiency of internet searches using publicly available data.

This study explored how reviewing PDFs

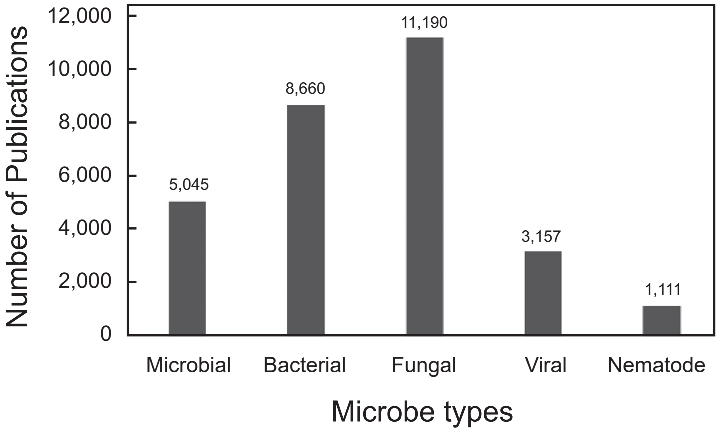

Fig. 1. Number of publications identified through search in PubMed database of National Center for Biotechnology Information (NCBI) using "microbial", "bacterial", "fungal", "viral", and "nematode" in combined keyword with "biopesticides" as of March 2025.

of scientific publications on microbial biopesticide research could be assisted by GenAI. Actual intelligence (AcI) based on our professional expertise was applied to curate and edit the draft version of this manuscript, which corrected output errors from the GenAI-aided literature search. Information was validated by closely reviewing selected references. Recent developments in genomics of biopesticide microorganisms and the research significance are highlighted. The assistance of GenAI technology in literature reviews is discussed.

PDF MANUSCRIPT DATA COLLECTION AND GENAI PROCEDURE

To test the use of GenAI procedure, 750 PDFs in the public domain were downloaded. A Langchain method was used with a retrieval augmented generation (RAG) protocol implemented to interface with an LLM. PDFs were parsed into chunks using an embedding tool (nomic-embed-text) and a vector store database (chromdb). A description of the process needed is available at the github repository (https://github.com/grlazo/AIRA/). Parameters of the vector store specified the chunksize to 800 with an overlap setting of 80. A variety of publicly available LLMs were screened via the ollama 0.5.7 (ollama.ai) framework. The LLM "Llama 3.18b" was chosen, which is in an 8 billion parameter trained model in a 4-bit quantized form with a 4K embedding length and 131K context window. Prompts retrieved the top 20 matches from the vector store and formulated a response based on the prompt presented to the LLM. Parameters can be adjusted for optimization of information received which in this case presented conditions apparently suitable for demonstration purposes.

Topics were resourced from subject matter experts and a series of prompts were presented and the answers scored (Table 1). The information retrieved was further evaluated, edited and updated based on responses available from public search engines including Google search, Microsoft Bing, and Meta AI. Selected PDFs were further examined by at least one coauthor. Corrections minimized possible hallucinations generated by AI. This enabled the application of AcI where humans correct GenAI outputs as needed. GenAI assistance to analyze and classify data adhered to guidance by the American Phytopathological Society (https://apsjournals.apsnet.org/page/authorinformation#AI).

Table 1. Initial topics and questions to sequester responses from GenAI based on 750 research publications related to microbial biopesticides.

Number	Topic	Questions
1	Historical perspective	"What is a microbial pesticide, and can you provide some historical perspective on how they came about?"
2	Economical and Academical Significance	"Why would microbial pesticides be used and are there any notable successful use cases?"
3	Biological natures of microbial biopesticides	"What types of microbial pesticides are in use and can you provide a ratio of the types that are currently in use?"
4	Bacterial pesticides	"Of the bacterial pesticides in use can you provide the mode of action and are there notable examples to be aware of?"
5	Fungal pesticides	"Of the fungal pesticides in use can you provide the mode of action and are there notable examples to be aware of?"
6	Viral pesticides	"Of the viral pesticides in use can you provide the mode of action and are there notable examples to be aware of?"
7	New technology	"Is there any mention of newer technologies in the use of microbial biopesticides in comparison to traditional application methods and can you provide some examples where success has been demonstrated?"
8	Microbial pesticides in genomic era	"Is there any mention of genome-based approaches which can be utilized for microbial biopesticide application, and can you provide examples?"

HISTORICAL PERSPECTIVE

The concept and practice of using microorganisms for pest control can be dated back to ancient times (Flint & van den Bosch 1981). Yet most documentational reports began in the 1800s. Ironically, many early works on microbial biopesticides started with pathogens on silkworm, a desirable insect. In 1835, an infectious disease of insect was observed by Agostino Bassi of Italy, in silkworm larvae caused by the fungus *Beauveria bassiana*. A Russian entomologist Elie Metchnikoff, reared *Metarhizium anisopliae*, a fungal pathogen, for suppression of the sugar beet curculio, *Cleonus punctiventris*, using fungal spores in 1884 (Hoddle & Van Driesche 2009). Both *B. bassiana* and *M. anisopliae* are still dominantly used fungal biopesticides nowadays. In 1911, the German scientist Berliner observed a disease of larvae of the flour moth, *Anagasta kuehniella*, and by 1938, a bacterium, *Bacillus thuringiensis*, was identified as the pathogen and being marketed for caterpillar pest control (Hoddle & Van Driesche 2009). These early works established the concepts of insect pathology, and, furthermore, procedures to rear the insect causative organisms that enabled the pest control practices.

As of March 2025, there are 446 microbial biopesticides registered by United States Environmental Protection Agency (EPA) (https://www.epa.gov/ingredients-used-pesticide-products/biopesticide-active-ingredients). The number of microbial biopesticide products is still in a trend of increasing mainly due to (1) regulators urging for sustainable plant protection, (2) food companies and consumers demanding zero-residue food, and (3) big agrochemical companies showing interest in biological alternatives as a new business opportunity (Preininger *et al*. 2018).

RELEVANCE AND ECONOMIC SIGNIFICANCE OF MICROBIAL BIOPESTICIDES

The justifications of using microbial biopesticides are that they are environmentally friendly, targeting only the intended pests but not beneficial insects or other natural enemies, and ecologically safer relative to chemical pesticides (Daraban *et al*. 2023). These are reflected in target-specific range, delayed mode of action, reduced persistence, low residue levels, and safe in practice handling (Oguh *et al*. 2019). One key feature of microbial pesticides is that they are derived from natural sources, i.e. they are already in the environment (El-Wakeil *et al*. 2006; Daraban *et al*. 2023). For this reason, they are considered natural products, in contrast to the non-natural synthetic chemical pesticides. However, in all circumstances, it is a good practice that the time and manner of application of biopesticides should be kept in mind and the precautions that must be strictly observed.

Various microorganisms including fungi, bacteria, viruses, nematodes, and protozoa have been studied and tested for their efficacy against various plant pests and pathogens (Ruiu 2018; Kvakkestad *et al*. 2020). A rough estimate from our literature search shows that bacteria are the most used biopesticide type (60–70%), followed by fungi (20–30%). In line with our current research interests, this review focuses on bacterial and fungal biopesticides. It is also not intended to be a comprehensive literature review, but to highlight some basic perspectives of pesticidal microorganisms through selected examples. A particular interest is in microbial genomics, a more recently developed technology which has potential to revolutionize microbial biopesticide research and application. Reviews of other biopesticides such as viral biopesticides can be found in multiple publications (Ruiu 2018; Mondal *et al*. 2021; Tadesse-Mawcha *et al*. 2024).

BACTERIAL BIOPESTICIDES

Bacterial biopesticides have been used to control both insect/mite pests (Glare *et al*. 2017; Ruiu 2018; Gangwar *et al*. 2022; Tomar *et al*. 2024) and plant pathogens (Bonaterra *et al*. 2012; Glare *et al*. 2012; Boro *et al*. 2022). Over 200 bacterial species of bacteria have been tested for use as biopesticides. Most entomopathogenic bacteria belong to the Bacillaceae family (Alsaedi *et al*. 2017; Tadesse-Mawcha *et al*. 2024). Other groups of entomopathogenic bacteria include gammaproteobacteria such as the endosymbionts of insecticidal nematodes *Photorhabdus*, *Xenorhabdus*, *Serratia* species (Hurst *et al*. 2000; Akhtar *et al*. 2025), *Yersinia entomophaga* (Landsberg *et al*. 2011), and *Pseudomonas entomophila* (Vodovar *et al*. 2006); betaproteobacteria such as *Burkholderia rinojensis* (Cordova-Kreylos *et al*. 2013), *Chromobacterium subtsugae* (Martin *et al*. 2007); and alphaproteobacterial such as *Agrobacterium radiobacter* (McCardell & Pootjes 1976). Actinobacteria is also an important source of biopesticides. Different *Streptomyces* species produces a variety of insecticidal toxins, such as the macrocyclic lactone (Copping & Menn 2000; Diab *et al*. 2024), and *Saccharopolyspora spinosa* produces spinosins (Kirst 2010; Ruiu 2018; Kumar *et al*. 2021; Tadesse-Mawcha *et al*. 2024).

Bacillus thuringiensis is the most widely used bacterial biopesticides (Schnepf *et al*. 1998; Bel *et al*. 2024). As an insect pathogen, *B. thuringiensis* insecticidal activity is attributed to parasporal crystals, which are toxic to a wide variety of insect species among the orders Lepidoptera, Coleoptera, Hymenoptera, Diptera, Homoptera, Orthoptera, and Mallophaga, and against nematodes, mites, and protozoa (Schnepf *et al*. 1998). Once the insect larva consumes a crop leaf containing crytals, the protoxin is activated by gut alkaline pH by action of protease. The activated protein binds to gut wall receptor and causes permeation in hemocoel. The toxin dissolves in body cavity and lava dies in 24–48 h from septicemia leading to dead larvae (Rajamani & Negi 2021).

In the context of biocontrol of plant diseases, the three families of *Bacillus* lipopeptides- surfactins, iturins, and fengycins are known for their antagonistic activity for a wide range of potential phytopathogens such as *B. subtilis* to apple gray mold disease caused by *Botrytis cinerea* (Ongena *et al*. 2005) and *B. amyloliquefaciens* to *Fusarium oxysporum* f. sp. *cubense* (Yuan *et al*. 2012). Iturin and Fengycin have antifungal activities, while surfactin has broad range of potent antibacterial activities (Meena & Kanwar 2015). Recent investigations have found that these lipopeptides can also influence the ecological fitness of the producing strain in root colonization and have a key role in the bacteria-plant interactions that stimulates host defense mechanisms (Ongena & Jacques 2008).

Many members of the *Pseudomonas fluorescens* complex are in fact known as effective biocontrol agents of plant pathogens and as plant growth promoters. For this reason, they are of great interest for biotechnological applications (Haas & Keel 2003; Haas & Défago 2005). The antagonistic activity of fluorescent *Pseudomonas* is mainly related to the production of several antibiotic compounds, lytic enzymes, lipopeptides, and siderophores. Several volatile organic compounds are also synthesized by fluorescent *Pseudomonas* including different kinds of molecules that are involved in antagonistic interactions with other organisms and in the induction of systemic responses in plants (García-Hidalgo *et al*. 2020; Raio 2024).

Agrobacterium radiobacter strains have been used to treat germinating seeds, roots and stems of stone fruit and nut trees for disease protection. *A. radiobacter* strain K84 occurs naturally in many types of soil. As a pesticide active ingredient, it is used in nurseries and greenhouses for controlling the closely related bacterium *A. tumefaciens*, which causes crown gall disease in plants due to the tumor-inducing (Ti) plasmid (McCardell & Pootjes 1976). *A. radiobacter* was formerly incorrectly synonymized with *A. tumefaciens* (Arahal *et al*. 2023). Unlike other members of its genus, it does not harbor a tumor-inducing (Ti) plasmid and is hence not plant pathogenic.

Bacterial pesticides demonstrate the diverse modes of action and their potential for large-scale commercialization. Their modes of action included (1) production of antibiotics that kill or inhibit the growth of plant pathogens; (2) direct interaction with plant pathogens, suppressing growth or reproduction; (3) competition with the pathogen for nutrients and niches in the host plant; (4) induction of host resistance, or the triggering of the plant's immune system to produce resistance against specific pathogens; and (5) plant growth promotion, or the enhancement of nutrient uptake to promote healthy growth and increase tolerance to stresses.

FUNGAL BIOPESTICIDES

Fungal biopesticides are commonly used to control insects, yet many fungi also work against other fungi, bacteria, and other plant pathogens (Leathers *et al*. 1993; Afshan 2023). Fungal biopesticides do not have to be ingested by target insects to cause infection. This is a distinct feature from bacterial and viral biopesticides. They can penetrate through the insect cuticle and cause infection either creating competition for space and nutrients or producing toxic secondary metabolites. Main fungal entomopathogens include species covering the following phyla: Chytridiomycota, Zygomycota, Oomycota, Ascomycota, and Deuteromycota (Ruiu 2018).

Beauveria bassiana is one of the most common fungal pesticides used to control insect pests. In taxonomy, *B. bassiana* is the anamorph (asexually reproducing form) of *Cordyceps bassiana*. The latter teleomorph (the sexually reproducing form) has been collected only in eastern Asia (Li *et al*. 2001). *B. bassiana* has been reported to control various insects, including termites, thrips, whiteflies, aphids and various beetles. In the disease cycle, the fungal spores/blastopores/conidia from insect killed by the fungus land on target insect, germinate through penetration peg. Inside the host hemocoel, fungi produce mycelium, blastopores and toxins. Mycelium inside host will germinate outward and kill the host (Tadesse-Mawcha *et al*. 2024).

Another popular fungal pesticide that has been used to control insects is *Metarhizium anisopliae* (Tang *et al*. 2019; Zekeya *et al*.

2022). One significant feature of the use of *M. anisopliae* is its compatibility with insecticides. In rice field, the fungus effectively infected two important planthoppers *Nilaparvata lugens* and *Sogatella furcifera*. In this case, *M. anisopliae* were used along with insecticides thiamethoxam and pymetrozine in field application. The combined use of *M. anisopliae* with insecticides resulted in more effective control of plant leafhoppers (Tang *et al*. 2019).

Members in the genus *Trichoderma* are noteworthy to mention for their extensive use as biopesticide against numerous aeronautical and soil-borne plant pathogens in field or greenhouse experiments (van Lenteren *et al*. 2018; Guzmán-Guzmán *et al*. 2023). Most biocontrol agents are from the species *T. asperellum*, *T. harzianum*, *T. viride*, and *T. hamatum*. In taxonomy, *Trichoderma* is the anamorphs of *Hypocrea*, an ascomycete. In general, *Trichoderma* spp. are non-plant pathogenic and opportunistic plant symbionts. The fungus establishes beneficial interactions with their hosts. However, many species of *Trichoderma* are pathogens of cultivated mushrooms refereed as “green mold” disease. Many fungal cell wall-degrading enzymes like chitinases, hydrolases, 1,3- proteases, glucanases, and mannanases are produced by different members of this genus (Thambugala *et al*. 2020; Saldaña-Mendoza *et al*. 2023). Other fungal species commercially exploited worldwide for pest management include *Verticillium lecanii*, *Lecanicillium* spp., *Hirsutella* spp., *Paecilomyces*, and *Isaria* spp. (Ruiu 2018).

Fungal pesticides are generally considered to be environmentally friendly in terms of low toxicity to mammals and birds. They can be applied through various methods, including spraying or immersing insects in fungal suspensions. The efficacy of fungal pesticides can be improved by optimizing application techniques, such as using the correct concentration and mode of delivery (Rajendran 2021).

MICROBIAL PESTICIDE RESEARCH AND DEVELOPMENT IN THE GENOMIC ERA

Various studies during the last century identified and characterized hundreds of bacterial and fungal species/strains with pesticidal properties from different hosts and regions around the world. However, conventional methodology often failed to reveal the genetic mechanisms of pesticidal activity, as well as many taxonomy issues among morphologically similar strains. Recent developments in next-generation DNA sequencing technology and genomics provided deeper insights into the genetic makeup of bio-pesticidal microorganisms. Two immediate and important impacts of genome sequencing discussed herein include: (1) accelerated identification of pathogenic genes and related regulatory factors; and (2) improved taxonomy systems for strain detection and selection through effective and accurate characterization of microbial populations.

Taking *B. thuringiensis* as an example. Since the first *B. thuringiensis* genome sequence was released in 2004 (Han *et al*. 2006), there are currently 1,410 genome sequences of this bacterium deposit in GenBank, including 105 complete and 5 metagenome-assembled genomes (MAGs). A full *B. thuringiensis* genome spans from 5.313 to 6.861 Mbp. The number of genes annotated varies from 5,343 to 7,227, and the number of plasmids ranges between 1 and 14. The guanine-cytosine content (GC) is between 31.4 and 35.48%. Analyses of these genome sequences confirm the vast genetic diversity among the studied strains. By exploring new strains, novel toxin genes and proteins have been detected (Jouzani *et al*. 2017; Pacheco *et al*. 2021; Yılmaz *et al*. 2025). An example genome of *B. thuringiensis* is shown in Table 2. The bacterial strain (IS5056) contains a circular chromosome of 5,491,935 bp and 14 plasmids ranging from 6,880 to 328,151 bp.

Table 2. List of sequenced genomes of selected bacteria and fungi used as active ingredient for microbial pesticide development.

Microbe names	Number of genome sequences	Bioactivity note	Example genomes	References
Bacteria				
Bacillus thuringiensis serovar *thuringiensis* (+ 16 other serovars)	1,410	Bt toxins against various insect pests	Strain IS5056, Chromosome: CP004123, 5,491,935 bp Plasmids: CP004137, pIS56-328, 328,151 CP004136, pIS56-285, 285,459 CP004135, pIS56-233, 233,730 CP004134, pIS56-107, 107,431 CP004133, pIS56-85, 85,134 CP004132, pIS56-68, 68,616 CP004131, pIS56-63, 63,864 CP004130, pIS56-39, 39,749 CP004129, pIS56-16, 16,206 CP004128, pIS56-15, 15,185 CP004127, pIS56-11, 11,331 CP004126, pIS56-9,9,671CP004125, pIS56-8, 8,251 CP004124, pIS56-6, 6,880 Total = 6,771,593 bp	Murawska *et al*. (2013)
Lysinibacillus sphaericus (*Bacillus sphaericus*)	65	Binary and mosquitocidal (Mtx) toxins	Strain DSM 28, CP019980.1, Chromosome, 4,681,723 bp	Lee *et al*. (2018)
Paenibacillus popilliae	2	Milky spore disease of the white grubs of Japanese beetles	ATCC 14706, ASM31523v1, chromosome (contig), 3,833,720 bp	Iiyama *et al*. (2023)
Agrobacterium radiobacter	27	Agrocin 84, a bacteriocin that inhibits the growth of *Agrobacterium tumefaciens* which causes crown gall disease	Strain LMG 267 CP169641.1, circular 2,974,514 bp CP169642.1, linear, 2,085,723 bp CP169643.1, pAtLMG267a 490,305 bp CP169644.1, pAtLMG267b 176,584 bp CP169645.1 pTiLMG267 194,263 bp Total = 5,921,389 bp	Weisberg *et al*. (2020)
B. subtilis	1,289	Antifungal and antibacterial lipopeptides	Strain 168, AL009126 4,215,606 bp	Kunst *et al*. (1997)
Pseudomonas fluorescens	321	Antibiotics and siderophores against fungal and nematode pathogens	Strain Pf0-1, CP000094, 6,438,405 bp	Silby *et al*. (2009)

Table 2. List of sequenced genomes of selected bacteria and fungi used as active ingredient for microbial pesticide development. (continued)

Microbe names	Number of genome sequences	Bioactivity note	Example genomes	References
Serratia marcescens	3,197	Antibiotics and siderophores against fungal pathogens	Strain ELP1.10 CP127881.1, Chr. 5,031,539 bp CP127882.1, pELP1.10 129,237 bp	Leung *et al*. (2023)
Fungi				
Beauveria bassiana	208	Toxins such as beauvericin, bassianin, bassianolide, beauverolides, tenellin, oosporein, oxalic acid, calcium oxalate crystals, and many beauvericin analogs	Strain HN6, Chr.1 CP045886.1; 6,424,025 bp Chr.2 CP045885.1; 5,597,631 bp Chr.3 CP045884.1; 5,431,745 bp Chr.4 CP045883.1; 4,613,348 bp Chr.5 CP045882.1; 3,702,357 bp Chr.6 CP045881.1; 3,525,630 bp Chr.7 CP045880.1; 3,028,184 bp Chr.8 CP045879.1; 1,996,242 bp Chr.9 CP045878.1; 1,357,674 bp Chr.10 CP045877.1; 615,663 bp Chr.11 CP045888.1; 454,462 bp Chr.12 CP045887.1; 387,253 bp Total = 37,134,214 bp	Guo *et al*. (2020) Wang *et al*. (2021)
Metarhizium anisopliae	9	Insecticidal cyclic peptides (destruxins).	Strain JEF-290, JABUBV; Chromosome: 42,848,098 bp	Lee *et al*. (2019)
Trichoderma harzianum	23	Antibiotics and cell wall-degrading enzymes	Strain BOL 12QD CP133624.1: Chr.1 CP133624.1; 7,677,970 bp Chr.2 CP133625.1; 7,232,126 bp Chr.3 CP133626.1; 6,421,825 bp Chr.4 CP133627.1; 4,804,947 bp Chr.5 CP133628.1; 4,726,887 bp Chr.6 CP133629.1; 4,458,692 bp Chr.7 CP133630.1; 4,296,277 bp Total = 39,618,724	Rocabado-Villegas *et al*. (2024)

Genomic studies on *B. thuringiensis* genomes have helped identify different genes encoding insecticidal proteins. The cry gene family, produced during the late exponential phase of growth in the bacterium, is a large and still-growing family of homologous genes. Each gene encodes a protein with strong specific activity against only one or a few insect species. The effectiveness and efficiency of genomic discoveries of insecticidal genes can be shown in the following example (Pacheco *et al*. 2021). In this work, the complete genome of *B. thuringiensis* strain GR007 that is toxic to *Spodoptera frugiperda* and *Manduca sexta* larvae were sequenced and annotated. Four replicons (one circular chromosome and three megaplasmids) were identified. The two largest megaplasmids (pGR340 and pGR157) contain multiple genes coding pesticidal proteins: 10 crystal (cry) toxin genes (cry1Ab, cry1Bb, cry1Da, cry1Fb, cry1Hb, cry1Id, cry1Ja, cry1Ka, cry1Nb, and cry2Ad), two vegetative insecticidal protein (vip) genes (vip3Af and vip3Ag), two binary toxin genes (vpa2Ac and vpb1Ca), five genes that codify for insecticidal toxin components (Tc's), and a truncated cry1Bd-like gene.

The whole genome sequencing fungal strains is more challenging because of the much larger genome size. With *B. bassiana* as an example, the first genome sequence was completed in 2012 with strain ARSEF 2860 (33.69 Mbp, 2 scaffolds). As of March 2025, there are 208 whole genome sequences submitted to the GenBank, compared to 1,410 genome sequences of *B. thuringiensis*. The full-length genome of *B. bassiana* spans from 28.68 to 38.31 Mbp with GC content between 48.5 and 53%. Of mentioning is the recent advancement of fungal genome sequencing at the resolution of chromosomal level as listed in Table 2. *B. bassiana* Strain HN6 was reported to have 12 chromosomes ranging from 387,253 to 6,424,025 bp with a total of 37,134,214 bp. The genome of *T. harzianum* Strain BOL 12QD harbors 7 chromosomes ranging from 4,296,277 to 7,677,970 bp with a total of 39,618,724 bp. It is expected that such chromosomal level sequencing will serve as significant references and genome structure frameworks facilitating genome research in both *B. bassiana* and *T. harzianum*, as well as other related fungi.

Another significant contribution of genome sequencing is the improvement of microbial taxonomy or systems for strain classification and identification. Taxonomy is critical for understanding the evolution of microorganisms and serves as the basis for evaluation of biocontrol agents. Accurate strain differentiation and collection are the first step towards identification of microbial strains with specific pest control properties. Genome sequencing techniques allowed for a more in-depth study of microorganisms with unclarified classification issues based on traditional methodologies such al morphology. For example, *B. bassiana* has long been used to describe a species complex of morphologically similar and closely related isolates. Recent molecular analyses indicated that *B. bassiana* consists of many distinct lineages that should be recognized as distinct phylogenetic species (Rehner & Buckley 2005; Rehner *et al*. 2011)

To evaluate the taxonomic status of *B. bassiana* with appropriate phylogenetic frameworks, whole genome sequences information was used in addition to morphological and secondary metabolites data, (Kobmoo *et al*. 2021). In this case, whole genome shotgun sequencing of 78 strains was performed. Clean reads were mapped to the *B. bassiana* reference genome ARSEF8028 (Valero-Jiménez *et al*. 2016). The selected single nucleotide polymorphisms (SNPs) (729,549 SNPs) from 1,132 genes were used for construction of phylogenetic tree. Three phylogenetic clades with high levels of divergence were found. Along with morphological data and secondary metabolite profiles, one clade remains to be *B. bassiana*, and two other clades were assigned to new species, *B. namnaoensis* and *B. neobassiana*.

The integration of genomics with microbial pesticide research holds great promise for research and discovering new microbial biopesticides. Future developments may include

(1) synthetic biology approaches to engineer microbes with custom pesticide properties; (2) CRISPR-based gene editing to enhance microbial effectiveness and specificity; (3) AI-driven genomic analysis to accelerate the discovery of new bio-pesticidal organisms; (4) metagenomic study by analyzing the genomes of soil and plant-associated microbes, new bacterial or fungal species with pesticidal properties can be discovered; and (5) investigation of RNA interference (RNAi) mechanism that could be used to produce a new generation of pesticides. By leveraging genomics, microbial pesticides can be optimized for efficiency, safety, and environmental sustainability, making them an effective and efficient tool for modern pest management.

CONCLUDING REMARKS

Public interests in microbial biopesticides as sustainable alternatives to chemical pesticides for agricultural pest management remains to be high in the years to come. The historical development of microbial biopesticides dates to ancient times and significant literature records started in the 1800s with landmark discoveries of *B. bassiana*, *M. anisopliae*, and *B. thuringiensis*. The relevance and economic significance of microbial biopesticides continues to benefit agriculture through their target specificity, reduced environmental persistence, and safety compared to chemical pesticides. These aspects are highlighted in the context of bacterial biopesticides such as *B. thuringiensis*, *Pseudomonas*, and *Agrobacterium*, and fungal biopesticides including *B. bassiana*, *M. anisopliae*, and *Trichoderma* species. Continued genomic research is expected to advance the development of technologies based on microbial biopesticides. Multi-omics including genomic approaches offer the opportunity to enhance our understanding of the diversity of microbes that could be used as pesticides by identifying pathogenic genes and finetuning the molecular taxonomy of bio-pesticidal bacteria and fungi.

Incorporating GenAI to screen publications in public databases facilitated the review process by identifying relevant PDFs from the voluminous scientific literature available electronically. However, this step occurring in the initial phase of the pursuit of knowledge with the assistance of GenAI resulted in mistakes that had to be corrected through AcI by the authors during the curation process. This interface of GenAI and AcI is critical for researchers to determine the reliability of what can be gained from topical literature reviews. Human oversight through AcI is fundamental to maximizing the benefit of using GenAI as a tool assisting in reviews like this one on microbial pesticide research for crop protection.

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生成式人工智慧輔助的作物保護微生物農藥研究簡述

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摘要

Lazo, G. R., S. C. Chang, H. T. Shih, H. Huang, C. M. Wallis, A. Á. Pérez de León, and J. Chen. 2025. Mini-review on microbial pesticide research for crop protection assisted by generative AI. J. Taiwan Agric. Res. 74(4):399–414.

開發更安全的害蟲防治技術對當前作物保護至關重要，公眾擔心大量使用合成化學農藥所產生的弊端加劇此一需求，此些弊端不僅會導致人類和非目標生物中毒，同時也會導致害蟲產生抗藥性。微生物農藥相較於合成化學農藥，對環境相對友善，且使用之後對目標害蟲族群產生抗藥性的風險也比較低。分析自 1950 年代以來累積的數千篇 PDF 格式 (電子格式) 的微生物農藥研究報告，可確認創新的微生物農藥是可以達到永續害蟲防治的研究領域。然而，受到能詳盡蒐尋資料的限制與提取巨量科學資料的潛在誤差，窮盡蒐尋大量研究文獻對研究人員而言，顯然是一項挑戰。使用生成式人工智慧 (GenAI) 工具可協助回顧並促進辨識跨學科的科學研究報告，為科學家提供可用於加速微生物農藥研究與創新的資訊。本研究使用 GenAI 工具輔助回顧從公共資料庫中所蒐集的 750 篇有關微生物農藥 PDF 格式報告，快速篩選出的相關論文摘要，經植物病理學與昆蟲學背景的共同作者檢視與歸納後，最終撰寫成稿。論文進一步運用實際智能，結合共同作者在基因體學研究的專業知識，對稿件進行進一步評估與修改，本文也討論使用 GenAI 技術進行文獻綜述的優缺點。本篇小型回顧報告著重介紹微生物農藥在農業的應用，以提供長期的蟲害管理方案，並闡述 GenAI 如何提供永續作物保護農業研究的實際智能構思。

關鍵詞：微生物農藥、生成式人工智慧 (GenAI)、PDF 出版品、細菌性生物農藥、真菌性生物農藥、基因組學。

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