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Endophytic Microorganisms Associated with the Biological Control of Insect Pests in Agriculture

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Abstract

Endophytes are microorganisms that form symbiotic relationships with their host and provide benefits to the host plant, such as protection against pests, resistance to unfavorable abiotic conditions, and improvement in growth. When insects cause excessive economic, social, and environmental damage, they are considered insect pests, and they need to be controlled. Often, they are controlled by chemical products, but their use is questionable due to potential risks to human health and the environment. Biological control using endophytic microorganisms is a more sustainable alternative; endophytic microorganisms are transferred between plants to stimulate the production of toxic metabolites against insects. This review summarizes the different genera and species of endophytic microorganisms, which show biocontrol capacity against insect pests, isolated from various plant species, as well as the secondary metabolites, which are characterized as having entomopathogenic activity against these insect pests, isolated from the endophytic microorganisms. The use of endophytic microorganisms that exhibit entomopathogenic action for the biological control of crops with high economic and environmental value is an effective and sustainable option for the control of insect pests.

Keywords: Endophytes; Insects; Biotechnology; Entomopathogenic

Introduction

Endophytic microorganisms are of interest to researchers because they can inhabit plant tissues, such as leaves, roots, and stems, without causing apparent damage [1], and benefit the host plant in several ways. Endophytic microorganisms such as fungi, bacteria, actinomycetes, and mycoplasma create a symbiotic association with plants, where the microorganisms obtain nutrients for survival and protection against abiotic factors [2], and receive protection against pathogens and harmful substances, benefiting from the primary and secondary metabolites produced by microorganisms [3,4].

Endophytic microorganisms function in different ways in plants, such as production of bioactive substances [5-7], tolerance to biotic and abiotic stresses [8,9], increase in nutrients acquired by plants [10,11], increase in stress tolerance in plants [12,13], and protection against herbivorism [14,15], phytopathogens [16-18], and insect pests.

Secondary metabolites produced by endophytes have several applications with great biotechnological potential, such as food, pharmaceuticals, medicine, chemistry, and agriculture, with the use of bioactive agents for the biological control of phytopathogens and insects [19].

Biological control using endophytic microorganisms is based on antagonism and parasitism, which produce metabolites against natural enemies and invasive plants, inhibiting their development and making it possible to control them, providing protection to the endophyte and host plant [3,20-24]. Damage caused by invasive insects in agriculture has been estimated to lead to income loss of approximately US$ 70 billion [21]. Endophytic microorganisms with entomopathogenic potential have been used for the biological control of agriculture in several regions globally. For example, endophytic fungi of the genus *Trichoderma* can contaminate and cause mortality in colonies of leaf-cutting ants (Hymenoptera: Formicidae) that are responsible for damage to *Eucalyptus urophylla* plantations [22].

Although advances have been made regarding the use of endophytic microorganisms as biological controls in agriculture and these microorganisms hold great promise for improving food production worldwide, caution should be exercised in terms of their application. Several of these fungi are insect generalists and can affect species other than the target pest, resulting in undesirable consequences for local ecology. For example, *Beauveria bassiana* conidia are known to effectively kill the predatory mite *Amblyseius swirskii*, which feeds on insects and other mites and is also used for biocontrol in agriculture [23]. Therefore, it is crucial to conduct adequate tests prior to field applications of the biological control to ensure that off-target infections are not harmful to local cultures and ecosystems.

This review summarizes the different genera and species of endophytic microorganisms, which show biocontrol capacity against insect pests, isolated from various plant species, as well as the secondary metabolites, which are characterized as having entomopathogenic activity against these insect pests, isolated from the endophytic microorganisms.

Biological Control

The first known use of biological control of agricultural pests was 1,200 years ago by Chinese farmers who placed bamboo among citrus plants to facilitate access to ants that prevented the defoliation of plants by caterpillars [25]. Observations of the natural way that species live and how they relate to other organisms, whose populations feed on and control other organisms in the environment, are important for determining which biological control is most suitable [26].

Biological control is based on alternative techniques where chemicals are not used, such as the removal of attacked branches and fruits, biotechnological development of resistant and predatory plants, bioinsecticides, male sterile insect pests, the use of traps, and the burning of crop remains. However, some experts in the field only consider the action of natural enemies, such as insect pests, microorganisms, and weeds, to control the populations of their hosts and predators, as biological control [26].

The term pest is used for insects, microorganisms, weeds, and other living things that adversely affect ecosystems and society, with or without economic damage [27]. In agriculture, insect pests cause economic damage to agricultural, forest, and urban areas and can be vectors of plant and animal diseases [26].

Chemical agents are the main form of insect pest control and have high efficiency, but present dangerous risks to the environment and human health. Dichloro-diphenyl-trichloroethane (DDT) has been banned for agricultural use in Brazil since 1971 and subsequently in approximately 40 other countries. DDT is the most powerful organochlorine insecticide in the world and can instantly eliminate hundreds of different insect species, while other insecticides are able to eliminate only a few insect species. Despite its high efficiency for elimination, several other risks were discovered; DDT penetrated the food chain and contaminated rivers, fish, animals, flora, and all life in the surrounding environment, causing their eventual mortality. DDT accumulates in the food and fatty tissues of humans and wild animals and can cause genetic damage. In plantations, it killed pests and countless species of insects for months after application, remaining toxic to the environment for decades [26]. The use of insecticides and other chemical agents to eliminate pests has been questioned because of the risks to human health and the environment. The overuse of chemicals has resulted in resistant insect populations, forcing the use of chemical agents at higher concentrations or the addition of new active ingredients not recommended by health and nature conservation organizations [28].

Insects are the largest class in the animal kingdom, including at least 1.2 million known species. Less than 1% of all insect species are considered pests, and only 15% of losses in agriculture are caused by herbivorous insects. However, when pest insect populations increase to the point of causing economic damage, their abundance must be reduced in an environmentally sensitive way [26,28].

There are three types of biological control: classic, applied, and natural. The classic technique involves the introduction of a natural predator or parasite into the environment affected by exotic pests and is well established [29].

The applied technique involves the release of natural enemies into the affected environment, but the natural enemies have a low population density or do not survive for long enough to control the pest. This technique aims to rapidly reduce pests by mass-releasing natural enemies that are often laboratory-produced. This large-scale release is called flooding, and the release of a small population is called inoculation. Natural biological control aims to manipulate the environment to conserve and increase natural enemies through food and shelter provisioning [28,29]. According to Nava, et al. [30], the first use of classic biological control with excellent results occurred in 1888, in the state of California, western USA, where the ladybird *Rodolia cardinalis* (Coleoptera: Coccinellidae) was introduced to control the mealybug *Icerya purchasi* (Hemiptera: Monophlebidae) in citrus groves.

Biological control has advantages over other techniques, including the protection of biodiversity because it does not leave chemical residues, causes pollution, or affects pollinating insects. Other advantages include specificity, eliminating only the desired pest and avoiding imbalance, increasing profits by reducing losses caused by pests, and costs less than chemical agents. The disadvantages include employing new technologies that require technical knowledge and the specificity of the control organism that limits the action of natural enemies, making it impossible to instantly control different pests [29].

Endophytic Microorganisms

The first reference on endophytic microorganisms was made by Heinrich Anton De Bary in 1866 and refers to any microorganism that inhabits the organs and tissues of host plants, differentiating them from pathogens. They can inhabit the plant throughout its life cycle or for only one of its developmental stages and can be found in one or more organs of the plant. Endophytes were largely forgotten until the end of the 70s, when a renewed interest in the microorganisms began, motivated by their innovative properties that offered protection to the host plant against pathogens, herbivores, and insect pests [1].

Endophytes form symbiotic relationships with their host, bringing advantages to the microorganisms (such as the supply of nutrients and compounds necessary for the end of their life cycle) and plants that use the compounds produced by the endophyte to increase their resistance to nematodes, insects, and animals, as well as leading to rapid growth due to the production of phytohormones [1,2,31,32]. The advantages of host plants are due to the symbiotic interactions that are related to the production or induction of secondary metabolites by endophytic microorganisms and have great biotechnological potential in the areas of food, pharmaceuticals, biological control, chemistry, and agriculture [33].

The endophyte-host plant relationship can sometimes be unfavorable, as some endophytes that do not cause disease in a given host may be pathogens for others, due to conditions of imbalance or change in the physiology of the host plant [1,34].

Some Historical Aspects and Facts on Endophytic Microorganisms Associated with the Biological Control of Insect Pests in Agriculture

In the search for sustainable and clean compounds for the control of pests in agriculture, several studies have been carried out to discover new products and methods of application in the field. However, in Brazil, a few decades ago, there was resistance toward the use of biological control due to the strong pressure of agrochemical companies and the culture of the Brazilian farmer who are accustomed to the application of chemical products to control pests. Thus, it is not only a culture that strongly prevents the “entry” of biological products into the Brazilian market, but also the belief that biological control is an easy measure and therefore lacks professionalism, unlike for chemical control. Another fact is that biological control is a long-term control measure, unlike chemicals that cause a “shock” action.

However, currently, the scenario is changing. In Brazil, biological control is advancing more than in other parts of the world. In 2020, the biologicals market was 100–200 million in China; it is expected to increase from US$ 3 billion this year to US$ 5 billion in the coming years globally [35].

Biological control with microorganisms began in the 1970s in Brazil with the fungus *Metarhizium anisopliae*, which is used to control sugar cane leafhopper (Hemiptera: Cercopidae), which attacks the leaves and roots of the cane [36].

In the 1980s, the first published evidence showed that endophytic microorganisms could have important functions within the host plant. These studies have shown that endophytic microorganisms protect the host plant from attack by herbivorous insects. According to Azevedo et al. [37], Webber, et al. [38] may have been the first to report an endophytic microorganism acting to protect a plant against an insect, whereby it was demonstrated that the endophytic fungus *Phomopsis oblonga* produces toxic compounds that affect the beetle larvae of the species *Physocnemum brevilineum* (Coleoptera: Cerambycidae), which causes disease in elm (*Ulmus* sp.) plants [38-54].

Currently, in Brazil, a large area of cultivated land is being treated with microorganisms for biological control of insect pests. Parra, et al. [35] reported that more than 5 million ha of land in Brazil is treated with *Trichoderma harzianum* Rifai;

1.5 million ha treated with *Beauveria bassiana* (Bals.-Criv.) Vuill; and 2.5 million ha treated with *Metarhizium anisopliae* (Metchnikoff) Sorokin.

Endophytes with Entomopathogenic Activity Applied in the Biological Control of Insect Pests

Entomopathogens are microorganisms that cause diseases in insects, and several reports of natural biological control of insect pests by the action of endophytic microorganisms have been described [1] (Table 1). For example, the fungus *Beauveria bassiana* is an endophyte found in corn that protects the host from attack by insect pests and is widely used in agriculture. Another endophytic fungus is *Rhabbdocline parkery*, which produces deadly toxins for insect larvae of the genus Contarinia (Diptera: Cecidomyiidae), which causes galls on pine trees [1].

Endophytic fungus	Plant Species	Target pest	Conclusion	Reference
Alternaria solani	*Pelargonium graveolens Melia azedarach*	*Spodoptera littoralis (Lepidoptera: Noctuidae)*	Increased mortality of larvae	[55]
Aspergillus nidulans	*Lantana camara*	*Spodoptera littoralis (Lepidoptera: Noctuidae)*	Increased mortality of larvae	[56]
	Musa spp.	*Cosmopolites sordidus (Coleoptera:Curculionidae)*	Reduction of larvae survival	[57]
	Papaver somniferum	*Iraella luteipes (Hymenoptera: Cynipidae)*	Increased mortality of larvae	[58]
	Corchorus capsularis	*Apion corchori (Coleoptera: Apionidae)*	Reduced infestation	[59]
Beauveria bassiana	*Gossypium hirsutum*	*Aphis gossypii (Hemiptera: Aphididae)*	Decreased reproduction	[60]
	Solanum lycopersicum	*Spodoptera exígua* (Lepidópteros: Noctuidae)	Decrease in the development of larvae	[61]
	Triticum aestivum	*Spodoptera littoralis (Lepidoptera: Noctuidae)*	Increased mortality of larvae	[62]
	Não relatado	*Thaumastocoris peregrinus (Hemiptera: Thaumastocoridae)*	Increased virulence in larvae	[63]
	Glycine max	*Helicoverpa gelotopoeon (Lepidoptera: Noctuidae)*	Decreased life cycle	[64]
	Capsicum annum	*Myzus persicae (Hemiptera: Aphididae)*	Increased mortality of larvae	[65]
	Fragaria sp.	*Myzus persicae (Hemiptera: Aphididae)*	Reduced feeding	[66]
	Zea mays	*Spodoptera frugiperda (Lepidoptera: Noctuidae)*	Increased mortality of larvae	[67]
	Vitis venifera	*Aphis illinoisensis (Hemiptera: Aphididae)*	Increased virulence in larvae	[68]
Curvularia lunata	*Pelargonium graveolens Melia azedarach*	*Spodoptera littoralis (Lepidoptera: Noctuidae)*	Increased mortality of larvae	[55]
Isaria fumosorosea	NR	*Thaumastocoris peregrinus (Hemiptera: Thaumastocoridae)*	Increased virulence in larvae	[63]
	Capsicum annum	*Myzus persicae (Hemiptera: Aphididae)*	Increased mortality of larvae	[64]
Metarhizium robertsii	*Zea mays*	*Agrotis ipsilon (Lepidoptera, Noctuidae)*	Growth decrease	[66]

Metarhizium anisopliae	*Brassica napus*	*Plutella xylostella (Lepidoptera:Yponomeutidae)*	Increased mortality of larvae	[69]
Metarhizium pingshaense	NR	*Thaumastocoris peregrinus (Hemiptera: Thaumastocoridae)*	Increased virulence in larvae	[63]
	Capsicum annum	*Myzus persicae (Hemiptera: Aphididae)*	Increased mortality of larvae	[64]
	Zea mays	*Spodoptera frugiperda (Lepidoptera: Noctuidae)*	Increased mortality of larvae	[65]
	Saccharum officinarum	Sugar cane leafhopper *(Hemiptera: Cercopidae)*	Increased mortality of larvae	[36]
	Zea mays	*Anomala cincta (Coleoptera: Melolonthidae)*	Increased virulence	[70]
Phomopsis oblonga	*Ulmus minor*	*Physocnemum brevilineum (Coleoptera: Cerambycidae)*	Reducing the spread of the disease	[71]
Purpureocillium lilacinum	*Gossypium hirsutum*	*Aphis gossypii (Hemiptera: Aphididae)*	Decreased reproduction	[60]
Rhizophagus sp.	*Solanum lycopersicum*	*Spodoptera exígua* (Lepidópteros: Noctuidae)	Decrease in the development of larvae	[61]
Sarocladium strictum	*Cynanchum acutum,*	*Spodoptera littoralis (Lepidoptera: Noctuidae)*	Deformity in adults	[56]
Trichoderma strigosellum	*Eucalyptus urophylla*	*Atta* spp. *Acromyrmex* spp. (Hymenoptera: Formicidae)	Infect the ant's nest	[22]
Epichloë spp	*Achnatherum robustum*	Aphids (Hemiptera: Aphidoidea)	Increased larval mortalitygonovina	[45]

Table 1: Endophytic fungi with entomopathogenic activity and their respective target insects in agriculture.

The entomopathogenic fungus attacks the target insect first, adhering to the conidia on the insect integument and, soon after, germination of these conidia begins (12 h at temperatures between 23 °C and 30 °C) [38]. In *M. robertsii*, adhesin MAD1 is essential for insect cuticle conidial spore adhesion [39], whereas hydrophobins play a role in *Beauveria* [40]. To successfully parasitize insects, the fungus needs to evade the immune system of the animal. The penetration process occurs through enzymatic action (lipases, proteases, amylases, etc.) or through the mechanical pressure exerted by the germinative and appressor tube on the insect integument. Colonization begins with the penetration of hyphae that initially thicken and branch out in the insect integument and then in the hemolymph, where they differentiate into blastospores (yeast-like asexual spores). Blastospores absorb nutrients in the hemocele and produce insecticidal metabolites, such as beauvericin and destruxins [41,42], resulting in insect death within a few days [15,42].

Recent research has shown that the endophytic fungus *Metarhizium robertsii* has the capacity to colonize parts of plants, especially the roots, and is able to promote plant root growth and infect soil larvae that feed on the roots. After penetrating the larvae, *M. robertsii* produces insecticidal metabolites such as beauvericin and destruxins, which kill the larvae, and together, the plant and fungi receive carbon, and nitrogen the decaying larvae [43,44]. Another example of entomopathogenic activity is found in the grass species *Achnatherum robustum*, which hosts endophytic microorganisms of the genus *Epichloë* that produce deadly alkaloid substances for aphids, specifically Ergonovina [45].

Endophytic microorganisms with entomopathogenic action can be inoculated into other plant species to protect against insect pests, thereby supporting the optimal development of the plant. Leaf-cutting ants of the genus *Atta* and *Acromyrmex* (Hymenoptera: Formicidae) are considered the main defoliating pests of the genera *Pinus* and *Eucalyptus*, causing large economic losses in plantations [22]. Some endophytic fungi can infect leaf-cutting ants, causing death or direct or indirect destruction of nests. This is achieved either by consumption of the symbiotic fungus *Leucoagaricus gongylophorus,* or through competition for the substrate [46]. Endophytic fungi of the genus *Trichoderma* adapt easily to environments, have high resistance to different environmental conditions, promote protection and growth for plants, out compete other fungi, and are antagonistic to the symbiotic fungus *Leucoagaricus gongylophorus* [47-49]. According to Batista, et al. [22], fungi of the genus *Trichoderma* can be inoculated into and colonize endophytic plants of the species *Eucalyptus urophyll*, thereby improving their development and receiving protection, suggesting that the plant material, colonized by endophytes, is transported to the nest, and can destroy the symbiotic fungus *L. gongylophorus* and, consequently, exterminate the colony of leaf-cutting ants. The result is not conclusive, as there may be other influencing factors; however, it may be an important strategy for the biological control of this pest, not only for eucalyptus, but also for other plantations that are intensely damaged by leaf-cutting ants.

In another study, the effects of colonization of endophytic fungi *Rhizophagus* and *Beauveria bassiana*, inoculated in tomato plants, were investigated in the defense of plants against herbivorous insects. Herbivore feeding tests using the *Spodoptera exigua* (Lepidoptera: Noctuidae) caterpillar, demonstrated that the caterpillars fed with the inoculated tomato gained significantly less weight in comparison to those fed with the non-inoculated plant, suggesting that the inoculated tomato plants had a stronger defense response against the caterpillar [50].

The application of biotechnology to endophytes has been an alternative for biological pest control, where plants receive genetically modified endophytes that act as vectors, expressing proteins with insecticidal activity, and providing increased resistance of plants to diseases or insect pests. The leafhopper of the species *Sogatella furcifera* (Hemiptera: Delphacidae) sucks the sap from rice plants (*Oryza sativa*) causing “burning” of the leaves. In this case, the recombinant DNA technique was used to transfer the gene that expresses the plant lectin of the species *Pinellia ternata* to the endophytic bacterium *Enterobacterium cloacae*. Subsequently, they were inoculated into rice seedlings to produce natural insecticides to obtain natural protection against *S. furcifera* [23].

Endophytic microorganisms can also have their genomes modified to obtain lineages with genetic characteristics that favor the host plant, such as high specificity, increased production, greater efficiency, and resistance, through exogenous genes that express proteins with insecticidal activity [1,23]. The first introduction of an exogenous gene into an endophytic microorganism, using recombinant DNA technology, aimed at insect control, was carried out by Fahey and colleagues in 1988. A gene from the bacterium *Bacillus thuringiensis* responsible for producing toxins against insects was introduced into the genome of the bacterium *Clavibacter xyli* subsp. *cynodontis* and inoculated into corn plants, improving protection of the plant against attack by insects sensitive to toxins [37].

Lectins are proteins present in most plants, especially vegetables, and can be considered a defense mechanism against microbial agents and insects, making them excellent pesticides in biological pest control [51]. Studies developed by Sharon N, et al. [52] demonstrated that herbs of the species *Pinellia ternata*, native to Korea, China, and Japan, expressed plant lectin by inhibiting aphids and sharpshooters of the species *Sogatella furcifera* (Hemiptera: Delphacidae), considered an important pest in rice culture and known as “burning by suctio” due to the ability to suck sap from the plants causing “burning” of the leaves. The gene responsible for the expression of plant lectin in *Pinellia ternata* was introduced into the endophytic bacterium *Enterobacterium cloacae*, which was reinoculated into rice seedlings as recombinant endophytes, successfully obtaining rice seedlings with the transformed endophytic bacteria exercising insecticidal activity against the leafhopper and developing a new biological control strategy for this insect pest [23,53].

The bacterium *Bacillus thuringiensis* acts as an endophytic microorganism in cabbages, controlling insects of the species *Plutella xylostella* (Lepidoptera: Plutellidae) that cause damage [43] and has been widely used in the biological control of pests to produce crystalline proteins that are toxic to insects that severely damage plantations and trees. Researchers cloned the cry218 gene responsible for expressing the toxic crystalline proteins of *Bacillus thuringiensis* and introduced the bacterium *Burkholderia pyrrocinia*, an endophytic microorganism of the tree species of the genus *Populus*. In bioassays, the bacterium *B. pyrrocinia* with the cry218 gene was inoculated into plants supplied as food for *Bombyx mori* (Lepidoptera: Bombycidae) caterpillars, demonstrating that the genetically modified bacterium was toxic to caterpillars of this species and has great potential for use in the control of *lepidopteran caterpillars* in agriculture (Table 2) [54].

Endophytic microorganisms with entomopathogenic action and genetically modified endophytic microorganisms, used to express toxins against insect pests within the host plant, are considered important biotechnological alternatives for the biological control of pest populations and should be used as part of an integrated pest control management program at levels that do not harm the environment and minimize the use of chemicals.

Endophytic bacteria	Plant Species	Target pest	Conclusion	Reference
Bacillus thuringiensis	*Brassica oleracea var. capitata*	*Plutella xylostella (Lepidoptera: Plutellidae)*	Increased larval mortality	[72]
Burkholderia pyrrocinia	*Populus* sp.	*Bombyx mori (Lepidoptera: Bombycidae)*	Increased toxicity	[54]
Enterobacterium cloacae	*Oryza sativa*	Sogatella furcifera (Hemiptera: Delphacidae)	Increased larval mortality	[23]
Pseudomonas marginalis	*Brassica olerace*	*Mamestra brassicae*	Reduced survival of the caterpillars	[73]
Pseudomonas azotoformans	*Brassica olerace*	*Mamestra brassicae*	Reduced survival of the caterpillars	[73]
Pseudomonas spp.	*Calotropis procera*	*Galleria mellonella*	Increased larval mortality	[74]

Table 2: Endophytic bacteria with entomopathogenic activity and their respective target insects in agriculture.

Conclusion

The existing plant biodiversity and the discovery of new strains of endophytic microorganisms with the potential to promote plant growth and protect against insect pests, must be used for the sustainability of resources instead of chemicals that are harmful to human health and the environment. Endophytic microorganisms can be utilized to control the populations of insect pests that cause economic damage to agriculture.

The capacity of these microbes to inhabit the same niche as that of these pests is an interesting approach to explore. In addition, endophytes can be systemically disseminated into the host plant, thereby inducing resistance in the plant or protecting it through metabolite production.

The use of biotechnological tools, such genetically engineered endophytic microorganisms used in a delivery system of toxic metabolites to pests, is an approach that requires further in-depth study, and can be an effective option to promote protection to high economic crops and to the environment. The use of endophytes is a promising tool that is beneficial to the host plant, reduces economic losses to the farmer, and decreases the use of harmful chemicals.

Conflict of Interest Statement

The authors declare that they have no conflict of interest.

Contribution

All the authors contributed substantially to the concrescence of the manuscript.

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