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Review

Rizobacteria in the control of pest insects in agriculture

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Biological control of pest insects in agriculture is the focus of many studies, because of the risks in the continuous use of synthetic insecticides, which can cause resistant pests or the occurrence of secondary pests. The use of microorganisms like endophytic bacteria has been performed separately or combined with other forms of control in the Integrated Pest Management. Endophytic bacteria live inside host plants, without causing any apparent damage or pathogenicity symptom. Besides performing many functions important to the host, these microorganisms are potentially useful in agriculture, since they are capable of substituting chemical products. By performing biocontrol actions and/or promoting plant growth, these microorganisms are favoring environmental preservation and are thus identified as a viable alternative for ecologically and economically sustainable agricultural production systems. Given the above, this review aimed to present a panorama of the potential application of plant growth-promoting bacteria in the control of pest insects in agriculture, in view of the great biotechnological advances.

Key words: Endophytic bacteria, plant growth promotion, biological control.

INTRODUCTION

The interest in the use of microorganisms in agriculture has increased significantly in the last years, because both in plant growth promotion and insect biological control, among other applications, they are potential substitutes of chemical products, thus favoring environmental preservation (Peixoto Neto et al., 2002; Souza, 2001). These microorganisms have the important property of providing protection to plants, either by the presence of endophytic microorganisms in host plants or by the application of biocontrol agents, which can result in the elimination of important agricultural pests (Souza, 2001). Due to this, there has been a great interest in the study on occurrence, colonization potential and the use of endophytic bacteria to promote plant growth and pest biological control in agriculture.

Microbial, especially bacteria-based, insecticides have been seen since the 1970s as an excellent alternative of biological control, considering the resistance developed by insects to chemical pesticides. Many products based on these microorganisms are available in the market to control a diversity of pests (Federici et al., 2010), and these products have reached a higher level in the global

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market because of the ecological and environmental demands.

The problems caused by the use of insecticides to guarantee high yields in agriculture over time have led to important
studies in the search for alternate, safe methods in the control of insects (Machado et al., 2012). The use of plant growth-promoting microorganisms is one of the alternatives for modern agriculture to face the challenge of increasing crop yield with sustainability. Among these microorganisms, there are the endophytic bacteria, also known as plant growth-promoting bacteria (PGPB). These bacteria, which are found inside plant tissues, especially leaves, branches and stem, without causing any apparent damage to the host, have a great potential for insect biological control, and the host-plant protection promoted by these endophytes is very complex (Azevedo et al., 2000). The capacity for biocontrol and plant growth promotion of these endophytic microorganisms can come from various mechanisms, such as: biological nitrogen fixation (Huergo et al., 2008; Han et al., 2005), phosphate solubilization (Rodriguez et al., 2004; Vasselev and Vassileva, 2003; Vassey, 2003), production of growth hormones like auxins, gibberellins and cytokinins (Donate-Correa et al., 2004; Radwan et al., 2004; Creus et al., 2004; Dobbelaere et al., 2003), synthesis of siderophores (Vessey, 2003), among others.

However, the role of these endophytes microorganisms in plants has been much discussed but little understanding and studies have been developed to further understand PGPB potentialities for the biological control of insects. Therefore, the use of PGPB will probably be one of the most important tactics in the current world, due to the emerging demand to decrease the dependence on chemical products and to the need for the development of a sustainable and productive agriculture (Moreira and Siqueira, 2006).

ENDOPHYTIC BACTERIA ASSOCIATED WITH PLANTS

The bacteria that colonize internal plant tissues without causing damages to the host are called endophytic (Schulz and Boyle, 2006; Strobel et al., 2004). This definition includes all bacteria living inside a plant in at least one period of the life cycle, and can be found in the rhizosphere, root surface or even inside root tissues, stems and leaves of plants (Berg et al., 2005; Okunishi et al., 2005; Sessitsch et al., 2002). Some of these bacteria have positive effects on plant development, producing substances that favor plant growth and/or avoid the development of pathogenic organisms (Pavlo et al., 2011; Hallmann, 2006; Ping and Boland, 2004).

Endophytic bacteria can develop their entire cycle in a host plant, depending on it for development and reproduction, in this case called obligatory endophytes, or develop part of their cycle outside a host plant, being called facultative endophytes. The division of the term into facultative and obligatory endophyte was proposed to distinguish, respectively, strains capable of colonizing both the surface and the inside of roots and able to survive in soil, from the ones that do not survive in the soil, but colonize the inside and the shoots of plant tissues without causing pathogenicity symptoms (Baldani et al., 1997).

The capacity to colonize the interior of a plant can provide endophytic bacteria with ecological advantages over the others, because internal plant tissues provide a more uniform and protected environment for micro-organisms compared with the surface, where they are exposed to extreme environmental conditions of temperature, ultraviolet radiation and microbial competition, which are the most limiting factors for the survival of bacteria over time (Cocking, 2003).

Despite systematically colonizing plants, endophytic bacteria have a preference to colonize certain tissues. Kuklinsky-Sobral et al. (2004) observed, in soybean, that the density and diversity of endophytic bacteria vary according to tissue, plant development stage, seasonal changes and host genotype, where the observed bacterial density was higher in roots in lower in leaves. Endophytic bacteria can change physiological and morphological conditions in the host, besides acting in other microorganisms living inside plants (Andreote et al., 2006).

The intimate plant-endophyte relationship has shown interesting characteristics for biotechnological and agricultural applications, like the promotion of plant growth, because both organisms benefit from it, that is, bacteria can provide nutrients to plants, through biological nitrogen fixation for instance, while plants provide carbon-rich exudates to bacteria. The plant-endophyte relationship is not fully comprehended yet, but apparently neutral and beneficial relationships are known to exist (Melo, 1998).

BIOTECHNOLOGICAL POTENTIAL OF ENDOPHYTIC BACTERIA

Endophytic microorganisms were first mentioned in the beginning of the 19th century, but Bary (1866) was the one who first outlined a possible distinction between them and plant pathogens. Defined as asymptomatic, they remained almost forgotten until the end of the 1970s, when, for a number of reasons, they started to draw attention. At this time, it was verified that, far from mere inhabitants of plant interior, they had properties of interest, such as providing protection against pest insects (Azevedo et al., 2000), which made evident their biotechnological potential.

Endophytic microorganisms are potentially useful to agriculture and industry, particularly to food and pharmaceutical sectors; many selected endophyte species have the potential to be used in agrochemical industries, besides being used as genetic vectors (Souza et al., 2004). These microorganisms can produce toxins, antibiotics and other pharmaceuticals, besides performing other functions important to the host, such as providing higher resistance to stress conditions, changing physiological properties and producing phytohormones (Azevedo et al., 2000).

The systematic resistance induced against a broad spectrum of pests is acquired after appropriate stimulation and has been reported as the explanation for the control of pest insects in agriculture. The modification in the structure of cell walls (deposition of lignin) and the biological and physiological changes lead to the synthesis of proteins and chemical substances involved in plant defense mechanisms.

The potential to use PGPB in the control of pest insects has been related to stimuli generated in the plant itself, through the action in different metabolic routes (salicylic acid, jasmonic acid and ethylene). These compounds act as elicitors to induce defense and/or resistance, which are kept inactive in their absence. This process, called resistance induction, causes plant to produce or increase the production of proteinase-inhibitor compounds (pathogens produce extracellular proteinases and, in response to their action, plants synthesize inhibitors like serine, cysteine and aspartate), glycoalkaloids, polyphenols etc.

In addition, the response induced by the plant can involve other mechanisms like the accumulation of secondary metabolytes (synthesis of siderophores, phytoalexins and phenylpropanoids) and biosynthesis of pathogenesis-related proteins (PR-protein). Phytoalexins, considered as a microbial property, accumulate in the infection site and around it. Phenylpropanoids catalyze the formation of trans-cinnamic acid – precursor of various plant defense compounds. Pathogenesis-relate proteins (peroxidase of phenols) are associated with processes related to cell wall and lignification of plant cells during the defense reaction against the pathogenic agent – the formation of papilla and hypersensitive response –, making difficult pathogen entrance, establishment and development in the host plant.

The elucidation of the ecological functions of these endophytic microorganisms can bring benefits, especially to the exploration of their biotechnological potential as plant-growth promoting agents (Peixoto Neto et al., 2002), and the characteristics of the growth-promoting potential can be analyzed through molecular techniques, with the quantification of genes involved in the desired characteristics. The biochemical tests are also useful in the search of microorganisms with the capacity to produce indoleacetic acid (IAA), phosphate solubilization, nitrogen fixation (Kuklinsky-Sobral et al., 2004), resistance induction, biological control of pests and diseases (Ramamoorthy et al., 2001) and production of siderophores (Compant et al., 2005).

BIOLOGICAL CONTROL AS A MECHANISM OF PLANT GROWTH PROMOTION

Chemical pesticides have been used in agriculture for a long time (Grigoletti Junior et al., 2000); however, besides their risks to human health, they also cause strong environmental imbalances, destructing the natural enemies of the different crop pests where they are applied. Chemical control can cause damage to the microbiota that is beneficial to plants, besides frequently leaving residues in the environment (Ethur et al., 2007) and in foods. Thus, the use of bacteria as an action of biocontrol and/or promotion of growth has been identified as a viable alternative for ecologically and economically sustainable agricultural production systems (Sousa et al., 2009; Compant et al., 2005), and the biological control through antagonists has allowed viable solutions for many pests considered difficult to control.

Bacteria, especially the genus *Bacillus*, have significant participation among the commercialized biological control products. Up to 50% of these products are bacterial formulations, from various species of *Bacillus.* The identification of different action mechanisms can lead to the combination of isolates to control a broad spectrum of pests (Lutz et al., 2004). Biotechnology can contribute to biological control, transforming microorganisms, so that they express more than one gene responsible for the desired characteristics, combining different action mechanisms (Timms-Wilson et al., 2000).

ENDOPHYTIC BACTERIA IN THE CONTROL OF PEST INSECTS IN AGRICULTURE

In Brazil and worldwide, many studies have described the use of different endophytic and/or plant growth-promoting bacteria in the control of pest insects references. In the study of Bong and Sikorowski (1991), it was found alteration in larval growth and reduction in the emergence of adults of *Helicoverpa zea* caused by *Pseudomonas maltophila*.

In 2006, Thuler et al. (2006) verified that the isolates EN4 of *Kluyvera ascorbata* and EN5 of *Alcaligenes piechaudii*, little reported in the literature in studies on insects, reduced the viability of *Plutella xylostella* in about 80 to 50%, respectively, indicating a broad field of research to explore the potentials of endophytic bacteria. Selecting and characterizing Brazilian strains of *Bacillus thuringiensis* toxic to *P. xylostella*, Praça (2012) found that the *B. thuringiensis* strains S1905 and S2122 caused

100% of mortality in caterpillars in the 3º instar of *Plutella xylostella* in evaluation performed 48 h after caterpillars had been exposed to selective bioassays, whereas S2124 caused 58.33% of mortality after the same period and 98.33% of mortality after 96 h. In these cases, CL_{50} values ranged from 2.33 to 4.84 ng/mL, with results of toxicity similar to the control. Viana et al. (2009) found that, from the 58 isolates of *B. thuringiensis* tested in caterpillars of *Plutella xylostella*, 12 caused 100% of mortality within 24-48 h: 3A.140, T3A.259, T08.024, E1, E26, 2.7L, 1.7L, E22, 22.7L, 49.19A and E2. Castelo Branco et al. (2003) observed 100% of mortality for larvae of *Plutella xylostella* in the 2º instar with the application of *B. thuringiensis.*

Recently, Macedo et al. (2012), selecting and characterizing native strains of *B. thuringiensis* toxic to *Diatraea saccharalis* (Lepidoptera: Crambidae), observed that the strains causing more than 75% of mortality after dilution of 50 times were: S602, S1264 and S1301. From these strains, the most toxic to *D. saccharalis* were S602 and S1264, with statistically similar CL_{50} values, but different from S1301. Melatti et al. (2010), selecting strains of *B. thuringiensis* for the control of cotton aphid (*Aphis gossypii*), found that the strains S29, S40, S616, S1576 (*Bacillus aizawai*) and S1168(*Bacillus kurstaki*) were the most toxic to *Aphis gossypii*, causing mortality higher than 50%. Among the analyzed strains, S29 and S1168 were the most effective in the selective bioassay, causing mortalities of 76 and 73% against *A. gossypii*, respectively.

Despite the various studies involving the lepidopteran *Spodoptera frugiperda* and its control, it is difficult to find bacterial isolates pathogenic to this species. This claim is confirmed in the study of Polanczyky et al. (2003), using 58 subspecies of *Bacillus thuringiensis* in *S. frugiperda*, which showed that only the *Bacillus thuringiensis morrisoni* caused 80% of mortality in caterpillars. Also, Berlitz et al. (2003), testing 24 isolates of *Bacillus thuringiensis* from many rice-growing regions of Rio Grande do Sul, Brazil, in the control of *S. frigiperda*, obtained the best mortality rates between 31.6 and 100% with only five isolates. Campanini et al. (2012), studying the pathogenicity of isolates of *B. thuringiensis* over *Spodoptera frigiperda* and *Sphenophorus levis* found that the isolates IB17.3 and IB8.2 are highly efficient in the control of caterpillars of *S. frugiperda*, and that the isolate IB26.2 is the most efficient in the control of larvae of *S. Levis*, all of them with average mortality rates higher than 75%.

However, the fact that an isolate causes mortality to caterpillars does not mean that they will be active in the insect when the toxic proteins are purified. The CL_{50} of Cry proteins of *Bacillus thuringiensis aizawai* for caterpillars of *S. frugiperda* in the 3º instar was determined by Lucho (2004). The obtained results indicated CL_{50} of 2.22; 0.41 and 0.18 µg/mL for 2, 3 and 4 days after treatment application, respectively, and revealed that the proteins Cry1Aa, Cry1Ab, Cry1C and Cry1D, synthesized by *Bacillus thuringiensis aizawai*, are highly toxic to *Spodoptera frugiperda*. For the same species, data from Knaak et al. (2007) show that the toxicity of the proteins Cry1Ab and Cry1Ac, synthesized by *Bacillus thuringiensis aizawai* 407 and *Bacillus thuringiensis kurstaki* HD73, respectively, revealed a CL_{50} of 9.29 and 1.79 $\mu g/cm^2$ to caterpillars in the 1º instar.

Analyses of mortality of caterpillars of *Anticarsia gemmatalis* caused by isolates of *Bacillus thuringiensis*, performed by Azambuja and Fiuza (2003), showed 37 and 50% of corrected mortality against velvetbean caterpillar using two natural isolates of *Bacillus thuringiensis* from rice-growing regions of Rio Grande do Sul, Brazil. In the pathogenicity evaluations performed with primitive isolates, Silva and coworkers (2004) revealed high numbers of isolates pathogenic to the same insect order. Praça et al. (2004) verified that, among the 300 tested strains of *Bacillus thuringiensis*, only S234 and S997 were simultaneously effective in the control of larvae of *Spodoptera frugiperda, Anticarsia gemmatalis, Anthonomus grandis, Aedes aegypti* and *Culex quinquefasciatus*.

For *Oryzophagus oryzae*, little data is reported in the literature with respect to the action of proteins of *Bacillus thuringiensis*. Pinto and coworkers 2003 selected 6 isolates of *Bacillus thuringiensis* with the presence of genes from the class *cry*3 or *cry*7, which synthesize proteins insecticidal to coleopterous, and evaluated their insecticide activity to *O. oryzae*. From the tested isolates, two caused corrected mortality of 100%, three between 59 and 67% and one around 50%. Steffens et al. (2001) obtained 53.41% of mortality of larvae of *O. oryzae* with an isolate of *B. thuringiensis* containing *cry3* genes, specific to coleopterous. Results obtained by the different authors confirmed the prediciton of the insecticide action of *B. thuringiensis*, possibly due to the presence of the *cry3* and *cry7* genes, which codify proteins specific to coleopterous.

References on endophytic bacteria against isopterous are restricted, with little data available, such as Castilhos-Fortes et al. (2002). Considering the pathogenicity of *Bacillus thuringiensis* for *Nasutitermes ehrhardti*, these authors tested 57 strains of this bacteria, and found the seven most effective: *B. thuringiensis sooncheon* (Bts) and *B. thuringiensis roskildiensis* (Btr) with a mortality of 100%; followed by the isolates *B. thuringiensis yunnanensis* (Bty) with 71.4%; *B. thuringiensis huazhongensis* (Bth) with 57.1%; *B. thuringiensis brasiliensis* (Btb) with 52.3%; *B. thuringiensis colmeri* (Btc) with 42.85% and *B. thuringiensis kurstaki* (Btk) with 28.57% of mortality at the 7th day after treatment application. For the determination of CL_{50} of *B. thuringiensis*, these authors used the isolates *B. thuringiensis sooncheon* and *B. thuringiensis roskildiensis*, which caused 100% of mortality in the pre-selective assays. The observed CL_{50} values of *B. thuringiensis sooncheon* were 46.98 × 10^8, 66.19 × 10^6 and 5.14 × 10^5 spores/mL, at 3, 5 and 7 days after treatment application. For *B. thuringiensis roskildiensis*, the values were 30.78 × 10^5, 48.40 × 10^6 and 16.80 × 10^4 spores/mL at 3, 5 and 7 days, respectively.

Besides all the above mentioned information, endophytic and/or plant growth-promoting bacteria can be used in combination with other microorganisms in the control of pest insects in agriculture. In this context, Broderick et al. (2000) identified an increase of 35% in the mortality of the lepidopterous *Lymantria dispar* (L.) when using *B. thuringiensis* and zwittermycin A of *Bacillus cereus*, which is responsible for the synergetic effect of the microorganisms. Results from Wraight and Ramos (2005) also show synergism of 35.2, 33.8, and 21.1% when commercial products based on *B. thuringiensis* and on the fungus *Beauveria bassiana* were simultaneously used in *Leptinotarsa decemlineata*. These authors reveal that the interaction may have resulted from the intoxication caused by entomopathogen, inhibiting insect feeding, thus causing stress, and physiological effects, which facilitated fungus penetration in the insect. Similar effects were also observed by Ma et al. (2008), when the Cry 1Ac protein of *B. thuringiensis* was used with *B. bassiana*. These authors observed deleterious effects in the mortality of larvae of *Ostrinia furnacalis* (Lepidoptera: Crambidae), besides the decrease in the formation of pupae and emergence of the adult insects.

These data indicate that studies on PGPB are increasingly important, since chemical insecticide application results in large impacts on the ecosystem, because they not only affect the natural enemies of the insects, but also contaminate soil and underground waters.

CONCLUSIONS

Given the information and the promising results already obtained with respect to the interaction between bacteria and host plants, the study on endophytic bacteria as biocontrol agents for inumerous pests and as plant growth promoters has been gaining special attention. The practical applications of these microorganisms tend to increase, as the aspects of this interaction become better understood. Therefore, it is essential to know the diversity of these endophytes, their presence, frequence and functions, because this understanding will allow expanding the spectrum of use of endophytes as a biotechnological tool, aiming to increase yields and decrease the use of agrochemicals, besides providing an efficient, economic and ecological alternative for the solution of damages caused by pest insects in agriculture.

Conflict of Interest

The authors have not declared any conflict of interest.

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