Successful and unexpected potential of entomopathogen *Bacillus thuringiensis* as plant growth promoting rhizobacterium

Exitoso e inesperado potencial del entomopatógeno *Bacillus thuringiensis* como rizobacteria promotora del crecimiento de las plantas

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Abstract

The microbiology of the rhizosphere has been investigated since 1904, revealing innumerable rhizomic microorganisms that promote growth in swamps (RPCPs), which have attracted a special interest to improve agroecological practices, crop productivity and protect soils from environmental contamination under a agricultural sustainability approach. This review addresses a topic as extensive as it is fascinating, beginning with the characteristics of soil microbiology. It also describes how RPCPs associated with plant roots offer greater crop growth through direct and indirect strategies. Also mention the complexity of plant growth and how it is done simultaneously through numerous metabolic activities. The bacterial genera of RPCP, the metabolic characteristics and the potential of entomopathogenic bacteria *Bacillus thuringiensis* (*Bt*) to be considered as RPCP are mentioned. Finally, the little known properties of *Bt* are exposed to benefit the growth of plants, their effectiveness in biocontrol of phytopathogens, as biofertilizers and / or biostimulants in crops. New perspectives are proposed to develop *Bt* products as a growth promoter in the near future. It is important to recognize that applied research of rhizosphere crops can be one of the key factors in achieving the sustainable yield of agriculture.

Resumen

La microbiología de la rizósfera se ha investigado desde 1904, revelando innumerables microorganismos rizómicos promotores del crecimiento en pantas (RPCPs), que han atraído un interés especial para mejorar las prácticas agroecológicas, la productividad de los cultivos y proteger los suelos de contaminaciones ambientales bajo un enfoque de sustentabilidad agrícola. Esta revisión trata un tema tan extenso como fascinante, iniciando con las características de la microbiología del suelo. Así mismo, describe cómo las RPCPs asociadas con raíces de las plantas ofrecen un mayor crecimiento de cultivos mediante estrategias directas e indirectas. También mencione la complejidad del crecimiento vegetal y cómo se realiza simultáneamente mediante numerosas actividades metabólicas. Se mencionan géneros bacterianos de RPCP, las características metabólicas y el potencial de la bacteria entomopatógena *Bacillus thuringiensis* (*Bt*) para considerarla como RPCP. Finalmente, se exponen las propiedades poco conocidas de *Bt* para beneficiar el crecimiento de plantas, su efectividad en biocontrol de fitopatógenos, como biofertilizantes y/o bioestimulantes en cultivos. Se plantean nuevas perspectivas para desarrollar en un futuro cercano, productos de *Bt* como promotor de crecimiento. Es importante reconocer que la investigación aplicada de los cultivos de rizósfera puede ser uno de los factores clave para lograr el rendimiento sostenible de la agricultura.

Rhizosphere, PGPRs, *Bacillus thuringiensis*

Rizósfera, RPCPs, *Bacillus thuringiensis*

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Introduction

The soil is an inexhaustible source of microorganisms that include bacteria, fungi, actinomycetes, protozoa and algae. Of these microorganisms, bacteria are the most abundant (~ 95%), with about 108 to 109 cells per gram of soil being reported, however, the number of bacterial cells cultivable in the soil is minimal, only 1% (Schoenborn et al., 2004). Bacteria produce and secrete numerous highly versatile and dynamic metabolic compounds to defend against microbial competitors (competition strategy) in the environment.

The most documented metabolites include a wide range of broad-spectrum antibiotics, lytic enzymes, metabolic by-products, proteinaceous exotoxin siderophores and antimicrobial peptides such as bacteriocins. Soil-isolated bacteria (rhizobacteria) and / or their metabolites have the ability to promote growth in different plants, which has been used as an alternative strategy to reduce the application of chemicals such as fertilizers and / or synthetic pesticides considered as the causes of catastrophic damage to the environment, human health and society (Subramanian and Smith, 2015; Kashyap et al., 2017).

Plant growth promoting rhizobacteria (RPCP)

Currently the rhizosphere concept refers to a soil that surrounds a root whose physical, chemical and / or biological properties are modified by the development, growth and activity of the root (Saharan and Nehra, 2011), although the term was coined for the first time in 1904 by Hiltner, the pioneer of research in microbial ecology of the rhizosphere and soil bacteriology. This activity is characterized by a high amount of exudates from the root mainly carbon secreted by the plant, thus promoting the growth of an amount of rhizobacteria even greater than in the soil surrounding the rhizosphere (Lugtenberg and Kamilova 2009; Kang et al., 2010).

Rhizobacteria have attracted scientific research for the ability to produce various plant growth promoting metabolites, and they are properly recognized as plant or plant growth promoting rhizobacteria (RPCV or RPCP) that act through various mechanisms applied as biofertilizer (direct) or in the biocontrol of phytopathogens (indirect) (Vacheron et al., 2013; Arellano, 2019).

Both mechanisms act simultaneously in the process of plant growth as seen in Figure 1

Figure 1 Mechanisms that perform rhizobacteria to promote plant growth directly as biofertilizers or indirectly for the biocontrol of phytopathogens

The activity of the RPCV has been described by both mechanisms, in the case of biofertilization the provision of nutrients (N, P, K), mineral salts is mentioned, as well as the regulation of the biosynthesis of plant hormones (auxins, gibberellins, cytokinins, ethylene). With respect to biocontrol, the action consists in the systematic inhibition or induction of resistance to various microorganisms caused by pathogen antagonist substances, developing effective biocontrol agents, root colonizers and environmental protectors (Figueiredo et al., 2016). Both mechanisms of action are evidenced through the interaction of the root of the plant with the RPCV. In the box of figure 1 they are observed in the CPR (*Bacillus thuringiensis*) in tomato root.

Plant growth is a very complex process and is carried out through numerous metabolic activities that are carried out simultaneously and that involve both bacteria and fungi for proper fertilization and nutrition of the plant (Figure 2). Likewise, during this process phosphates are solubilized and ribosomal peptides (bacteriocins) and non-ribosomal metal chelates (siderophores) are produced. In addition, an antagonistic activity is exerted by different genera of bacteria (*Bacillus*, *Paenibacillus*, among others), as well as detoxification caused by heavy metals, and the specific activity of growth phytohormones, ethylene synthesis inhibitors (C₂H₄), the chelating action of siderophores, and a marked systematic defense response such as antibiosis, antibiotics, hydrolytic enzymes as shown in Figure 2 (Glick, 2012; García-Fraile, et al., 2012).

Figure 2 Metabolic activities carried out simultaneously by bacteria and fungi for the fertilization and nutrition of a plant

Despite the advantages of using the CPR inoculums mentioned above, there are a number of requirements that must be met to achieve a registration for marketing. Priority requirements include demonstrating the optimal production of the crop by means of some commercially fermentative bioprocess without modifying stability and without compromising the activity of the microorganism. Likewise, the biosecurity of the product, the ease of application in the crops and confirm the shelf life must be guaranteed. All of the above should be considered in the cost that will depend on the type of product, whether it is a biofertilizer (or supplement) or a biological control agent, and in both cases the information must be submitted to the corresponding federal agency to request registration and patent in each country (Nelson, 2004).

Some of the international companies that market products with RPCV reported are: Novozymes, Loveland Products, Inc. BASF Canada Inc., EVL Inc., Labiofam SA, Institute of Biological Phosphate (IFB) Ltda, Brett-Young Seeds, Tokachi Federation of Agricultural Cooperatives (TFAC), Biomax, Symborg, Amnrite A 100®, Armor-Zen®, RhizoVital®, 42 Sonata®, Trichobacter®, TwinN®, and many more products (García-Fraile et al., 2015; Bishnoi, 2015; Azizoglu, 2019; Alcantara, 2019). It is worth mentioning that the success of each product with RPCV will depend on soil survival, soil ecology, host compatibility, microbial competitiveness and biotic and abiotic factors of the micro-environment (Martínez-Viveros, et al. 2010).

Plant growth promoting bacteria

Root or rhizobacterial colonizing bacteria that exert beneficial effects on plant development through the aforementioned mechanisms and also as promoters of seed germination and plant growth (Nelson, 2004; Fernández et al., 2012) cover various bacterial genera.

Recently in (Kashyap et al., 2017) the bacterial genera of *Bacillus*, *Streptomyces*, *Pseudomonas*, *Burkholderia*, *Agrobacterium*, among others, were reported considering *Azotobacter*, *Azospirillum*, *Enterobacter*, *Klebsiella*, *Serratia*, *Streptomyces*, *Variovorax* (*Rhizobia*, Glick, reported 2012), as the most studied and most commercialized biological control agents such as RPCV inoculants that benefit plant growth. RPCVs act by at least one mechanism as bioprotectors reducing the development of phytopathogens, they also function as biofertilizers that capture and translocate nutrients, and also as biostimulants of phytohormones levels (Kashyap et al., 2017).

The microbial community of growth promoting bacteria / rhizobacteria (BPCV / RPCV) includes a unique member, the most recognized entomopathogenic microorganism worldwide: *Bacillus thuringiensis* (De la Fuente-Salcido et al., 2013; 2016). This physiologically bacillus has a broad biochemical capacity to synthesize heterogeneous metabolites that allow it a multifaceted activity, which even distinguishes it as a promoter of crop growth due to the effects demonstrated as a biocontrol agent, biofertilizer and biostimulant (Azizoglu, 2019; Qi et al., 2016; Lyngwi et al., 2016; Bai et al., 2003).

Multifaceted bacteria: *Bacillus thuringiensis* as a promoter of plant growth

The bacterium *B. thuringiensis* (Bt) belongs to the genus *Bacillus*, is gram-positive, aerobic, mobile (perimeter), sporulated, synthesizes a parasporal crystal made up of insecticidal proteins (Cry). These proteins have been used for decades to formulate powerful bioinsecticides against pests of diptera, lepidoptera, beetles, mites, among others. In addition to the toxic crystal proteins (Cry), Bt also synthesizes inclusion proteins called cytotoxic (Cyt) (Lacey et al. 2015).

The multifaceted physiological capacity of Bt is recognized not only by Cry and Cyt proteins, but also synthesizes various proteins with very prominent bioactivities. These proteins include potent chitinases (Chi) that enhance the insecticidal effect of Cry, vegetative insecticidal proteins (Vip), also bacteriocins (Bac) that are antimicrobial peptides to inhibit / control pathogens in food and also, cytotoxic proteins known as parasporins (Ps) with specific activity against some types of cancer in humans (Palma et al. 2014; Hollensteiner, et al., 2016; López-De la Cruz et al., 2018).

Several species of the genus *Bacillus* to which Bt belongs have been reported as beneficial microbes to increase the productivity of crops affected by environmental and / or genetic factors, and some subspecies of Bt are considered as BPCV as some of the metabolites they synthesize (chitinases, bacteriocins, ACC, etc.) promote plant growth by acting as natural biostimulants and biofertilizers (Gray et al. 2006; Lee et al. 2009; Subramanian and Smith, 2015; Azizoglu, 2019).

There are some reports of Bt as an adjunct in plant growth and seed germination and include the application of bacillus as a co-inoculant of nitrifying bacteria such as *Bradyrhizobium japonicum* that stimulates growth and nodulation in soybean plants (*Glycine max*) (Bai et al., 2013; Bais et al., 2006).

Bacterialization of tomato seeds with Bt cells is another novel application with biostimulant effects for tomato growth (*Solanum lycopersicum*), increasing the percentage of germination in the seeds and promoting root and seedling elongation in seedlings (Qi et al. 2016). Also, the direct dosing of Bt cell suspensions on the tomato root system stimulates the growth of crops under normal conditions and in plants infected with phytopathogens, modifying the synthesis of antioxidants and the induction of genes related to systemic defense (Akram et al., 2013; Hyakumachi et al., 2013).

With respect to bacteriocins (antimicrobial peptides produced by bacteria as a competition strategy for space and / or nutrients), they have application as bioconservatives in food.

In addition, they have been tested in veterinary medicine as antimicrobials and in agriculture as biostimulants and biocontrol agents (Subramanian and Smith, 2015; López-De la Cruz et al. 2019; De la Fuente-Salcido et al. 2013).

In agriculture, the bacteriocin thuricina 17 (Th17) is synthesized by the endophytic bacterium not *Bradyrhizobium* 17 (*B. thuringiensis* NEB 17) and is the most widely studied to determine its effect on plant growth (Subramanian and Smith, 2015). Th17 supplied in low concentrations (nM) by sprinkling the leaves and by irrigation roots of soybean and corn plants, increased growth in both types of plants. Soy and corn differ in soybeans have no photosynthetic adaptations (C3) and corn performs light dependent reactions and Calvin Cycle (C4) (Lee et al., 2009).

In other investigations, it has been shown that both Th17 bacteriocin and the *B. thuringiensis* NEB17 producing strain are not toxic to some nitrifying bacteria such as *Bradyrhizobium japonicum* 532C (Gray et al. 2006), so they could be used in set, that is, as co-inoculants to increase root nodulation and growth in soy (Bai et al., 2003).

Th17 belongs to the class II bacteriocins of the genus *Bacillus*, they are highly effective as elicitors or activators of enzymes involved in the defense of plants against pests (Damián, 2017). Activation consists in inducing the production of phytoalexins or stimulating any defense mechanism of the plant to protect itself, increasing the activity of the enzymes phenylalanine ammonia-lyase (PAL), peroxidase (POD), ascorbate peroxidase (APX), superoxide dismutase (SOD) and Protoporphyrinogen oxidase (PPO) in soy (Jung et al. 2011).

There are three case reports that relate various subspecies of Bt with plant growth promoting properties acting as biofertilizer and biostimulant (Azizoglu, 2019). The enzyme deaminase of 1-aminocyclopropane-1-carboxylic acid (ACC) or ACC-deaminase, is a mechanism that some RBCVs use to promote the growth of plants in environmental stress, with the advantages of reducing ethylene concentrations in the plant and increase the availability of ammonia in the rhizosphere (Esquivel-Cote et al. 2013), therefore, the activity of ACC-deaminase improves plant nutrition and resistance to stressors.

In (Praca et al., 2012) confirms the effective colonization of four Bt strains native to Brazil verifying the presence of vegetative cells, spores and crystals in buds, leaves and predominantly in cabbage roots.

The above demonstrated for the first time the ability of Bt to colonize cabbage seedlings and control the *P. xylostella* pest and other endophytic insect pests. *B. thuringiensis* SNKr10 has also been reported with significant ACC deaminase activity that contributes to the growth of the spinach plant (*Vigna radiata*) proven in in vitro germination studies of seeds under conditions of heavy metal contamination stress (Sharma and Saharan, 2016).

Mixtures of mycorrhizal fungal strains and Bt strains confirmed their efficiency to improve the growth of toothed lavender (*Lavandula dentata*) in drought conditions. In addition, it was found in the same in vitro experiment under osmotic stress that the Bt strains synthesize ACC-deaminase and indole acetic acid (AIA) without affecting phosphate solubilization (Armada et al., 2016).

It was also confirmed that each fungal strain maintained a particular interaction with Bt reflecting the diversity, intrinsic abilities and inherent compatibility of these microorganisms.

Conclusions

It is evident that the mechanisms of action of RPCV and other little studied bacteria such as *B. thuringiensis*, represent benefits in agriculture. The use of these bacteria represents a novel and very promising strategy to increase plant growth and can be one of the key factors to achieve greater agricultural profitability with a purely sustainable approach.

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