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Actinobacteria as Plant Growth-Promoting Rhizobacteria

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Abstract

Actinobacteria commonly inhabit the rhizosphere, being an essential part of this environment due to their interactions with plants. Such interactions have made possible to characterize them as plant growth-promoting rhizobacteria (PGPR). As PGPR, they possess direct or indirect mechanisms that favor plant growth. Actinobacteria improve the availability of nutrients and minerals, synthesized plant growth regulators, and specially, they are capable of inhibiting phytopathogens. Different activities that are performed by actinobacteria have been studied, such as phosphate solubilization, siderophores production, and nitrogen fixation. Furthermore, actinobacteria do not contaminate the environment; instead, they help to maintain the biotic equilibrium of soil by cooperating with nutrients cycling. The aforementioned is directly related to the quality and productivity of crops. Moreover, different aspects of these microorganisms have been studied, such as production of metabolites that improve plant growth, resilience against unfavorable environmental conditions, and beneficial and synergic interactions with arbuscular mycorrhizal fungi. Taking into account the above-mentioned activities, actinobacteria can be considered as possible plant fertilizers.

Keywords: Actinobacteria, PGPR, nutrients, solubilization, growth

1. Introduction

Actinobacteria are one of the major components of microbial populations present in soil. They belong to an extensive and diverse group of Gram-positive, aerobic, mycelial bacteria that play an important ecological role in soil nutrient cycling [1-4]. These bacteria are known for their economic importance as producers of biologically active substances, such as antibiotics,

vitamins, and enzymes [5]. Actinobacteria are also an important source of diverse antimicrobial metabolites [6-8].

Historically, the most commonly described actinobacterial genus has been *Streptomyces* and *Micromonospora*. In fact, the genus *Streptomyces* is known as one of the largest sources of bioactive natural products [7-9]. Particularly, it has been estimated that approximately two-thirds of natural antibiotics have been isolated from actinobacteria, and about 75% of them are produced by members of the *Streptomyces* genus [10, 11].

In the past decade, research has focused on minor groups of actinobacteria, including species that are difficult to isolate and cultivate, and those that grow under extreme conditions, i.e., alkaline and acidic conditions [6, 12]. However, the vast majority of soil actinobacteria show their optimum growth in neutral and slightly alkaline conditions; thus, the methods of isolation have been traditionally based on this neutrophilic character. Actinobacteria have attracted special interest because these filamentous sporulating bacteria are able to thrive in extremely different soil conditions and they play an ecological role of importance in nutrient cycling. Moreover, they were recently considered as plant growth-promoting rhizobacteria [13-15].

Plant growth-promoting rhizobacteria (PGPR) are free-living beneficial bacteria of agricultural importance. The presence of PGPR produces beneficial effects on plant health and growth by suppressing disease-causing microbes and accelerating nutrient availability and assimilation. Hence, in the quest to improve soil fertility and crop yield and reducing the negative impact of chemical fertilizers on the environment, there is a need to exploit PGPR for beneficial agricultural uses. In that regard, we propose to characterize actinobacteria as PGPR.

2. Mechanisms involved in the PGPR activity

2.1. Production of plant growth regulators

Plant organ formation and their subsequent development are mediated by internal factors of vital importance. Growth regulators in plants (PGR “Plant Growth Regulators”) are known as plants hormones. PGR are small molecules that affect plant growth and development at very low concentrations [16].

One of the parameters used to determine the effectiveness of certain rhizosphere bacteria is the ability to promote the development of characteristic root system of this type of plant growth regulators [17, 18]. The rapid development of roots, either by primary root elongation or secondary lateral root emergence, allows young seeds to have easy access to nutrients and water from their environment [19].

Different mechanisms are involved such as production of siderophores and indole acetic acid, nutrient solubilization, antagonistic or beneficial synergistic effects. These mechanisms have been studied in our collection of actinobacteria and they will be further explained as follows.

2.2. Siderophores production

Microorganisms have been forced by environmental restrictions and biologic imperatives to produce specific molecules that can compete efficiently with hydroxyl ions for ferric state of iron. Siderophores are compounds produced by various microorganisms in soil. These organisms rely on chelation phenomena to support their biological activity. Siderophores are extracellular fluorescent pigments that possess affinity to iron (III), they are water-soluble and have low molecular weight (500-1,000 Da) [20]. Furthermore, siderophores are produced by a great variety of microorganisms that grow in scarce iron conditions [21, 22]. These compounds act as specific chelate agents of ferric ion, leaving available the ionic form (Fe^{+2}), which is easily absorbed by microorganisms [23].

Chelation is a usual phenomenon of the biologic systems. This refers to formation of chelates that can be described as a polydentate ligand in coordination with a central ion by two or more atoms [24]. When siderophores form a complex with Fe^{+3} , these are recognized by cell membrane receptors [25, 26]. This facilitates the inclusion of the formed complex to cell. Once in the cell, Fe^{+3} ions are reduced to a Fe^{+2} becoming available to be used in different biological processes. Finally, the siderophore [27] is released again. Siderophores are classified into three groups based on their chemical nature of the bounds created with metals. They are known as catecholates, hydroxamates, and hydroxide-carboxylates [24]. Actinobacteria and Enterobacteria are among the microorganisms able to produce siderophores. Actinobacteria is one the most important groups in terms of siderophores production.

The vast majority of nitrogen-fixing microorganisms produce siderophores so as to obtain iron. This is necessary to perform the enzyme nitrogenase. The enzyme is composed of several protein units; a total of 36 iron atoms are required for operating properly [28]. According to determinations made by our research group, the highest levels of siderophores production were obtained by *Streptomyces* MCR24 that was maintained over the time; conversely, the lowest recorded levels were observed in *Streptomyces* MCR30. Despite the fact that in its great majority, the analyzed siderophores produced by actinobacteria contained high concentrations of hydroxamates, it can be assumed that some strains show a possible advantage as PGPR mechanism. The strains tested for production of siderophores hydroxamate were capable of growing in culture media without addition of any source of nitrogen. These results are similar to those found by Carson *et al.* [29] and indicate that the selected strains had such capacity and in one-way or another (high or low) produced siderophores.

Studies performed by Díaz [30] evidenced a higher performance of siderophores production when a monosaccharide like glucose is used as a source of carbon. Studies with actinobacteria have shown that the metabolic rate of this group of bacteria is higher when there is an excess of carbon. This favored the production of some organic compounds related to the physiology of the species [31-34]. In the presence of an easily assimilable source of carbon, some species of *Streptomyces* are capable of synthesizing organic acids. Those acids return to metabolic routes to produce energy and result in various secondary metabolites that in such case can be considered as siderophores [34-36].

Authors like Diaz [30] proved that strains of actinobacteria such as *Streptomyces* MCR3 and *Thermobifida* showed a great synthesis of siderophores of the hydroxamate-type using glucose as the only source of carbon, in contrast to what occurred when sodium succinate was utilized to the same purpose. Concerning *Streptomyces* sp., the production of hydroxamate-Desferrioxamine [37, 38] B by this microorganism is well documented. On the other hand, *Thermobifida* has been recently reported as the producer of a siderophore known as fuscachelin A [39].

2.3. Indole acetic acid production

Indole acetic acid (IAA) is a plant growth regulator and active form of auxins. It plays an important role in plant development through its life cycle [40, 41]. IAA stimulates the growth of the radicular system [28, 42-44], thanks to the development of lateral roots and divisions of the apical meristem that derives in roots elongation [43, 44]. This increases the access of soil nutrients to the plant [40, 46]. IAA has proved to be the main one responsible for plant growth promotion over the nitrogen fixation related to diazotrophic bacteria activity [45].

The production of IAA has been widely studied in actinobacteria [47-53]. Authors like El-Shanshoury [47] suggest that IAA can act as endogen regulator of spore germination of *Streptomyces atroolivaceus* and can be involved in the differentiation of actinobacteria [46].

Streptomyces genus [47-53], *Frankia* sp. [47, 51, 54], *Nocardia* sp. [47, 54], *Kitasatospora* sp. [52] have been widely studied as IAA producers. Studies performed by our research group confirmed the ability of genus *Streptomyces* sp. and *Thermobifida* sp. to produce IAA. Duque and Quintana [50] affirmed that MCR14 (*Streptomyces sannanensis*) was the most important microorganism for IAA production.

2.4. Non-symbiotic nitrogen fixation

The actinobacteria are heterotrophic organisms that require carbon sources to obtain the energy necessary for nitrogen fixation. Therefore, each of the different bacteria differs in the carbon metabolism and the intrinsic ability of nitrogen fixation, showing different rates of acetylene reduction assay (ARA). This test is based on detecting indirectly the presence of nitrogenase enzyme, which is in charge of reducing nitrogen (N_2) to ammonium. This assay evaluates the enzymatic reduction of acetylene to ethylene (NH_4^+) [55]. Likewise, ARA indirectly measures the microorganism capacity to fixate nitrogen, given that nitrogenase is an unspecific enzyme that catalyzes the reduction of steric analogs of N_2 [56].

Actinobacteria log phase was evaluated in free-nitrogen media. From those experiments, it was established that the best period for ARA measurement was found after 10 days of culturing. Strains MCR14, MCR27, and MCR31 were selected from the 10 evaluated strains as they turn the pH indicator in Nfb culture media. This fact did not occur for the rest of the evaluated strains. High rates of nitrogen fixation were expected in the vials where color change was observed (Figure 1) and then positively confirmed by ARA. De los Santos et al. [57] described a similar behavior when a semi-solid media inoculated with the bacteria *Burkholderia* did not display changes of pH in all vials; however, some of them showed a slight increase of pH due to microbial growth. In those vials, it was possible to observe a blue-green color.

This confirms the need of carrying out ARA test in order to guarantee diazotrophic characteristics of these microorganisms. In Nfb semi-solid media, actinobacteria grew as a thin white film placed few millimeters below the agar surface. Bacteria were able to move through the media, thanks to Nfb consistency and found the optimum place to balance the respiration rate with diffusion oxygen rate [58].

Certain evidence indicates that diazotrophs organisms are capable of nitrogen biologic fixation in considerable wide ranges of pH. Despite the fact that Nfb has a neutral pH, hydrolysis of carbon derived from the metabolism will cause products that may change the pH. These pH values interfere with nitrogen fixation, making it difficult to alkalinize the culture media, and therefore, changing the color to blue. This behavior was evidenced in most of the isolated actinobacteria (Figure 1).



Figure 1. Actinobacteria evaluated in NFB modified media

After ARA evaluation, it was observed that isolated MCR24, MCR26, and MCR31 recorded the highest rates of ethylene production at the three incubation times. However, no statistically significant differences were noted. The highest nitrogenase activities were observed in MCR31 cultures. It is highly important to detect nitrogenase activity among our strains of free-living diazotroph actinobacteria by using ARA. The high microbial diversity present in soils of high Andean forest of Colombia (Cundinamarca and Boyacá states) derives in the ability of nitrogen fixation obtained by the isolates.

Frankia sp. have an outstanding feature related to vesicles specialized in nitrogen fixation. These vesicles are in charge of protecting the nitrogenase complex. These actinobacteria have been extensively reported by several authors [59-61], due to its fixation characteristics that have been confirmed by acetylene reduction method [62-64]. The morphology of the bacteria cultured in our laboratory facilities using Nfb media did not display any similarity with *Frankia* sp, since different microscopic descriptions such as long and short filaments, tortuous or spiral-

shaped filaments were found. The above mentioned can be an evidence of the presence of new species that have the ability to grow in nitrogen-free conditions.

Gen *nifH* detection was performed. This gene codifies for nitrogenase enzyme and is the molecular marker most widely used for detection of nitrogen-fixing bacteria and phylogenetic studies [65]. We decided to use the primers purposed by Diallo et al. [66]. These primers are very similar to those used by Valdés et al. [64] in non-Frankia actinobacteria for amplifying the *nifH* gen. Based on this protocol was possible to amplify the gen in all the isolated bacteria. Figure 2 displays a band that corresponds to a gen fragment with an expected size of 320 bp. These amplified genes were purified by means of QiaGEN Minelute PCR Purification Kit and then sequenced. The size of the observed bands is similar to that reported by Valdés et al. [64] for *nifH* gene. Furthermore, background can be observed. Studies performed by Soares et al. [67] affirmed that the “background” or “smearing” could be related with the use of degenerate primers such as PolR and PolF during nested PCR.

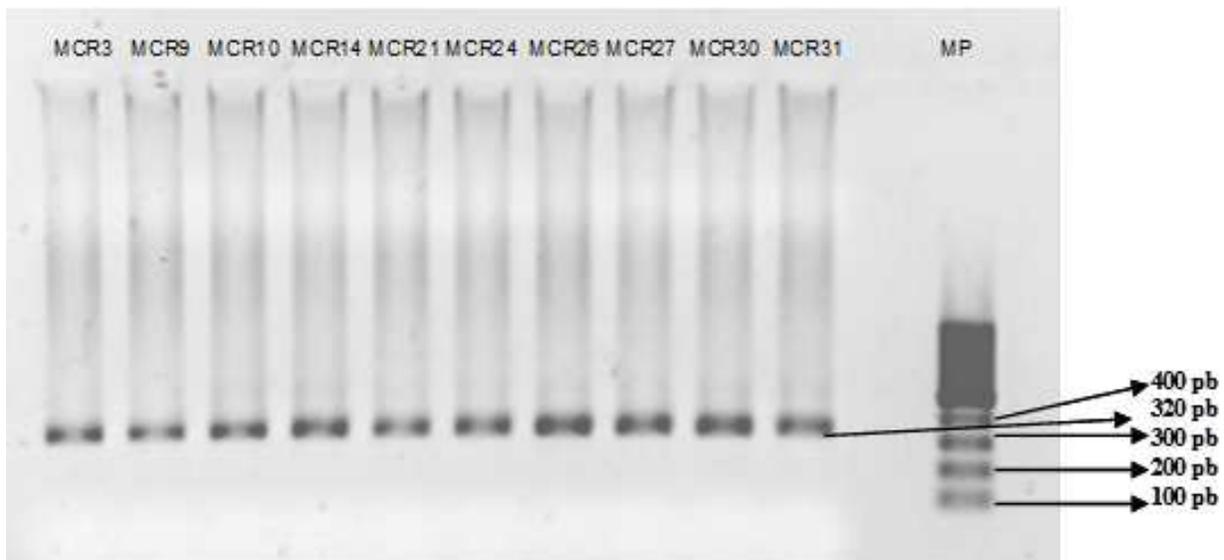


Figure 2. PCR amplification of an intern fragment of gen *nifH* of 320 bp, in 10 of the isolated diazotrophic actinobacteria MP (100 bp Invitrogen).

It is important to emphasize that the research performed by Valdés et al. [64] corresponds to one of the first studies that have recorded the use of degenerate primers to detect the presence of *nifH* gene in free-living actinobacteria different from *Frankia* and isolated from *Casuarina equisetifolia*. As *nifH* gene can act as a molecular marker, there are other genes that belong to nitrogenase complex that are capable of indicating such activity in nitrogen-fixing microorganisms. Studies performed by Fedorov et al. [68] on a new primer system for detecting and amplifying gene *nifDK* portion have pointed out the possible use of such gene as a molecular marker. The progress in the development of new primer systems that target different sites in *nif* operon can be efficiently used for searching nitrogen-fixing actinobacteria in which no nitrogenase activity has been detected before. However, the presence of *nifH* gene by itself does not indicate active nitrogen fixation since nitrogenase activity is regulated at pre- and

post-transcriptional level [69] and its activity depends on environmental conditions. Probably, the most suitable indicator of nitrogenase activity complex is ARNm of *nifH* [70].

BLAST search was carried out using GenBank in order to find the gene *nifH* sequence closely related to that found in our study. It was noted that they were similar to the sequence assigned to *Frankia* sp. According to the access produced by GenBank, some of the strains (MCR 3 and MCR24) showed similarity score between 96 and 98%. The other strains recorded *nifH* gene alignments similar to genes found in nitrogen-fixing bacteria such as *Rhizobium* and *Bradyrhizobium*, and others. This suggests that PCR fragments probably belong to the *nifH* gene. Among the noted sequences, the majority of the strains did not record any similarities with diazotrophic bacteria; this was expected to take into account that *Frankia* sp. represent the only nitrogen-fixing bacteria by means of actinorrhizal symbiosis. *Streptomyces thermoautotrophicus* UBT1 is capable of fixing atmospheric nitrogen and grows in media free of nitrogen; however, it is not capable of acetylene reduction. This type of nitrogen reduction is unusual and it is believed to be coupled to carbon monoxide and dependent of oxygen [71]. These types of microorganisms are not associated to plants and they do not have other characteristics of agronomic interest. In contrast, the atmospheric nitrogen fixed by the actinobacteria studied in our laboratory can influence plant growth. Nitrogenase activity was confirmed after analyzing the ability to reduce acetylene to ethylene and the presence of *nifH* genes by PCR amplification [64, 72].

2.4.1. Phosphorus solubilization

Phosphorus (P) is one of the major essential macronutrients for plants, which is applied to the soil in the form of phosphatic manure. However, a large portion of the applied phosphorus is rapidly immobilized, becoming unavailable for plants [73]. The free phosphatic ion in soil plays a crucial role. The orthophosphatic ion form is the only ion that can be assimilated by plants in considerable amounts [74]. Soil microorganisms are involved in a wide range of biological processes, including phosphorus transformation of soil. They solubilize soil phosphorus for plants growth [75].

Although the mechanisms used by actinobacteria to solubilize phosphorus are not fully understood, it is known that in the solubilization process, inorganic phosphorus and some organic acids (oxalic and citric, principally) are involved [76-78]; our group performed solubilization quantifications and determined organic acids associated with solubilizing of P [79]. Similarly, Hamdali et al. in 2010 and 2012 [80, 81] have reported that compounds different from organic acids, more specifically metabolites of the viridomicenas and siderophores families, are capable of solubilizing P from various sources, which lead us to explore more about the process of solubilization of inorganic phosphorus generated by this group of organisms.

2.4.2. Organic phosphate solubilization

The organic P is composed of various fractions, compromising the most labile and most resistant to mineralization. However, the main organic component of phosphorus cycle is the

microbial biomass [82]. Organic phosphate is mineralized by the phosphatase enzyme, which is excreted by some microorganisms, and is released [83, 84].

Studies performed by our research group evidenced that *Streptomyces* MCR26 has the capacity of secreting acid phosphatases, and therefore, mineralizing sources of organic phosphorous [15]. Additionally, actinobacteria not previously reported as phosphate-mineralizing microorganisms were identified and were related to genus *Saccharopolyspora*, *Thermobifida* and *Thermonospora*. Actinobacteria from the genus *Micromonospora* sp., *Nocardia* sp., *Actinomadura* sp., *Rhodococcus* sp., *Actinoplanes* sp., *Microbispora* sp. and *Streptosporangium* sp. produce phosphatase enzymes which have been classified according to their alkaline or acid activity, depending on reaction conditions [85].

2.4.3. Inorganic phosphate solubilization

The growth of phosphate-solubilizing bacteria (PSB) growth often causes soil acidification, playing a key role in phosphorus solubilization [86]. Therefore, PSB are considered important solubilizers of insoluble inorganic phosphate. In turn, plants reimburse PSB with carbohydrates [87].

Prada et al. [79] isolated 57 strains of actinobacteria from different sampling areas. Soil characterization showed the following: pH ranges from 4.0 to 5.9, total P from 360 to 2830 mg/kg, available P from 8.7 to 118.4 mg/kg, and organic matter from 2.95 to 13.52%. The results of two qualitative assessments are not totally consistent. Seven of the tested strains F1A, F1B, F1C, F4C, T1A, T1D, and T3A were the best solubilizing strains, in both solid and liquid evaluation media. We performed a quantitative assessment in order to find the strain that has the highest solubilizing capacity and to evaluate which of the two methods is more reliable. The strains T1C, T1H, T3A, T3C, P3E, F1A, F2A, and V2B are as good as *Streptomyces* sp. MCR24 for $\text{Ca}_3(\text{PO}_4)_2$ and these strains solubilized significantly more phosphorus than the other strains. Strains T1H, T1C, T3A, T3C, and F1A are only present in the selection obtained with the methodology reported by Mehta and Nautiyal [88], suggesting that this test can select more strains with true solubilizing ability, and thus it is more reliable.

Perez et al. [89] claimed that isolates that cause a shift of > 1.5 units could be selected for further studies. In order to confirm the usefulness of this cut-off point proposed by Perez et al. [89] and therefore, to select the best strains, we implemented a quantitative assay by measuring the release of soluble phosphorus using the NBRIP broth [90-91]. Figure 3 shows strains T1C, T1H, T3C, P3E, and V2B. They have significantly higher activity than other isolates. However, this result was not observed in the plate assay, probably because one or more acids involved in the process did not diffuse into the agar and, therefore, there was no presence of a solubilization halo. On the other hand, the evaluation in NBRIP-BPB broth revealed that isolates capable of decolorizing the broth more than 1.5 units were also more efficient in the quantitative assay. Additionally, Mehta and Nautiyal [88] assay contribute to reduce costs and efforts in microorganisms with bio-fertilizing potential screening. Studies focusing on actinobacteria physiology in Colombia are scarce, especially those focused on agriculture [15, 90-92].

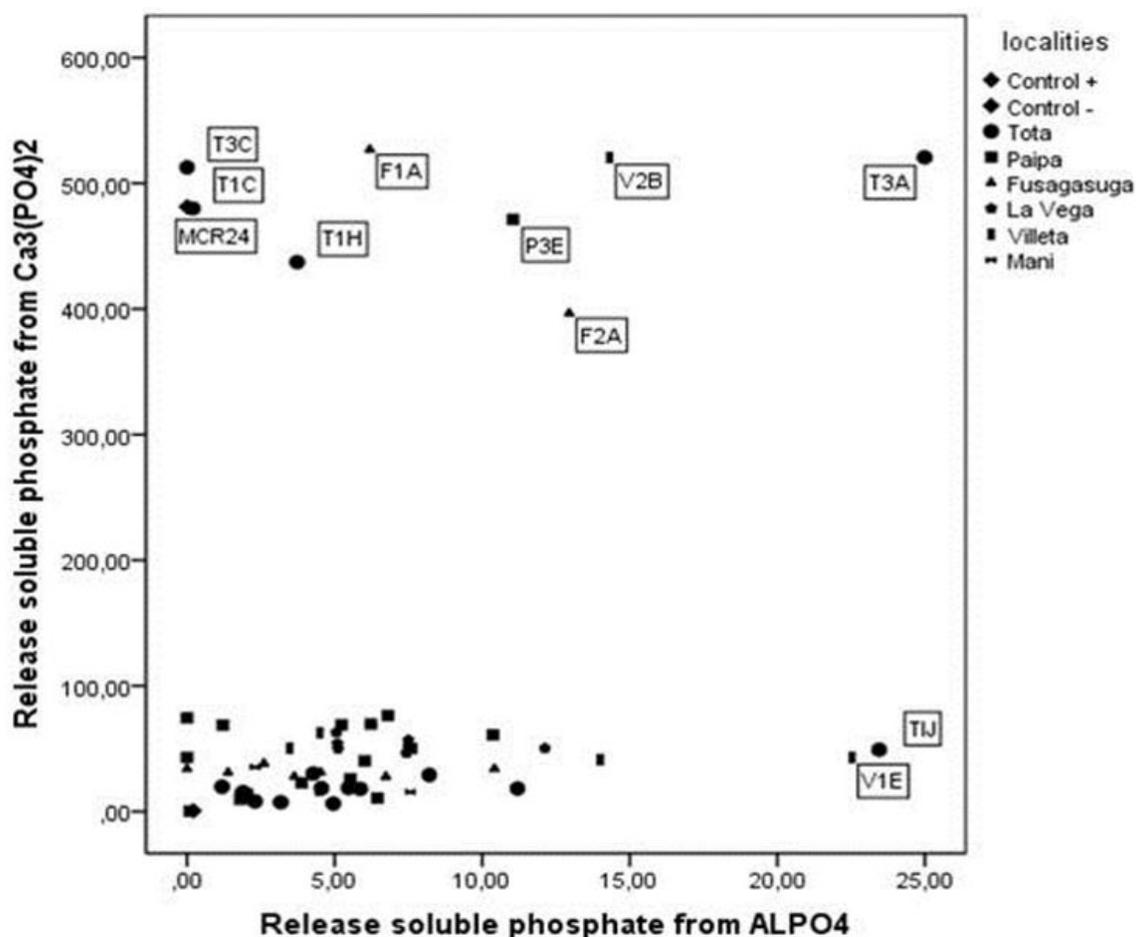


Figure 3. Released soluble phosphate activity with two sources of P. $\text{Ca}_3(\text{PO}_4)_2$ 5g L^{-1} is displayed in Y axis and activity with ALPO_4 1g L^{-1} source is displayed in X axis.

2.5. Chitinases production

The chitin is a homopolymer comprised of N-acetyl-D-Glucosamine residues with α -1, 4 bonds. It is widely spread in nature as a structural component of fungi (22-44%), insects and crustaceans (25-58%), and protozoa [93-96]. The chitin is hydrolyzed by a complex chitinase that comprises three enzymes such as exochitinase, endochitinase and N-acetyl-D-Glucosamine.

Actinobacteria are considered as the dominant organisms involved in the decomposition of chitin in soil [1] and also promising antagonist agents for biocontrol due to the hydrolysis reaction over the fungi mycelium [97]. The species that belong to *Streptomyces* genus are considered as the principal chitinolytic microbial group in soil, due to its capacity to degrade this polymer [98].

Within a group of 30-isolated actinobacteria, 20 were able to hydrolyze chitin after 3 days of growth on mineral agar supplemented with chitin, as the sole source of carbon. The clearing

zones were observed around bacteria following 9 days of growth, suggesting the presence of chitinolytic activity with secreted proteins into the culture medium [99].

2.6. Antagonistic activity against phytopathogenic fungi

Antagonism is defined as a mechanism of action based primarily on the direct inhibitory activity between two microorganisms [100] that have opposite actions within the same system. In order to evaluate the chitinolytic activity of seven strains of actinobacteria against *Fusarium oxysporum*, *Phytophthora infestans*, *Rhizoctonia solani* and *Verticillium dahliae*, a set of experiments were performed. Additionally, its ability as plant growth regulator was also considered.

It was observed that the strains had a high, medium, or low inhibition on tests of antagonism against phytopathogenic fungi, but *F. oxysporum* was the most resistant fungal strain. Diverse actinobacteria may act as antagonistic microorganisms of *F. oxysporum* by producing antibiotics (antibiosis). These compounds diffuse through the medium inhibiting the growth of phytopathogenic fungus. Molano et al. [101] determined *in vitro* inhibition of *Fusarium oxysporum* growth by actinomycin, an antibiotic produced by *Nocardia* sp., strain isolated from rhizosphere soil sample lichen (Mosquera, Colombia). Production of such secondary metabolites was toxic to the phytopathogenic fungus.



Figure 4. Dual culture confrontation method. (a) MCR26 vs *Verticillium dahliae*. (b) MCR10 vs *Phytophthora infestans*. (c) MCR14 vs *Rhizoctonia solani*

Strains MCR26, MCR10, and MCR24 proved to be the best as fungal antagonists (Figure 4). Based on these results, it can be inferred that mycelial growth inhibition is not caused by chitinase production, but rather by antifungal products. No inhibition of mycelial growth was observed by strains with chitinolytic activity. Using these results, we moved to the interaction phase with the mycorrhizal fungi, considering that chitinolytic enzymes did not directly affect fungi.

Actinobacteria that belong to *Streptomyces* genus have been commercially used to control plant damages. This genus have demonstrated antagonistic activity against *Alternaria* sp., *Pythium aphanidermatum*, *Colletotrichum higginsianum*, *Acremonium lactucum*, and *Fusarium oxysporum* [102,103]

Experiments performed at Unidad de Investigaciones Agropecuarias (UNIDIA) have proved the ability of *Streptomyces cuspidosporus* to inhibit the phytopathogen fungus *Fusarium oxysporum* following 8 days of incubation [50]. Additionally, we evidenced antibacterial activity

present in actinobacteria. Complete inhibition was observed when *Streptomyces* MCR26 was tested against *Bacillus cereus* and *Escherichia coli*, conversely, *Thermobifida* MCR24 strain which was completely inhibited by *Bacillus cereus* [104].

The antagonistic potential of the compounds produced by these strains was previously reported by our research group (UNIDIA). We evaluated the antagonistic activity in vitro of no mycorrhizal fungi generally found in soil. It was found that *Streptomyces* MCR26 and *Thermobifida* MCR24 partially inhibited *Rhizoctonia solani* and *Phytophthora infestans* growth [15].

2.7. Mycorrhiza (MA) helper bacteria

In general, the ability of certain microorganisms to influence the formation and functioning of the symbiosis MA through various kinds of activities, such as activation in fungal propagules infective pre-symbiotic stages [93,105], facilitate formation of inputs point into the root [106-108] and they increase the growth rate [109-111].

In our studies, it can be seen that the two strains of *Streptomyces* (MCR9 and MCR26) cause a stimulation of spores germination of the fungus MA, while *Thermobifida* MCR24 reduces significantly the germination of spores. It was also observed that isolated from *Streptomyces* and *Thermobifida* improved the growth of the mycelium of *Glomus* FC8 sp. Actinobacteria behavior evaluated in this study confirmed the results obtained by *Streptomyces globisporus* 1-K-4 [112], which showed that the concentration of the bacteria increases in rhizoplane seedling rice almost immediately after the inoculation.

Following the methodology described by Azcón-Aguilar et al. [113] and Barea et al. [114] we determine in this study, with, whether or not germination of *Glomus* sp spores were inhibited by the three isolated actinobacteria. Each petri dish was individually inoculated with actinobacteria (MCR9, MCR24, or MCR26) and the spores. Spores and the correspondent actinobacteria were placed on the apex of a hypothetical hexagon keeping a distance of approximately 3.5 cm between each other. After being inoculated, the germination of the spores was observed after 32 days of incubation at 25°C in dark conditions. Percentage of germination was calculated in each treatment. It was recorded that the two strains of *Streptomyces* (MCR9 and MCR26) improved germination of the spores of fungus MA. In contrast, *Thermobifida* MCR24 notably decreases spores germination. Furthermore, *Streptomyces* and *Thermobifida* improved mycelium development of *Glomus* FC8 sp.

Carpenter-Boggs et al. [111] found that actinobacteria such as *Streptomyces orientalis* have a beneficial effect on spores of *Gigaspora margarita*. They also observed that the amount of volatile compounds produced by the isolated ones have a good correlation with the germination of MA spores. Such research can explain why the actinobacteria that belong to our collection improve spore germination of *Glomus* FC8 sp. Moreover, Mousse [109] and Azcón-Aguilar & Barea [115] described that some mycorrhizosphere bacteria were capable of promoting the MA settle. This improves germination of spores.

Through confocal microscopy was observed that chitinolytic strains and strains that showed antagonistic capability against non-mycorrhiza fungi with chitin wall did not cause degradation of the mycelium wall of *Glomus* FC8 sp. or to the commercial witness. These results are

consistent with other studies that have observed bacteria inside of MA and colonizing fungal hyphae [116-118]. Different studies have proved that microbial antagonists of fungal pathogens do not cause any inhibitory effect against MA [114,119-121].

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