Interactions between beneficial microorganisms: Endophytic fungi and rhizobacteria

Interacciones entre microorganismos benéficos: Hongos endófitos y rizobacterias

GÓMEZ-LUNA, Blanca Estela†*, MORALES-VARGAS, Adán Topiltzin, DÍAZ-PÉREZ, César and RAMÍREZ-GRANADOS, Juan Carlos

Universidad de Guanajuato, Agroindustrial Engineering Department

ID 1st Author: *Blanca Estela, Gómez-Luna* / **ORC ID**: 0000-0001-6345-0461, **CVU CONACYT ID**: 101592

ID 1st Co-author: *Adán Topiltzin, Morales-Vargas* / **ORC ID**: 0000-0003-0490-5934, **CVU CONACYT ID**: 176575

ID 2nd Co-author: *César, Díaz-Pérez* / **ORC ID**: 0000-0001-7847-1062, **Researcher ID Thomson**: X-5157-2019, **CVU CONACYT ID**: 101579

ID 3rd Co-author: *Juan Carlos, Ramírez-Granados* / **ORC ID**: 0000-0001-6460-6472, **Researcher ID Thomson**: S-5874-2018, **CVU CONACYT ID**: 167866

DOI: 10.35429/JANRE.2021.9.5.11.16 Received October 14, 2021; Accepted December 03, 2021

Abstract

For many years, the obvious benefits of using agrochemicals without restriction were exploited, causing irreversible damage. Currently, it is known that the vast majority of used agrochemicals cause damage to the health of those who handle them, as well as cause deterioration in the soil and contaminate aquifers. For this reason, it is important to create new alternatives that reduce the use of agrochemicals and that fulfil the same functions. The objective of this work was to carry out the interaction between beneficial organisms such as endophytic fungi and plant growth-promoting bacteria, in order to observe positive mutualistic interactions, in order to test beneficial consortia in plant material in future research. In vitro interactions were carried out in PDA medium, inoculum of fungi and bacteria were placed, they were incubated at 28 ° C in a period of time of approximately 8 to 10 days and the photo and type of positive or negative interaction were taken. Around 950 interactions were carried out and of these, 402 were positive interactions. These positive interactions can be used in combination for better plant development.

Beneficial microorganisms, Plants, Environment

Resumen

Durante muchos años se aprovecharon los beneficios evidentes que tenía el utilizar agroquímicos sin restricción, causando daños irreversibles. Actualmente, se sabe que la gran mayoría de agroquímicos usados causan daño a la salud de quien los maneja, al igual que causan deterioro en el suelo y contaminan mantos acuíferos. Por ello, es importante crear nuevas alternativas que reduzcan el uso de agroquímicos y que cumplan con las mismas funciones. El objetivo de este trabajo fue realizar la interacción entre organismos benéficos como hongos endófitos y bacterias promotoras del crecimiento vegetal, con el fin de observar interacciones positivas de tipo mutualista, para en futuras investigaciones probar los consorcios benéficos en material vegetal. Se realizaron las interacciones in vitro en medio PDA, se colocaron inóculos de hongos y bacterias, se incubaron a 28 °C en un periodo de tiempo de aproximadamente 8 a 10 días y se tomó la foto y tipo de interacción positiva o negativa. Se realizaron alrededor de 950 interacciones y de éstas, 402 fueron interacción positiva. Estas interacciones positivas pueden ser utilizadas en combinación para un mejor desarrollo de las plantas.

Microorganismos benéficos, Plantas, Ambiente

Citation: GÓMEZ-LUNA, Blanca Estela, MORALES-VARGAS, Adán Topiltzin, DÍAZ-PÉREZ, César and RAMÍREZ-GRANADOS, Juan Carlos. Interactions between beneficial microorganisms: Endophytic fungi and rhizobacteria. Journal-Agrarian and Natural Resource Economics. 2021. 5-9: 11-16

* Correspondence to Author (email: be.gomez@ugto.mx)
† Researcher contributing as first author.

Introduction

Rhizosphere and soil

In a pinch of soil there are millions of bacteria that can transform certain elements into essential nutrients for the survival of living beings present in the rhizosphere. The rhizosphere is of great importance, because in it the exchange of nutrients between the atmosphere and the soil takes place, which involves the microbiota and the plants present in it, that is, ecological-biological processes, and surrounds the system root of the plant. Various authors suggest that the presence of the microbiota is essential for the proper growth of crops and to maintain an ecological balance (Pedraza et al., 2010; Espinosa et al., 2020). In the rhizosphere there are various microorganisms that feed on the exudates of the plant and in turn, strengthen the biological and environmental balance of the soil. The rhizosphere is usually divided into the endorizosphere, which is the intercellular space between the radical tissues colonized by microorganisms, the ectorizosphere, which is the compartment of soil associated with the root up to a distance of 5 mm, and finally, the rhizoplane, which is the interface between the soil and the root (Espinosa et al., 2020).

The concentration of bacteria in the rhizosphere is approximately 10 to 1000 times higher than in bulk soil, but lower than in a laboratory medium. To maintain their beneficial effects in the root environment, bacteria must compete well with other microbes in the rhizosphere for nutrients secreted by the root (Gouda et al., 2018).

There are groups of bacteria capable of transforming atmospheric nitrogen, reducing it into compounds available to other microorganisms and plants. These types of bacteria are often used as parameters of soil quality. Soil quality in ecosystems, whether natural or modified, is evaluated by the ability to maintain or improve plant and animal productivity, water-air quality and habitability, as well as human health (Benjumeda, 2017). In order for the crops and the soil to have a good response to the inoculants, it is necessary to know the interrelation between the inoculant and the native microbiota, since if there is no good interrelation, there may be drawbacks in the cycling of nutrients and organic compounds (Del Puerto et al., 2014).

The participation of bacteria in the transformation depends on their physiological state and enzymatic activity, in addition to the bioavailability of the elements to be transformed and the competition between the microorganisms present in the soil (Pedraza et al., 2010).

Endophytic fungi

Endophytic fungi are microorganisms that inhabit plants without causing apparent symptoms of disease. They are a group of microorganisms that can belong to the genus *Ascomycota*, *Basidiomycota*, *Zygomycota* and *Oomycota*. They manage to inhabit the leaves, stems, flowers or roots of a plant and the location of the fungus in the plant depends on the species. Generally, the concept of endophytic association is associated with mycorrhizae, since they describe a mutualistic relationship, however, endophytic fungi that do not form mycorrhizae have been found and have the same benefits (Ordóñez et al., 2012).

The relationship they form with the plant can be mutualistic, neutral or antagonistic. The classification of these fungi depends on where they are located in the host plant. One of the most common classifications is: claviciptacean endophytes and non-claviciptaceous endophytes (Jambon et al., 2018).

The presence of endophytic fungi was documented in 1898, when they observed that certain animals after consuming grasses presented symptoms of intoxication, but it was until 1977 that Bacon related the presence of *Neotyphodium coenophialum* with summer syndrome (Sánchez et al., 2013). In 1988 Clay proposed the mutualistic interaction that exists between certain fungi and grasses.

The relationship between an endophytic fungus and its host plant is related to the production of virulence factors and defense metabolites produced by the plant either by biochemical or mechanical means, environmental factors and the development stages of both organisms (Frey et al., 2011). When there is balance between the factors already mentioned, an endophytic relationship occurs. However, if the plant is in senescence or under stress, the fungus will be detected as a pathogen, therefore, it will manage to infect the plant and thus produce symptoms (Sánchez et al., 2013).

ISSN 2524-2091

The relationship that exists between the host and the host is of great interest, since the fungus is capable of producing bioactive metabolites, as well as modifying the defense mechanisms of its host, allowing and increasing the survival of both organisms (Gamboa, 2006).

Endophytic fungi can protect the plant against biotic and abiotic factors, in addition to producing allelopathic metabolites, this means that it prevents the growth of other microorganisms around it. The protection mechanisms are divided into three, direct, indirect and ecological (Sánchez et al., 2013). The direct protection mechanism is characterized by the production of enzymes or secondary metabolites, on the other hand, the indirect mechanism has the ability to induce the chemical and physiological defense mechanisms of the plant. The ecological protection mechanism is through predation or hyperparasitism (Sánchez et al., 2013).

Fungi contribute to the mineralization of carbon in the soil, they also have an antagonistic role against phytopathogenic fungi. They generally degrade compounds formed by cellulose since the nitrogen requirement is usually low (Sánchez et al., 2013). Yeasts are a group of microorganisms belonging to the fungi kingdom, the main genera used are *Saccharomyces* and *Candida*. Their main function as beneficial microorganisms are the production of enzymes and hormones that can be used by lactic acid bacteria.

In addition, they have a wide spectrum in their carbon sources and are not capable of assimilating nitrites or nitrates as a nitrogen source and their phosphorus sources are in the form of sulfates (Ordóñez et al., 2012). The main fermenting fungi are *Aspergillus oryzae* (it has uses in western cuisine as a fermenter of various cereals and legumes, it is a filamentous, aerobic fungus), *Penicillum* sp (they have an important function in the degradation of cellulose and lignin, in addition, it has a good adaptation in acidic environments and with low water levels) and *Trichoderma* sp (produces various enzymatic compounds capable of degrading organic matter, it can also be found almost anywhere) (Morocho & Leiva, 2019).

Plant growth promoting bacteria

Plant growth promoting bacteria (PGPR) can be aerobic anaerobic or facultative anaerobic (Loredo et al., 2004). They are found in the rhizosphere and are microorganisms that have the ability to stimulate the growth of certain plant species, they are characterized by their efficiency by fixing nitrogen, solubilizing phosphates (organic and inorganic), producing indole compounds, as well as in the decomposition of crop residues, mineralization of organic matter and immobilization of mineral nutrients (Espinosa et al., 2020). PGPRs modify the physiology of plants and the nutritional properties of the soil. In addition, it has been shown that they increase the absorption of compounds such as calcium, potassium, iron, copper and zinc, through the production of organic acids by the plant and the decrease in pH by PGPR (Loredo et al., 2004; Espinosa et al., 2020).

The mechanisms of the PGPR can be direct or indirect, the direct effects are the fixation of atmospheric nitrogen, production and synthesis of siderophores, solubilization of minerals, the synthesis of phytohormones, as well as the production of ACC deaminase, antibiotics, enzymes, competition, hydrogen cyanide, RSI, and quorum extinction (Oluwaseyi et al., 2017). The indirect mechanisms of PGPR are through the inhibition of the growth of other microorganisms, generally pathogens. That is, the bio-control of phytopathogens mainly by the production of antibiotics and iron reduction. Currently, the application of these bacteria can be through inoculation of seeds, substrates, seedlings, foliage and fruits (Pedraza, et al., 2010; Virgen, 2011).

Some authors such as Gouda (2018), have classified PGPR as growth promoting rhizobacteria (ePGPR) and intracellular plant growth promoting rhizobacteria (iPGPB). Within the ePGPR are those that inhabit the rhizosphere (in the rhizoplane) or in the spaces between the cells of the root cortex. The main ePGPRs are *Azotobacter*, *Serratia*, *Azospirillum*, *Bacillus*, *Agrobacterium*, *Flavobacterium*, *Arthrobacter*, *Micrococcous*, *Pseudomonas* and *Burkholderia*. On the other hand, iPGPBs inhabit nodular structures, the main endophytic bacteria are Allorhizobium, Bradyrhizobium, *Mesorhizobium* and *Rhizobium* (Gouda et al., 2018).

ISSN 2524-2091

In regions where climatic conditions have high humidity, the soil tends to have an acid pH, due to the leaching of cations. This type of soil tends to produce chemical compounds like iron and aluminum oxide. In regions with a dry climate, the soil tends to have high concentrations of alkaline cations, that is, a basic pH (Scherlach et al., 2013). Therefore, pH plays a very important role, since in the case of having acidic soils, plant growth, production, yield, population and microbial activity, both beneficial and pathogens, can be affected. As well as the availability of nutrients in the soil (Espinosa et al., 2020).

The main function of *Actinomycetes* consists in the solubilization of the cell wall or components of plants, insects or fungi. Therefore, they are of great help in composting and improving soil quality (Pérez et al., 2015). The main genera of *Actinomycetes* are *Streptomyces*, some of these species have important roles in biological control, since they produce hydrolytic enzymes and thus can inhibit the growth of phytopathogenic fungi (Morocho & Leiva, 2019). Bacteria of the genus *Pseudomona* promote plant nutrition, regulate hormone levels and the expression of genes related to growth, as well as the induction of antioxidants and osmolytes. In general, rhizobacteria improve the photosynthetic rate due to the excellent assimilation of CO_2 and due to its function at the photochemical level in photosystems (Pérez et al., 2015; Morocho & Leiva, 2019).

Methodology

Obtaining strains of bacteria and fungi

From existing collections of endophytic fungi and plant growth-promoting bacteria, a duplicate of the 24 fungal strains and 52 bacterial strains was generated. Obtaining the duplicates of fungi was done by using papers containing the inoculum, subsequently, under sterile conditions, the inoculum was placed in a Petri dish with PDA medium. The growth conditions for the fungi were: a temperature of 28 ° C for a period of 7 days in an incubator, avoiding the change in temperature and controlling the surrounding light. Finally, they were stored in a refrigerator at a temperature of 18 ° C.

The bacteria were obtained from an existing collection; the inoculum was contained in Petri dishes with PDA medium.

To generate the copies of the PGPR, the inoculum was taken and seeded in a potato dextrose medium, contained in sterile Petri dishes. The growth and storage conditions were similar to those of the fungi, with the difference that the growth period was 1 to 3 days, with an approximate temperature of 28 ° C.

Sowing and conditions of interactions

About 950 interactions were made in vitro, with a duplicate of each. For this, potato dextrose medium (PDA) was prepared and subsequently emptied into sterile Petri dishes. The boxes with PDA medium were used to place inoculum of fungi and bacteria. Finally, a fungi was placed in the center of the box and 4 bacteria around it, as shown in the figure 1.

Once the bacteria and fungi had been planted in the boxes prepared with PDA, they were sealed with a plastic film and labeled, later they were stored in a room with little light, at an approximate temperature of 24 ° C in a period of time approximately 8 to 10 days.

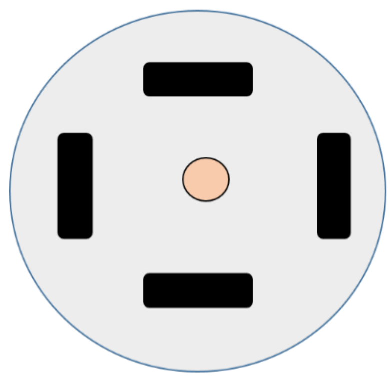

Figure 1 The interaction between 1 fungus - 4 bacteria

Take picture

Once the incubation time of the interactions had passed, their analysis was carried out. Once the interactions obtained were observed, photos were taken of the boxes that contained the interactions, for this, two backgrounds were used, a black and a white background. To take the photo a Sony camera of 32 mega pixels were used. It is important to take into account the background in which the interactions will be placed for the image taking, because the different strains of fungi and bacteria have different colorations, therefore, to facilitate the visualization of the interactions and provide an accurate analysis, it is they selected two backgrounds (black and white).

In the present work, genera of fungi *Aspergillus*, *Fusarium*, *Penicillium* and other fungi that are not yet characterized were used. These genera can pigment the medium with red, purple, green and black colors.

Results

The interactions occur through signals produced by being in contact with certain volatile compounds or that diffuse in the medium. However, the invasion of the fungus by means of spores or mycelia towards the bacterial strain present in the medium was considered a positive interaction. That is, if the development of the fungus was allowed from the center of the Petri dish towards the walls. In several cases, the fungus managed to colonize various parts of the Petri dish, until it reached the bacteria, covering it completely or partially.

On the other hand, in some cases the bacteria generated larger colonies preventing the invasion of the fungus, which was considered a negative interaction, since the inhibition of both microorganisms was observed. In some cases, the interaction of the fungus is perceived above and below the bacteria, with the generation of mycelia and / or spores. It should be noted that generally the mycelium was observed more clearly on the black background, while on the white background it was possible to visualize the sporulation present in the medium.

Figure 2 shows the growth of fungi and the interactions of fungi and bacteria. A total of 932 interactions were carried out and of these 402 were positive, that is, they could form consortia.

The strains used in in vitro interactions have been studied and their effectiveness in terms of benefits in the growth of plant material has been proven. In this study, the interaction of the strains as a mutualistic community was observed and so far they have not been applied in plant models, therefore, the effectiveness of each of the interactions obtained has not been evaluated. However, after having information on the mutualistic interaction of these microorganisms individually, that is, in a fungus-plant interaction, or plant bacteria, it is expected that by being in a tripartite interaction (fungus-bacteria-plant) the development and the benefits are tangible.

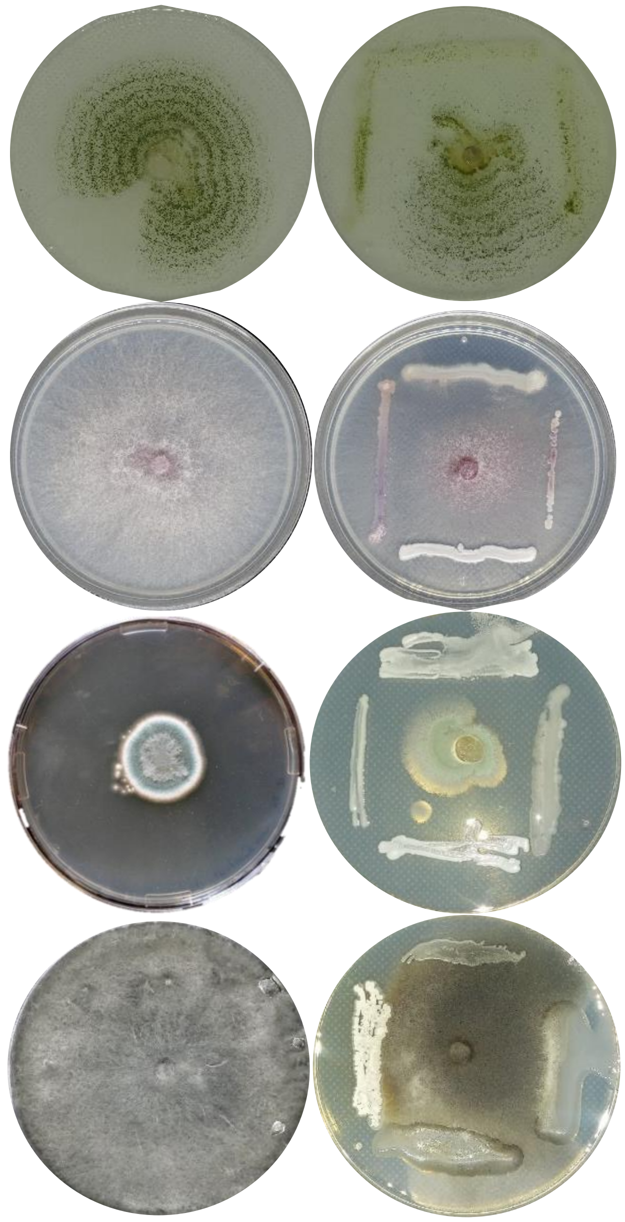

Figure 2. Fungus *Aspergillus*, *Fusarium*, *Penicillum* and H-01 and Interactions positive whit bacterial strain

Conclusions

The interactions obtained can have an important place in the market or in research, since not only can they function as an alternative to agrochemicals, but also have diverse functions such as bioremediation and increase in the quality of the ground and with it brings benefit, after benefit. There is still a long way to go in terms of the application of plant material, among other studies. For the application of consortia, it is necessary to take into account that all microorganism communities have a peculiar microhabitat that depends on the host species, type of root and the composition of the root exudates. These factors influence the production of various volatile compounds, which involves the growth of plants.

Thanks

This was carried out thanks to the support of the project for Strengthening Academic Teams 2018 call, SEP-PRODEP and University of Guanajuato.

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