Biocontrol Potential of Rhizobacteria Isolated from Aquaponic Systems with Eruca vesicaria Against Fusarium sp.

Edwin Ramirez Gómez

Edwin.gomez@unimilitar.edu.co

Universidad Militar Nueva Granada https://orcid.org/0000-0002-6700-9603

Aura Camila Tello Pachón

Edwin Gómez Ramírez

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Abstract

The agroecosystems are affected by phytopathogens such as *Fusarium sp*. Nevertheless, rhizobacteria isolated from different crops have demonstrated effective biocontrol against various phytopathogens. The *in vitro* biocontrol effect of rhizobacteria isolated from arugula (*Eruca vesicaria*) in an aquaponic system against *Fusarium sp*. was evaluated. Thirty rhizobacteria were isolated from the rhizosphere and characterized phenotypically and biochemically. Most isolates were Gram-positive bacilli, showing variable enzymatic activity, motility, and low sulfate and indole reduction capacity. Antagonism tests revealed four morphotypes that exceeded 50% inhibition of the phytopathogen. These were molecularly identified as *Bacillus pumilus* (61.29%), *Bacillus cereus JCM* (55.56%), *Brucella pecoris* (52.40%), and *Bacillus cereus ATCC*(50.00%). The results demonstrate a high diversity of rhizobacteria in aquaponic systems. Furthermore, four of these isolates exhibited significant biocontrol potential against the phytopathogen *Fusarium sp*. These findings suggest that aquaponic systems represent a promising source of beneficial microorganisms for biological control strategies. Further studies are required to evaluate their efficacy under *in vivo* conditions and to elucidate the mechanisms involved in pathogen suppression. In conclusion, the rhizobacteria isolated from *E. vesicaria* in aquaponics constitutes a valuable alternative for sustainable management of *Fusarium* diseases.

Introduction

Plant diseases caused by fungal pathogens represent a major and persistent constraint on global food security, with estimates indicating that more than 80% of plant diseases are attributable to fungi (Tiann et al. 2020). Among phytopathogenic fungi, the genus *Fusarium* is one of the most economically significant, encompassing numerous species responsible for severe crop losses worldwide. In addition to their phytopathogenicity, many *Fusarium* species produce mycotoxins that pose substantial risks to human and animal health (Ma et al. 2013; Dean et al. 2012).

Fusarium species infect a wide range of economically important crops and are responsible for destructive diseases, including vascular wilt, root rot, crown rot, seedling blight, and seed decay, resulting in substantial reductions in crop yield and quality worldwide. Their capacity to survive for extended periods in soil through resistant chlamydospores, combined with a broad host range and remarkable genetic variability, makes disease management particularly difficult (Rahman et al. 2021). Although chemical fungicides remain one of the principal control strategies, their long-term use has raised concerns regarding environmental contamination, fungicide resistance, negative effects on non-target organisms, and increasingly restrictive regulatory policies (Biswas et al. 2023). Consequently, the development of environmentally sustainable disease management strategies has become a priority in modern plant protection. Consequently, the development of environmentally sustainable disease management strategies has become a priority in modern plant protection, with increasing emphasis on exploiting beneficial rhizosphere microorganisms as biological control agents capable of suppressing *Fusarium* and improving plant resilience (Pankaj et al. 2025; Yang et al. 2025).

Arugula (*Eruca vesicaria*) is an annual species native to the Mediterranean region that has gained global importance due to its increasing commercial demand, short production cycle, high nutritional value, and suitability for intensive horticultural production systems (Zanin, 2021). Optimal growth occurs in well-drained soils rich in organic matter, with neutral to slightly acidic pH. However, this crop is susceptible to several phytopathogens, including *Fusarium* spp., *Pythium* spp., and *Botrytis* spp., which can significantly reduce productivity (Lopez, 2013).

Root-associated pathogens directly compromise plant establishment and marketable yield, therefore, the identification of effective biological control agents has become an important strategy for sustainable arugula production. Previous studies have identified taxa such as *Delftia acidovorans* and members of the genus *Pseudomonas* associated with the rhizosphere of *Eruca vesicaria* (Khalifa and AlMalki 2019; Wassermann et al. 2017), highlighting the need for a deeper understanding of plant microbe interactions in this crop. However, the diversity, functional traits, and disease suppressive potential of rhizobacterial communities associated with *Eruca vesicaria* remain poorly understood, particularly in soilless cultivation systems as hydroponics or aquaponics, thereby limiting the exploitation of these microorganisms as potential biological control agents.

Aquaponic systems have emerged as sustainable alternatives to conventional agriculture, integrating aquaculture and hydroponics in a recirculation aquaculture system. These systems offer advantages such as increased productivity per unit area, efficient water use, and nutrient recycling. However, the recirculating nature of aquaponic systems limits the use of conventional chemical pesticides and fungicides because of their potential adverse effects on fish health, beneficial microorganisms, and overall system stability, thereby increasing the need for environmentally compatible disease management strategies (Aguirre et al. 2023; Debroy et al. 2025). Microorganisms play a central role in aquaponic systems, mediating essential biogeochemical processes such as nitrification, denitrification, and sulfur cycling (Jifiriya et al. 2025).

Beyond their fundamental role in nutrient cycling, rhizosphere microbial communities contribute substantially to plant health by enhancing nutrient acquisition, producing phytohormones and other growth-promoting metabolites, improving nutrient use efficiency, suppressing phytopathogens through competitive and antagonistic interactions, and strengthening plant resilience against biotic and abiotic stresses (Molefe et al. 2023; Singh et al. 2022). Consequently, the rhizosphere of plants grown in aquaponic systems represents a potentially valuable reservoir of beneficial microorganisms with biocontrol capabilities (Naranjo et al. 2024). Despite this, the diversity and functional potential of rhizosphere microorganisms inhabiting aquaponic systems remain considerably less explored than those associated with conventional agricultural soils, particularly regarding their application in plant disease management (Liu et al. 2016).

Biological control using plant growth-promoting rhizobacteria (PGPR) and other beneficial microorganisms has emerged as one of the most promising environmentally friendly alternatives for managing soil borne plant pathogens. These microorganisms promote plant growth through direct

mechanisms, such as phytohormone production, and indirect mechanisms, including nutrient solubilization, induction of systemic resistance, and production of antimicrobial compounds (Sana et al. 2016; Ramírez et al. 2017; Moreno et al. 2018).

In addition to these general plant growth-promoting traits, numerous PGPR suppress *Fusarium* spp. through multiple antagonistic mechanisms, including the production of hydrolytic enzymes (chitinases and β-1,3-glucanases), siderophores, antimicrobial lipopeptides, volatile organic compounds (VOCs), and competition for ecological niches and nutrients. Furthermore, many PGPR activate induced systemic resistance (ISR) by modulating plant defense signaling pathways, thereby enhancing host resistance and reducing disease severity under pathogen pressure (Meena et al. 2020; Yang et al. 2025). *In vitro* studies have demonstrated the ability of rhizospheric bacteria to inhibit pathogens such as *Fusarium oxysporum* and *Sclerotinia sclerotiorum* (Wael et al. 2014).

Despite extensive research on rhizobacteria from conventional agricultural soils, the diversity and biocontrol potential of rhizobacterial communities associated with plants grown in aquaponic systems remain largely unexplored. The unique ecological conditions of aquaponic systems including continuous water recirculation, biofilm formation, high microbial diversity, and persistent interactions among plants, fish, and microorganisms may favor the establishment of bacterial communities with enhanced antagonistic activity against phytopathogens (Khalil et al. 2021; Stouvenakers et al. 2023). However, evidence supporting this hypothesis remains scarce, particularly for *Eruca vesicaria*, whose aquaponic associated rhizobacteria have been scarcely investigated as a source of biological control agents.

Considering the limited knowledge regarding aquaponic associated rhizobacteria as biological control agents, this study evaluated the *in vitro* antagonistic activity of rhizobacteria isolated from the rhizosphere of *Eruca vesicaria* cultivated in an aquaponic system against *Fusarium* sp. microbiological, molecular, and biochemical analyses were performed to characterize traits associated with biocontrol and plant growth promotion. This work provides new insights into the disease suppressive potential of aquaponic rhizobacteria and their application as sustainable biological control agents against soil borne plant pathogens.

Materials and methods

Study Area

The study was carried out in the aquaponic systems of the Ichthyology Greenhouse and the Multiple Laboratory 1 of the Nueva Granada Military University at the headquarters of Cajicá municipality of Cundinamarca, Colombia (4° 56´29.18" N and 74° 00´4200´42.92" W)

Obtaining rhizobacteria

The bacterial strains were obtained from the rhizosphere of *E. sativa* from an aquaponic system. The seedlings used in this work were acquired at the Agricultural Research and Development Center (CAIDVANDRE SAS) to later be transplanted into aquaponic systems at a flow rate of 200 L/h, together with tilapia produced from the Surala trout fish farm.

Isolation of bacterial strains

The bacteria were isolated from the roots, and after a vegetative growth stage of 5 weeks, they were transferred to a paper bag for subsequent conservation at the Animal Physiology Laboratory of Nueva Granda Military University. The samples were subsequently washed with distilled water to remove excess soil. The roots were cut into 8 cm pieces to be transferred to 200 ml of 0.95% saline. From these suspensions, serial dilutions were carried out, which were up to 10 − 7, seeded in triplicate in nutrient agar (OXOID®) and incubated at 35°C in an oven (BIOBASE).

Characterization of the microbial activity of rhizobacteria.

Morphology

To perform microscopic and macroscopic characterization of possible genera isolated from the rhizosphere, Gram staining was used. The type of grouping, structure, sporulation status, morphology, growth, surface, edge and color of the colonies were determined microscopically.

Biochemical tests

The tests were performed under these characteristics and applied in the microbiology laboratory for the characterization of rhizobacteria:

Catalase

This test indicates the presence of the enzyme catalase in a bacterium. This enzyme can convert hydrogen peroxide (hydrogen peroxide) into water and oxygen, causing bubbles to form. An object holder was used, the strain was grown, and a few drops of 3% hydrogen peroxide were added. The test is positive if bubbling is generated and negative if the colony does not have any type of reaction.

Oxidase

A special paper impregnated with Kovacs reagent was used, which was impregnated with a seeding handle in the center of the paper. With the sewing handle, a cologne is spread on the impregnated paper; for 5 to 10 s, a purple turn is produced, which indicates a positive test; in contrast, if the paper does not turn, the test is negative.

Motility

A semisolid nutrient broth, a very mild medium that allows mobile bacteria to pass easily, causing cloudiness, was used. When a sterile inoculation needle is used, the inoculum is stuck in the center of the agar; a diffuse growth zone extending from the inoculation line indicates positive bacterial motility. Some organisms grow throughout the medium, whereas others grow only small areas or nodules from the inoculation line. The immobile bacteria grew only in the area where the sample was inoculated.

Respiration: The isolates were seeded in a liquid broth with a small amount of agar at a ratio of 3:1, which reduces the entry of oxygen, as it approaches the interior of the tube, and the availability of oxygen is limited. This phenomenon results in a low oxidation–reduction potential, allowing the growth of strict aerobic bacteria, which present turbidity only in the upper part of the agar; facultative aerobics, which present turbidity throughout the environment, from the aerobic zone to the anaerobic zone; and, finally, strict anaerobic bacteria, which only grow in the basal zone of the environment.

Analysis of the antagonistic percentage of Rhizobacteria against Fusarium sp

Two types of tests were used to measure antagonism; the first consists of a dual seeding in which the fungus and the bacterium are directly confronted from a distance measured in the Petri dish to be able to make the respective measurements (Aquino et al., 2008). The second consists of a seeding by quadrants, in which the four equidistant points with bacterial wells are observed and in the center of an explant of the fungus (Ríos et al., 2016). These tests were carried out at an incubation temperature of 25°C, which allows adequate growth of fungi and bacteria.

Antagonistic evaluation

The diameter of fungal growth (mm) was taken at the two cardinal points referring to inhibition, and the percentage of inhibition of the phytopathogen was determined from the equation proposed by Etesami (2017):

Radial growth inhibition percentage (PRGI) = [(R1 - R2)/R1] × 100, where R1 represents the radial growth of the control colony (pathogen) and R2 represents the radial growth of the pathogenic colony in the in vitro confrontation.

The radial growth of fungal colonies of both pathogenic and antagonistic microorganisms was measured every 24 hours. After 120 hours of incubation, the inhibition halo between colonies of phytopathogens and antagonists was measured up to the cardinal points of the Petri dish.

Molecular identification of the bacterial species with the highest antagonistic percentage.

Amplification of the 16S ribosomal gene was performed via the *polymerase chain reaction (PCR*) technique. The fragments were purified via PCR and sequenced via the Sanger method via the 337F, 518F, 800R, and 1100R primers of the 16S ribosomal gene. For phylogenetic analysis, a taxonomic

analysis of the problem sequence was performed via the *Basic Local Alignment Search Tool* (BLAST) of the *National Center for Biotechnology Information (NCBI*), which was compared against the reference RNA database "refseq_rna", leBIBI IV SSU-rDNA (16S) (*Automated ProKaryotes Phylogeny*) of the University of Lyon (France). This tool uses the BIBI DB mk45 database (updated as of December 2022) and the Kalign v3.3, FastTree v2.1.11, CIAlign 1.0.9, Fastroot v1.5 and ToyTree v1 tools to generate the phylogenetic relationships of the sample. For the multiple alignment, the MUSCLE (*Multiple Sequence Comparison by Log-Expectation*) algorithm was used, from the problem sequence with the thirty sequences of greatest similarity reported by BLAST. Finally, for the generation of a phylogenetic tree, the Tamura–Nei genetic distance model (TN93) was used, with the "neighbor-joining" method and the "bootstrap" method with a thousand replicates.

Statistical analysis

The percentage of inhibition and the diameter of the phytopathogen were validated for normality via the Shapiro–Wilk test. An ANOVA and a Tukey–Kramer HSD test were subsequently performed to determine the differences between the different morphotypes ($P < 0.05$). All tests were carried out with tinn-R 4.2.0 software (Blanco & Castro, 2021).

Results

Isolation of bacterial strains

The bacterial strains were isolated and seeded in a nonselective medium to allow the growth of all types of microorganisms; thus, the strains whose antagonistic effects were evaluated were isolated and purified. In total, 30 vines were isolated, which were planted in triplicate.

Characterization of the microbial activity of rhizobacteria

Phenology

The micro- and macroscopic characterization of each of the isolated strains, which were seeded in nutrient agar (AN) and observed separately, was carried out. These observations were made 48 hours later to obtain a greater percentage of colonization (Table 1).

Biochemical Testing

The values of the tests performed in the laboratory for the enzymatic characterization of the rhizobacteria were recorded (Table 2).

Analysis of the percentage of Rhizobacteria antagonistic to Fusarium sp

The evaluation of bacterial growth in the face of the phytopathogen was established via the measurement of halos inhibited at 24 h, 48 h and 120 h after inoculation of the fungal explant (Figure 1).

Rapid growth of the bacterium was observed; although the PDA medium is a promising medium for the growth of the phytopathogen, the morphotypes with a relatively high percentage of inhibition are characterized by expansion of the colony throughout the box.

Antagonistic evaluation

Radial growth (mm) of the phytopathogen versus the biocontrol agent (mm) and its percentage of total invasion inhibition at 120 hours (Table 3).

Molecular characterization of the 16S rRNA gene and phylogeny

The phylogenetic trees of the bacterial isolates, which were molecularly identified to demonstrate the highest percentage of inhibition against *Fusarium sp.*, are shown in the antagonistic evaluation. Four different morphotypes were obtained, characterized as *Bacillus pumillus, Brucella pecoris,* and two species, *B. cereus JCM* and *B. cereus ATCC,* belonging to the *Bacillus cereus group.* The values shown in (Figure 2) correspond to the descriptions of the scientific names and identification percentages provided by the BLAST platform, this demonstrates the differences among the four isolated species.

Below are the phylogenetic trees of each of the molecularly identified species. The numbers in red, characterized as (10-1), (10-2), (10-3) and (10-4), correspond to the *Bacillus pumillus strains* NBRC 12092, *Brucella pecoris strain* 08RB2639, *B. cereus* ATCC 14579, and *B. cereus* JCM 2152, respectively.

Discussion

Bacterial characterization

A total of 30 bacterial morphotypes were isolated from the rhizosphere of *Eruca sativa* (arugula), with a predominance of Gram-positive bacilli (70%) over Gram-negative coccobacilli (30%). Most isolates exhibited whitish, translucent colonies with stable phenotypic traits over a 120h incubation period. These morphological characteristics are consistent with rhizospheric bacteria described as metabolically versatile, predominantly aerobic, and capable of colonizing diverse environments such as soil, water, and organic substrates (Wackett et al., 2014),

The predominance of Gram-positive bacteria observed in this study partially contrasts with previous reports describing Gram-negative taxa as dominant in rhizosphere communities (Ahmad et al., 2008; Kuklinsky-Sobral et al., 2004). However, this observation is consistent with previous findings in aquaponic and hydroponic systems, where microbial community structure is influenced by the distinct system components (fish tanks, biofilters, clarifiers, and plant-growing units) and nutrient flux dynamics. For instance, Schmautz et al. (2022) reported distinct bacterial assemblages across aquaponic components, with root-associated microbiomes enriched in taxa such as *Planctomycetaceae, Methylobacteriaceae, Herpetosiphonaceae, and Comamonadaceae*.

The higher relative abundance of Gram-positive bacteria in the present study may be attributed to their enhanced capacity for rhizosphere colonization and persistence. These microorganisms efficiently utilize root exudates and establish stable plant microbe interactions, as reported in crops such as strawberry, wheat, and potato (Prashar et al., 2013). Additionally, Gram-positive bacteria, particularly members of the genus *Bacillus*, are frequently recovered in culture dependent studies due to their physiological robustness and ability to grow under a wide range of culture conditions (Binnian et al., 2018).

The rhizosphere microbial composition is highly host dependent. Plant species selectively recruit microorganisms through the release of specific exudates, which vary according to developmental stage and physiological condition (Dukare et al., 2021). This selective pressure likely contributes to the variability in microbial community structure reported across different studies. A notable finding in the present study was the high frequency of endospore forming bacteria among the isolates. Endospore formation represents a key adaptive strategy that enables bacterial survival under adverse environmental conditions, including nutrient limitation, high salinity, temperature fluctuations, and pH stress (Martínez et al., 2014; Vimal, 2016). These conditions are relevant in aquaponic systems, which are characterized by dynamic nutrient availability and microbial competition (Sholtes et al., 2016).

Endospore forming bacteria are mainly associated with genera such as *Bacillus*, *Clostridium*, and *Desulfomonas*, with *Bacillus* being the most extensively studied due to its ecological and biotechnological relevance (Goddek, 2015). The members of this genus are widely recognized as plant growth promoting bacteria (PGPB), contributing to plant development through mechanisms such as phytohormone production, nutrient solubilization, and biocontrol of phytopathogens (Stegelmeier et al., 2022). These results suggest that the dominance of Gram-positive, endospore forming bacteria reflects a rhizosphere microbiome adapted to fluctuating environmental conditions and potentially enriched in functionally relevant taxa involved in plant growth promotion and stress mitigation within aquaponic systems.

Biochemical testing

Motility

Among the isolates, only morphotype SP7, identified as *Brucella pecoris*, was non motile. This trait is consistent with the genus *Brucella*, whose members are characteristically non-flagellated coccobacilli and therefore lack active motility (Whatmore & Foster, 2021). In contrast, all remaining morphotypes exhibited motility, particularly those assigned to the genus *Bacillus*, which commonly possess flagella that facilitate movement through aqueous and semi solid environments, including the rhizosphere associated with aquaponic systems (Piedrahita, 2003).

Bacterial motility is an ecologically relevant trait, as it enhances colonization, surface attachment, and migration toward favorable microhabitats. In aquaponic systems, motile bacteria may more effectively disperse among distinct system components, such as fish tanks, biofilters, clarifiers, and plant

rhizospheres, where physicochemical conditions and nutrient availability vary spatially. In addition to flagella mediated motility, other surface appendages such as pili can contribute to attachment, twitching motility, and horizontal gene transfer, thereby promoting genetic exchange and adaptive potential within microbial communities (Kim et al., 2016).

Catalase

Most isolates were catalase positive, a trait commonly associated with the genus *Bacillus*. This result is also consistent with rhizobacteria inhabiting environments with continuous water flow, where oxidative stress and reactive oxygen species may occur more frequently. In bacteria and fungi, catalases include catalase peroxidases containing a heme group in their structure (Murshudov et al., 2002). High molecular weight catalases may also bind NADPH, which increases resistance to H_2O_2 mediated inactivation (Melik-Adamyan et al., 2001). In aerobic environments such as aquaponic systems, catalase detoxifies hydrogen peroxide through dismutation into water and oxygen, thereby limiting the formation of highly reactive species such as hydroxyl radicals and singlet oxygen (Fang et al., 2021).

These findings may have functional relevance for aquaponic performance. Mao et al. (2023), comparing a fish only recirculating system with a fish plant integrated system, reported significant differences in catalase and superoxide dismutase activities. The plant containing system showed lower catalase activity, possibly due to antioxidant compounds associated with plant roots or root associated microorganisms that modulate oxidative balance (Lledias et al., 1998). Such plant microbe interactions may reduce oxidative stress, thereby improving fish welfare and growth under aquaponic conditions (Mate et al., 2000).

Oxidase

A high proportion of the rhizobacterial isolates showed positive oxidase activity, suggesting an active aerobic respiratory metabolism within the aquaponic rhizosphere. These results may be due to changes in the pH of the aquaponic system and the variations that occur in the system due to the simultaneous management of fish and plants. This could be attributed to the defense against oxidative stress (Wei et al, 2011).

In aquaponic and recirculating systems, pH is a key factor governing microbial stability and plant health. Regarding the ability of phytopathogens to spread in the environment, studies such as those of Myeong et al. (2018) revealed that the wilt disease caused by *Fusarium* in strawberry plants varies in terms of the incidence and growth of microorganisms. At low pH values (5.0) an effective and dynamic response of the enzyme oxidase present in the rhizobacteria of an aquaponic system would eventually increase the ability to control phytopathogens.

Oxidoreductases are enzymes that are involved in various cellular processes that use enzymatic oxidation/reduction processes. Among the potential determinants of resistance to the chromate ion present in bacteria, an enzyme of this class, encoded by the *yieF gene*, has been found in the intestinal

bacterium *Escherichia coli* (Ramírez et al; 2017). In culture media, microorganisms have very varied sensitivity to the potential for oxide reduction. Therefore, it is believed that redox potential is an important factor in each of the environments where the substrate probably determines the presence of a variety of microorganisms and their metabolic variation (Kinsinger et al., 2003).

The isolates *Brucella pecoris*, *Bacillus cereus ATCC 14579 and JCM 2152* showed significant differences (Fig. 10), which affirms and correlates with inhibition percentages greater than 50%, which were taken as assumptions for molecular identification. Despite *Bacillus pumillus* being the isolate with the highest percentage of inhibition (61.29%), the ANOVA results did not significantly differ. Most of the rhizobacteria isolated with percentages greater than 25% differed and values with a negative percentage were not recorded to avoid generating possible noise in the presentation of the data, which indicates that, despite not presenting higher percentages, the values obtained are consistent with the evaluation carried out against the phytopathogen.

Analysis of the antagonistic percentage of rhizobacteria against Fusarium sp. and molecular characterization

The inhibition percentages varied across the 30 strains evaluated, 25 of which yielded positive percentages, which indicates that the microorganisms grew in the medium, generating different percentages of inhibition. On the other hand, isolates that yielded negative percentages indicate that the fungus quickly joined the control colony, preventing the growth of the bacterium. The rhizobacteria that were subjected to molecular identification had inhibition percentages greater than 50%, with an antagonistic percentage of 61.29% for *Bacillus pumillus*, 55.56% for *Bacillus cereus JCM*, 52.44% for *Bacillus cereus ATCC*, and 50.00% for *Brucella pecoris*.

The molecularly identified morphotypes are related to the inhibition and antagonistic activity that these rhizobacteria present against *Fusarium* sp. However, antagonistic activity may be due to several factors, such as the production of volatile metabolites, lytic enzymes, and antifungals (Compant et al., 2005). The inhibition of growth generated by the rhizospheric bacteria isolated in the present study is highly important since the presence of a new species of the genus *Brucella* as an inhibitor and controller of *Fusarium* in aquaponic systems has been reported, which has greatly contributed to the study of this microorganism in unconventional systems associated with plants of national horticultural interest, such as arugula (Egea et al, 2009).

The ability of Bacillus to adapt to different environments encourages plant growth and inhibits various phytopathogens makes it a good biocontrol agent for agricultural crops against pests and diseases that affect their crops. In addition, it is estimated that 85% of the microbial biopesticides found on the market are produced with bacteria of the genus *Bacillus* and are applied to plants of horticultural and fruit interest (Martínez, 2021).

The results of the molecular identification of bacteria with a relatively high antagonistic percentage demonstrated the potential of the genus *Bacillus* by identifying three different species of the four

analyzed bacteria. The species of the *genus Bacillus* associated with the rhizosphere exhibit wide metabolic and functional diversity by synthesizing several metabolites that allow the control of phytopathogens and the promotion of plant growth (Gao et al, 2017). It has been reported that the variety of *Bacillus sp*. present in plants produces many antifungal oligopeptides, such as iturins, bacillomycins and surfactins (Kundan et al, 2015). Therefore, it cannot be ruled out that these types of molecules are synthesized and contribute to the antagonistic activity observed in this genus of bacteria (Santoyo et al., 2010).

The *Bacillus subtilis* group includes several species that are highly valuable in agricultural areas (Villareal, 2018). For example, *B. subtilis, B. pumillus* and *B. licheniformis* have been approved by the European Food Safety Authority *(QPS*), which ensures their safety in the use and application of food products suitable for human consumption (EFSA, 2015).

Bacillus pumilus is a nonpathogenic microorganism found in various environments, such as water, soil, and air, and in decaying organic matter (Moreno et al., 2011). This bacterium can produce proteases and other enzymes that allow it to degrade different types of natural substrates and contribute to nutrient recycling (Gutiérrez, 2008). The *Bacillus pumilus* strain *NBRC 12092* prevents the germination of spores through the formation of a physical barrier and subsequent colonization, interrupting cell metabolism by destroying the cell walls of pathogens, resulting in their elimination (Vimal, 2016). Its mechanism of action makes it an effective fungicide capable of preventing the development of pathogen resistance (Zanin, 2021).

The cellular structure of *B. pumilus* is like that of other *Bacillus species*, such as *B. subtilis, B. megaterium and B. cereus*. Like most other gram-positive bacteria, the outer layer of peptidoglycan cross-links is covered by teichoic and lipoteichoic acids (Gerceker et al., 2009). These acids contain polyglycosyl phosphates with mono- and disaccharides as monomers, which can help host cells bind to the membrane (Madhaiyan, 2010). On the other hand, phosphate groups present on the surface of *B. pumilus* can create a net negative charge on the cell surface, allowing the capture of cations such as calcium and magnesium (Ca2 + and Mg2+) (Soto et al., 2018). Calcium plays an important role in protein expression, followed by Mg2+, which regulates the aggregation and formation of extracellular polymeric substances (SPE) and the synthesis of polysaccharides (Xiaoyan et al., 2022). All these mechanisms generate extra protection to the membrane against external damage, making these bacteria the most recent in the face of environmental variations and therefore the most commonly used bacteria in the agricultural sector (Kuklinsky-Sobral et., 2004).

This is evident from the research of Calderón (2013), who reported the effectiveness in the control of several phytopathogens, such as *Mildew* sp., *Powdery mildew* sp., *Sclerotinia* sp., *and Cercospora* sp., in numerous crops, among which the following stand out: Brassica, peanuts, cereals, cucurbits, strawberries, green leafy legumes, hops and corn. Additionally, the genus *Bacillus* has been shown to promote plant growth, and *Bacillus cereus MJ-1, B. macroides CJ-29* and *B. pumilus* CJ-69, when inoculated into the rhizosphere of red pepper seedlings, considerably promoted growth.

The molecular identification of the bacterial isolates revealed two species associated with the *Bacillus cereus* group; the members of this group are genetically related; however, they differ in their metabolic activities (Layton et al., 2011). Proof of this was the work carried out by Helganson et al. (2000), who identified specific peptides of different strains that allowed the creation of a diagnostic tool to differentiate the species of the *B. cereus group.* An analysis of soil isolates of *B. cereus* and *B. thuringiensis* revealed great diversity in the multilocus genotypes, indicating that *B. cereus* and *B. thuringiensis* exhibit a low degree of clonality and that the exchange of genetic material frequently occurs in the wild. The close similarity of the genomes of the *B. anthracis* strains with those of the *B. thuringiensis* and *B. cereus* strains shows that the *B. anthracis* strains should be considered to belong to the same species, which distinguishes them functionally as genes transported in plasmids. Horizontal transfer of plasmids can drastically alter their phenotypes.

The second genus identified as biocontrollers was *Brucella*, whose metabolic activity as an antagonist is largely unknown. However, this strain exhibited a good inhibitory percentage (50.00%), which is the first report of this bacterium in an isolate of *Eruca vesicaria* arugula cultured in an aquaponic system. For *Brucella*, only Ravi et al. (2021), who evaluated bacterial endophytes isolated from the banana cultivar Ney Poovan and identified the species *Brucella mellitensis* as an antagonist of *Fusarium oxysporum f.sp. cubense (foc)*, with a percentage antifungal activity of 63.3%. This result is like that obtained in this research, which generates great expectations when working with this species as a potential biocontrol agent of the phytopathogen *Fusarium*.

CONCLUSIONS

This study demonstrated the diversity of bacterial communities from the rhizosphere of *Eruca vesicaria* grown in an aquaponic system. Four of the rhizobacteria isolated from this vegetable demonstrated a biocontrol effect against the agriculturally important phytopathogen *Fusarium* sp., with inhibition percentages greater than 50%. The biocontrol nature of these bacteria was also confirmed through enzymatic characterization, as they are catalases, oxidases and have positive motility, which allows them to resist the stress of the environment more efficiently and act through various mechanisms of action against phytopathogens. In this way, the potential of the genus *Bacillus* and a new report on the genus *Brucella* present in aquaponic systems are highlighted. The perspective of the communities associated with the rhizosphere of the plants from these systems is broadened so that they can be used as potential bio controllers and evaluated as possible consortia, which verifies the beneficial effects of rhizobacteria in different crops of interest.

Declarations

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Disclosure statement

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Tables

Tables 1 to 3 are available in the supplementary files section

Figures

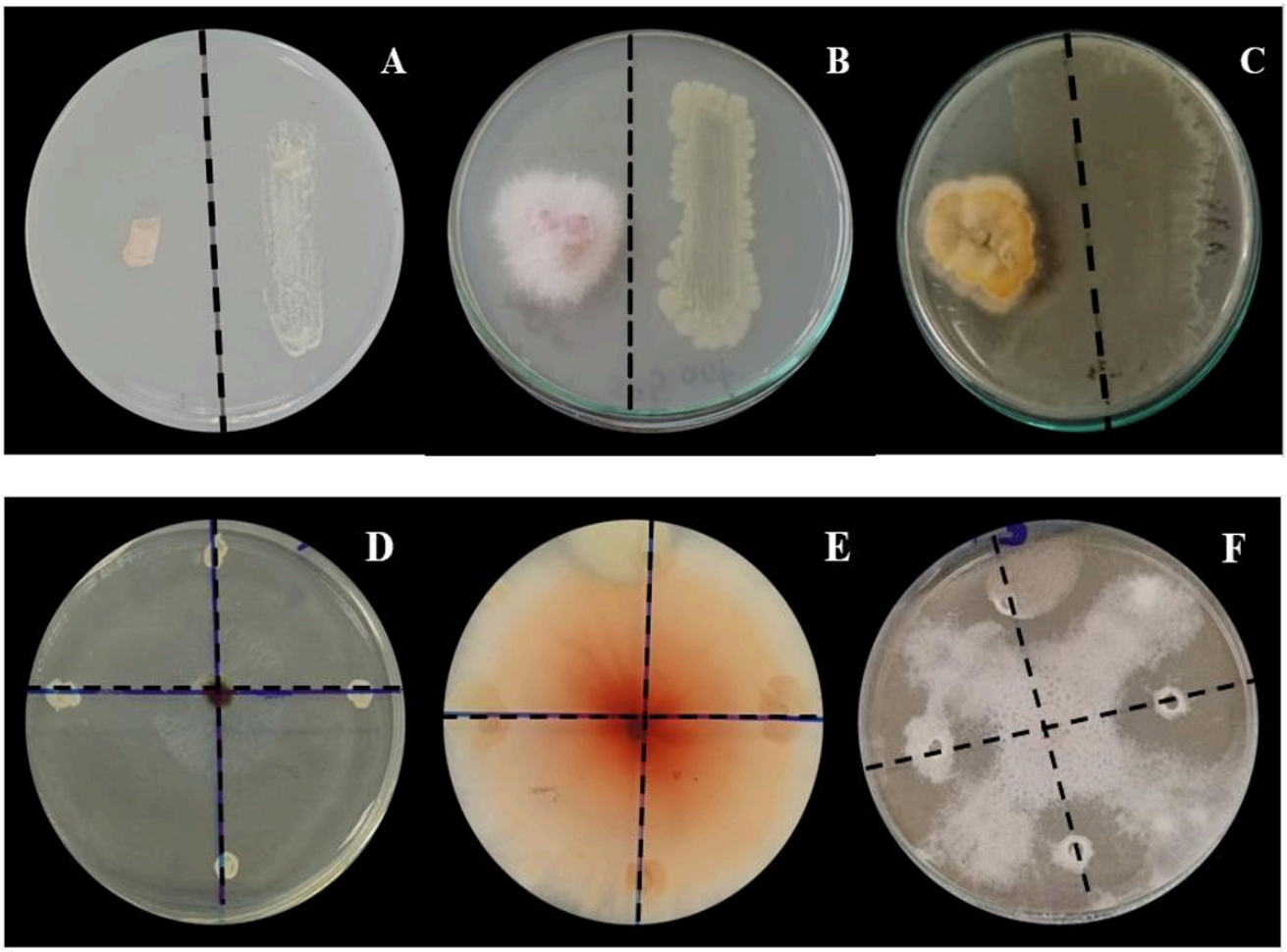

Figure 1

Comparison of the two methods of antagonistic seeding of types of bacteria with relatively high percentages of invasion of the phytopathogen. (A) 24-hour confrontation method (B) 48-hour confrontation method (C) 120-hour confrontation method (D) 24-hour quadrant method (E) 48-hour quadrant method. (F) 120-hour quadrant method

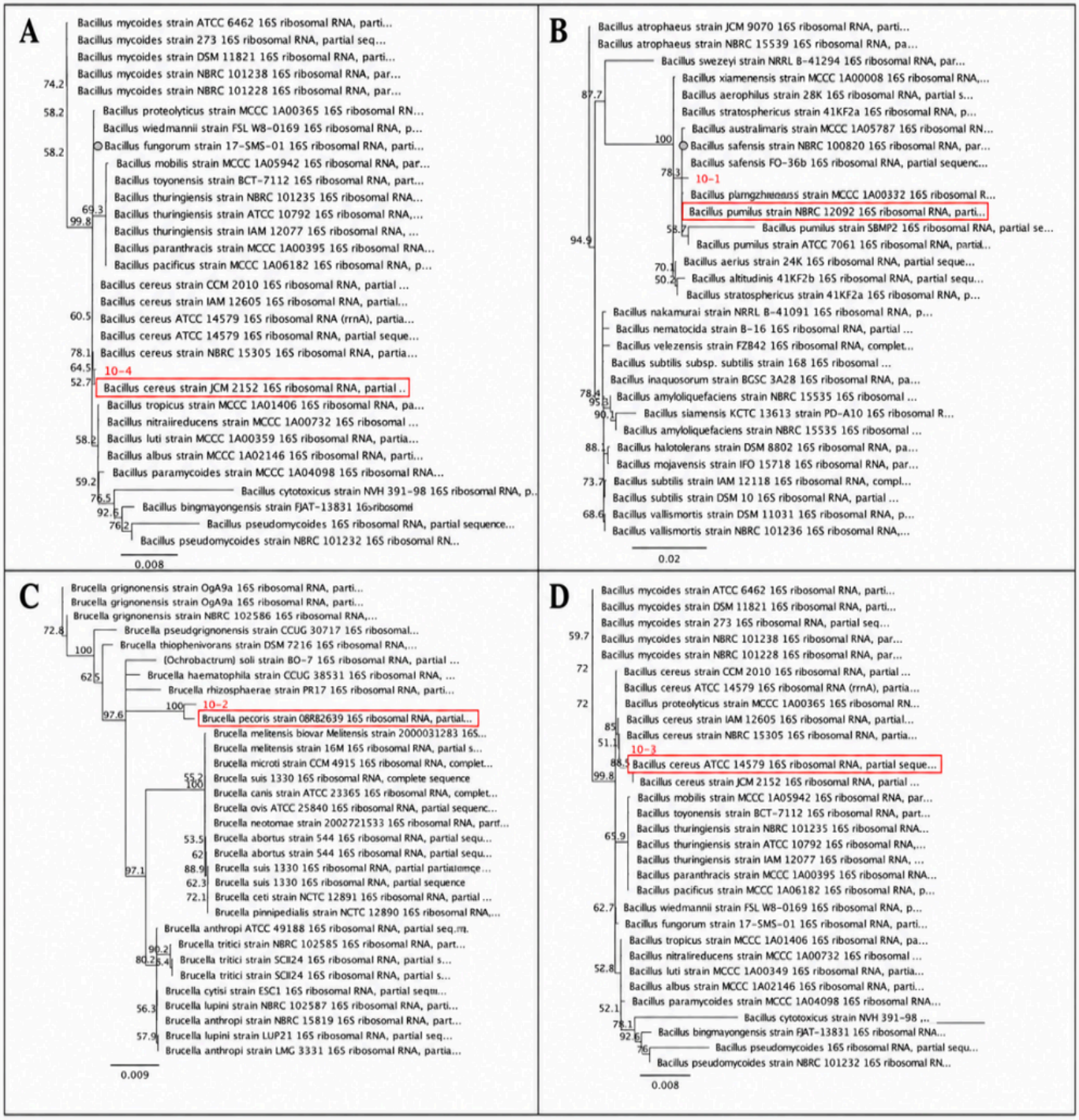

Figure 2

Phylogenetic analysis based on partial 16S rRNA gene sequences of the bacterial isolates obtained in this study. The phylogenetic trees show the taxonomic relationships of isolates 10-1 (B), 10-2 (C), 10-3 (D), and 10-4 (A)

Supplementary Files

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