# Epiphytic Microorganisms Associated with Banana Phyllosphere with Potential Antagonism to Black Sigatoka (*Pseudocercospora fijiensis*) in Los Ríos, Ecuador

## Identificación de microorganismos epífitos asociados a la filósfera de banano con potencial antagonismo a Sigatoka Negra (*Pseudocercospora fijiensis*) en la provincia de Los Ríos, Ecuador

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**ABSTRACT**

Black Sigatoka (*Pseudocercospora fijiensis)* is the most important leaf spot disease of bananas worldwide, particularly affecting Cavendish banana, the most exported variety. Additionally, this pathogen has developed resistance to some effective fungicides, making its management increasingly difficult. Epiphytic microorganisms with potential antagonism to *P. fijiensis* were identified in conventional banana farms in the province of Los Ríos. Sampling areas were determined through zoning processes and selecting the cantons of Mocache, Valencia, Baba and Pueblo Viejo. Leaf tissue samples were collected from three farms per zone. Microorganisms were isolated and morphologically and molecularly characterised in nine farms in the cantons of Valencia (63 bacteria), Baba (39 bacteria), Pueblo Viejo (8 bacteria) and 8 genera of fungi including 15 species. The isolated bacteria presented macroscopic and microscopic characteristics with different shapes, elevations, edges, consistencies and pigmentations. Taxonomically, they belonged to the genera *Bacillus* and *Cocos*, 81% Gram-negative and 19% Gram-positive. The analysis conducted for sampling-site selection allowed the identification of different microbial behaviours.

**Keywords**
microorganisms • fungi • bacteria • *Musa* spp.

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**Resumen**

Sigatoka negra es la enfermedad de la mancha foliar más importante del banano a nivel mundial. La variedad de banano Cavendish, es considerada la más común y la más exportada; sin embargo, presenta una alta susceptibilidad frente a la enfermedad. Existen fungicidas altamente efectivos para su control; sin embargo, el patógeno ha logrado generar resistencia a algunos de estos, lo que ha dificultado cada vez más su manejo. Se identificaron microorganismos epífitos con potencial antagonismo a *Pseudocercospora fijiensis* en fincas de banano convencionales de la provincia de Los Ríos. Las zonas de muestreo fueron determinadas a través de procesos de zonificación, seleccionando los cantones Mocache, Valencia, Baba y Pueblo Viejo. Se recolectó muestras de tejido foliar en tres fincas por zona. Se aislaron microorganismos y se caracterizaron morfológica y molecularmente en nueve fincas en los cantones de Valencia (63 bacterias), Baba (39 bacterias) y Pueblo Viejo (8 bacterias) y 8 géneros de hongos que incluyen 15 especies. Las bacterias aisladas presentaron características macroscópicas y microscópicas con diversas formas, elevaciones, bordes, consistencias y pigmentaciones, así como diversas taxonomías pertenecientes a los géneros *Bacillus* y Cocos, siendo 81% Gram negativas y 19% Gram positivas. El análisis realizado para la selección de los sitios de muestreo fue apropiado ya que se observó un comportamiento diferencial de los microorganismos en estas zonas.

**Palabras clave**
microorganismos • hongos • bacterias • *Musa* spp.

**Introduction**

*Musaceae* is a family of monocotyledonous plants that include bananas and plantains, often called giant herbs (31). These plants belong to the genus *Musa,* cultivated in tropical and subtropical regions (36).

The banana sector in Ecuador has 167,893 hectares, with a productivity of 6,684,916 tons. Los Ríos province has the highest participation in the national production of fresh fruit with 38.47% (2 571 356 t), with a contribution of 1 328 537 964.98 US dollars (45).

Investment in production and related industries (goods and services needed for banana production) and the current banana export process created jobs for more than one million households in Ecuador, benefiting around 2.5 million people in nine provinces heavily dependent on the banana industry. Compared to other non-oil sectors in the country, this sector is the backbone of economic activity, generating higher incomes and providing more employment opportunities (16).

Black Sigatoka is the most economically important leaf spot disease of *Musaceae*, affecting many plantations and resulting in forced early harvesting (27). This disease is caused by the fungus *Pseudocercospora fijiensis*, exclusive of banana foliage with sexual and asexual reproduction. It infects the plants, hindering photosynthesis and causing gradual leaf necrosis and death. Disease severity is determined by the Stover scale modified by Benavides-López (2019), Gauhl (1994) and Muimba-Kankolongo (2018).

Black Sigatoka is mainly controlled by technical management and appropriate fungicide rotation. However, given climatic variability, the disease shows different behaviours around the country. Los Ríos province is the most affected, with 74% of production losses. Twenty-two to 29 annual aerial spraying cycles are used to fight the disease, representing costs between $430 and $800 (8, 9). In addition, surgical practices like excision of mottled areas and leaf removal are carried out (9, 17).

International markets for plant protection products are dominated by synthetic pesticides (30). These chemical substances seriously affect the ecosystem and induce resistance, altering ecological equilibriums (28). Therefore, searching for alternative control strategies is relevant worldwide (24).

The search for antagonistic microorganisms for biological control of pathogens in economically important crops has aroused particular interest due to their potentialities (4, 54). Microorganisms of agricultural importance represent a key ecological strategy

towards the integrated development of practices such as nutrient, disease and pest management, reducing chemicals and improving crop yield (42). Several microorganisms showing beneficial effects on plants may constitute potential biocontrol agents (3, 41) and important actors in sustainable agriculture (51).

Biological control of Black Sigatoka has received relatively little attention due to the availability of highly effective fungicides. However, the emergence of pathogen isolates resistant to systemic fungicides and the need for cleaner production technologies have increased interest in biological control (22).

The search for effective biological products against this disease has studied different microorganisms associated with these crops (12). Therefore, this research aimed to collect, isolate and characterise microorganisms from the phyllosphere of *Musaceae.*

## MATERIALS AND METHODS

### Study Area

This research was conducted at the Pichilingue Tropical Experimental Station (EETP) of the National Institute of Agricultural Research (INIAP) with samples obtained from conventional banana farms of Cavendish Williams cultivar in the cantons of Mocache, Valencia, Baba and Pueblo Viejo, Los Ríos province.

### Field Methodology

Sampling sites were chosen by zoning with cartographic charts from the IGM (Military Geographic Institute) database. The climate micro-zonation map was generated by satellite images with ArcGIS 10.8, at a scale of 1:25 000 for geo pedological conditions considering soil pH, organic matter and surface texture, with 1:50 000 scale, considering geopedology, geomorphology, CUT (soil usage capacity) and isotherms, including climatic zones, temperature and cover use (figure 1, page 4).

Ten subsamples were collected from each farm, constituting one composite sample. In selected plants, the third and fourth leaves were identified for tissue to be obtained from the central third, both on the right and left side of the midrib. Samples were identified by recording origin and date (41).

Fungal identification from leaf tissue was conducted in Mocache, Baba and Pueblo Viejo. Bacteria were identified from leaves in Valencia, Baba and Pueblo Viejo.

### Microorganism Isolation

For the isolation of bacteria, the samples obtained were processed according to Intriago Mendoza (2010). Agar culture medium was prepared in flasks, sterilised in auto-clave for 30 minutes and distributed in petri dishes. Twenty-five g of leaf tissue were washed in 100 mL of sterile distilled water (SDW). Product water was used for serial dilutions up to -3. One ml of each dilution was seeded by Digralsky loop, and plates were incubated at room temperature for 5 days for growth evaluation.

In order to isolate fungi, plant tissue samples were washed with distilled water, cut into small portions of tissue (3 to 5 mm) and immersed in a 1-3% hypochlorite solution for one minute, followed by rinsing with sterile water. Tissue portions were seeded in Petri dishes with PDA (potato dextrose agar) + chloramphenicol medium and incubated at 28°C for 5 days. Isolates were purified and preserved at 5°C (20).

### Morphological Characterization

After biochemical Gram staining and catalase tests, macroscopic and microscopic characterisation was carried out on the isolated microorganisms, described by their shape, colour, edges, elevation and consistency (52).

### Number of Colonies

Number of colonies on the plates is expressed as CFU/ml (Colony Forming Units) according to Casas *et al.* (2017).
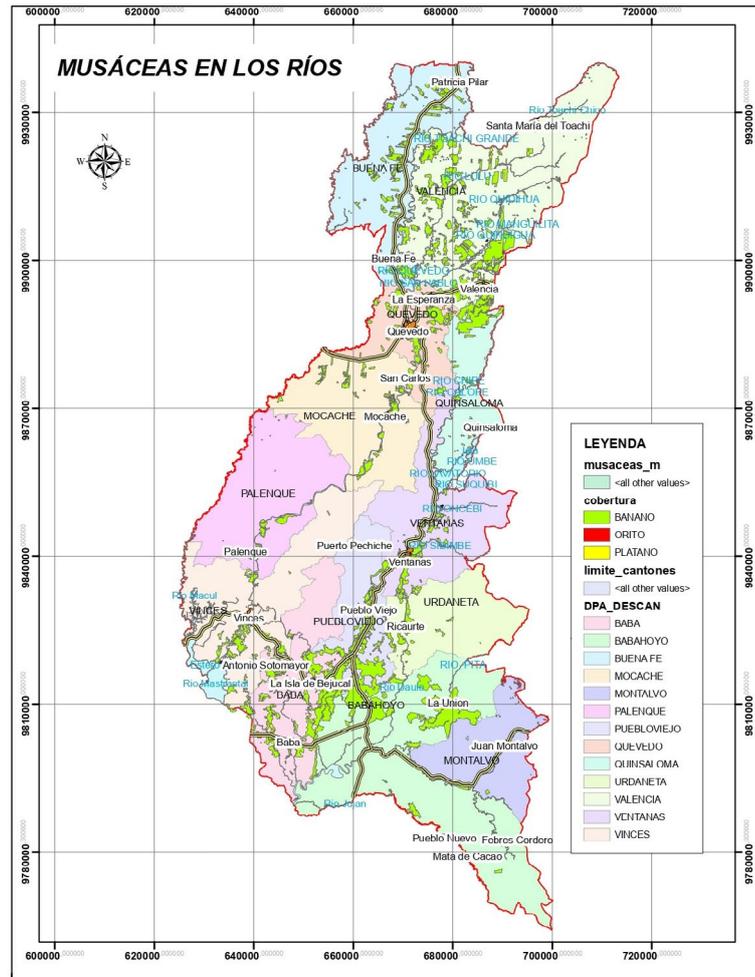
**Figure 1.** Climate micro-zonation map for sampling sites in the Province of Los Ríos, generated by ArcGIS 10.8 software.

**Figura 1.** Mapa de microzonificación climática para sitios de muestreo en la Provincia de Los Ríos, generado con el software ArcGIS 10.8.

### Extraction of Fungal Genomic DNA

According to Doyle & Doyle (1987) modified by Faleiro *et al.* (2002), samples were split into two boxes per sample with 14 daysqold mycelium and triturated with liquid nitrogen. The homogenate was mixed with 800 µL extraction buffer (7% cetyltrimethylammonium bromide [CTAB], 5 M NaCl, 0.5 mM ethylenediaminetetraacetic acid [EDTA], 1 M Tris-HCl pH = 8, Polyvinylpyrrolidone (PVP-40), 1% (v/v) ß-mercaptoethanol and milliQ water). Five µL of proteinase K (concentration 20 mg/uL) was added to the homogenate and incubated at 65°C for 1 hour in a water bath. Then, it was centrifuged at 14 000 rpm for 15 minutes and the supernatant was collected into 2 mL tubes, added 55 µL of 7% CTAB and 700 µL chloroform: isoamyl alcohol (25: 1; v: v), mixed with inversion and vortexed until an emulsion was formed and centrifuged at 14 000 rpm for 16 minutes. Again, the supernatant was extracted to 2 mL tubes by adding 700 µL chloroform: isoamyl alcohol (25: 1; v: v), mixed and centrifuged. The supernatant was recovered in 1.5 mL tubes and 700 µL (2/3 of the tube) was added with ice-cold absolute ethanol (-20°C), for storage at -20°C for 1 to 2 hours. Centrifugation was performed at 14 000 rpm for 5 minutes, obtaining a white pellet and the supernatant was removed by washing the pellet 3 to 4 times with 70% ethanol at -2°C. (500 µL). Finally, the pellet was dried in a thermoblock at 55°C and DNA was resuspended in 100 µL of TE with RNAsa (concentration 20 mg/ml).

### PCR Amplification of Ribosomal ITS Region

To verify the extracted DNA, amplification was performed with markers ITS1 (TCCGTAGGTGAACCTGCGG) and ITS4 (TCCTCCGCTTATTGATATATGC) and the amplification cocktail proposed by Morillo & Miño (2011). All samples were amplified on the Applied Biosystems thermal cycler in a total reaction volume of 25 µl, including 2.50 µL of 5x Green GoTaq® Flexi Buffer (Promega), 1.5µL of MgCl2 (25 mM), 0.50 µL of dNTPs (5mM), 2 µL of each primer (5 µmol), 0.50 µL of DNA polymerase (Thermo Scientific DreamTaq) (5 U/ µl), 1 µL of genomic DNA (5 ng/ µl) and 15 µL of ultrapure water. PCR included initial denaturation at 95°C for 5 min, followed by 35 cycles of denaturation at 94°C for 1 min, hybridisation at 55°C for 2 min, elongation at 72°C for 1 min and a final elongation step at 70°C for 10 min.

DNA amplicons were analysed on a 1.5% (w/v) agarose gel using Syber Safe for 30 minutes at 100V. Amplicon sizes were estimated by comparison with a TrackIt™ 1 Kb Plus DNA Ladder molecular weight marker and visualised in a photodocumenter.

### Sequence Analysis

PCR products were shipped to the research laboratories of the Universidad de las Américas (UDLA), according to the university guidelines, which consisted of 10 µL of PCR product, 2 µL of each primer (ITS 1/ ITS4) (2 µM concentration) per sample and cold chain storage at 4°C or below. Sequence editing was performed using the Unipro UGENE software and the BLAST programme at the Centro Nacional de Información Biotecnológica (http://www.ncbi.nlm.nih.gov/) to obtain consensus sequences. Sequence alignment was performed by MUSCLE algorithm with MegaX software. The matrix obtained was used to assemble the phylogenetic tree based on the Maximum-Likelihood (ML) algorithm, with genetic distance calculated by the Jukes-Cantor model, Bootstrap-100 and Uniform Rates.

## RESULTS

### Colony Count

Considering the three farms evaluated in each canton, in Valencia, the Bellavista farm had the highest number of colonies with $9.18 \times 10^4$ CFU; in Baba the Soledad farm had the highest number of colonies with $3.468 \times 10^4$ CFU and in Pueblo Viejo, Valdivia and Viviana farms had $6.12 \times 10^3$ CFU, obtaining a total of 63 bacteria in Valencia, 39 bacteria in Baba and 8 bacteria in Pueblo Viejo (table 1).

**Table 1.** Bacterial colony count (CFU) per farm.

**Tabla 1.** Recuento de colonias bacterianas (CFU) por finca.

| Estate | Colony Forming Units |
|---|---|
| Bellavista | $9.18 \times 10^4$ CFU |
| El Zapote | $4.08 \times 10^4$ CFU |
| Buenos Aires 2 | $6.528 \times 10^4$ CFU |
| San Luis | $1.224 \times 10^4$ CFU |
| Carolina | $2.244 \times 10^4$ CFU |
| Soledad | $3.468 \times 10^4$ CFU |
| Carolina | $4.08 \times 10^3$ CFU |
| Valdivia | $6.12 \times 10^3$ CFU |
| Viviana | $6.12 \times 10^3$ CFU |

### Strain Characterisation

Colonial morphological characterisation, microscopic morphology and biochemical tests were performed on the bacteria isolated in the province of Los Ríos (table 2, page 6).

**Table 2.** Characterisation of microorganisms associated with the banana phyllosphere, Los Ríos province.
**Tabla 2.** Caracterización de microorganismos asociados a la filósfera de banano, provincia de Los Ríos.

| Cantons | Estate | Strain | Colonial morphology | | | | | Microscopic morphology | Biochemical Test |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| | | | Shape | Color | Borders | Elevation | Consistency | Gram stain | Catalase |
| Valencia | Bellavista | VF1C5 | circular | Orange | whole | elevated | Viscous | coconuts (+) | (+) |
| | Bellavista | VF1C6 | irregular | Yellow | wavy | elevated | Viscose | coconuts (+) | (+) |
| | Bellavista | VF1C9 | irregular | Milky | wavy | Flat | Viscose | coconuts (+) | (+) |
| | Bellavista | VF1C15 | irregular | Orange | wavy | Flat | Viscose | coconuts (-) | (+) |
| | Bellavista | VF1C16 | irregular | Red | whole | Flat | Viscose | coconuts (+) | (+) |
| | Bellavista | VF1C17 | circular | Red | whole | convex | Viscose | coconuts (-) | (+) |
| | Bellavista | VF1C24 | irregular | Yellow | wavy | convex | Viscose | coconuts (-) | (+) |
| | Bellavista | VF1C29 | circular | Yellow | whole | convex | Viscose | coconuts (+) | (+) |
| | El Zapote | VF2C3 | circular | Red | wavy | convex | Viscose | coconuts (-) | (+) |
| | El Zapote | VF2C5 | irregular | Yellow | lobed | Flat | Viscose | bacillus (-) | (+) |
| | El Zapote | VF2C8 | circular | Orange | whole | convex | Viscose | bacillus (-) | (-) |
| | El Zapote | VF2C10 | circular | Orange | whole | Flat | Viscose | coconuts (-) | (-) |
| | El Zapote | VF2C12 | circular | Orange | whole | convex | Viscose | coconuts (-) | (+) |
| | Buenos Aires 2 | VF3C13 | circular | Pink | whole | convex | Viscose | coconuts (-) | (-) |
| | Buenos Aires 2 | VF3C18 | circular | White | wavy | Flat | Viscose | coconuts (-) | (+) |
| | Buenos Aires 2 | VF3C24 | circular | pink | whole | elevated | Viscose | coconuts (-) | (+) |
| | Buenos Aires 2 | VF3C31 | circular | milky | wavy | convex | Viscose | coconuts (-) | (+) |
| Baba | San Luis | BF1C2 | circular | milky | Wavy | Flat | Viscose | bacillus (-) | (-) |
| | San Luis | BF1C3 | irregular | white | Wavy | Flat | dry | cocobacillus (+) | (+) |
| | San Luis | BF1C4 | circular | pink | Wavy | convex | Viscose | bacillus (-) | (+) |
| | San Luis | BF1C5 | circular | milky | Wavy | elevated | Viscose | bacillus (-) | (+) |
| | San Luis | BF1C8 | circular | yellow | Wavy | convex | Viscose | bacillus (-) | (+) |
| | Carolina | BF2C3 | punctate | yellow | Wavy | Flat | Viscose | coconuts (+) | (+) |
| | Carolina | BF2C7 | irregular | white | Wavy | Lisa | Viscose | bacillus (-) | (+) |
| | Carolina | BF2C8 | irregular | milky | Irregular | Lisa | Viscose | bacillus (-) | (+) |
| | Carolina | BF2C9 | circular | yellow | Lobed | Flat | Viscose | bacillus (-) | (+) |
| | Carolina | BF2C15 | irregular | white | Irregular | Flat | Viscose | streptobacillus (-) | (+) |
| | Carolina | BF2C18 | irregular | orange | Lobed | elevated | Viscose | bacillus (-) | (+) |
| | Soledad | BF3C1 | circular | milky | Whole | convex | Viscose | coconuts (-) | (+) |
| | Soledad | BF3C2 | spindle | white | Whole | Flat | Viscose | coconuts (-) | (+) |
| | Soledad | BF3C3 | circular | white | Whole | convex | Viscose | coconuts (-) | (+) |
| | Soledad | BF3C5 | circular | white | Whole | Flat | Viscose | coconuts (-) | (+) |
| | Soledad | BF3C9 | irregular | milky | Lobed | Flat | Viscose | bacillus (-) | (+) |
| | Soledad | BF3C12 | punctate | milky | Wavy | Flat | Viscose | coconuts (-) | (+) |
| Pueblo Viejo | Viviana | PvF1C1 | circular | milky | whole | elevated | Viscose | coconuts (-) | (+) |
| | Viviana | PvF1C2 | circular | milky | filamentous | Flat | Viscose | bacillus (-) | (+) |
| | Viviana | PvF1C3 | circular | milky | whole | convex | Viscose | cocobacillus (-) | (+) |
| | Valdivia | PvF2C1 | circular | milky | whole | convex | Viscose | cocobacillus (-) | (+) |
| | Valdivia | PvF2C2 | circular | milky | whole | convex | Viscose | cocobacillus (-) | (+) |
| | Valdivia | PvF2C3 | circular | milky | whole | convex | Viscose | cocobacillus (+) | (+) |
| | Valdivia | PvF2C4 | circular | milky | wavy | elevated | Viscose | cocobacillus (-) | (+) |
| | Carolina | PvF3C1 | circular | milky | lobed | Flat | Viscose | coconuts (-) | (+) |

Eight fungal strains were isolated in Mocache, 5 in Baba and 13 in Pueblo Viejo. Eight PCR products were amplified from Mocache, 5 from Baba and 13 from Pueblo Viejo. Twenty-six sequences were obtained. Similarity percentages were 90-100 % with NCBI homologous sequences (table 3).

**Table 3.** Collection and ITS rDNA sequences of fungi isolated in the cantons of Mocache, Baba and Pueblo Viejo.

**Tabla 3.** Detalles de la colección y secuencias ITS del ADNr de hongos aislados en los cantones de Mocache, Baba y Pueblo Viejo.

| Canton | Estate | Identity in GenBank | | Identity |
| --- | --- | --- | --- | --- |
| | | Accession | Description | |
| Mocache | INIAP Lot 1 | MN173821.1 | *Colletotrichum fructicola* | 100% |
| | INIAP Lot 1 | MT982177.1 | *Fusarium oxysporum* | 99.41% |
| | INIAP Lot 1 | KM519998.1 | *Colletotrichum gloeosporioides* | 100% |
| | INIAP Lot 1 | KU350718.1 | *Nectria haematococca* | 98.66% |
| | INIAP Lot 2 | JN649339.1 | *Cymatoderma dendriticum* | 98.34% |
| | INIAP Lot 2 | MH483999.1 | *Miyakei microdiplody* | 99.61% |
| | INIAP Lot 2 | MK041519.1 | *Colletotrichum fructicola* | 98.89% |
| | INIAP Lot 2 | HM855208.1 | *Nodulisporium sp.* | 99.27% |
| Baba | San Luis | KY488352.1 | *Cladosporium uredinicola* | 99.41% |
| | San Luis | KT240141.1 | *Cladosporium cladosporioides* | 99.22% |
| | San Luis | MN515070.1 | *Nigrospora oryzae* | 98.80% |
| | Carolina | MW016510.1 | *Fusarium subglutinans* | 99.21% |
| | Carolina | MT645782.1 | *Nigrospora osmanthi* | 98.64% |
| Pueblo viejo | Viviana | MH864880.1 | *Diatrypella vulgaris* | 99.27% |
| | Viviana | KY488352.1 | *Cladosporium uredinicola* | 99.22% |
| | Viviana | KY488352.1 | *Cladosporium uredinicola* | 99.41% |
| | Viviana | MH864880.1 | *Diatrypella vulgaris* | 99.45% |
| | Viviana | MH270558.1 | *Cladosporium tenuissimum* | 99.22% |
| | Viviana | MN704703.1 | *Cladosporium cladosporioides* | 99.60% |
| | Viviana | MH270558.1 | *Cladosporium tenuissimum* | 99.42% |
| | Viviana | MW723859.1 | *Cladosporium cladosporioides* | 93.79% |
| | Valdivia | KY488352.1 | *Cladosporium uredinicola* | 98.83% |
| | Valdivia | KY488352.1 | *Cladosporium uredinicola* | 99.41% |
| | Valdivia | MW624421.1 | *Nigrospora sphaerica* | 98.84% |
| | Carolina | MW113408.1 | *Cladosporium cladosporioides* | 99.80% |
| | Carolina | MN959990.1 | *Fusarium oxysporum* | 99.21% |

The identified fungi were from the genera *Fusarium* sp., *Colletotrichum* sp., *Diatrypella* sp., *Nodulisporium* sp., *Nigrospora* sp., *Microdiplodia* sp., *Cymatoderma* sp. and *Cladosporium* sp. *Fusarium* sp. is a candidate biological control agent against *Pseudocercospora fijiensis*. *Nodulisporium* sp. is an endophyte capable of producing insecticidal nodilosporic acids and volatile antifungal substances (Suwannarach *et al.*, 2013). *Nigrospora* sp. produce bioactive secondary metabolites with antifungal activity (23) (figure 2).
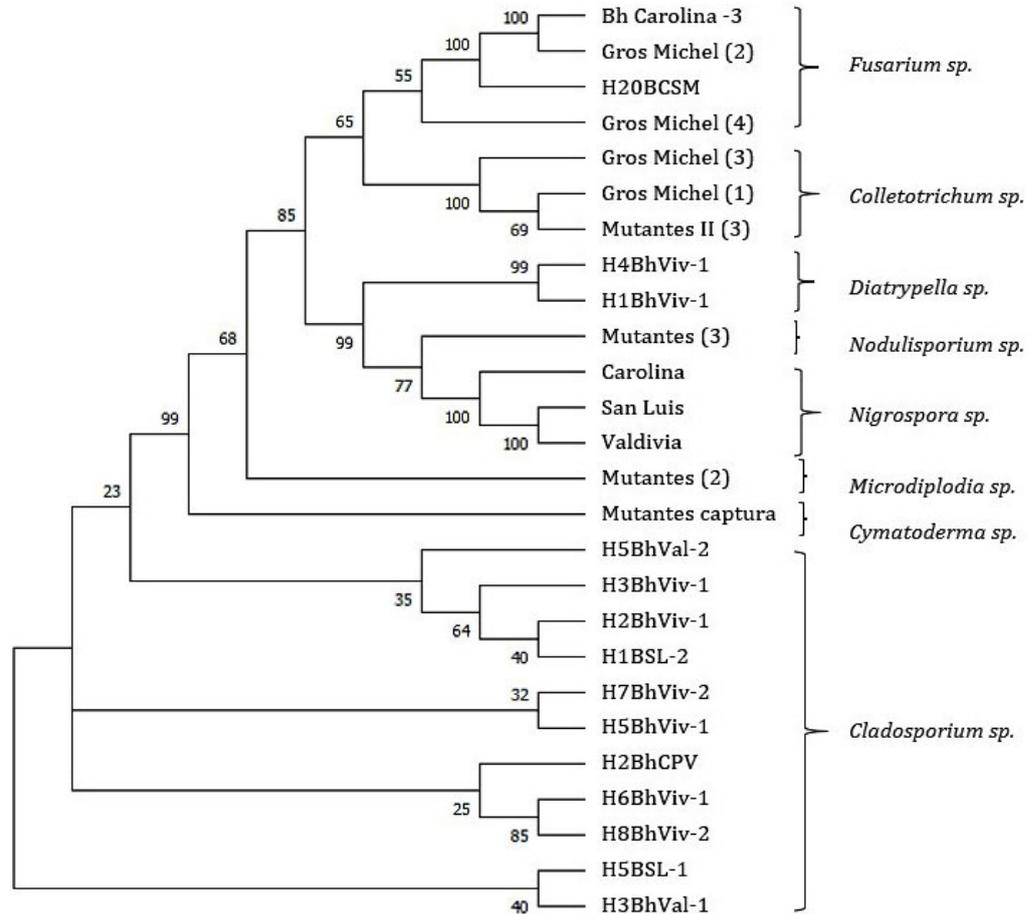


**Figure 2.** Phylogenetic tree of fungal isolates associated with banana phyllosphere with potential antagonism to Black Sigatoka in the cantons of Mocache, Baba and Pueblo Viejo. Phylogenetic construction by *Maximum- Likelihood* method, Jukes-Cantor model, Bootstrap-100 and Uniform Rates.

**Figura 2.** Árbol filogenético de aislamientos de hongos asociados a la filósfera de banano con potencial antagonismo a Sigatoka Negra en los cantones de Mocache, Baba y Pueblo Viejo. Construcción filogenética por el método Maximum-Likelihood, Jukes-Cantor model, Bootstrap-100 y Uniform Rates.

## DISCUSSION

Fungal disease control mainly relies on the application of agrochemicals. However, this practice causes pathogen resistance after prolonged application, generating public concern about the effects of toxic residues on human health and the environment.

This research identified *Bacillus* bacteria. According to Cruz-Martín *et al.* (2018) *Bacillus pumilus* CCIBP-C5 decreases fungal biomass, induces phytodefense mechanisms in the plant, and may constitute a potential biological control agent against *P. fijiensis*. Based on this finding, *B. pumilus* CCIBP-C5 constitutes a guideline for further research.

Contrary to other studies, proportions of Gram-negative bacteria (81%) exceeded that of Gram-positive bacteria (19%). Previously, Ceballos *et al.* (2012) found higher proportions of Gram-positive bacteria (67%) than Gram-negative bacteria from three banana and plantain cultivars in Urabá (Northwest Colombia). Regarding the inhibitory capacity of the isolated microorganisms, the results showed that *Bacillus* bacteria have antagonistic activity against the fungus *P. fijiensis*, as seen by Villegas-Escobar *et al.* (2013) when isolating 649 strains of aerobic endospore-forming bacteria. The strain *Bacillus subtilis* showed the highest inhibition (89±1%), proving its bioactive potential against *P. fijiensis*.

After isolation, colonies showed different shapes (circular, pointed, irregular, spindle), elevations (flat, convex, smooth, raised), edges (entire, wavy, lobulated, irregular, filamentous), consistencies (viscous, dry) and pigmentations (orange, yellow, red, pink, milky, white), as reported by Alfaro (2013) when isolating and quantifying epiphytic bacteria from the banana phylloplane *Musa* AAA cv. Grande Naine.

Considering the 26 isolated fungi, 25 belonged to Ascomycota and one to Basidiomycota. Ascomycota fungi grow in subtropical conditions, as bananas (44).

Fungal molecular identification is frequently assessed with the internal transcribed spacer (ITS) region (47). The primers ITS1 and ITS4 have broad utility and presence in universal databases, with successful amplification rates of fungal lineages (53). Based on in silico analysis (49) the primer ITS1 represents 73.8% of Ascomycota and 85.6% of Basidiomycota in the SSU region, while the primer ITS4 represents 97.6% in Ascomycota and 96.9% in Basidiomycota in the LSU region. This means ITS primers allowed amplifying sequences from both Ascomycota and Basidiomycota fungi.

In a study of pathogenic taxa in wild banana (*Musa acuminata*), Brown *et al.* (1998) identified potential endophytic pathogen genera and species that may remain dormant, such as *Colletotrichum* sp. and *Nigrospora* sp. Zakaria & Aziz (2018) isolated fungi of the genus *Nigrospora* sp., *Fusarium* sp. and *Colletotrichum* sp. on bananas. Very similar results were obtained by Horra (2014) and in the present study, including *Diatrypella* sp., *Nodulisporium* sp., *Microdiplodia* sp., *Cymatoderma* sp. and *Cladosporium* sp.

*Fusarium oxysporum* is present among the rhizosphere microflora, and some strains cause wilting or total root rot of banana plants (19). It should be noted that all *F. oxysporum* strains are saprophytes, surviving for long periods in both soil organic matter and the rhizosphere. This possibly explains their latent presence in leaf tissue and the sampled areas. We also isolated *Nectria haematococca* (sexual morph of *F. solani*), a filamentous type of fungus. *F. solani* is part of a complex with 60 phylogenetic species (46). These two *Fusarium* sp. strains could constitute candidate biological control agents against *P. fijiensis* (2), encouraging future antagonistic tests for evaluations against black Sigatoka.

*Colletotrichum* sp. predominates in the tropics and subtropics with heavy rainfall and high relative humidity (43). Two species were found in this study, *C. gloeosporioides* and *C. frutícola*. The former causes banana anthracnose and leaf spot (38), while *C.* frutícola has been identified on mango plants (29). One of the main characteristics of *Colletotrichum* sp. infection in bananas is the difficulty in detecting the disease before fruit generation, given latency (37).

*Diatrypaceae* members like the genus *Diatrypella* are saprobes and pathogens associated with different hosts in both terrestrial and aquatic environments (14). Species of this genus are separated according to their entostrophic morphology and the number of ascospores they present (50). Fungal pathogenicity is moderate for this genus. This study firstly reports *Diatrypella vulgaris*, in banana. However, it is commonly isolated from diseased *Vitis vinifera*, causing necrotic lesions on the plant (40).

The fungal isolate *Nodulisporium* sp. produces nodilosporic acids with insecticidal properties and volatile antifungal substances used against other microorganisms through mycofumigation (48).

Fungi of *Nigrospora* sp. are host-specific phytopathogenic, endophytic and saprophytic species that produce bioactive secondary metabolites with antifungal activity (23). During isolations, *N. sphaerica* and *N. osmanthi* were isolated from the environment. *N. sphaerica* exhibits a violent spore-discharging mechanism that projects spores over long distances (57). Similarly, *Nigrospora oryzae* and *N. sphaerica* were identified from banana leaves (58). This study also firstly reports *N. osmanthi* in bananas.

Isolated fungi of the genus *Microdiplodia* sp. and *Cymatoderma* sp. have not previously been recorded in bananas. However, Pinheiro da Costa *et al.* (2021) detail the presence of *Microdiplodia* sp. with antifungal activity on Brugmansia suaveolens and *Cymatoderma* sp. as the only Basidiomycota reported in tropical rainforest (1).

Finally, the genus *Cladosporium* sp. comprises more than forty species, including pathogenic species causing leaf spot and saprophytic species acting on vegetation and soil (35). Isolated *C. cladosporioides* confirms this fungus as an endophyte isolated from foliar culture tissues of banana plants (58). In addition, *C. uredinicola*, also isolated in this study, presents small conidia formed with branched chains that facilitate its propagation over long distances (6), explaining its higher prevalence over other fungi. Another species identified, *C. tenuissimum* is an abundant saprobe in the tropics (56). Moubasher *et al.* (2016) state that the frequency of *Cladosporium* sp. isolation is moderate and peaks during winter, when humidity benefits banana development.

## Conclusions

Considering the differential behavior of microorganisms and the number of strains found in the studied sites, the analysis focused on selecting sampling sites was appropriate.

The isolated microorganisms presented macroscopic and microscopic characteristics with different shapes, elevations, borders, consistencies and pigmentations. Different taxonomies belonged to the *Bacillus* and Coccus genera, 81% being Gram-negative and 19% Gram-positive.

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