



Plant Growth Promoting Bacteria Associated with *Langsdorffia hypogaea*-Rhizosphere-Host Biological Interface: A Neglected Model of Bacterial Prospection

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Soil is a habitat where plant roots and microorganisms interact. In the region of the Brazilian Iron Quadrangle (IQ), studies involving the interaction between microbiota and plants have been neglected. Even more neglected are the studies involving the holoparasite plant *Langsdorffia hypogaea* Mart. (Balanophoraceae). The geomorphological peculiarities of IQ soil, rich in iron ore, as well as the model of interaction between *L. hypogaea*, its hosts and the soil provide a unique niche that acts as selective pressure to the evolution of plant growth-promoting bacteria (PGPB). The aim of this study was to prospect the bacterial microbiota of holoparasitic plant *L. hypogaea*, its plant host and corresponding rhizosphere of IQ soil, and to analyze the potential of these isolates as PGPB. We obtained samples of 11 individuals of *L. hypogaea* containing fragments of host and rhizosphere remnants, resulting in 81 isolates associated with Firmicutes and Proteobacteria phyla. The ability to produce siderophores, hydrocyanic acid (HCN), indole-3-acetic acid (IAA), nitrogen (N₂) fixation, hydrolytic enzymes secretion and inhibition of enteropathogens, and phytopathogens were evaluated. Of the total isolates, 62, 86, and 93% produced, respectively, siderophores, IAA, and were able to fix N₂. In addition, 27 and 20% of isolates inhibited the growth of enteropathogens and phytopathogens, respectively, and 58% were able to produce at least one hydrolytic activity investigated. The high number of isolates that produce siderophores and indole-3-acetic acid suggests that this microbiota may be important for adaptation of plants to IQ. The results demonstrate for the first time the biological importance of Brazilian IQ species as reservoirs of specific microbiotas that might be used as PGPB on agricultural land or antropized soils that needs to be reforested.

Keywords: *Langsdorffia hypogaea*, bioprospecting, biotechnological potential, plant growth-promoting bacteria, Brazilian Iron Quadrangle, IAA and siderophores

INTRODUCTION

Throughout evolution, plants have developed adaptive mechanisms related to interactions with microorganisms (Zilber-Rosenberg and Rosenberg, 2008). Accordingly, plants comprise a complex host system, made up of different microhabitats that can be simultaneously colonized by a great diversity of endophytic and epiphytic microorganisms (Lodewyckx et al., 2002). This microbial community is essential for the development of plants since it facilitates the absorption of nutrients and at the same time provides protection against phytopathogens (Fungi, oomycetes, bacteria, viroses, protozoa, and nematodes) and herbivores (Lynch and Whipps, 1990).

The rhizosphere or portion of soil that has close contact with the plant roots represents a highly dynamic environment that enables the interaction of roots with beneficial and pathogenic microorganisms, invertebrates and even root systems of other plants (Bais et al., 2006; Raaijmakers et al., 2009). The communication between the plant roots and organisms present in the rhizosphere is based on the production and secretion of chemicals that can cause different responses depending on the sensitivity or responsiveness of microorganisms present in this highly dynamic environment (Jones et al., 1994; Bertin et al., 2003; Badri et al., 2009).

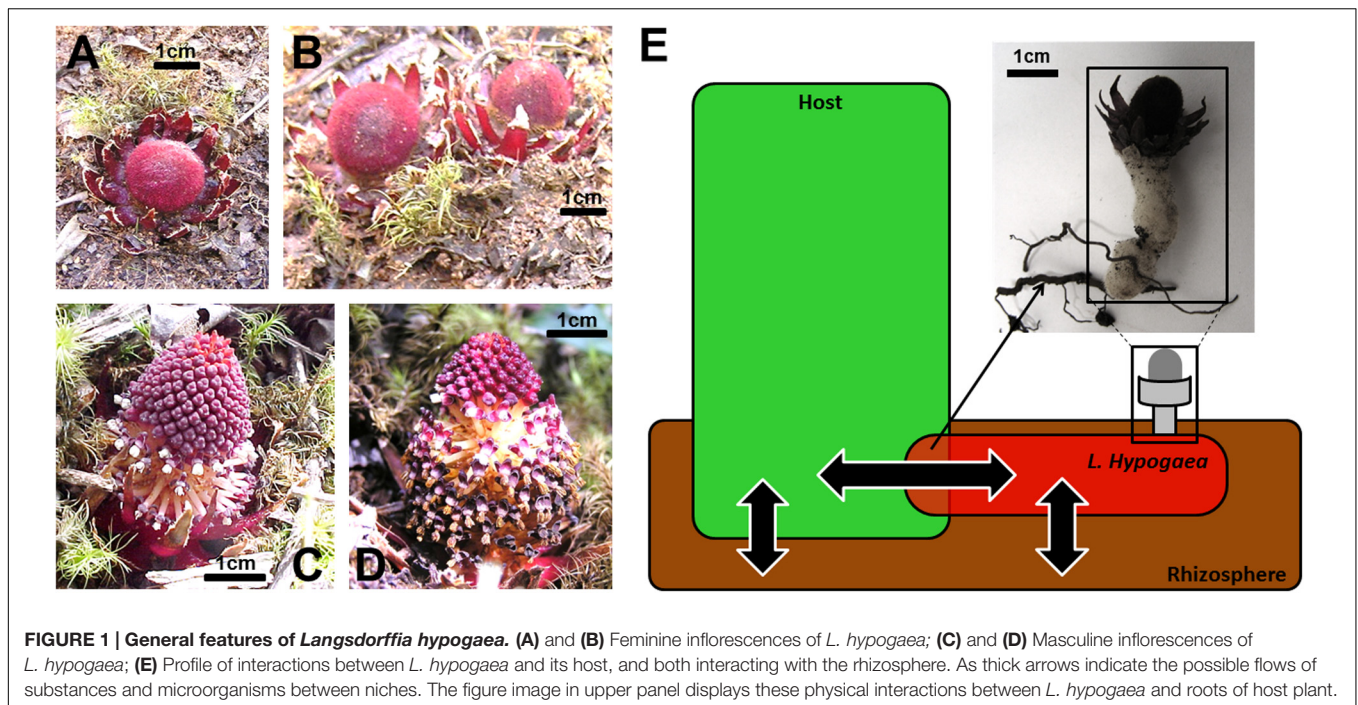
Some microorganisms present in this plant rhizosphere-interface have the ability to solubilize mineral phosphates, among other soil nutrients (Rodríguez and Fraga, 1999). Many of them synthesize, provide or increase the production of plant hormones such as indole-3-acetic acid (IAA), gibberellic acid, cytokines and ethylene (Costacurta and Vanderleyden, 1995); promote associative nitrogen fixation (Richardson et al., 2009); and produce siderophores (Kloepper et al., 1980), hydrolytic enzymes such as glucanases, chitinases, proteases, cellulases, and amylases (Bashan and de-Bashan, 2005), hydrocyanic acid (HCN) (Voisard et al., 1989), and even antimicrobial agents (Compant et al., 2005). All these features allow classify them as plant growth-promoting bacteria (PGPB) (Bashan and Holguin, 1998). Accordingly, plant growth is favored by the influence of the direct or indirect action of these microorganisms, which features them as important biotool of agronomic and environmental interest (Mirza et al., 2001; Ramamoorthy et al., 2001; Vessey, 2003). Besides this potential (Moore et al., 2003), these microorganisms are commercially important when capable of producing enzymes with different applicabilities in specific sectors. In the same way, secondary metabolites produced by these microorganisms have been used in medicine, when they have antibiotic, antitumor, antifungal, or antiparasitic activity (Bertin et al., 2003; Glick, 2010).

Therefore, understanding distinct interactions of the microbiota with soil and plants allow not only a better understanding of the biological models studied, but also prospecting potential uses of this microbiota or even its biomolecules in a biotechnological perspective. In this context, the search for new microorganisms and natural processes in environments with unique characteristics and that are neglected in biological studies are fundamental, and this is the case of the Brazilian Iron Quadrangle. The geomorphological peculiarities

of this soil rich in iron ore as well as the model of interaction with plants provide a unique niche that acts as a selective pressure to the evolution of PGPR. Furthermore, these peculiarities make this environment a potential hotspot of microbial diversity. Belonging to a geologically very old craton that covers about 7200 km², the IQ extends between southeast of Ouro Preto and northeast of Belo Horizonte, continuing to the south of Serra do Espinhaço. In this region, there are rocky outcrops that have a naturally high contamination of soil with heavy metals, which makes the environment very adverse for many plant species. Despite this adverse condition, the IQ presents a great floristic diversity with high levels of endemism (Jacobi and do Carmo, 2008). Due to its association with an extensive deposit of iron ore, and since it is one of the least studied ecosystems in Brazil, IQ has a seriously threatened biodiversity due to the intense mining activity associated with its iron outcrops.

Among the plant species threatened by this anthropic activity in IQ, there have been few studies particularly on holoparasitic plants, as in the case of *Langsdorffia hypogaea* MART, the model of this study, being Asteraceae (*Guatteria* genus), Fabaceae (*Dalbergia* genus), Melastomataceae (*Miconia* genus), and Myrsinaceae (*Myrsine* genus) the most representative families of potential host plants from *L. hypogaea* (Vale, 2013). There are approximately 4200 species of parasitic plants distributed in 18 families and 274 genera (Nickrent, 2002). *Langsdorffia hypogaea* is one of the 44 species of plants described belonging to the family Balanophoraceae, which includes herbaceous angiosperms, achlorophyllous plants and holoparasites of roots of trees, shrubs, and even herbaceous plants (Hsiao et al., 1995). In Brazil, this holoparasite is found in the Amazon, Caatinga, Cerrado, and Atlantic Forest (Cardoso, 2014), and although it is not threatened by extinction, due to its wide distribution, it is considered at risk because of substantial habitat loss, due to global warming (Miles et al., 2004) and human use for obtaining wax (Pott et al., 2004). In some places, however, it is considered “Rare,” and it is on the list of threatened flora of the state of Paraná, Brazil (Sema/Gtz, 1995). In fact, a series of local compromising extinctions can be disrupting the gene flow of this species so vagile in its biology of dispersion, and historical events may no longer correspond to the effective state of extinction threat. Morphologically, *L. hypogaea* has two regions that are well distinguishable anatomically, i.e., a basal vegetative body and an apical reproductive region. The vegetative body or rhizome is irregularly cylindrical, elongate and epigeal, with tomentose and fleshy appearance (Hsiao et al., 1995). The reproductive region is represented by dioecious, fleshy, unisexual inflorescences that erupt from ascending vegetative branches, encircle at the base by a sheath of bracts where the fruits are drupaceous and small (Hansen, 1980; Cardoso et al., 2011) (Figures 1A–D). Haustoria extend from the vegetative body and attach to the roots of host plants (Nickrent, 2002). Although there is direct connection with its host, part of vegetative body stays surrounded by soil, allowing intimate contact with organisms that live in the rhizosphere (Figure 1E).

Taking into account this anatomic-physiological perspective and composition of fixation substrate of the plant described above, some questions involving this model of interaction are



yet to be answered to better comprehend the biology of the species: understand if the microbiota living in association with *L. hypogaea* is shared to the host plant as well as with the rhizosphere, and verify if the potential of these bacterial isolates contribute to the adaptive mechanism of these plants in such a hostile environment as the soil from ferruginous fields.

In an attempt to answer these and other related questions, we carried out bacterial prospecting. The main objective was to identify which bacterial species were associated with *L. hypogaea*, the host and specific rhizosphere of the soil of the semidunal seasonal forests of Brigida hill, basically composed by sandy-clay textures, low concentration of P and K, high concentration of Ca, Mg, Fe, As, and Sb (Vale, 2013), and pH values ranging from 3.9 to 6.2 in the first 20 cm deep (Filho et al., 2010). In parallel, biochemical assays were used to investigate the biotechnological potential of these isolates, which could be eventually used for various purposes.

MATERIALS AND METHODS

Location and Sampling of Plants and Rhizosphere Soil

The collections were made in Serra da Brigida (central point: 20°21'35''S, 43°30'11''W), which is part of Parque Natural Municipal das Andorinhas and is in southern part of Environmental Protection Area Cachoeira das Andorinhas, within IQ (Ferreira, 2011), municipality of Ouro Preto, Minas Gerais – Brazil (**Supplementary Figure S1**). The topography of the region is sustained by itabirites and quartzites. Itabirites are iron formations, metamorphic and strongly oxidized, showing discontinuous bodies with high ore content (>64% Fe) (Rosière

and Chemale, 2000). Sandy and flooded soils are absent and they have large amounts of humic substances (Jacobi and do Carmo, 2008). In this forest fragment, we randomly collected 11 individuals of *L. hypogaea*, containing fragments of parasitized roots and remnants of corresponding rhizosphere. The samples were stored in sterile plastic bags and processed on the same day of collection.

Isolation of Bacteria, Media and Culture Conditions

The samples of *L. hypogaea* were externally disinfected using chlorine solution 2.5% by 2 min. We used for each sample five inner fragments of *L. hypogaea* root (~1.0 × 0.5 cm) inoculated in Luria-Bertani (LB) medium (Maniatis et al., 1982) containing 0.03 mg/L thiophanate, with pH adjusted to 6.0. The host root was washed following a standardized sequence of solutions for surface disinfection (9 g/L NaCl – 2 min, 70% alcohol – 2 min, 2,5% sodium hypochlorite – 2 min and 9 g/l NaCl – 2 min), and similarly, five fragments of each root of plant host were inoculated in LB medium. For the isolation of microorganisms present in the rhizosphere and in fragments of plant host root containing traces of soil (approximately 2 g), the samples were placed in a 10 mL of saline solution (0,5 NaCl g/L) for 10 min. Next, an aliquot of 100 μL of this solution was inoculated in selective LB medium. All plates were incubated at 25–28°C for a period of up to 10 days, and the microorganisms grown were isolated in new 60 mm diameter Petri dishes containing the same culture medium. The colonies isolated were photographed (front and back, data not shown) and grouped according to their origin. All isolates were cataloged and preserved in 30% glycerol and stored at –80°C.

DNA Extraction, Amplification, and Sequencing

DNA of the isolates was extracted using the CTAB/NaCl protocol (Doyle and Doyle, 1987). The primers 27f and 1492r were used for amplification of bacterial 16S rRNA gene (Lane et al., 1985). The 50- μ L PCR mixture contained 20–50 ng of DNA, 250 pmol of each primer, 5 μ L 10x PCR buffer, 2.5 U rTaq DNA polymeraseTM (Invitrogen), and 100 μ M deoxynucleoside triphosphate mixture. The PCR program consisted of initial denaturation at 95°C for 5 min, followed by 35 cycles of 1 min of denaturation at 95°C, 45 s of annealing at 47°C and 2 min of extension at 72°C and a final extension for 10 min at 72°C, utilizing a 2720 ThermalCyclerTM (Applied Biosystems). The amplicons generated by PCR were verified in 1% agarose gels and purified using 20% PEG-8000 in 2.5 M NaCl (Arbeli and Fuentes, 2007). The product obtained was quantified by spectrophotometry using a NanoDrop ND 1000TM (NanoDrop Technologies). Sequencing was carried with the DYEnamicTM kit (Amersham Biosciences, USA) in combination with the MegaBACE 1000TM automated sequencing system (Amersham Biosciences, USA). The sequencing reactions were performed with 100–150 ng purified DNA and the reagents in the DYEnamicTM kit (Amersham Biosciences, USA), using the manufacturer's recommendations. The program consisted of 36 cycles with an initial denaturation at 95°C for 25 min, followed by 15 s of annealing at 50°C and 3 min of extension at 60°C. After cycling, the reaction product was transferred to a 96-well sequencing plate to be precipitated.

For precipitation, 1 μ L of 7.5 M ammonium acetate was added to each well. Next, 28 μ L of absolute ethanol (Merck, USA) were added. The plate was vortexed and incubated for 20 min at room temperature, protected from light. Afterward, the plate was centrifuged for 45 min at 3200 \times g and the supernatant was discarded. Next, 150 μ L of 70% ethanol were added. The plate was centrifuged again for 15 min at 3200 \times g and the supernatant was then discarded. The plate was allowed to stand for 20 min, protected from light, para evaporation of ethanol. Precipitated DNA in each well of the plate was then resuspended in 10 μ L of loading buffer (present in sequencing kit). The plate was vortexed for 2 min, centrifuged for 1 s at 800 \times g and stored at 4°C, protected from light, until injection of samples in a MegaBACE 1000TM sequencer (Amersham Biosciences, USA).

Determination of Sequences and Phylogenetic Analysis

The contigs were assembled using the forward and reverse sequences of each 16S rRNA gene amplicon using Phred (Ewing and Green, 1998; Ewing et al., 1998). The DNA sequences were analyzed utilizing the BLASTn program (Altschul et al., 1997). The sequences were aligned using the program Muscle (Edgar, 2004) and then curated by the program G-block (Castresana, 2000). A neighbor-joining phylogenetic tree was then determined using PhyML 3.0 (Anisimova and Gascuel, 2006) followed by TreeDyn (Chevenet et al., 2006), and the statistical robustness of the analysis was estimated by bootstrapping with 1,000 replicates.

Nucleotide Sequence Accession Numbers

All sequences obtained in this study were deposited in GenBank, according to the accession numbers given in **Table 2**.

Production of IAA

The production of IAA was determined by the method of Bric et al. (1991), with modifications. Bacterial producers of IAA were identified by the change in the color of the nitrocellulose disk from yellow (negative result) to red (positive result). The assays were made in triplicates and only those that achieved a positive result in at least two of them were accounted in the analysis.

Production of Ammonium Ions

The production of ammonium ions was determined by the indophenol method (Verdouw et al., 1978), using *Proteus* sp. as positive control (Vince et al., 1973). The samples were read at 690 nm, and the absorbance values were compared with a control condition. The assays were made in triplicates and only those that the absorbance values were greater than or equal to the *Proteus* values in at least two of the assays were accounted in the analysis.

Production of Siderophores

The production of siderophores was based on the work of Schwyn and Neilands (1987), with modifications. To remove traces of iron in medium it was made a pretreatment with hidroxiquinolone followed by separation using dropping funnel (Pierre et al., 2003). An orange shade of the culture medium around the regions where bacteria grew was indicative of production of siderophores. The assays were made in triplicates and only those that achieved a positive result in at least two of them were accounted in the analysis.

Nitrogen Fixation

Nitrogen fixation capacity was investigated using two serial methods: Nitrogen-free combined carbon (NFCC) semi-solid medium, free of N₂ (pH 5.7), supplemented with 5 g/L mannitol and 5 g/L sucrose (Dobereiner et al., 1976) and PCR using universal *nifH* primers (Burgmann et al., 2004). The Change in color of medium from yellow to green was indicative of the capacity to fix N₂. This assay was made in triplicates and only those that achieved a positive result in at least two of them were accounted in the analysis, which was confirmed by amplification of *nifH* fragment evaluated in 1.2% agarose gel. *Bradyrhizobium elkanii* BR96 was used as positive control.

Production of Hydrocyanic Acid (HCN)

The production of HCN was determined by the method of Bakker and Schippers (1987), with modifications. A change in yellow color of filter paper (negative result) to brown (positive result) indicated production of HCN. The assays were made in triplicates and only those that achieved a positive result in at least two of them were accounted in the analysis.

TABLE 1 | Distribution of bacterial representativeness of taxa found in *Langsdorffia hypogaea*, host and rhizosphere.

| Phylogenetic group | Bacteria | Number of isolates (Shared with other niches) | | |
|---------------------|-------------------------------------|---|---------|-------------|
| | | <i>L. hypogaea</i> | Host | Rhizosphere |
| Firmicutes | <i>Bacillus cereus</i> | 1 | 2 (2) | 3 (2) |
| | <i>Bacillus mycoides</i> | 1 | | |
| | <i>Bacillus</i> sp. | 3 (2) | 4 (3) | 2 (1) |
| | <i>Lysinibacillus sphaericus</i> | 4 (2) | 1 | |
| | <i>Lysinibacillus xylanilyticus</i> | 1 | 1 | 1 |
| | <i>Lysinibacillus</i> sp. | | 3 (2) | 3 (3) |
| | <i>Viridibacillus arenosi</i> | | | 1 |
| | <i>Paenibacillus taichungensis</i> | | 3 (2) | |
| Gammaproteobacteria | <i>Citrobacter freundii</i> | | 1 | |
| | <i>Enterobacter aerogenes</i> | 1 | | |
| | <i>Enterobacter</i> sp. | 1 | 1 | 2(1) |
| | <i>Klebsiella oxytoca</i> | 1 | | |
| | <i>Klebsiella</i> sp. | 1 | | 1 |
| | <i>Pseudomonas fluorescens</i> | | | 1 |
| | <i>Shewanella</i> sp. | | 1 | |
| | <i>Raoultella terrigena</i> | | | 1 |
| | <i>Rahnella</i> sp. | 1 | | |
| | <i>Rahnella aquatilis</i> | | | 1 |
| | <i>Serratia marcescens</i> | | 2 (2) | 1 |
| | <i>Serratia proteamaculans</i> | 3 (2) | 5 (2) | 8 (2) |
| | <i>Serratia</i> sp. | | 1 | 6 (2) |
| TOTAL | 21 | 18 (6) | 25 (13) | 31 (11) |

Production of Amylase, Cellulase, and Protease

Amylase activity was determined in 90-mm Petri dishes containing Yeast nitrogen base (YNB) medium (2%) containing 2 g/L soluble amide, 5 g/L peptone, and 1 g/L yeast extract, with pH adjusted to 6.0 (Strauss et al., 2001). Cellulase activity was determined in 90-mm Petri containing YNB medium (2%) supplemented with 0.5 g/L cellobiose and 1 g/L carboxymethyl cellulose (Teather and Wood, 1982). Protease activity was determined in 90-mm Petri dishes containing culture medium composed of 20 g/L casein, 5 g/L peptone, 3 g/L yeast extract, 10 g/L glucose, and 20 g/L agar, with pH adjusted to 5.0 (Strauss et al., 2001). After 3 days of growth at 28°C, the bacterial isolates in all assays producing a transparent halos determined the amylase, cellulase, or protease activity in the respective isolates and assays.

Inhibition of Entero- and Phytopathogens

To investigate a possible antimicrobial activity of the isolates, antimicrobial assays were performed against the following targets: *Staphylococcus aureus* ATCC 29213 (opportunistic human pathogen), *Bacillus cereus* ATCC 11778 (associated with food poisoning), *Klebsiella pneumoniae* ATCC 4352 (causal agent of pneumonia), *Shigella flexneri* (associated with dysentery) and *Xanthomonas citri* subsp. *citri* 306 (causal agent of citrus canker) using solid LB in a direct inhibition test. The assays were made in triplicates and only those that achieved a

positive result in at least two of them were accounted in the analysis.

To investigate the capacity of isolates to inhibit the growth of the phytopathogenic fungus *Fusarium oxysporum* f.sp. *lini* (causal agent of various diseases in plants), the isolates were grown in LB and then transferred to 60-mm Petri dish containing potato agar. The bacterial isolates were inoculated to make a square of approximately 2 cm on the culture medium. After 2 days of growth of these isolates at 28°C, a fraction of 4 mm² of culture medium containing *F. oxysporum* was inoculated exactly at the center of the plate (center of square), starting at a pre-growth of 5 days at 28°C. The bacteria capable of producing some anti-*Fusarium* substance hindered the growth of the fungus, compared to the growth profile of *Fusarium* under control conditions in the absence of bacterial isolate. The values to determine per cent inhibition were obtained by the formula (%) = (C × E)/C × 100, where C is the diameter of the *Fusarium* culture in control, and E is the diameter in the presence of bacterial isolate (Zhao et al., 2014). The assays were made in triplicates and the average of per cent inhibition was plotted in radar graph.

RESULTS

Characterization of Bacterial Population

Of the 11 samples collected in four different points of Serra da Brígida-MG, we selected and preserved 81 isolates (Table 1).

TABLE 2 | List of isolates representing the 75 OTUs from *L. hypogaea*, host and rhizosphere.

| Representative isolate (Accession number) | Nearest type strain | Seq. Id (%) | Q. Cover (%) | Accession number |
|---|---|-------------|--------------|------------------|
| <i>L. hypogaea</i> | | | | |
| L6 (KU057004) | <i>Bacillus mycoides</i> UrCA07 | 73 | 89 | KC618478 |
| L7 (KU057005) | <i>Bacillus</i> sp. SG19 | 96 | 99 | JX402434 |
| L18 (KU057014) | <i>Bacillus</i> sp. SG19 | 96 | 99 | JX402434 |
| L19 (KU057015) | <i>Bacillus</i> sp. WYT007 | 95 | 98 | JQ807855 |
| L22 (KU057017) | <i>Bacillus cereus</i> HKG201 | 69 | 85 | KF947110 |
| L8 (KU057006) | <i>Lysinibacillus sphaericus</i> R7 | 95 | 98 | HQ259956 |
| L9 (KU057007) | <i>Lysinibacillus sphaericus</i> R7 | 96 | 95 | HQ259956 |
| L10 (KU057008) | <i>Lysinibacillus sphaericus</i> R7 | 94 | 94 | HQ259956 |
| L14 (KU057011) | <i>Lysinibacillus sphaericus</i> DS11 | 96 | 99 | EU835735 |
| L20 (KU057016) | <i>Lysinibacillus xylanilyticus</i> TAX5 | 95 | 99 | JX280924 |
| L11 (KU057009) | <i>Enterobacter aerogenes</i> PSB28 | 95 | 99 | FJ360760 |
| L17 (KU057013) | <i>Enterobacter</i> sp. SPj | 95 | 93 | FJ405369 |
| L16 (KU057012) | <i>Klebsiella oxytoca</i> ALK313 | 97 | 98 | KC456529 |
| L28 (KU057023) | <i>Klebsiella</i> sp. 38 | 96 | 90 | EU294412 |
| L12 (KU057010) | <i>Rahnella</i> sp. DmB | 95 | 97 | KF720908 |
| L1 (KU057001) | <i>Serratia proteamaculans</i> 568 | 97 | 99 | NR074820 |
| L2 (KU057002) | <i>Serratia proteamaculans</i> PW172 | 94 | 98 | JF494823 |
| L3 (KU057003) | <i>Serratia proteamaculans</i> PW172 | 93 | 98 | JF494823 |
| L4 | No significant similarity found. | -- | -- | |
| Host | | | | |
| H61 (KU057055) | <i>Bacillus cereus</i> M2 | 95 | 99 | JF836882 |
| H65 (KU057059) | <i>Bacillus</i> sp. SG19 | 96 | 94 | JX402434 |
| H69 (KU057063) | <i>Bacillus</i> sp. SG19 | 97 | 99 | JX402434 |
| H72 (KU057066) | <i>Bacillus</i> sp. S11714 | 99 | 99 | KF956655 |
| H77 (KU057071) | <i>Bacillus cereus</i> strain D7 | 97 | 99 | KF500919 |
| H80 (KU057073) | <i>Bacillus</i> sp. N4/130 | 95 | 98 | LN680100 |
| H56 (KU057051) | <i>Lysinibacillus</i> sp. NSI08 | 42 | 79 | AB811363 |
| H73 (KU057067) | <i>Lysinibacillus</i> sp. E15 | 72 | 94 | JN082735 |
| H63 (KU057057) | <i>Lysinibacillus</i> sp. E15 | 95 | 94 | JN082735 |
| H78 (KU057072) | <i>Lysinibacillus xylanilyticus</i> fwzy21 | 95 | 99 | KF208475 |
| H81 (KU057074) | <i>Lysinibacillus sphaericus</i> STNG28 | 95 | 92 | KF312283 |
| H59 (KU057053) | <i>Paenibacillus taichungensis</i> B2 | 95 | 99 | JX010966 |
| H66 (KU057060) | <i>Paenibacillus taichungensis</i> B2 | 96 | 98 | JX010966 |
| H70 (KU057064) | <i>Paenibacillus taichungensis</i> A80 | 86 | 79 | JX010963 |
| H64 (KU057058) | <i>Citrobacter freundii</i> H1-2 | 71 | 95 | KC210870 |
| H60 (KU057054) | <i>Shewanella</i> sp. XH15 | 81 | 87 | KJ922531 |
| H58 (KU057052) | <i>Enterobacter</i> sp. RA-15 | 97 | 95 | KJ152098 |
| H55 (KU057050) | <i>Serratia proteamaculans</i> KB22 | 96 | 94 | JF327454 |
| H62 (KU057056) | <i>Serratia proteamaculans</i> 568 | 95 | 99 | NR_074820 |
| H67 (KU057061) | <i>Serratia proteamaculans</i> KB22 | 95 | 99 | JF327454 |
| H68 (KU057062) | <i>Serratia proteamaculans</i> 568 | 94 | 93 | NR074820 |
| H71 (KU057065) | <i>Serratia proteamaculans</i> 568 | 96 | 99 | NR074820 |
| H74 (KU057068) | <i>Serratia marcescens</i> S418 | 96 | 99 | GQ202220 |
| H75 (KU057069) | <i>Serratia marcescens</i> subsp. <i>sakuensis</i> RK26 | 96 | 99 | KC790279 |
| H76 (KU057070) | <i>Serratia marcescens</i> subsp. <i>sakuensis</i> RK26 | 95 | 90 | KC790279 |
| Rhizosphere | | | | |
| R24 (KU057019) | <i>Bacillus</i> sp. SG19 | 95 | 99 | JX402434 |
| R31 (KU057026) | <i>Bacillus cereus</i> M2 | 95 | 99 | JF836882 |
| R32 (KU057027) | <i>Bacillus cereus</i> S72 | 96 | 99 | FJ763650 |
| R35 (KU057030) | <i>Bacillus cereus</i> S72 | 96 | 99 | FJ763650 |
| R49 (KU057044) | <i>Bacillus</i> sp. SG19 | 95 | 99 | JX402434 |
| R40 (KU057035) | <i>Viridibacillus arenosi</i> Hc6 | 95 | 99 | JF899298 |

(Continued)

TABLE 2 | Continued

| Representative isolate (Accession number) | Nearest type strain | Seq. Id (%) | Q. Cover (%) | Accession number |
|---|--|-------------|--------------|------------------|
| R23 (KU057018) | <i>Lysinibacillus</i> sp. O-E16 | 95 | 99 | JN613478 |
| R29 (KU057024) | <i>Lysinibacillus xylanilyticus</i> fwzy21 | 71 | 87 | KF208475 |
| R33 (KU057028) | <i>Lysinibacillus</i> sp. T1-9 | 95 | 89 | KJ127177 |
| R34 (KU057029) | <i>Lysinibacillus</i> sp. E15 | 97 | 89 | JN082735 |
| R39 (KU057034) | <i>Klebsiella</i> sp. D81 | 96 | 97 | DQ923489 |
| R48 (KU057043) | <i>Enterobacter</i> sp. Hg4-01 | 94 | 97 | EU304247 |
| R38 (KU057033) | <i>Enterobacter</i> sp. Hg4-01 | 83 | 96 | EU304247 |
| R37 (KU057032) | <i>Raoultella terrigena</i> strain PK35 | 97 | 97 | KC790281 |
| R47 (KU057042) | <i>Rahnella aquatilis</i> 2B-CDF | 95 | 99 | FJ811859 |
| R25 (KU057020) | <i>Serratia</i> sp. PT3 | 95 | 99 | GU458285 |
| R26 (KU057021) | <i>Serratia</i> sp. PT3 | 95 | 99 | GU458285.2 |
| R27 (KU057022) | <i>Serratia</i> sp. PT3 | 94 | 98 | GU458285.3 |
| R30 (KU057025) | <i>Serratia</i> sp. S3.MAC.008 | 70 | 90 | HM063908 |
| R36 (KU057031) | <i>Serratia proteamaculans</i> KB22 | 95 | 99 | JF327454 |
| R41 (KU057036) | <i>Serratia marcescens</i> KtMC2-16 | 97 | 99 | KC122200 |
| R42 (KU057037) | <i>Serratia</i> sp. PT3 | 95 | 99 | GU458285 |
| R43 (KU057038) | <i>Serratia proteamaculans</i> KB22 | 94 | 99 | JF327454 |
| R44 (KU057039) | <i>Serratia proteamaculans</i> 568 | 94 | 96 | NR074820 |
| R45 (KU057040) | <i>Serratia</i> sp. PT3 | 96 | 96 | GU458285 |
| R46 (KU057041) | <i>Serratia proteamaculans</i> KB22 | 96 | 98 | JF327454 |
| R50 (KU057045) | <i>Serratia proteamaculans</i> KB22 | 94 | 99 | JF327454 |
| R51 (KU057046) | <i>Serratia proteamaculans</i> 568 | 95 | 98 | NR074820 |
| R52 (KU057047) | <i>Serratia proteamaculans</i> 568 | 95 | 98 | NR074820 |
| R53 (KU057048) | <i>Serratia proteamaculans</i> 568 | 95 | 96 | NR074820 |
| R54 (KU057049) | <i>Pseudomonas fluorescens</i> Y5 | 74 | 97 | KJ882377 |

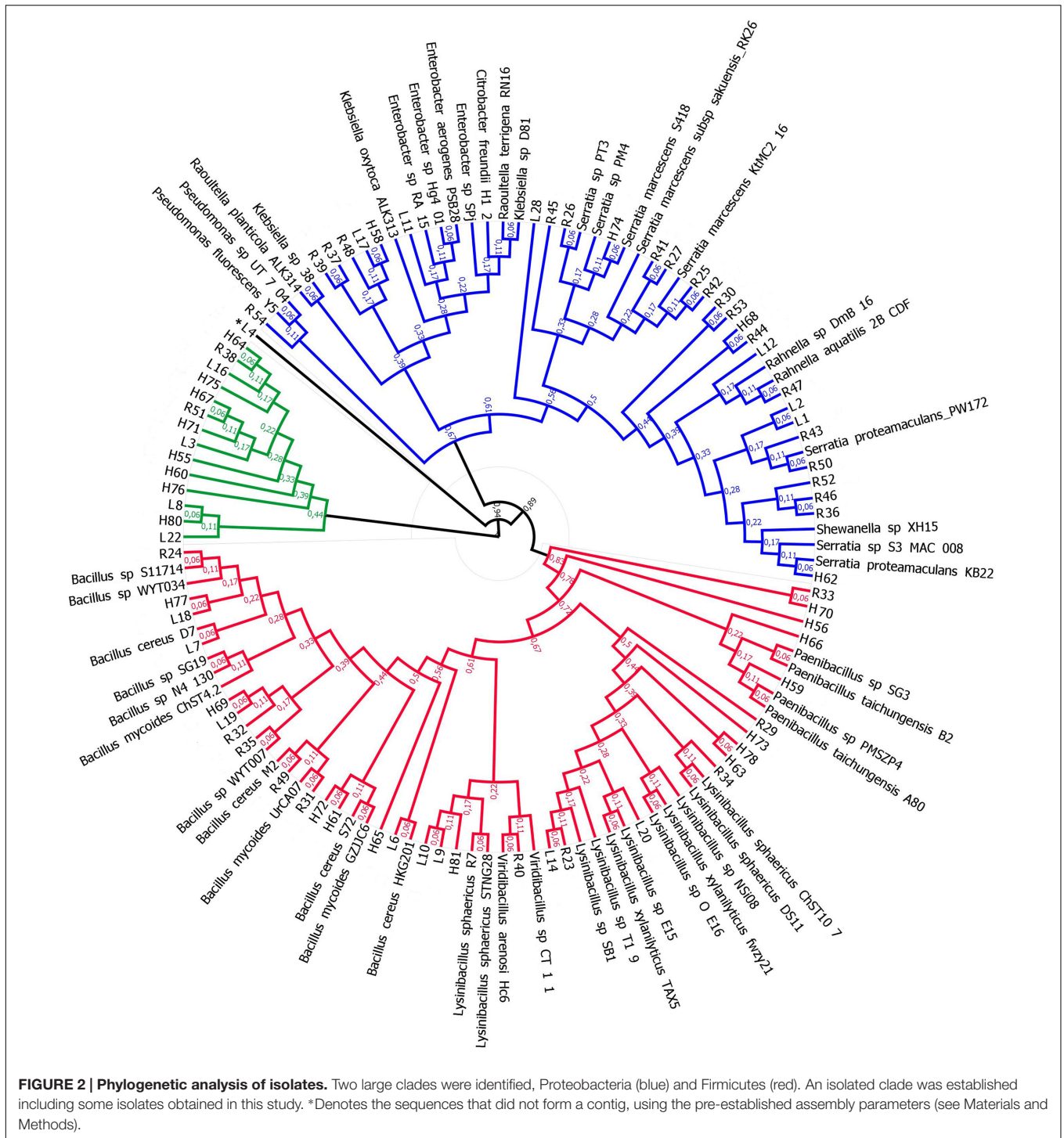
However, isolate L5 was non-viable after storage in glycerol. The growth rate of isolates L13, L15, L21, H57, and H79 were unsatisfactory, resulting in the extraction of insufficient DNA and sequencing of low quality. Although sample L4, isolated from *L. hypogaea*, showed good quality of sequencing and satisfactory assembly of contigs using the assembly parameters (see Materials and Methods), it still showed low percentage of similarity (76.8%) and identity (77%) compared with sequences deposited in GenBank of NCBI, featuring it as a potential new organism to be investigated rigorously.

In the three niches analyzed, we found isolates of the phyla Firmicutes (34), belonging to the genera *Bacillus* (17), *Lysinibacillus* (13), *Paenibacillus* (3), and *Viridibacillus* (1), and Proteobacteria (40), belonging to the genera *Serratia* (26), *Klebsiella* (3), *Rahnella* (2), *Citrobacter* (1), *Enterobacter* (5), *Shewanella* (1), *Raoultella* (1), and *Pseudomonas* (1) (Table 2). Four of these genera (*Serratia*, *Bacillus*, *Lysinibacillus*, and *Enterobacter*) were found in all three niches analyzed, where the first genera was the most representative. Isolates of the genera *Citrobacter*, *Paenibacillus*, and *Shewanella* were found only in the plant host. Isolates of the genera *Viridibacillus*, *Pseudomonas*, and *Raoultella* were found only in the rhizosphere (Table 2). Isolates of the genera *Klebsiella* and *Rahnella* were found exclusively shared between *L. hypogaea* and the rhizosphere.

Phylogenetic analysis of the isolates confirmed the sequencing results and allowed the grouping of the bacterial isolates from different niches to the taxa represented, Firmicutes and

Proteobacteria. The isolates from *L. hypogaea* (L3, L16, L22, and L8), the isolates from the plant host (H55, H60, H64, H67, H71, H75, H76, and H80), and the isolates from the rhizosphere (R38 and R51) were grouped in a different clade (Figure 2).

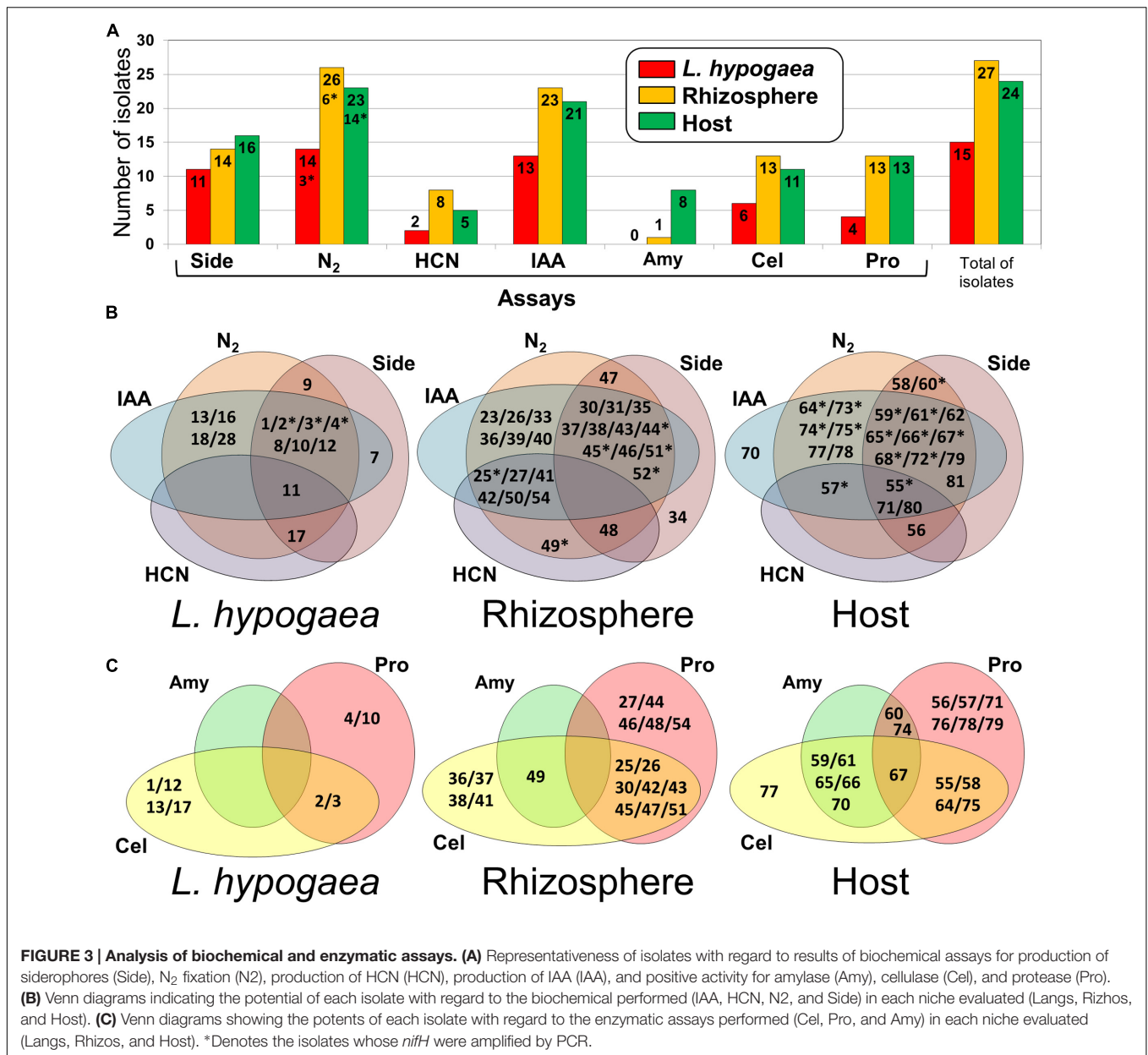
For the biochemical tests, 66 isolates were analyzed since nine of these isolates showed reduced growth rate, prevented an accurate analysis of the results in the proposed biochemical assays. Of these, 63 isolates showed growth in nitrogen-free medium, representing 14 (93.4%) of the 15 isolates of *L. hypogaea*, 23 (95.8%) of the 24 isolates from plant host and 26 (96.3%) of the 27 isolates from rhizosphere (Figure 3A). From these isolates, the presence of *nifH* was confirmed in 23 genomes by PCR analysis (Supplementary Figure S2). With regard to siderophore production, more than 62% of the isolates were capable of producing these compounds. All isolates were able to produce ammonium ions, at different concentrations. Meanwhile, only 15 isolates (22.72%) were capable of producing hydrocyanic acid. More than 86% of the isolates were capable of producing IAA, of which 13 were from *L. hypogaea* (Figure 3A). In attempt to understand the potential of each of these isolates with regard to the capacity to fix atmospheric N₂, to produce siderophores, IAA, and HCN, Venn diagrams were prepared for each of the three niches analyzed (Figure 3B). Especially the isolates L11, H55, H71, and H80 yielded positive results for all analyses. Of the isolates obtained from *L. hypogaea*, four were capable of to fix N₂ and to produce IAA (L13, L16, L18, and L28), and seven were able to produce IAA and siderophores, and to fix N₂ (L1,



L2, L3, L4, L8, L10, and L12). Of the isolates obtained from the rhizosphere, six were capable of producing IAA and fixing N₂ (R23, R26, R33, R36, R39, and R40). Eleven were capable of producing IAA and siderophores, and even fixing N₂ (R30, R31, R35, R37, R38, R43, R44, R45, R46, R51, and R52), while another six were capable of producing IAA and HCN, and fixing N₂ (R25, R27, R41, R42, R50, and R54). Of the isolates obtained from the

host plant, six were capable of producing IAA and fixing N₂ (H64, H73, H74, H75, H77, and H78). Two were capable of producing siderophores and fixing N₂ (H58 and H60), whereas ten were able to produce IAA and siderophores, and to fix N₂ (H59, H61, H62, H65, H66, H67, H68, H72, H79, and H80).

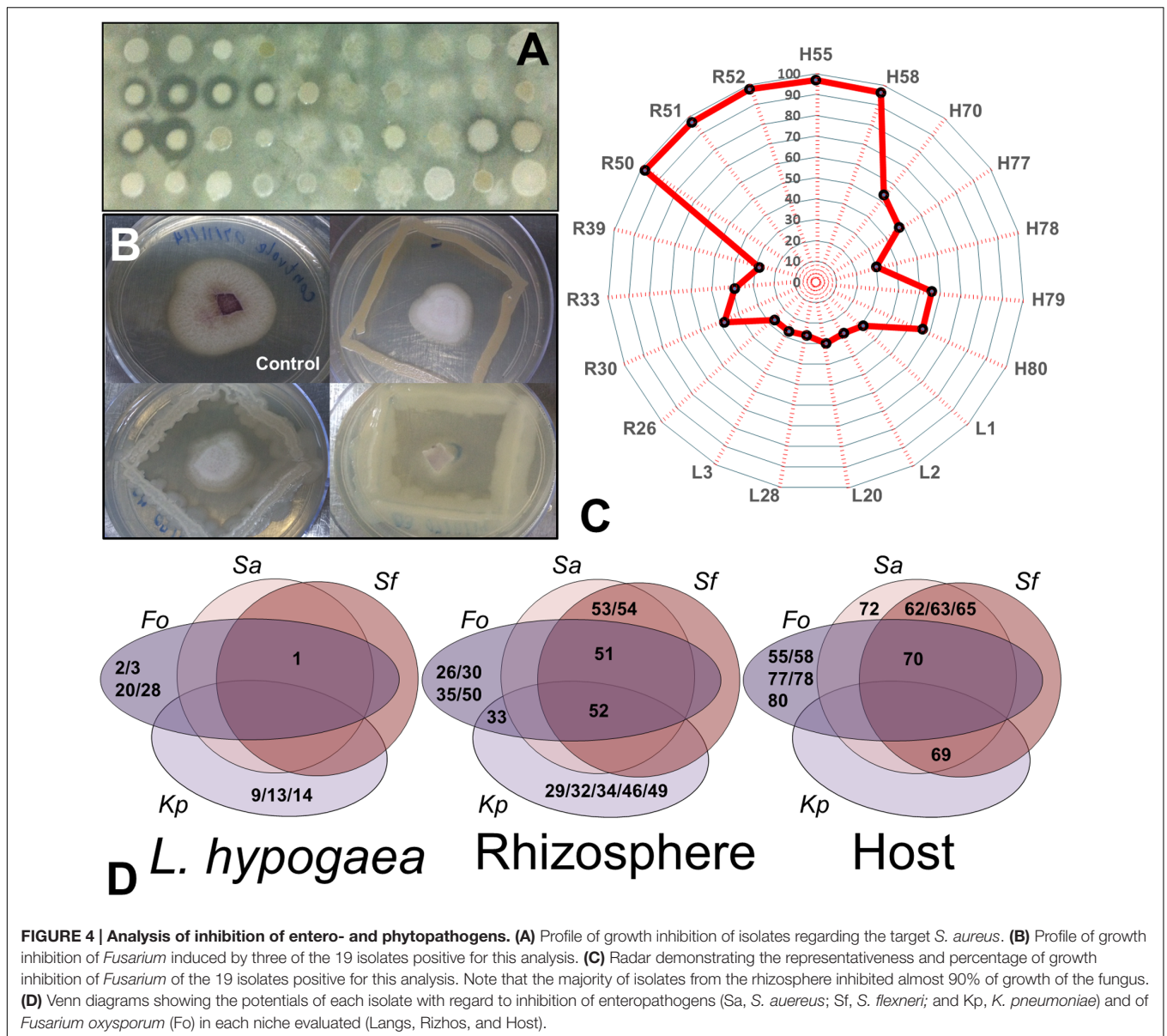
Regarding hydrolytic enzymes assay, 11 isolates were able to produce amylase. None of these isolates was obtained from



L. hypogaea, the isolates obtained from the plant host were the most representative in this analysis. Thirty isolates were capable of producing cellulase, with greatest representativeness among the isolates from the rhizosphere. Thirty isolates were able to produce proteases, with equal representativeness among the isolates from the plant host and rhizosphere. Similarly, a Venn diagram pointing out the isolates and their origin with respect to the production of hydrolytic enzymes was constructed (Figure 3C). Notably, isolate H67 was capable of producing all three types of hydrolytic enzymes investigated.

Twenty isolates were able to inhibit the growth of enteropathogens. They include 11 inhibitors of *Staphylococcus aureus* (L1, R51, R52, R53, R54, H62, H63, H65, H69, H70, and H72), 11 inhibitors of *Klebsiella pneumoniae* (L9, L13,

L14, R29, R32, R34, R46, R49, and H69), and 10 inhibitors of *Shigella flexneri* (L1, R51, R52, R53, R54, H62, H63, H65, H69, and H70) (Figure 4A). No isolate was capable of inhibiting the growth of *Bacillus cereus*, and only one isolate inhibited the growth of *Xanthomonas citri* strain 306 (H65). Nineteen isolates inhibited the growth of *Fusarium* (Figure 4B), 5 of them were obtained from *L. hypogaea* (L1, L2, L3, L20, and L28), eight from the rhizosphere (R26, R30, R33, R39, R50, R51, R52, and R55) and six from the plant host (H58, H70, H77, H78, and H80) (Figure 4D). The potential of some of these isolates is indicated in Figure 4C, ranging from 28% in isolates from *L. hypogaea* to above 95% in the isolates R50, R51, R52, H55, and H58 from rhizosphere and host, respectively.



DISCUSSION

Identification of Microbiota Associated with *L. hypogaea* and Its Interactions

The importance and complexity of the rhizosphere in the interaction with plants and other organisms in which they live have been reported in many studies (Bais et al., 2006; Vacheron et al., 2013; Zhang et al., 2014). Similarly, the modifications in the physiological profile of plants as a result of alterations in the chemical composition of the rhizosphere or the microbiota contained therein have also been characterized, especially with regard to the interaction between plants of agricultural interest (Saleem et al., 2007; Bhattacharyya and Jha, 2012; Dodd and Ruiz-Lozano, 2012; Nadeem et al., 2014). Contrary to these advances, studies seeking to understand the identification of microbiota

associated with parasitic plants, especially holoparasitic ones are limited or lacking. This relation of strict dependence on its host makes it a very interesting target for understanding the degree of importance of microbiota associated with the adaptation and resultant survival of species in the most diverse environments where they occur. Accordingly, this is the first work aimed at understanding the composition and importance of microbiota in the biological interface holoparasitic plant-host-rhizosphere, using *L. hypogaea* as a model. Not only is the biological model distinctive for studies bioprospecting, the sampling site, the IQ, is characterized as an environment also neglected in studies involving its microbiota. Because it is an environment enriched in iron compounds and other heavy metals, whose soils are extremely compacted, adaptation of this microbiota can be directly related not only to the soil features, but also related to the adaptation of plants in this environment, and may uncover

new organisms and the biotechnological potential of this specific microbiota.

Although there are no known genera that are unique for *L. hypogaea*, isolate L4 did not show significant identity (over 80%) with any other isolate with sequence deposited in databanks and therefore deserves attention. This isolate was capable of producing ammonium ions in high concentration, IAA and siderophores and fix nitrogen. This activity could generate better or complementary nutritional conditions compared to those offered by the plant host such as for a tree growing on soils lacking nutrients. A key adaptive aspect of this would be the decrease in risk of mortality of the plant host. Regardless of nutrients that it provides, physical sustentation and supply of water and carbohydrates to the parasite is totally dependent on host plants, and their death is a great adaptive disadvantage (Lopez Pascua et al., 2014). Thus, the co-association with a microbiota capable of providing the necessary nutrients saves the parasitized tree from irreversible stress. However, more studies are necessary to confirm these preliminary results.

Similarly, isolates of the genera *Klebsiella* and *Rahnella* were found only in *L. hypogaea* and the rhizosphere. This allows us to infer that there may be a direct association of soil bacteria with the holoparasitic plant, and that a possible relation of complementarity makes the parasitic plant not only dependent on the plant host for survival but also dependent on specific soil bacteria. This allows us to raise the prospect that perhaps the concept of botanical holoparasitism needs to be rethought, taking the scientific community to undertake further research in this area of knowledge. Although need the host plant for acquisition of carbohydrates, since it does not have the capacity of producing them itself (by the chlorophyll absence), *L. hypogaea* could also depend closely on PGPB to assist in the development of its roots, acquisition of ions from the rhizosphere or even in the interaction with its host plant through their haustoria. Finally, isolates of the genera *Serratia*, *Bacillus*, *Lysinibacillus*, and *Enterobacter* were found in the three niches, reinforcing this exchange of microbiota, now a more complex perspective. These results reinforce the prospect that in a community, the ecological interaction of plants and microorganisms is directly related to the rigor of the habitat and the ability of colonization. In other words, the mutualistic interactions with microorganisms would be the basis of evolution of adaptations to inhospitable environments. The theoretical concept of species “A” of adversity strategists [sensu (Greenslade, 1983)], in contrast to the artificial r-k continuum of Pianka (1970; Taylor et al., 1990), or the understanding of “template habitats” (Southwood, 1977; Korfiatis and Stamou, 1999) and specialization in habitats (consequently, the whole concept and use of bio-indicators) can make sense only in the light of these interactions. Therefore, a specific habitat provides characteristic conditions that promote the growth of certain microorganisms, which in turn facilitate the development of other species associated with them.

Thus, much less time adjustment to selective pressures imposed by oligotrophic and contaminated soils would be necessary to ensure the invasion of environment, always done by species that evolve from less hostile niches. This possibility of having one microbiological micro-habitat that minimizes the

natural hostility of the environment can change the ecological-evolutionary perception of biodiversity evolution, and even the basic theoretical models that guide our understanding of these processes.

PGP Activities of the Isolates

About 95% of isolates showed growth in medium combined nitrogen, thereby demonstrating diazotrophic activity. This may have a direct relation with an environment whose soil is highly leached, oligotrophic and contaminated, like these montane ecosystems or any other soil of Brazilian savannas (Goodland, 1971; Goodland and Pollard, 1973; Batmanian and Haridasan, 1985; Haridasan, 2008). The understanding of the evolutionary costs of colonizing these environments are well studied (Ribeiro and Brown, 2006) and consistent with the existing theoretical propositions (Herms and Mattson, 1992; Fine et al., 2004). This is the first time that the role of mutualistic microbiota was taken into consideration as a fundamental adaptive mechanism.

Iron can be available in the soil as Fe^{2+} or Fe^{3+} , where the latter is less soluble but more abundant. Siderophores are molecules secreted by some bacterial species that chelate Fe^{3+} converting it to Fe^{2+} , which as a consequence is internalized by specific cellular receptors for these ions (Neilands, 1995). From a competition point of view, microorganisms that are able to utilize siderophores as a mechanism of acquisition of Fe^{3+} make it available for them consequently decreasing the availability of iron for possible competing microorganisms in the same niche (Hibbing et al., 2010). Often when this competition occurs because of a phytopathogenic organism, this resource becomes used as an indirect mechanism of plant growth since it controls the growth of these phytopathogens (Perez-Montano et al., 2014). In the same perspective, many plants only have receptors for siderophores, depending intimately on the their production by microorganisms that live in symbiosis or cooperation with these plants for acquiring iron from the environment, essential for their growth (Crowley et al., 1991). More than half of the bacterial isolates obtained here were producers of siderophores (Figure 3). Although this number was high, it was expected since the environment favors the adaptation of bacteria capable of surviving in such high concentrations of iron. Therefore, further study of the structural composition and regulation of the synthesis of these substances in these microorganisms is necessary and may lead to the discovery of new biomolecules with ion-chelating activity. With regard to the region where the plants were isolated, this perspective becomes even more interesting, because it is an environment classically reported as rich in arsenic, and it is possible that these microorganisms make use of these molecules as an adaptive alternative to the presence of this element (Gonçalves and Lena, 2013).

Similarly, about 86% of isolates were capable of producing IAA. Since this hormone helps to increase root growth, this could explain the adaptation of plants of these niches to extremely hard soil, which provides surface branching of roots, favoring not only anchoring but also procurement of water and minerals. These bacteria may have great importance in the study model proposed since it is a holoparasitic plant whose capacity to produce this hormone can be rather low in relation to an autotrophic

plant (Magnus et al., 1982), which could explain its intimate dependence on IAA-producing bacteria.

Far less representative, but of similar importance were the isolates in which we identified hydrolytic enzyme activities. These enzymes are of great industrial interest, since they can optimize the manufacture of products of economic interest such as in the case of glucose for fermentation processes obtained through cellulolytic or amylolytic activity, or of amino acids and peptides widely used in the food, pharmaceutical and chemical industry, obtained from proteolytic activity (Dalmaso et al., 2015). In an environmental microbiological context, all these enzymes can be of fundamental importance in the process of adaptation to a specific niche.

Individually, some genera deserve attention because of the previous results described in the literature. R54, for example, was isolated from the rhizosphere and showed similarity to *Pseudomonas fluorescens*. A recent study involving the strains PA4C2 and PA3G8 of *P. fluorescens*, also isolated from the rhizosphere, were found to be able to inhibit the growth of the phytopathogen *Dickeya* (Cigna et al., 2015), which causes diseases in herbaceous plants. In our study, R54 was also found to be a potential inhibitor of enteropathogens since it blocked the growth of *Staphylococcus aureus* and *Shigella flexneri*. Specific strains of *Pseudomonas* have also been described as inducing systemic resistance in cloves, cucumbers, radishes, tobacco, and *Arabidopsis*, which raises the possibility of these bacteria being a potential growth inhibitors of phytopathogens in wild plants. Similarly to *Pseudomonas*, induced systemic resistance has also been described for different strains of *Bacillus* spp., including the specific species *B. amyloliquifaciens*, *B. subtilis*, *B. pasteurii*, *B. cereus*, *B. pumilus*, *B. mycoides*, and *B. sphaericus* (Choudhary et al., 2007). When inoculated or present in specific organisms, they induce a significant reduction in the incidence or severity of diseases in various hosts (Choudhary et al., 2007). In this study, various isolates showed similarity with bacteria of the genus *Bacillus*, including the strains *B. cereus* and *B. mycoides*. Some of these isolates showed positive results for all the biochemical assays performed (H80) or inhibited the growth of three of the four enteropathogens investigated (H69).

Bacteria of the genus *Enterobacter* has been associated with numerous biological models. *Enterobacter* sp. strain EJ01 isolated from *Dianthus japonicus thunb* (China Sea rose) was described as a bacterium capable of aiding vegetative growth, besides alleviating salt stress in tomato and *Arabidopsis* (Kim et al., 2014). Of the five isolates similar to *Enterobacter* identified in this study, all produced siderophores and fixed N₂, and only two were capable of producing IAA. In another study, *Serratia marcescens* isolated from the rhizosphere of the coconut tree was found to fix nitrogen and to produce IAA and siderophores, among other compounds investigated (George et al., 2013), highlighting the importance of these genera in the support and growth of these plants. Of the 26 isolates that showed similarity to *Serratia*, 25 isolates were capable of fixing N₂ and producing IAA, and 15 were capable of producing siderophores.

Paenibacillus yonginensis DCY84 was evaluated in growth with *Arabidopsis thaliana* subjected to salt, drought and heavy metal stress, and the study showed that plants treated with

this bacterial isolate were more resistant than the untreated control plants (Sukweenadhi et al., 2015). Our isolates H59, H66, and H70 showed similarity to this genus and were able to produce siderophores and IAA and to fix N₂, besides inhibiting enteropathogens. Another recent work isolated bacteria from the rhizospheric soil of *Populus euphratica* and identified ten strains that induced a significant increase in dry weight of buds and roots of wheat (Wang et al., 2014). These isolates were identified as being from the genera *Pseudomonas*, *Bacillus*, *Stenotrophomonas*, and *Serratia*. Among these strains, *Serratia* sp. 1–9 and *Pseudomonas* sp. 23/05 were the most effective strains. Both produced auxin, and significantly increased production when grown under simulated dry conditions, leading to a direct effect on promoting plant growth under drought stress (Wang et al., 2014). Similarly, a work identified 12 endophytic bacteria characterized as diazotrophic, two species belonging to the genus *Paenibacillus*, three to the genus *Mycobacterium*, three to the genus *Bacillus*, and four to the genus *Klebsiella* (Ji et al., 2014). Rice seeds treated with these bacteria showed improved growth, increase in height and dry weight and antagonistic effects against pathogenic fungi (Ji et al., 2014). Our isolates that showed high identity to *Paenibacillus* (H59 – 99% and H66 – 98%), were also capable of fixing N₂ and were thus diazotrophic. The isolate H70, although showing similarity to *Paenibacillus*, was not able to fix N₂, but did inhibit the growth of enteropathogens and *Fusarium*. All isolates that showed similarity to *Klebsiella* (L16, L28, and R39) were also capable of fixing N₂, besides producing IAA.

Perspectives of Use of Isolates

In an agroecological context, there is currently an emerging demand for the development of sustainable agriculture, to decrease our dependence on agrochemical farming and its harmful consequences to the environment (Bhardwaj et al., 2014). The utilization of PGPB to increase farm production has become an important alternative. Similarly, alternative methods for pest control attracted attention, and biological control has been considered a viable solution for various diseases that are difficult to control (Cespedes et al., 2015). This practice aims to maintain a balance in the agroecosystem, so that the host, in the presence of a pathogen or pest, does not suffer significant damage due to the controlling action exerted by non-pathogenic organisms (Meldau et al., 2012). Thus, understanding microbial relations in soils and plants can lead to the discovery of microorganisms with great agricultural potential and other applications as well.

Besides agroecological importance, all potential presented by microbiota isolated from these neglected biological niches drives the search for new products and processes with potential pharmacological and for environmental bioremediation. This was evident by the ability of some isolates to inhibit three out of four investigated enteropathogenic species, besides *Xanthomonas* and *Fusarium* strains. Analysis of genomic composition and metabolites produced by these isolates could uncover new metabolic pathways associated with the synthesis of new biomolecules of pharmaceutical interest. In the same way, the isolates associated with production of IAA and siderophores may be used as bacterial consortia to induce native plant growth in areas degraded by human action (de-Bashan et al., 2012).

CONCLUSION

The integration of biological data found in this study suggests a hypothetical complex network of interaction and mutual dependence between the niches analyzed and the isolated bacteria. Classically, holoparasitic plants draw all necessary nutrients from their host plant. However, the results of this study show that the microbiota in *L. hypogaea* can also be of benefit by supplying nutrients essential for the survival of the plant. This hypothesis needs further and in-depth studies to become valid. From an ecological perspective, this is the first report of the potential of bacteria isolated from the IQ region in producing these siderophores and IAA, which allowed us to infer that part of the adaptive process of these plants in ferroginous fields can be a result of the ability of a large percentage of isolates to produce these compounds. Siderophores would be key for the chelation of iron in the Fe³⁺ state, existing in high concentrations in these ferroginous fields, and the production of IAA by a large number of isolates demonstrated how essential this compound would be for the induction of extensive root system of plants that survive in this environment. The characteristics of the soil from this area are extremely adverse, with low supply of water, requiring the plants to obtain nutrients from more superficial regions. Thus, it is possible that the survival of plants in this environment have some relationship with the presence and interaction with these microorganisms, which could also justify the large endemic plant found in this environment. From a biotechnological aspect, the perspectives and results obtained with this work point to the potential of developing a bacterial consortium that could be used as an indispensable tool in the recovery of areas degraded by anthropic actions, especially in this region where mining activities are eliminating important species of the biome of ferroginous fields. Therefore, the results presented in this work emphasize the importance of studying biological models neglected and differentiated such as the holoparasite plants and ferruginous soils from IQ since they are propitious sources for finding new compounds with biotechnological potential.

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AUTHOR CONTRIBUTIONS

ÉF and LF collected samples. ÉF and LM conceived and designed the experiments. ÉF and LM performed all experiments and analysis. ÉF, LM, IS, and LR contributed with reagents, materials and analysis tools. ÉF and LM prepared the figures and tables. ÉF, LM, and SR wrote the paper.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: <http://journal.frontiersin.org/article/10.3389/fmicb.2017.00172/full#supplementary-material>

FIGURE S1 | Geographical location of Serra da Brigida, collection site of *L. hypogaea*. Serra da Brigida is located around the city of Ouro Preto, state of Minas Gerais – Brazil. The stars indicate the sampling points for *Langsdorffia hypogaea*. Adapted from Atlas Digital GeoAmbiental (<http://institutopristino.org.br/atlas/>).

FIGURE S2 | Presence of *nifH* confirmed by PCR analysis.

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Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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