


STRAINS OF *Paraburkholderia* ORIGINATED FROM RUPESTRIAN FIELDS PROMOTE THE GROWTH OF *Mimosa foliolosa*

Mariana Gonçalves Souza² , Fernanda de Carvalho³ , Silvia Maria de Oliveira-Longatti³ , Leonardo Paiva Barbosa⁴  and Fatima Maria de Souza Moreira^{3*} 

¹ Received on 21.08.2019 accepted for publication on 10.02.2020.

² Universidade Federal de Lavras, Mestrado em Engenharia Agrícola, Lavras, MG-Brasil. E-mail: <marigsouza@gmail.com>.

³ Universidade Federal de Lavras, Departamento de Ciência do Solo, Lavras, MG-Brasil. E-mail: <fernandacarva@gmail.com>, <sylmarya@yahoo.com.br> and <fmoreira@ufla.br>.

⁴ Instituto Federal Minas Gerais, Ponte Nova, MG-Brasil. E-mail: <ldpaiva@gmail.com>.

*Corresponding author.

ABSTRACT – *Mimosa foliolosa* is a promising native species of rupestrian fields for revegetation of degraded areas in this ecosystem. The symbiosis between leguminous plants and N₂-fixing bacteria may play an important role in the recovery of these areas, since these plants have better development and are more resistant to the attack of pathogens. In addition to the biological nitrogen fixation (BNF), these bacteria can promote plant growth through other processes, such as phosphate solubilization and siderophore production. We studied the cultural and genetic characteristics of 11 bacterial strains, isolated from rupestrian field soils using *Mimosa tenuiflora* as “trap” plant. We evaluated these strains considering their symbiotic characteristics such as nodulation, and growth-promotion of *Mimosa foliolosa*, as well as their ability for siderophore production and phosphate solubilization. Native N₂-fixing bacterial strains belonging to the *Paraburkholderia* genus (UFLA01-750, UFLA01-728, UFLA01-725, and UFLA01-757), showed high symbiotic efficiency with *M. foliolosa*. These strains also solubilized calcium phosphate and produced siderophores, exhibiting high functional diversity and potential for use in revegetation projects.

Keywords: Nitrogen fixing bacteria; Recovery of degraded areas; Phosphate solubilization.

ESTIRPES DE *Paraburkholderia* ORIUNDAS DE CAMPOS RUPESTRES PROMOVEM O CRESCIMENTO DE *Mimosa foliolosa*

RESUMO – *Mimosa foliolosa* é uma espécie nativa de campos rupestres promissora para revegetação de áreas degradadas neste ecossistema. A simbiose entre leguminosas e bactérias fixadoras de N₂ pode desempenhar um papel importante na recuperação dessas áreas, uma vez que estas plantas têm melhor desenvolvimento e são mais resistentes ao ataque de patógenos. Em adição à fixação biológica de N₂ (-FBN), essas bactérias podem promover o crescimento vegetal por meio de outros processos, tais como solubilização de fosfatos e produção de sideróforos. Nós estudamos as características culturais e genéticas de 11 estirpes de bactérias isoladas de solo de campos rupestres utilizando *Mimosa tenuiflora* como planta isca. Nós avaliamos estas estirpes em relação as suas características simbióticas como nodulação e promoção de crescimento de *Mimosa foliolosa*, assim como sua habilidade de solubilizar fosfato e produzir sideróforos. Estirpes bacterianas fixadoras de N₂ nativas pertencentes ao gênero *Paraburkholderia* (UFLA01-750, UFLA01-728, UFLA01-725 e UFLA01-757), apresentaram alta eficiência simbiótica com *M. foliolosa*, além de solubilizar fosfato de cálcio e produzir sideróforos, evidenciando uma alta diversidade funcional e potencial para uso em projetos de revegetação.

Palavras-Chave: Bactérias fixadoras de nitrogênio; Restauração de áreas degradadas; Solubilização de fosfato.



1. INTRODUCTION

Rupestrian fields are known for the high diversity of plant and animal species that inhabit them, many of which are endemic. Given this high diversity above ground level, a large diversity of soil-dwelling organisms is expected. Studies have shown that the rupestrian fields can be considered a hotspot of diversity of an important group that composes the soil biota – the arbuscular mycorrhizal fungi (Coutinho et al., 2015; Carvalho et al., 2012).

Symbiosis between leguminous plants and Nodulating Bacteria may play an important role in processes for recovery of these areas, since these plants have better development and are more resistant to attack from pathogens (Martins et al., 2018; Ferreira et al., 2020). In addition, through improving soil fertility, they may favor the growth of other non-leguminous species (Zotarelli et al., 2012). The multifunctionality of this group of microorganisms is well-known, i.e., in addition to biological nitrogen fixation (BNF), these bacteria can promote plant growth through other processes, such as phosphate solubilization (Marra et al., 2012), which results in an increase in the supply of phosphorus available for uptake by plants, and siderophore production, which makes iron available for plant growth through the iron/siderophore bacterial complex and limits iron acquisition by plant pathogens, preventing their proliferation in this ecological niche (Neilands, 1952; Neilands, 1995). For that reason, we studied the bacterial culture and genetic characteristics of 11 native bacterial strains, among which are rhizobia and endophytic bacteria, that may have characteristics of plant growth-promoting rhizobacteria (PGPR) (Oliveira-Longatti et al., 2014). We evaluated the strains regarding their nodulation- and growth-promoting ability in *Mimosa foliolosa* (a leguminous plant species endemic to the rupestrian fields), as well as their ability for siderophore production and phosphate solubilization.

2. MATERIAL AND METHODS

The strains were isolated from nodules of *Mimosa tenuiflora* grown in pots containing soil from different habitats (Cerrado-tropical savanna; Peat bogs; Rocky outcrops; Quartz gravel field) of the rupestrian field ecosystem collected from the private reserve Vellozia, in the Serra do Cipó, Minas Gerais.

Soil samples from each of the 4 habitats constituted the 4 treatments that were arranged in completely

randomized design with three repetitions. The experimental unit corresponded to a 1kg pot with one plant per pot. Each pot contained 75% of sterile inert sandy substrate and 25% of soil from each habitat studied. Before planting *Mimosa tenuiflora* seeds, used as trap plant, were superficially disinfected using 70% alcohol for 3min, 1% sodium hypochlorite for 3min and successive washing in running water. After 90 days, nodules were collected from the plants for isolation. The nodules obtained from the trap plants were first immersed in 95% ethyl alcohol for 30 seconds to break the surface tension, then immersed in 30% hydrogen peroxide for 1 to 3 minutes according to the size of the nodules. Disinfect the surface of the lump, and then washed several times in sterile water. The nodules were then crushed with the aid of properly sterilized forceps in plates containing culture medium 79 (Fred and Waksman, 1928) with bromothymol blue, pH 6.8. The plates were incubated at 28 °C. After the appearance of colonies, subculturing was performed and incubated again until obtaining isolated colonies which were described according their growth characteristics in 79 medium.

Genetic characterization of the bacterial strains was obtained by means of partial sequencing of the gene 16S rRNA. The DNA of each strain was isolated using the ZR Fungal/Bacterial DNA Kit (Zymo Research Corp.). The 16S rRNA of the strains was amplified by PCR using the primer set 27F (50-AGAGTTTGATCCTGGCTCAG-30) and 1492R (50-GGTTACCTTGTTACGACTT-30) (Lane, 1991). Sequences were compared to the GenBank database to obtain the most similar accessions and their similarities (Table 1). The 11 strains sequences were deposited in the GenBank under accession numbers MK649676 to MK649686.

The nodulating and plant growth-promoting ability of the strains in *Mimosa foliolosa* plants were evaluated in a greenhouse using seedling plug pots (240 cm³) containing sand and vermiculite (1:2). The experiment consisted of 15 treatments: 12 treatments involved inoculations with the 12 strains, each one inoculated separately, and irrigation with Hoagland nutrient solution (Hoagland and Arnon, 1950) containing a low concentration of mineral N (5.25 mg L⁻¹); two negative controls without inoculation, one irrigated with nutrient solution with the low concentration (5.25 mg L⁻¹) of mineral N and the other with a high concentration of mineral N (52.5 mg L⁻¹); and one positive control with

the strain BR 3460 (*Burkholderia* sp.) authorized by the Brazilian Ministry of Agriculture (MAPA) as an inoculant for *Mimosa bimucronata*, since there is no strain authorized for *M. foliolosa*. One of the strains tested (UFLA 01-750) had already proven to be effective in *M. bimucronata* in a previous study (Araújo et al., 2017). The sources of mineral N used were $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$, KNO_3 , and $\text{NH}_4\text{H}_2\text{PO}_4$. The experimental design was completely randomized, with eight replications. The seedling plug pots and the nutrient solution (Hoagland and Arnon, 1950) were autoclaved for 60 min at a pressure of 1.5 cm^2 at 121°C .

Before sowing, the *M. foliolosa* seeds were disinfected and scarified, in accordance with Silveira and Fernandes (2006). After that, the seeds were pre-germinated in moistened filter paper in petri-dishes for 5 days in a growth chamber at 28°C . For each inoculated treatment, the seedlings received 1 mL, containing 1×10^8 cells of the strain grown in 79 medium (Vicent, 1970).

At 70 days after setting up the experiment, the plants were collected to determine the number of nodules (NN), nodule dry matter (NDM), shoot dry matter (SDM), root dry matter (RDM), and total dry matter (TDM). For determination of NDM, SDM, and RDM, the nodules, the shoots, and the roots were placed in paper bags and then dried in a forced air circulation oven at 60°C until reaching constant weight. Analysis of variance was conducted on the data from the trial using the SISVAR 5.6 statistical analysis program (Ferreira, 2011). The effects of the treatments were compared by the Scott-Knott test at 5% probability. The NN and NDM data were transformed in square root of $Y + 0.5$.

The solubilization of calcium phosphate (Ca-P), aluminum phosphate (Al-P), and iron phosphate (Fe-P) was evaluated in solid NBRIP medium (Nautiyal, 1999), in solid GES medium (Sylvester-Bradley et al., 1982), and in solid GELP medium (Sylvester-Bradley et al., 1982), respectively. The evaluation followed the procedure described by Marra et al. (2012). Based on the solubilization index (SI), the strains were classified as having low ($\text{SI} < 2 \text{ mm}$), medium ($2 \text{ mm} \leq \text{SI} < 4 \text{ mm}$), or high ($\text{SI} \geq 4 \text{ mm}$) solubilization capacity (Berraquero et al., 1976).

Siderophore production was evaluated in King B medium (King et al., 1954), called “poor medium” since it does not contain any source of iron. The strains were grown in liquid 79 medium for 48 hours under shaking

of 100 rpm. Aliquots of 100 μL of the bacterial cultures were transferred to microtubes and centrifuged (10,000 rpm) for 3 minutes. The supernatant was discarded and the cell mass was washed with 1.0 ml of salt solution (0.85% NaCl) three consecutive times to remove residues of the culture medium. The strains were then inoculated in King B medium and shaken at 110 rpm for 48 hours more. After that period, 1.5 ml of these bacterial cultures was pipetted in microtubes and centrifuged (10,000 rpm) for 5 minutes. Then 300 μL of the supernatant was removed and added to test tubes containing 300 μL of the transport solution called CAS (Alexander and Zuberer, 1991). A change from blue color to a pinkish color constituted a positive test for siderophore production. All the material used was cleaned with HCL (30%) and running, distilled, and ultrapure water, respectively, so as not to leave any trace of contaminating material that could generate a false-positive result.

3.RESULTS

The origin (habitats, Global Positioning System) and Growth characteristics of twelve isolated strains are shown in Table 1.

Sequences obtained varied from 560 to 1073bp. Most strains (7) belonged to *Paraburkholderia* genus with similarity to sequences in GenBank varying from 98-100%. The other five strains belong to genera do not recognized as legume- nodulating bacteria, with similarities varying from 99 to 100%.

The species *Mimosa foliolosa* nodulated with 50% of the strains evaluated (UFLA 01-750, UFLA 01-725, UFLA 01-728, UFLA 01-757, UFLA 01-794, and UFLA 01-802); these strains belong to the *Paraburkholderia* genus. Among the *Paraburkholderia* strains identified (Table 1), UFLA 01-786 (*P. nodosa*) was not able to nodulate *M. foliolosa* (Table 2).

The strains that significantly promoted the largest number of nodules (NN) were UFLA 01-750, UFLA 01-794, and UFLA 01-802, followed by strains UFLA 01-725, UFLA 01-728, and UFLA 01-757. In regard to nodule dry matter (NDM), the highest production was obtained with strains UFLA 01-750, UFLA 01-725, and UFLA 01-794, followed by strains UFLA 01-728 and UFLA 01-757, which differed significantly from the others (Table 2). Although the strain UFLA 01-802 promoted high production of nodules, the dry matter of these nodules did not differ from the other treatments.

Table 1 – Original habitats, cultural characteristics, and identification of bacterial strains isolated from rupestrian field soils using *Mimosa tenuiflora* as trap plants.
Tabela 1 – Habitats de origem, características culturais e identificação de bactérias isoladas de estirpes de *Mimosa tenuiflora* como planta isca.

Strains	Habitats/GPS	Growth characteristics in 79 medium				Identification based in the 16S rRNA most similar sequence found in the GenBank		
		pH	Colony colour	Ø(mm)	NBP	Species	SI%	Accession number
UFLA 01-750	Ce / 19°16' 55,0" S 43°35' 35,8" W	acid/alkali	white	<1	560	<i>Burkholderia</i> sp.	99	FNS43721
UFLA 01-725	Ce / 19°16' 57,7" S 43°35' 40,0" W	alkali	white	<1	1070	(syn. <i>Paraburkholderia</i> sp.) <i>Paraburkholderia</i> sp.	99	MG182917
UFLA 01-728	Ce / 19°16' 45,0" S 43°35' 28,9" W	neutral	yellow	2	865	<i>Paraburkholderia</i> sp.	98	MG182917
UFLA 01-757	Pb / 19°16' 53,0" S 43°35' 26,6" W	alkali	white	<1	951	<i>Paraburkholderia nodosa</i>	99	MF495772
UFLA 01-794	Ro / 19° 17' 15,2" S 43° 35' 39,2" W	acid/alkali	white	1	998	<i>Paraburkholderia nodosa</i>	99	MF495782
UFLA 01-802	Qf / 19°17' 09,0" S 43°35' 20,0" W	acid/alkali	white	<1	947	<i>Paraburkholderia nodosa</i>	99	MF495781
UFLA 01-786	Ce / 19°16' 55,0" S 43°35' 35,8" W	neutral	white	1,5	1073	<i>Paraburkholderia nodosa</i>	100	MF495782
UFLA 01-690	Ce / 19°16' 57,7" S 43°35' 40,0" W	acid	cream	1	883	<i>Lysinibacillus sphaericus</i>	100	JQ744621
UFLA 01-758	Qf / 19°17' 09,0" S 43°35' 20,0" W	alkali	white	<1	888	<i>Brevibacillus</i> sp.	99	FJ719350
UFLA 01-759	Ro / 19° 17' 15,2" S 43° 35' 39,2" W	alkali	white	<1	1042	<i>Stenotrophomonas</i> sp.	100	MH883921
UFLA 01-760	Ce / 19°16' 45,0" S 43°35' 28,9" W	acid	yellow	<1	1030	<i>Paenibacillus polyxyxa</i>	100	MH021190
UFLA 01-789	Ce / 19°16' 45,0" S 43°35' 28,9" W	neutral	Incolor	<1	1002	<i>Paenibacillus</i> sp.	100	KX959692

Ce: Cerrado (tropical savanna); Pb: Peat bogs; Ro: Rocky outcrops; Qf: Quartz gravel field. All the strains showed rapid growth (2 to 3 days) in 79 medium, except for UFLA 04-265, which had intermediate growth (4 to 5 days). Ø: Colony diameter; NBP: Number of base pairs of the 16S rRNA sequences; SI: Similarity. *Strain identified by Araújo et al. (2017).
 Ce: cerrado; Pb: brejo arenoso; Ro: afloramentos rochosos; Qf: campos pedregosos. Todas as estirpes mostraram rápido crescimento (2 ou 3 dias) em meio de cultura 79, exceto para a UFLA 04-265, que apresentou crescimento intermediário (4 a 5 dias). Ø: ; diâmetro da colônia. NBP: número de pares de sequências de 16S rRNA. SI: similaridade. *Estripe identificada por Araújo et al. (2017)

Table 2 – Number of nodules (NN), nodule dry matter (NDM), shoot dry matter (SDM), root dry matter (RDM), and total dry matter (TDM) obtained in *Mimosa foliolosa* plants inoculated with bacterial strains isolated from rupestrian field soils, as well as their plant growth-promoting characteristics.

Tabela 2 – Número de nódulos (NN), matéria seca de nódulos (NDM), matéria seca da parte aérea (SDM), matéria seca das raízes (RDM), e matéria seca total (TDM) de plantas de *Mimosa foliolosa* inoculadas com estirpes de bactérias isoladas de campos rupestres assim como suas características de promoção de crescimento vegetal.

Treatments	NN					Phosphate Solubilization				Siderophore production
	NDM	SDM	RDM	TDM	mg plant ⁻¹	CaHPO ₄	Solubility capacity	Al(H ₂ PO ₄) ₃	FePO ₄ ·2H ₂ O	
*Mineral-N (52.5 mg L ⁻¹)	-	0,262 c	0,177 c	0,440 c	-----					
BR 3460	-	0,277 c	0,150 c	0,426 c	-----					
UFLA 01-750	28 a	0,035 a	0,678 a	0,371 a	1,048 a	1.20	Low	NG	GNS	+
UFLA 01-725	20 b	0,032 a	0,525 b	0,254 b	0,777 b	1.48	Low	GNS	GNS	+
UFLA 01-728	18 b	0,018 b	0,451 b	0,207 c	0,658 b	1.76	Low	NG	GNS	+
UFLA 01-757	21 b	0,026 b	0,456 b	0,260 b	0,716 b	2.26	Medium	GNS	GNS	+
UFLA 01-794	29 a	0,038 a	0,274 c	0,187 c	0,461 c	GNS	-	GNS	GNS	-
UFLA 01-802	26 a	0,012 c	0,166 c	0,132 c	0,297 d	GNS	-	GNS	GNS	-
UFLA 01-786	-	-	0,270 c	0,165 c	0,434 c	1.54	Low	GNS	GNS	-
UFLA 01-690	-	-	0,316 c	0,166 c	0,482 c	NG	-	NG	GNS	-
UFLA 01-758	-	-	0,242 c	0,161 c	0,403 c	GNS	-	GNS	GNS	-
UFLA 01-759	-	-	0,322 c	0,186 c	0,510 c	GNS	-	GNS	GNS	+
UFLA 01-760	-	-	0,300 c	0,154 c	0,455 c	GNS	-	GNS	GNS	-
UFLA 01-789	-	-	0,290 c	0,170 c	0,460 c	NG	-	NG	GNS	-
*Small dose of mineral-N	-	-	0,204 c	0,078 d	0,282 d					
CV %	26,4	1,06	25,7	24,16	18,1					

*Treatments without inoculation. Inoculated treatments received a low application rate of mineral N (5.25 mg L⁻¹). S.I. Solubilization index = halo diameter (mm) / colony diameter (mm); NG: the strain did not grow; GNS: the strain grew but was unable to solubilize. Values followed by the same letter in the column did not differ (Scott-Knott test, p < 0.05).

*Tratamentos sem inoculação. Tratamentos inoculados receberam solução nutritiva com baixa concentração de nitrogênio mineral (5.25 mg L⁻¹) de . S.I. Índice de solubilização = diâmetro do halo (mm) / diâmetro da colônia (mm); NG: as estirpes não cresceram; GNS: as estirpes cresceram mas não solubilizaram. Valores seguidos pela mesma letra na coluna não diferem pelo teste de Scott-Knott, p < 0.05.

The strains UFLA 01-690, UFLA 01-758, UFLA 01-759, UFLA 01-760, and UFLA 01-789 did not form nodules in *M. foliolosa*.

The experiment to evaluate the ability of strains to promote growth of *M. foliolosa* had significant effects for all the variables analyzed. In relation to shoot dry matter, most of the strains, including two that were able to nodulate (UFLA 01-794 and UFLA 01-802), did not differ from the control with low concentration of mineral N, which also did not differ from the control with high concentration of mineral N, even though the control with high concentration of mineral N had a 28% increase in relation to the control with low concentration of mineral N (Table 2).

The strain UFLA 01-750 stood out not only for NN and NDM, but also for production of shoot dry matter (SDM), root dry matter (RDM), and total dry matter (TDM) (Table 2). Following that strain, three other nodule-forming strains, UFLA 01-725, UFLA 01-728, and UFLA 01-757, also led to a significant increase in production of these three parameters (Table 2).

The other root-nodule-forming strain (UFLA 01-794) and non-root-nodule-forming strains (UFLA 01-690, UFLA 01-758, UFLA 01-759, UFLA 01-760, UFLA 01-786, UFLA 01-789) also promoted growth of *M. foliolosa* since their production of RDM and TDM were superior to the control with low concentration of mineral N (Table 2).

In production of plant growth-promoting compounds, five strains were able to solubilize Ca-P *in vitro* and none was able to solubilize Fe-P and Al-P (Table 2). Nevertheless, all strains were able to grow in Fe-P and four did not grow in Al-P (Table 2). The strains that solubilized Ca-P had a low solubilization index, except for the strain UFLA 01-757, which stood out from the others since it was the only one that had medium solubilization capacity of Ca-P (Table 2).

The strains UFLA 01-750, UFLA 01-725, UFLA 01-728, and UFLA 01-757 that were able to solubilize Ca-P (Table 2) were also able to produce siderophores (Table 2) and were precisely those that led to the highest plant growth. The strain UFLA 01-786 solubilized Ca-P; however, it was not able to produce siderophores (Table 2). The strain UFLA 01-759 was able to produce siderophores; however, it did not solubilize phosphate (Table 2).

4.DISCUSSION

The species *Mimosa foliolosa* was recently recommended for ecological recovery of rupestrian fields due to its high relative growth rate and its growth in low fertility soils (Negreiros et al., 2009). In addition, symbiosis with efficient bacterial strains represents a viable strategy for preventing mortality in the initial stages of setting up environmental recovery programs (Negreiros et al., 2009). Our study shows that *M. foliolosa* establishes symbiosis with nitrogen fixing bacteria of the *Paraburkholderia* genus. Among these bacteria, the strain UFLA 01-750 had already been reported as efficient in *M. foliolosa*; however, at that time, the *Paraburkholderia* genus had not yet been described as including the symbiotic species of the *Burkholderia* genus (Dobritsa and Samadpour, 2016). Our study shows other strains of this genus also efficient in growth promotion of *M. foliolosa* and they are also able to perform other plant growth-promoting processes, such as phosphate solubilization and siderophore production. The use of species that are able to establish symbiosis with diazotrophic bacteria in recovery programs is fundamental since they have a high capacity for growth in low fertility soils and they endure severe conditions (Chaer et al., 2011), as is the case of rupestrian fields.

Therefore, we suggest that production of seedlings for setting up these programs consider inoculation of strains that are promising for promoting their development, as is the case of the strains UFLA 01-750, UFLA 01-728, UFLA 01-725, and UFLA 01-757. These nodule-forming strains that are effective in promoting the growth of *M. foliolosa* perform other processes beyond biological N₂ fixation, which are important for development of seedlings and success in establishing them in the field, especially in tropical soils; because they can provide nitrogen and phosphorus directly, and iron indirectly.

In addition to nodule-forming strains, bacterial strains of other genera already reported as plant-growth promoters (*Lysinibacillus*, *Brevibacillus*, *Stenotrophomonas*, and *Paenibacillus*) were isolated and may be also promising for the development of *M. foliolosa* seedlings. *Lysinibacillus sphaericus* is known for resistance to toxic metals (Velásquez and Dussán, 2009), potential for phytoremediation processes and in the growth and nutrition of plants grown in polluted soils and soils with low nutrient content (Martínez and Dussán, 2017). *Brevibacillus* sp. promotes plant growth

(increase in shoot and root dry matter), accumulation of N and P, and increase in number of nodules, as well as mycorrhizal infection when co-inoculated with *Rhizobium leguminosarum* in *Trifolium repens* (Vivas et al., 2006). Some species of *Stenotrophomonas* can produce antimicrobial compounds that protect plants and produce ACC deaminase, gibberellic acid, indole acetic acid (IAA), and siderophores and have the capacity for solubilization of low solubility phosphates that can promote plant growth (Singh and Jha, 2017). Furthermore, many *Stenotrophomonas* spp. have a high level of intrinsic resistance to heavy metals and antibiotics and have shown the ability to degrade a wide range of compounds, including pollutants. For that reason, they may be used in bioremediation and phytoremediation (Ryan et al., 2009). *Paenibacillus* is another important genus like PGPR that can directly affect plant growth through production of indole-3-acetic acid (IAA) and other auxins and phosphate solubilization. Some species can also fix atmospheric nitrogen (Weselowski et al., 2016). As experimental conditions were adequate for expression of biological N₂ fixation, i.e., there was deficiency of N in the mineral form, if these strains are growth promoters by other processes, growth promotion of *M. foliolosa* would be manifested in a greater degree in concentrations of mineral N adequate for plant growth, what should be tested in further experiments. *Lysinibacillus*, *Brevibacillus*, *Stenotrophomonas*, and *Paenibacillus* are not considered as legume symbionts according to the Subcommittee on Taxonomy of Rhizobia and Agrobacteria of the International Committee on Systematics of Prokaryotes (Lajudie et al., 2019). However, they can be found as nodule endophytes on other legume species (Lima et al., 2009; Oliveira-Longatti et al., 2014).

5. CONCLUSIONS

The nodule-forming strains UFLA 01-750, UFLA 01-728, UFLA 01-725, and UFLA 01-757 isolated from *M. tenuiflora* originating from rupestrian field soils proved to be able to solubilize Ca-P and produce siderophores, and were the most effective in promoting the growth of *M. foliolosa*, showing high functional diversity and potential in revegetation projects.

The *M. foliolosa* species establishes symbiosis with bacteria of the *Paraburkholderia* genus; however, other strains of non-nodule-forming genera, such as *Paenibacillus*, *Brevibacillus*, and *Lysinibacillus*, also favor *M. foliolosa* development.

6. ACKNOWLEDGMENTS

We thank the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) (CAPES/PROEX AUXPE 593/2018), the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) (Process: 304527/2016-5; Process: 431504/2016-4), and the Fundação de Amparo e Pesquisa de Minas Gerais (Fapemig) (PACCSS/PPGCS - 2009/C2012) for financial support and for granting scholarships. This research is associated with the Brazilian "Instituto Nacional de Ciência e Tecnologia - Biodiversidade do Solo" (Soil Biodiversity/INCT-CNPq).

7. REFERENCES

- Alexander DB, Zuberer DA. Use of chrome azurol S reagents to evaluate siderophore production by rhizosphere bacteria. *Biology and Fertility of Soils*. 1991;12(1):39-45. doi: 10.1007/BF00369386
- Araújo KS, Carvalho F, Moreira FMS. *Burkholderia* strains promote *Mimosa* spp. growth but not *Macroptilium atropurpureum*. *Revista Ciência Agronômica*. 2017;48(1):41-48. doi: 10.5935/1806-6690.20170005
- Berraquero FR, Baya AM, Cormenzana AR. Establishment of indices for the study of phosphate solubilization by soil bacteria. *Ars Pharmaceutica*. 1976;17:399-06. doi: 10.1590/0001-3765201720160111
- Carvalho F, Souza FA, Carrenho R, Moreira FMS, Jesus EC, Fernandes GW. The mosaic of habitats in the high-altitude Brazilian rupestrian fields is a hotspot for arbuscular mycorrhizal fungi. *Applied Soil Ecology*. 2012;52:9-19. doi: 10.1016/j.apsoil.2011.10.001
- Chaer GM, Resende AS, Campello EFC, Faria SM, Boddey RM. Nitrogen-fixing legume tree species for the reclamation of severely degraded lands in Brazil. *Tree Physiology*. 2011;31(2):139-49. doi: 10.1093/treephys/tpq116
- Coutinho ES, Fernandes GW, Barbara RLL, Valério HM, Goto BT. Variation of arbuscular mycorrhizal fungal communities along an altitudinal gradient in rupestrian grasslands in Brazil. *Mycorrhiza*. 2015;25(8):627-38. doi: 10.1007/s00572-015-0636-5
- Dobritsa AP, Samadpour M. Transfer of eleven

- species of the genus *Burkholderia* to the genus *Paraburkholderia* and proposal of *Caballeronia* gen. nov. to accommodate twelve species of the genera *Burkholderia* and *Paraburkholderia*. *International Journal of Systematic and Evolutionary Microbiology*. 2016;66(8):2836-46. doi: 10.1099/ijsem.0.001065
- Ferreira DF. Sisvar: A computer statistical analysis system. *Ciência Agrotecnologia*. 2011;35(6):1039-42. doi: 10.1590/S1413-70542011000600001
- Ferreira LVM, Carvalho F, Andrade JFC, Oliveira DP, Medeiros FHV, Moreira FMS. Co-inoculation of selected nodule endophytic rhizobacteria strains with *Rhizobium tropici* promotes plant growth and controls damping off in common bean. *Pedosphere*. 2020;30(1):98-108. doi:10.1016/S1002-0160(19)60825-8
- Fred EB, Waksman SA. *Laboratory manual of general microbiology*. New York. McGraw- Hill Book; 1928. 142 p.
- Hoagland HS, Arnon DI. *The water culture method for growing plants without soil*. Berkeley: California Agriculture Experimental Station; 1950. 32 p.
- King EO, Ward MK, Raney DE. Two simple media for the demonstration of pyocyanin and fluorescein. *Journal of Laboratory and Clinical Medicine*. 1954;44(2):301-7.
- Lajudie PM, Andrews M, Ardley J, Eardly B, Jumas-Bilak E, Kuzmanović N, et al. Minimal standards for the description of new genera and species of rhizobia and agrobacteria. *International Journal of Systematic and Evolutionary Microbiology*. 2019;69(7):1852-63. doi: 10.1099/ijsem.0.003426
- Lane DJ. 16S/23S rRNA sequencing. In: Stackebrandt E, Goodfellow M. *Nucleic acid techniques in bacterial systematics*. New York, USA: Wiley; 1991. p. 115-48.
- Lima AS, Nóbrega RSA, Barberi A, Silva K, Ferreira DF, Moreira FMS. Nitrogen-fixing bacteria communities occurring in soils under different uses in the Western Amazon Region as indicated by nodulation of siratro (*Macroptilium atropurpureum*). *Plant and Soil*. 2009;319:127-45. doi: 10.1007/s11104-008-9855-2
- Marra LM, Soares CRFS, Oliveira SM, Ferreira PAA, Soares BL, Carvalho RF, et al. Biological nitrogen fixation and phosphate solubilization by bacteria isolated from tropical soils. *Plant Soil*. 2012;357(1-2):289-307. doi: 10.1007/s11104-012-1157-z
- Martínez SA, Dussán J. *Lysinibacillus sphaericus* plant growth promoter bacteria and lead phytoremediation enhancer with *Canavalia ensiformis*. *Environmental Progress & Sustainable Energy*. 2017;37(1):276-82. doi: 10.1002/ep.12668
- Martins SA, Schurt DA, Seabra SS, Martins SJ, Ramalho MAP, Moreira FMS, et al. Common bean (*Phaseolus vulgaris* L.) growth promotion and biocontrol by rhizobacteria under *Rhizoctonia solani* suppressive and conducive soils. *Applied Soil Ecology*. 2018;127:129-35. doi: 10.1016/j.apsoil.2018.03.007
- Nautiyal CS. An efficient microbiological growth medium for screening phosphate solubilizing microorganisms. *FEMS Microbiology Letters*. 1999;170(1):265-70. doi:10.1111/j.1574-6968.1999.tb13383.x
- Negreiros D, Fernandes GW, Silveira FAO, Chalub C. Seedling growth and biomass allocation of endemic and threatened shrubs of rupestrian fields. *Acta Oecologica*. 2009;35(2):301-10. doi: 10.1016/j.actao.2008.11.006
- Neilands JB. A Crystalline Organo-iron Pigment from a Rust Fungus (*Ustilago sphaerogena*). *Journal of the American Chemical Society*. 1952;74(19):4846-47. doi: 10.1021/ja01139a033
- Neilands JB. Siderophores: Structure and Function of Microbial Iron Transport Compounds. *Journal of Biological Chemistry*. 1995;270(45):26723-726. doi: 10.1074/jbc.270.45.26723
- Oliveira-Longatti SM, Marra LM, Soares BL, Bomfeti CA, Silva K, Ferreira PAA, et al. Bacteria isolated from soils of the western Amazon and from rehabilitated bauxite-mining areas have potential as plant growth promoters. *World Journal of Microbiology and Biotechnology*. 2014;30(4):1239-50. doi: 10.1007/s11274-013-1547-2
- Ryan RP, Monchy S, Cardinale M, Taghavi S, Crossman L, Avison MB, et al. The versatility

and adaptation of bacteria from the genus *Stenotrophomonas*. *Nature Reviews Microbiology*. 2009;7(7):514-25. doi: 10.1038/nrmicro2163

Silveira FAO, Fernandes GW. Effect of light, temperature and scarification on the germination of *Mimosa foliolosa* (Leguminosae) seeds. *Seed Science and Technology*. 2006;34(3):585-92. doi: 10.15258/sst.2006.34.3.05

Singh RP, Jha PN. The PGPR *Stenotrophomonas maltophilia* SBP-9 Augments Resistance against Biotic and Abiotic Stress in Wheat Plants. *Frontiers in microbiology*. 2017;8:1-15. doi: 10.3389/fmicb.2017.01945

Sylvester-Bradley R, Asakawa N, La Torraca S, Magalhães FMM, Oliveira LA, Pereira RM. Quantitative survey of phosphate solubilizing microorganisms in the rhizosphere of grasses and legumes in the Amazon. *Acta Amazonica*. 1982;12:15-22.

Velásquez L, Dussán J. Biosorption and

bioaccumulation of heavy metals on dead and living biomass of *Bacillus sphaericus*. *Journal of Hazardous Materials*. 2009;167(1-3):713-16. doi: 10.1016/j.jhazmat.2009.01.044

Vivas A, Biró B, Ruíz-Lozano JM, Barea JM, Azcón R. Two bacterial strains isolated from a Zn-polluted soil enhance plant growth and mycorrhizal efficiency under Zn-toxicity. *Chemosphere*. 2006;62(9):1523-33. doi: 10.1016/j.chemosphere.2005.06.053

Weselowski B, Nathoo N, Eastman AW, MacDonald J, Yuan ZC. Isolation, identification and characterization of *Paenibacillus polymyxa* CR1 with potentials for biopesticide, biofertilization, biomass degradation and biofuel production. *BMC Microbiology*. 2016;16(1):244. doi: 10.1186/s12866-016-0860-y

Zotarelli L, Zatorre NP, Boddey RM, Urquiaga S, Jantalia CP, Franchini JC, et al. Influence of no-tillage and frequency of a green manure legume in crop rotations for balancing N outputs and preserving soil organic C stocks. *Field Crops Research*. 2012;132:185-95. doi: 10.1016/j.fcr.2011.12.013