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Research Article

Multifunctional characteristics of *Acinetobacter lwoffii* Bac109 for growth promotion and colonization in micropropagated sugarcane[1]

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ABSTRACT

Endophytic bacteria with multifunctional characteristics can benefit plants through different mechanisms, as well as promoting growth in an efficient, low-cost and ecofriendly way. This study analyzed the potential of the multifunctional endophytic isolate *Acinetobacter lwoffii* Bac109 in promoting the early *in vitro* growth of sugarcane seedlings. The Bac109 strain showed potential to solubilize phosphate in a solid medium (solubilization index: 3.73). In addition, the bacterium was an efficient biocontrol agent against the phytopathogenic fungi *Rhizoctonia* sp., *Fusarium oxysporum*, *Phoma* sp. and *Bipolaris papendorfii*, showing a performance equal to or better than the commercial antifungal hygromycin B. An *in vitro* assay confirmed the biofilm production, which increased in the presence of sugarcane root extract. Additionally, *A. lwoffii* Bac109 showed a strong adhesion to the sugarcane roots. The inoculation of this bacterium in micropropagated sugarcane seedlings increased the shoot length (35 %) and regulated the nonphotochemical energy dissipation after 28 days of cultivation. At the end of the experiment, the bacterium showed a great potential for survival, with 5.72 × 107 CFU mL^{-1} recovered from the substrate, what is crucial for plant interaction. The results showed the potential of the biotechnology application for *A. lwoffii* Bac109 by evaluating multifunctional traits of plant growth promotion and by specific interactions with sugarcane, which may help to improve micropropagation protocols for this crop.

KEYWORDS: *Saccharum* sp., biofilm, biocontrol, cross-inoculation, plant growth promoting bacteria.

RESUMO

Características multifuncionais de *Acinetobacter lwoffii* Bac109 para promoção de crescimento e colonização em cana-de-açúcar micropropagada

Bactérias endofíticas com características multifuncionais podem beneficiar as plantas por meio de diferentes mecanismos e promover o crescimento de forma eficiente, econômica e ecologicamente correta. Neste estudo, foi investigado o potencial do isolado endofítico multifuncional *Acinetobacter lwoffii* Bac109, com o objetivo de promover o crescimento de mudas de cana-de-açúcar *in vitro*. A linhagem Bac109 demonstrou capacidade de solubilização de fosfato em meio sólido (índice de solubilização: 3,73). Além disso, a bactéria foi um eficiente agente de biocontrole contra os fungos fitopatogênicos *Rhizoctonia* sp., *Fusarium oxysporum*, *Phoma* sp. e *Bipolaris papendorfii*, apresentando desempenho superior ou igual ao antifúngico comercial higromicina B. A produção de biofilme foi confirmada em ensaio *in vitro* e foi maior na presença de extrato de raiz de cana-de-açúcar. Além disso, *A. lwoffii* Bac109 apresentou forte adesão às raízes da cana-de-açúcar. A inoculação da bactéria em mudas micropropagadas de cana-de-açúcar aumentou o comprimento da parte aérea (35 %) e a dissipação de energia não fotoquímica regulada após 28 dias de cultivo. Ao término do experimento, a bactéria apresentou grande potencial de sobrevivência, com 5,72 × 10^7 UFC mL^{-1} recuperadas do substrato, o que é crucial para a interação da planta. Os resultados mostraram potencial de aplicação biotecnológica para *A. lwoffii* Bac109 pela avaliação dos traços multifuncionais para a promoção do crescimento vegetal e por interações específicas com a cana-de-açúcar, as quais podem auxiliar no aprimoramento de protocolos de micropropagação da cultura.

PALAVRAS-CHAVE: *Saccharum* sp., biofilme, biocontrole, inoculação cruzada, bactérias promotoras de crescimento de plantas.

INTRODUCTION

Brazil is the world's largest producer and exporter of sugarcane (*Saccharum* sp.) (Conab 2019). This crop is of great importance to the Brazilian economy, generating millions of dollars annually through the production of sugar, ethanol, bagasse and other derivatives (Barnabas et al. 2015). In this context, researchers have delevoped new sugarcane propagation methods to reduce planting material, improve seedling health and increase yield (Ali et al. 2008).

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Micropropagation protocols have been developed for the large scale production of sugarcane, with techniques that allow to obtain uniform clones with high phytosanitary standards, as well as reduced time and space requirements (Kumari et al. 2017). However, their use may affect plant growth promoting microorganisms by reducing the endophytic bacteria population. These bacteria inhabit the interior of plant tissues, and their (re)introduction can benefit plant growth, accelerating its development, protecting against pathogens and reducing production costs (Pereira et al. 2019).

Beneficial plant-microorganism interactions can occur through different mechanisms, such as nutrient solubilization, phytohormone production, nitrogen fixation and production of antimicrobial compounds for pathogen biocontrol (Rohini et al. 2018).

Phytopathogenic fungi like *Fusarium* sp., *Phoma* sp., *Rhizoctonia* sp. and *Bipolaris* sp. can cause several diseases in sugarcane, generating yield losses (Raza et al. 2019). *F. oxysporum* causes rot and vascular wilt in several crops, including tomato, onion and cotton (Armitage et al. 2018). Moreover, *Rhizoctonia* sp. causes damping-off and root rot in a wide range of hosts (Ajayi-Oyetunde & Bradley 2018). In a study in southern China, *Bipolaris* and *Phoma* were in the top five common genera of plant pathogenic fungi affecting sugarcane (Raza et al. 2019). Inhibiting the growth of such fungi by bacteria via competition or through the production of bioactive secondary metabolites can benefit plant development by indirectly promoting its growth.

As observed in micropropagated sugarcane (Barreto et al. 2019) and cashew trees (Faria et al. 2021), the introduction of growth-promoting endophytic microorganisms in micropropagated plants can decrease contamination losses and increase seedling survival from their establishment and acclimatization to field planting. This bacterium-plant interaction contributes to sustainable agriculture, since it minimizes the use of inducing compounds and chemical pesticides, also resulting in economic gains for the producer (Silveira et al. 2018).

Phosphorus is an essential macronutrient for plant growth. However, the element is not readily available in the soil for plants (Lobo et al. 2019). Thus, the use of phosphorus-solubilizing microorganisms can be a useful tool for agriculture to reduce the use of mineral fertilizers. Several bacterial genera can solubilize phosphate (Pande et al. 2017), with the genus *Acinetobacter* standing out for having multiple functional traits for plant growth promotion. These gram-negative bacteria can colonize diverse habitats, including water, soil, plants, animals and humans (Nemec et al. 2019).

In addition to phosphate solubilization, these bacteria act in the production of indole-3-acetic acid (IAA); biological nitrogen fixation (Kuan et al. 2016); production of siderophores and biocontrol against plant pathogens (Zhao et al. 2014); synthesis of the enzyme ACC (1-aminocyclopropane-1-carboxylate) deaminase, thus reducing ethylene levels in plants to mitigate adverse stresses while indirectly promoting plant growth (Ahemad & Kibret 2014); and growth promotion of monocots and dicotyledons (Zhang et al. 2017).

Several studies have shown that bacteria of the genus *Acinetobacter* have multiple functional traits for plant growth promotion, such as calcium phosphate solubilization, siderophore production, antibiosis and auxin synthesis (Rokhbakhsh-Zamin et al. 2011, Oliveira-Longatti et al. 2014, Kandel et al. 2017). *Acinetobacter* occurs in association with the rhizosphere and as endophytic bacteria of sugarcane roots, stems and leaves (Velázquez et al. 2008). This indicates the existence of a bacterium-plant interaction that developed throughout evolutionary history (Awais et al. 2017, Santos et al. 2017, Armanhi et al. 2018). However, *A. lwoffii* Bac109 has never been tested for its ability to promote sugarcane growth.

The *Acinetobacter lwoffii* strain Bac109 is an endophytic isolate from the roots of *Anacardium othonianum* Rizzini (Faria et al. 2021). This plant, native to Brazil and popularly known as *Cerrado* cashew, has a great biotechnological potential for plant growth promotion.

Considering the potential of *Acinetobacter* in promoting plant growth, the inoculation of this bacterium into sugarcane may represent a viable and fast alternative for the sustainable development of this crop. Thus, the hypothesis of the present study is that the *A. lwoffii* strain Bac109 has growth-promoting characteristics that can improve the *in vitro* development of sugarcane. In this context, the study evaluated the functional traits and potential of the strain Bac109 for promoting growth in sugarcane seedlings.

MATERIAL AND METHODS

The *A. lwoffii* strain Bac109 used in the present study was isolated by Faria et al. (2021) and

belongs to the collection of the Instituto Federal Goiano, Rio Verde, Goiás state, Brazil (17º48'15.9"S, 50º54'19.5"W). The sugarcane plants (cultivar CTC 04) used in this study were collected at 14 months of field cultivation at the São Martinho Mill (Quirinópolis, Goiás state, Brazil).

The antagonistic capacity of the bacterial strain Bac109 was tested against the phytopathogenic fungi *Rhizoctonia* sp., *Fusarium oxysporum*, *Phoma* sp. and *Bipolaris papendorfii*, which belong to the collection of the Instituto Federal Goiano. The bacterial culture was grown in nutrient broth and adjusted to OD_{600} 1.0. Phytopathogenic fungi were inoculated and incubated with the bacteria as proposed by Felestrino et al. (2017).

The Bac109 and sugarcane roots were prepared according to Paungfoo-Lonhienne et al. (2016). The controls consisted of media with PBS or root extract without the bacterium. The plates were incubated for 48 h, at 28 ºC, with shaking at 3 *g*. Biofilm formation was evaluated by staining with 0.1 % crystal violet. The measurement of the OD_{595} value followed the method by Schembri & Klemm (2001). The assessment of the adhesion of Bac109 to sugarcane roots followed Hozore & Alexander (1991), with 2.48 x 10^8 CFU mL^{-1}.

Sugarcane apices were removed and disinfected according to Dutra et al. (2011). After disinfection, the explants were inoculated into test tubes containing 50 % MS medium (Murashige & Skoog 1962) supplemented with 0.22 mg L^{-1} of 6-benzylaminopurine (BAP) and 0.11 mg L^{-1} of kinetin. The test tubes were kept in the dark for five days, being then kept at 25 ± 2 ºC, with a 16-hour photoperiod and a photon flow density of 27 mmol m^{-2} s^{-1}. Subcultures were performed every 30 days, for 11 months.

The inoculation experiment lasted from May 01 to May 29, 2017. In the experiment, sugarcane seedlings were kept under micropropagation conditions with the bacterial isolate Bac109 in autoclaved sand. The seedlings were cut such that each had shoots of 4 cm and roots of 0.5 cm. At 10 days after subculture, the seedlings were placed in magenta vessels containing sterile sand. The *in vitro* plant growth bioassay had a completely randomized design and consisted of two treatments (with Bac109 inoculation - "Bac109"; and without bacterial inoculation - "control"), with four replicates each, with a plot consisting of two seedlings.

The nutrient solution (Hoagland & Arnon 1939) was used without phosphate salts and with the addition of inorganic calcium phosphate for plant nutrition. Moreover, the Bac109 treatments received 500 µL of saline solution containing 1.0 x 10^8 CFU mL^{-1} of the bacterial strain per sugarcane seedling, by drenching. When the field capacity of the substrate reached 80 %, the seedlings were irrigated with sterile distilled water for the substrate to return to 100 % of field capacity. To this end, the vessels were weighed every two days and maintained in plant growth chambers (Fitotron, SGC 120) at 26 ºC, with 16-h photoperiod, 80 % humidity and light intensity of 50 µmol m^{-2} s^{-1}.

Biometric evaluations were performed 28 days after the inoculation and consisted of measurements of the number of leaves, shoot length, leaf area and root length. The leaf area was measured from images that were processed using the ImageJ software (Schneider et al. 2012). Subsequently, the seedlings were dried in a forced-air oven at 65 ºC to a constant weight, to determine the shoot and root dry weight.

For the determination of inoculum survival, the total bacteria of the substrate were reisolated. Samples (100 mg) of sand near the roots were collected from each treatment 24 h after planting. Serial dilutions of the soil samples were plated on nutrient agar and incubated at 28 ºC, to enumerate the CFUs. A new reisolation was performed at the end of the experiment (28 days).

To confirm the ability of the reisolated bacteria to solubilize calcium phosphate, 10 µL of the 10^{-2} dilution were inoculated in triplicate onto GL medium plates (10 g L^{-1} of glucose, 0.05 g L^{-1} of yeast extract and 15 g L^{-1} of agar), supplemented with $CaHPO_4$. The latter was generated by the addition of 50 mL of a 10 % K_2HPO_4 solution and 100 mL of a 10 % $CaCl_2$ solution. The total phosphorus added to the culture media corresponded to 1.85 g L^{-1}, in the form of insoluble phosphates. The solubilization capacity was determined after three days of incubation at 28 ºC by the formation of a clear halo around the colony (Katznelson & Bose 1959). The experiment was conducted in triplicate and the solubilization index (SI) was calculated according to the formula: SI = halo diameter (cm)/colony diameter (cm).

Chlorophyll *a* fluorescence was assessed using an Imaging-PAM fluorometer and the Imaging Win software (Heinz Walz, Effeltrich, Germany). For this assay, the plants were dark-adapted for at least

30 min; then, 5 cm of the central region of the apical sugarcane leaves were used for analysis. Fluorescence signals at all points of the analyzed leaf area were captured using a charge-coupled device camera. Fluorescence parameters of chlorophyll *a* were determined according to Oxborough & Baker (1997). The calculation of the regulated nonphotochemical energy dissipation [Y (NPQ) = $(F/F_m') - (F/F_m)$] followed Genty et al. (1989). Five replicates were used for this evaluation.

Antagonism, motility and biofilm production tests were performed in triplicate and statistically analyzed using Anova (GraphPad Prism v. 7.03). Biometric data were subjected to the Shapiro-Wilk normality test, using the Sisvar 5.6 software (Ferreira 2011). All analyses were followed by the Tukey test, with $p < 0.05$ considered significant.

RESULTS AND DISCUSSION

A. lwoffii Bac109 showed a biocontrol potential against all the phytopathogens under study (Figure 1). The bacterium inhibited the growth of the fungus *Rhizoctonia* sp. by 27.59 %, in relation to the control (Figures 1A and 1B), whereas hygromycin B inhibited 10.02 % of the fungal growth. Thus, *A. lwoffii* Bac109 significantly inhibited this fungus (more than two-fold), in relation to the commercial antifungal ($p < 0.01$). Moreover, Bac109 and hygromycin B inhibited the *F. oxysporum* growth by 32.28 and 18.74 %, respectively ($p < 0.001$) (Figures 1A and 1C). The bacterium also inhibited the growth of the fungus *Phoma* sp. by 22.12 %, which was statistically equal to the fungal inhibition caused by the antibiotic (19.25 %) (Figures 1A and 1D). For the phytopathogen *B. papendorfii*, the growth inhibition by *A. lwoffii* (34.69 %) was almost four times greater than that by hygromycin B (8.85 %) ($p < 0.0001$) (Figures 1A and 1E).

The coculture assay of *A. lwoffii* Bac109 with the phytopathogenic fungi *Rhizoctonia* sp., *F. oxysporum*, *Phoma* sp. and *B. papendorfii* showed inhibition of fungal growth in relation to the control without bacteria, which was superior or similar to the inhibition caused by the commercial antifungal hygromycin B. Rokhbakhsh-Zamin et al. (2011) had previously shown the biocontrol potential of *Acinetobacter* sp. In their study, 27 bacterial strains of this genus, isolated from the rhizosphere of *Pennisetum glaucum*, inhibited the growth of *F. oxysporum* by 17-86 %. This *in vitro* result for fungal control by Bac109 is an indicative of a plant inoculant.

Biofilm production is a mechanism that bacteria use to colonize plant tissues. Biofilms consist of bacterial communities embedded in a matrix composed of exopolysaccharides, DNA and proteins (Meneses et al. 2011, Paungfoo-Lonhienne et al. 2016). Bacterial biofilms on plant surfaces may promote plant growth, protection or pathogenesis (Bogino et al. 2013). The ability of *A. lwoffii* Bac109 to form biofilms was tested in the absence and presence of sugarcane root extract (Figure 2).

Bac109 produced biofilm in LB. However, the treatment showed a significantly denser crystal violet staining when the bacterium grew in a medium supplemented with sugarcane extract than in the absence of the extract ($p < 0.01$). This result indicates that *A. lwoffii* interacts with sugarcane and that the plant extract induces the formation of even more biofilm by the bacterium. This demonstrates the colonization ability of *A. lwoffii* amongst microorganism-plant interactions. Syed-Ab-Rahman et al. (2018) have also reported the biofilm capacity of *Acinetobacter* sp.

The results of the assays evaluating the adherence of *Acinetobacter* to sugarcane roots demonstrated that an average of 3.54×10^9 and 1.99×10^5 CFU mL^{-1} of the isolate weakly and strongly adhered to the roots, respectively, confirming the interaction between *A. lwoffii* and sugarcane. Researches in the field have aimed to find the efficiency of endophytic bacteria inoculants for application in sugarcane (Naveed et al. 2014, Bach et al. 2016). Among the factors considered in these studies, bacterial adherence to roots is an important characteristic for predicting the ability of a microorganism to colonize roots. *A. lwoffii* Bac109 bacterial cells strongly adhered to sugarcane roots, demonstrating their colonization ability, as well as microorganism-plant interactions. These results agree with those of the biofilm production assay, which revealed an increased *A. lwoffii* Bac109 biofilm formation in the presence of sugarcane root extract.

After 28 days of cultivation, the sugarcane seedlings inoculated with the Bac109 strain increased their shoot length by 35 %, in relation to the control treatment (Figures 3A and 3B), what may be a differential feature for better development in an acclimatization phase. This result may be due to the

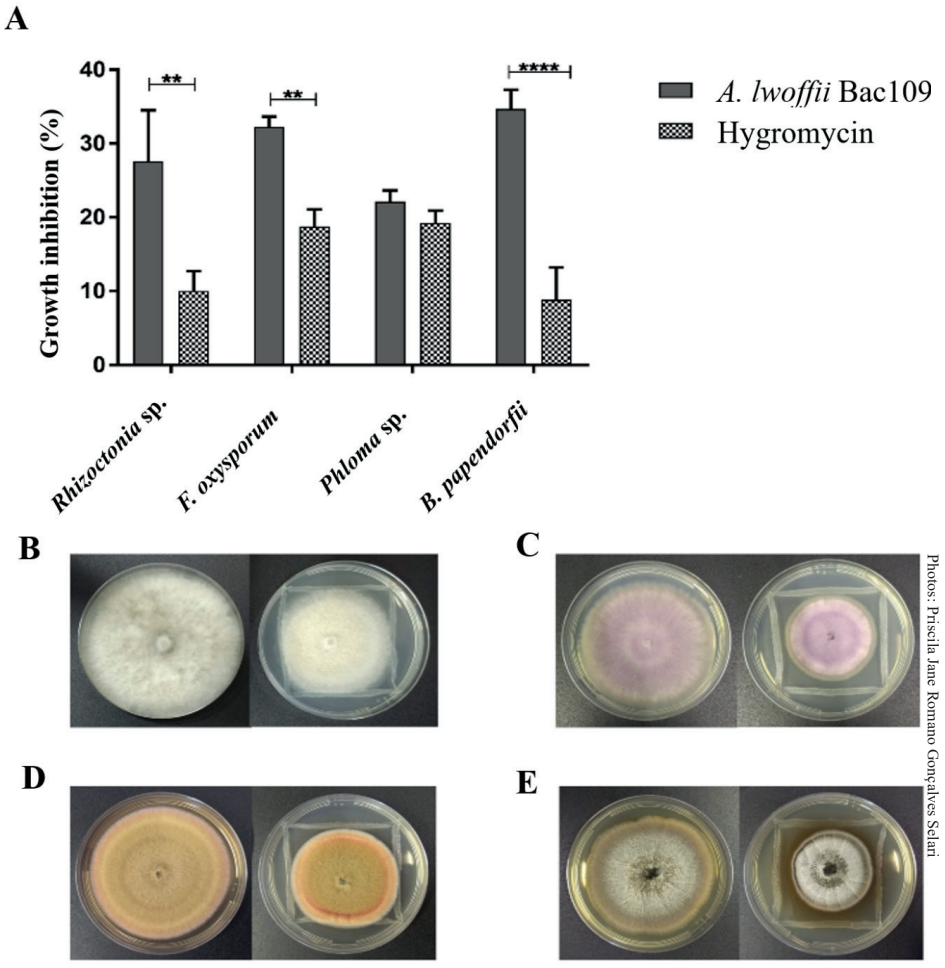

Figure 1. Phytopathogen inhibition analysis. A) percentage of phytopathogenic fungi inhibition in the presence of the bacterium *Acinetobacter lwoffii* strain Bac109 and hygromycin B, relatively to the fungi alone in the control plates. Error bars represent the standard deviation. Asterisks above the lines indicate a significant difference between the bacterial and antibiotic treatments (** $p < 0.001$; **** $p < 0.00001$). Tests without hygromycin B: B) *Rhizoctonia* sp. control plate and *Rhizoctonia versus* Bac109; C) *Fusarium oxysporum* control plate and *Fusarium versus* Bac109; D) *Phoma* sp. control plate and *Phoma versus* Bac109; E) *Bipolaris papendorfii* control plate and *Bipolaris versus* Bac109.

auxin biosynthesis, since *A. lwoffii* Bac109 produces IAA, responsible for promoting cell elongation and increasing the length and number of root hairs (Faria et al. 2021). However, neither the shoot dry weight and shoot/root ratio nor other biometric parameters differed between the treatments. According to the literature, *Acinetobacter* sp. RSC9 also improves sugarcane growth parameters such as plant height, number of leaves, and shoot and root fresh and dry weight (Patel et al. 2021), demonstrating the ability of the bacteria from this genus to promote plant growth.

For the physiological parameters, the nonphotochemical quenching [Y (NPQ)] was higher in inoculated seedlings than in control treatments (Figures 3C and 3D). Chlorophyll *a* fluorescence,

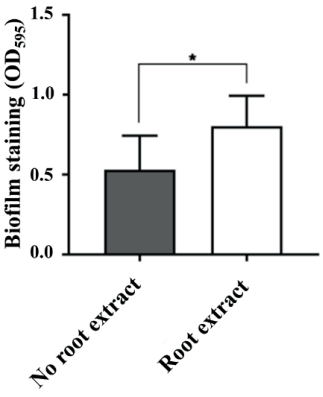

Figure 2. Biofilm formation assay for *Acinetobacter lwoffii* Bac109 cultured in LB broth in the absence or presence of sugarcane root extract. Bars represent the standard deviation. * Significant difference at $p < 0.01$.

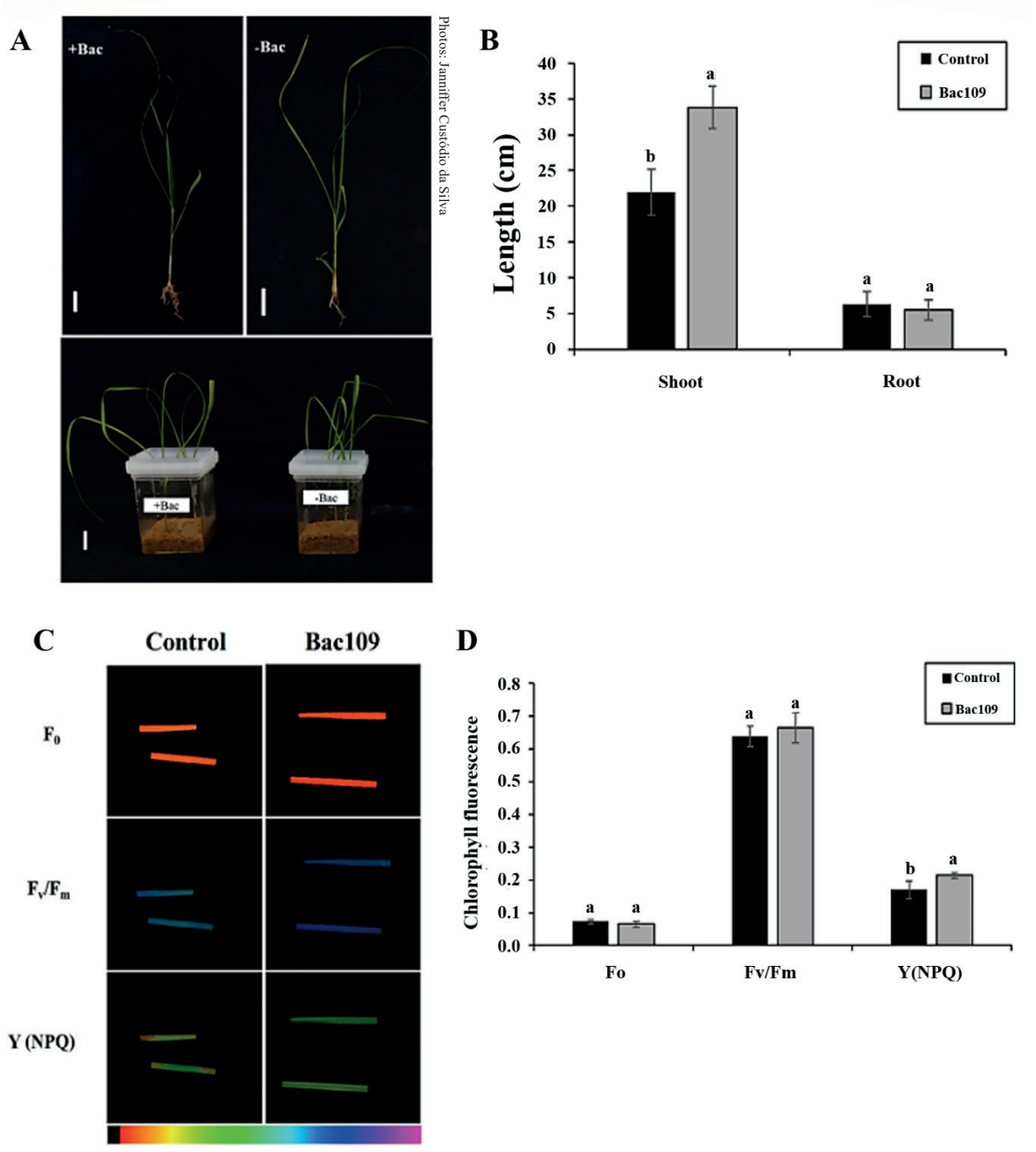

Figure 3. Micropropagated sugarcane seedlings after 28 days of cultivation in chambers inoculated with *Acinetobacter lwoffii* Bac109 (+Bac) or without bacterium (-Bac/Control). A) Seedlings in growth chambers (bar scale: 3.55 cm); B) biometric data: shoot and root length; C) chlorophyll *a* fluorescence [F_0: minimal fluorescence; Fv/Fm: maximum quantum efficiency; Y (NPQ): regulated nonphotochemical energy dissipation]. The color code, from 0 (black) to 1 (pink), is shown at the bottom of the image; D) F_0, Fv/Fm and Y (NPQ) data. Means followed by the same letter do not differ by the Tukey test at 0.05 of probability.

minimum (F_0) and maximum (F_m) fluorescence and maximum quantum efficiency of photosystem II (Fv/Fm), as well other analyzed parameters, did not differ between the treatments.

The physiological performance evaluation of micropropagated sugarcane seedlings showed that the plants did not have a functional photosynthetic apparatus in all treatments. This characteristic is common for micropropagated plants because the plants are in an environment that favors their growth, limiting the development of the photosynthetic apparatus (Matysiak & Gabryszewska 2016).

This characteristic is a major constraint for the adaptation of plants to greenhouse and field conditions. For this reason, the inoculation of beneficial microorganisms before acclimatization could aid in adapting plants to the conditions of the *in vivo* environment.

Despite the evaluations being conducted under *in vitro* conditions, the seedlings showed higher rates of nonphotochemical quenching when inoculated with Bac109. This result could suggest an increase in energy dissipation that improves plant development in a new condition, such as a substrate. The photosystem II efficiency, including NPQ, increased in pepper plants inoculated with *Bacillus* spp., suggesting a better way of disposing of excess light energy, in relation to uninoculated plants (Samaniego-Gámez et al. 2016). Furthermore, the interaction between endophytic bacteria and sugar beets (*Beta vulgaris* L.) improves the photosynthetic capacity of the crop, increasing the carbohydrate synthesis (Shi et al. 2010). In the present study, the sugarcane seedling with higher nonphotochemical quenching also showed an increase in shoot length that may be a consequence of a higher photosynthesis rate.

In addition, the *A. lwoffii* survival was tested at 24 hours after the inoculation and at the end of the experiment by reisolation of the bacteria from the substrate. Counts were 1.53×10^8 and 5.72×10^7 CFU mL^{-1} of *A. lwoffii* Bac109, respectively, confirming the ability of the isolate to survive throughout the experiment. Besides presenting characteristics of plant growth-promoting bacteria, the survival, abundance and persistence of the microorganism in the substrate or plant are fundamental characteristics for the development and establishment of an inoculant for crops (Ambrosini et al. 2016).

Reisolated colonies were semiquantitatively tested for the ability to solubilize phosphate. The results showed that the strain maintained this functional trait, with a mean solubilization halo, colony diameter and SI of 3.11 cm, 0.883 mm and 3.73, respectively.

Phosphate solubilization by microorganisms is a mechanism that directly promotes plant growth. This is because the microorganisms that have this ability can make phosphorus available to the plants from insoluble phosphate, contributing to the plant development (Zeng et al. 2017). A report in the literature shows that *Acinetobacter* sp. UFLA 03-09 solubilizes approximately 20 mg mL^{-1} of $CaHPO_4$ in a liquid medium via acidification of the medium, with a solubilization index similar to that of the Bac109 (3.73) (Marra et al. 2012).

The amount of phosphate solubilized by members of the genus *Acinetobacter* may vary among strains. For example, *Acinetobacter calcoaceticus* D10 solubilized 10.07 mg mL^{-1} of $CaHPO_4$ (Zhao et al. 2014), while *Acinetobacter* sp. ST02 solubilized a maximum of 329.9 µg mL^{-1} of phosphate (Ogut et al. 2010). According to these data, *A. lwoffii* Bac109 stands out within the genus as being highly efficient in phosphate solubilization, improving fertilization by making this nutrient available to plants.

CONCLUSIONS

1. *Acinetobacter lwoffii* Bac109 presents multiple functional traits to promote *in vitro* plant growth. The strain solubilizes inorganic phosphate, presenting an efficient biocontrol against phytopathogenic fungi and increasing biofilm production and the initial sugarcane growth;
2. The isolate adheres to sugarcane roots, allowing the population to survive under micropropagation conditions;
3. The combination of the beneficial traits of the Bac109 strain and its ability to efficiently colonize the plant shows a potential for formulating an inoculant that contributes to sustainable agriculture from the early stages of *in vitro* multiplication.

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REFERENCES

AHEMAD, M.; KIBRET, M. Mechanisms and applications of plant growth promoting rhizobacteria: current perspective. *Journal of King Saud University - Science*, v. 26, n. 1, p. 1-20, 2014.

AJAYI-OYETUNDE, O. O.; BRADLEY, C. A. *Rhizoctonia solani*: taxonomy, population biology and management of Rhizoctonia seedling disease of soybean. *Plant Pathology*, v. 67, n. 1, p. 3-17, 2018.

ALI, A.; NAZ, S.; SIDDIQUI, F. A.; IQBAL, J. An efficient protocol for large scale production of sugarcane through micropropagation. *Pakistan Journal of Botany*, v. 40, n. 1, p. 139-149, 2008.

AMBROSINI, A.; SOUZA, R. de; PASSAGLIA, L. M. P. Ecological role of bacterial inoculants and their potential impact on soil microbial diversity. *Plant and Soil*, v. 400, n. 1-2, p. 193-207, 2016.

ARMANHI, J. S. L.; SOUZA, R. S. C. de; DAMASCENO, N. B.; ARAÚJO, L. M.; IMPERIAL, J.; ARRUDA, P. A community-based culture collection for targeting novel plant growth-promoting bacteria from the sugarcane microbiome. *Frontiers in Plant Science*, v. 8, e2191, 2018.

ARMITAGE, A. D.; TAYLOR, A.; SOBCZYK, M. K.; BAXTER, L.; GREENFIELD, B. P. J.; BATES, H. J.; WILSON, F.; JACKSON, A. C.; OTT, S.; HARRISON, R. J.; CLARKSON, J. P. Characterisation of pathogen-specific regions and novel effector candidates in *Fusarium* 309 *oxysporum* f. sp. *cepae*. *Scientific Reports*, v. 8, e13530, 2018.

AWAIS, M.; TARIQ, M.; ALI, A.; ALI, Q.; KHAN, A.; TABASSUM, B.; NASIR, I. A.; HUSNAIN, T. Isolation, characterization and inter-relationship of phosphate solubilizing bacteria from the rhizosphere of sugarcane and rice. *Biocatalysis and Agricultural Biotechnology*, v. 11, n. 1, p. 312-321, 2017.

BACH, E.; SEGER, G. D. S.; FERNANDES, G. C.; LISBOA, B. B.; PASSAGLIA, L. M. P. Evaluation of biological control and rhizosphere competence of plant growth promoting bacteria. *Applied Soil Ecology*, v. 99, n. 1, p. 141-49, 2016.

BARNABAS, L.; RAMADASS, A.; AMALRAJ, R. S.; PALANIYANDI, M.; RASAPPA, V. Sugarcane proteomics: an update on current status, challenges, and future prospects. *Proteomics*, v. 15, n. 10, p. 1658-1670, 2015.

BARRETO, M. C. S.; FIGUEIREDO, M. V. B.; SILVA, M. V.; OLIVEIRA, J. P.; ANDRADE, A. G.; ALMEIDA, C. M. A.; FERREIRA JÚNIOR, M. U.; SANTOS, C. E. R. S.; REIS JÚNIOR, O. V.; LIMA, V. L. M. Inoculation of endophlytic diazotrophic bacteria in micropropagated seedlings of sugarcane (*Saccharum officinarum* sp.). *Environmental Sustainability*, v. 2, n. 1, p. 5-12, 2019.

BOGINO, P. C.; OLIVA, M. M.; SORROCHE, F. G.; GIORDANO, W. The role of bacterial biofilms and surface components in plant-bacterial associations. *International Journal of Molecular Sciences*, v. 14, n. 8, p. 15838-15859, 2013.

COMPANHIA NACIONAL DE ABASTECIMENTO (Conab). *Safra brasileira de cana-de-açúcar*. 2019. Available at: https://www.conab.gov.br/info-agro/safras/cana. Access on: July 06, 2020.

DUTRA, L. F.; DONINI, L. P.; SILVA, S. D. A.; SILVA, N. D. G. da; THIEL, F. B.; VITORIA, J.; ZACARIAS, F. *Protocolo de micropropagação de cana-de-açúcar*. Pelotas: Embrapa Clima Temperado, 2011.

FARIA, P. S. A.; MARQUES, V. O.; SELARI, P. J. R. G.; MARTINS, P. F.; SILVA, F. G.; SALES, J. F. Multifunctional potential of endophytic bacteria from *Anacardium othonianum* Rizzini in promoting *in vitro* and *ex vitro* plant growth. *Microbiological Reasearch*, v. 242, e126600, 2021.

FELESTRINO, É. B.; SANTIAGO, I. F.; FREITAS, L. D.; ROSA, L. H.; RIBEIRO, S. P.; MOREIRA, L. M. Plant growth promoting bacteria associated with *Langsdorffia hypogaea* rhizosphere-host biological interface: a neglected model of bacterial prospection. *Frontiers in Microbiology*, v. 8, e172, 2017.

FERREIRA, D. F. Sisvar: a computer statistical analysis system. *Ciência e Agrotecnologia*, v. 35, n. 6, p. 1039-1042, 2011.

GENTY, B.; BRIANTAIS, J. M.; BAKER, N. R. The relationship between the quantum yield of photosynthetic electron transport and quenching of chlorophyll fluorescence. *Biochimica et Biophysica Acta (BBA) - General Subjects*, v. 990, n. 1, p. 87-92, 1989.

HOAGLAND, D. R.; ARNON, D. I. *The water-culture method for growing plants without soil*. Berkeley: California Agricultural Experiment Station, 1939.

HOZORE, E.; ALEXANDER, M. Bacterial characteristics important to rhizosphere competence. *Soil Biology and Biochemistry*, v. 23, n. 8, p. 717-723, 1991.

KANDEL, S.; JOUBERT, P.; DOTY, S. Bacterial endophyte colonization and distribution within plants. *Microorganisms*, v. 5, n. 4, e77, 2017.

KATZNELSON, H.; BOSE, B. Metabolic activity and phosphate-dissolving capability of bacterial isolates from wheat roots, rhizosphere, and non-rhizosphere soil. *Canadian Journal of Microbiology*, v. 5, n. 1, p. 79-85, 1959.

KUAN, K. B.; OTHMAN, R.; RAHIM, K. A.; SHAMSUDDIN, Z. H. Plant growth promoting rhizobacteria inoculation to enhance vegetative growth, nitrogen fixation and nitrogen remobilisation of maize under greenhouse conditions. *Plos One*, v. 11, n. 3, e0152478, 2016.

KUMARI, K.; LAL, M.; SAXENA, S. Enhanced micropropagation and tiller formation in sugarcane through pretreatment of explants with thidiazuron (TDZ). *3 Biotech*, v. 7, n. 5, e282, 2017.

LOBO, C. B.; TOMÁS, M. S. J.; VIRUEL, E.; FERRERO, M. A.; LUCCA, M. E. Development of low-cost

formulations of plant growth-promoting bacteria to be used as inoculants in beneficial agricultural technologies. *Microbiological Research*, v. 219, n. 1, p. 12-25, 2019.

MARRA, L. M.; SOARES, C. R. F. S.; OLIVEIRA, S. M. de; FERREIRA, P. A. A.; SOARES, B. L.; CARVALHO, R. F.; LIMA, J. M.; MOREIRA, F. M. S. Biological nitrogen fixation and phosphate solubilization by bacteria isolated from tropical soils. *Plant and Soil*, v. 357, n. 1-2, p. 289-307, 2012.

MATYSIAK, B.; GABRYSZEWSKA, E. The effect of *in vitro* culture conditions on the pattern of maximum photochemical efficiency of photosystem II during acclimatisation of *Helleborus niger* plantlets to *ex vitro* conditions. *Plant Cell, Tissue and Organ Culture*, v. 125, n. 3, p. 585-593, 2016.

MURASHIGE, T.; SKOOG, F. A revised medium for rapid growth and bio assays with tobacco tissue cultures. *Physiologia Plantarum*, v. 15, n. 3, p. 473-497, 1962.

NAVEED, M.; MITTER, B.; YOUSAF, S.; PASTAR, M.; AFZAL, M.; SESSITSCH, A. The endophyte *Enterobacter* sp. FD17: a maize growth enhancer selected based on rigorous testing of plant beneficial traits and colonization characteristics. *Biology and Fertility of Soils*, v. 50, n. 2, p. 249-262, 2014.

NEMEC, A.; RADOLFOVÁ-KŘÍŽOVÁ, L.; MAIXNEROVÁ, M.; NEMEC, M.; CLERMONT, D.; BZDIL, J.; JEŽEK, P.; SPANELOVÁ, P. Revising the taxonomy of the *Acinetobacter lwoffii* group: the description of *Acinetobacter pseudolwoffii* sp. nov. and emended description of *Acinetobacter*. *Systematic and Applied Microbiology*, v. 42, n. 2, p. 159-167, 2019.

OGUT, M.; ER, F.; KANDEMIR, N. Phosphate solubilization potentials of soil *Acinetobacter* strains. *Biology and Fertility of Soils*, v. 46, n. 7, p. 707-715, 2010.

OLIVEIRA-LONGATTI, S. M.; MARRA, L. M.; SOARES, B. L.; BOMFETTI, C. A.; SILVA, K.; FERREIRA, P. A. A.; MOREIRA, F. M. S. Bacteria isolated from soils of the western Amazon and from rehabilitated bauxite-mining areas have potential as plant growth promoters. *World Journal of Microbiology & Biotechnology*, v. 30, n. 4, p. 1239-1250, 2014.

OXBOROUGH, K.; BAKER, N. R. Resolving chlorophyll *a* fluorescence images of photosynthetic efficiency into photochemical and non-photochemical components: calculation of qP and Fv'/Fm' without measuring Fo'. *Photosynthesis Research*, v. 54, n. 2, p. 135-142, 1997.

PANDE, A.; PANDEY, P.; MEHRA, S.; SINGH, M.; KAUSHIK, S. Phenotypic and genotypic characterization of phosphate solubilizing bacteria and their efficiency on the growth of maize. *Journal of Genetic Engineering and Biotechnology*, v. 15, n. 2, p. 379-391, 2017.

PATEL, P.; GAJJAR, H.; JOSHI, B.; KRISHNAMURTHY, R.; AMARESAN, N. Inoculation of salt-tolerant *Acinetobacter* sp. (RSC9) improves the sugarcane (*Saccharum* sp. hybrids) growth under salinity stress condition. *Sugar Tech*, v. 23, n. 4, p. 1-8, 2021.

PAUNGFOO-LONHIENNE, C.; LONHIENNE, T. G. A.; YEOH, Y. K.; DONOSE, B. C.; WEBB, R. I.; PARSONS, J.; LIAO, W.; SAGULENKO, E.; LAKSKMANAN, P.; HUGENHOLTZ, P.; SCHMIDT, S.; RAGAN, M. A. Crosstalk between sugarcane and a plant-growth promoting *Burkholderia* species. *Scientific Reports*, v. 6, n. 1, p. 1-14, 2016.

PEREIRA, L. B.; ANDRADE, G. S.; MENEGHIN, S. P.; VICENTINI, R.; OTTOBONI, L. M. M. Prospecting plant growth-promoting bacteria isolated from the rhizosphere of sugarcane under drought stress. *Current Microbiology*, v. 76, n. 11, p. 1345-1354, 2019.

RAZA, M.; ZHANG, Z. F.; HYDE, K. D.; DIAO, Y. Z.; CAI, L. Culturable plant pathogenic fungi associated with sugarcane in southern China. *Fungal Diversity*, v. 99, n. 1, p. 1-104, 2019.

ROHINI, S.; ASWANI, R.; KANNAN, M.; SYLAS, V. P.; RADHAKRISHNAN, E. K. Culturable endophytic bacteria of ginger rhizome and their remarkable multi-trait plant growth-promoting features. *Current Microbiology*, v. 75, n. 4, p. 505-511, 2018.

ROKHBAKHSH-ZAMIN, F.; SACHDEV, D.; KAZEMI-POUR, N.; ENGINEER, A.; PARDESI, K.; ZINJARDE, S.; DHAKEPHALKAR, P.; CHOPADE, B. A. Characterization of plant-growth-promoting traits of *Acinetobacter* species isolated from rhizosphere of *Pennisetum glaucum*. *Journal of Microbiology and Biotechnology*, v. 21, n. 6, p. 556-566, 2011.

SAMANIEGO-GÁMEZ, B. Y.; GARRUÑA, R.; TUN-SUÁREZ, J. M.; KANTUN-KAN, J.; REYES-RAMÍREZ, A.; CERVANTES-DIAZ, L. *Bacillus* spp. inoculation improves photosystem II efficiency and enhances photosynthesis in pepper plants. *Chilean Journal of Agricultural Research*, v. 76, n. 4, p. 409-416, 2016.

SANTOS, C. M.; SOUZA, D. G.; BALSANELLI, E.; CRUZ, L. M.; SOUZA, E. M.; BALDANI, J. I.; SCHWAB, S. A culture-independent approach to enrich endophytic bacterial cells from sugarcane stems for community characterization. *Microbial Ecology*, v. 74, n. 2, p. 453-465, 2017.

SCHEMBRI, M. A.; KLEMM, P. Biofilm formation in a hydrodynamic environment by novel FimH variants and ramifications for virulence. *Infection and Immunity*, v. 69, n. 3, p. 1322-1328, 2001.

SCHNEIDER, C. A.; RASBAND, W. S.; ELICEIRI, K. W. NIH image to ImageJ: 25 years of image analysis. *Nature Methods*, v. 9, n. 7, p. 671-675, 2012.

SHI, Y.; LOU, K.; LI, C. Growth and photosynthetic efficiency promotion of sugar beet (*Beta vulgaris* L.) by endophytic bacteria. *Photosynthesis Research*, v. 105, n. 1, p. 5-13, 2010.

SILVEIRA, A. P. D. da; IÓRIO, R. de P. F.; MARCOS, F. C. C.; FERNANDES, A. O.; SOUZA, S. A. C. D.; KURAMAE, E. E.; CIPRIANO, M. Exploitation of new endophytic bacteria and their ability to promote sugarcane growth and nitrogen nutrition. *International Journal of General and Molecular Microbiology*, v. 112, n. 2, p. 283-295, 2018.

SYED-AB-RAHMAN, S. F.; CARVALHAIS, L. C.; CHUA, E.; XIAO, Y.; WASS, T. J.; SCHENK, P. M. Identification of soil bacterial isolates suppressing different *Phytophthora* spp. and promoting plant growth. *Frontiers in Plant Science*, v. 9, e1502, 2018.

VELÁZQUEZ, E.; ROJAS, M.; LORITE, M. J.; RIVAS, R.; ZURDO-PIÑEIRO, J. L.; HEYDRICH, M.; BEDMAR, E. J. Genetic diversity of endophytic bacteria which could be find in the apoplastic sap of the medullary parenchym of the stem of healthy sugarcane plants. *Journal of Basic Microbiology*, v. 48, n. 2, p. 118-124, 2008.

ZENG, Q.; WU, X.; WANG, J.; DING, X. Phosphate solubilization and gene expression of phosphate-solubilizing bacterium *Burkholderia multivorans* WS-FJ9 under different levels of soluble phosphate. *Journal of Microbiology and Biotechnology*, v. 27, n. 4, p. 844-855, 2017.

ZHANG, J.; WANG, P.; FANG, L.; ZHANG, Q. A.; YAN, C.; CHEN, J. Isolation and characterization of phosphatesolubilizing bacteria from mushroom residues and their effect on tomato plant growth promotion. *Polish Journal of Microbiology*, v. 66, n. 1, p. 57-65, 2017.

ZHAO, L.; WANG, F.; ZHAO, J. Identification and functional characteristics of chlorpyrifos degrading and plant growth promoting bacterium *Acinetobacter calcoaceticus. Journal of Basic Microbiology*, v. 54, n. 5, p. 457-463, 2014.