

Biochemical characteristics of potential beneficial microorganisms

Características bioquímicas de microrganismos potencialmente benéficos

Gabriella A. Duarte^{1*}, Maria F. G. G. Moura¹, Juliana de F. Machado¹, Rafael B. Oliveira², Dennis R. C. Cruz³, Akintunde A. Ajulo¹, Martha C. C. de Filippi⁴, Adriano S. Nascente⁴

¹Department of Agronomy, Universidade Federal de Goiás, Goiânia, GO, Brazil. ²Pontifícia Universidade Católica de Goiás, Goiânia, GO, Brazil. ³Universidade Estadual de Goiás, Posse, GO, Brazil. ⁴Embrapa Arroz e Feijão, Santo Antônio de Goiás, GO, Brazil.

ABSTRACT - The use of plant growth-promoting bacteria can significantly increase crop grain yield while reducing synthetic inputs and production costs, representing a sustainable agricultural practice. This study aimed to biochemically characterize bacterial strains with potential plant growth-promoting activity. For each biochemical characteristic evaluated (nitrogen fixation, nutrient solubilization, and production of hydrogen cyanide, indole-3-acetic acid (IAA), and biofilm), an experiment was conducted with twenty-nine treatments, namely M01 (*Leclercia adecarboxylata*), M02 (*Priestia megaterium*), M03 (*Pseudomonas* sp.1), M04 (*Pseudomonas* sp.2), M05 (*Enterobacter hormaechei*), M06 (*Bacillus toyonensis*), M07 (*Serratia nematodiphila*), M08 to M28 (combination of microorganisms), and M29 (control - without microorganisms). *Bacillus toyonensis* (M06), *Serratia nematodiphila* (M07), *Enterobacter hormaechei* (M05), and *Priestia megaterium* (M02) exhibited multiple plant growth-promoting traits, including nutrient solubilization, nitrogen fixation, biofilm formation, and IAA production. Among the evaluated treatments, M05, M11, M16, M20, and M24 exhibited the highest IAA production (42.95–49.08 µg/mL). Treatments M12, M17, and M28 showed strong multi-nutrient solubilization capacity. Overall, the results indicate that specific bacterial combinations can enhance biochemical functions and may be strategically selected to improve plant growth-promoting activity.

RESUMO - O uso de bactérias promotoras de crescimento em plantas pode proporcionar incrementos significativos na produtividade das culturas, reduzir o uso de insumos sintéticos e os custos de produção, sendo considerada prática sustentável. Este estudo teve como objetivo caracterizar bioquimicamente bactérias com potencial para a promoção de crescimento de plantas. Para cada característica bioquímica (assimilação de nitrogênio; solubilização de fósforo, óxido de zinco e potássio; produção de ácido cianídrico, ácido indol acético e biofilme) foi realizado um experimento com vinte e nove tratamentos, sendo eles M01 (*Leclercia adecarboxylata*), M02 (*Priestia megaterium*), M03 (*Pseudomonas* sp.1), M04 (*Pseudomonas* sp.2), M05 (*Enterobacter hormaechei*), M06 (*Bacillus toyonensis*), M07 (*Serratia nematodiphila*), M08 a M28 (combinação entre os microrganismos) e M29 (controle – sem microrganismo). Todos os microrganismos, exceto o M01, assimilaram nitrogênio. O fósforo foi solubilizado por todos os microrganismos, isolados e em mistura. Os microrganismos dos tratamentos M01, M06, M07, M08, M11, M12, M13, M17, M18, M26, M27 e M28 solubilizaram zinco. Potássio foi solubilizado pelos microrganismos M02, M03, M04, M06, M08, M09, M10, M12, M14, M15, M17, M19 e M21. Os microrganismos M01, M05 e M07 tanto isolados como combinados produziram biofilme. Com exceção dos microrganismos M01, M06 M12 e M13, todos os outros produziram HCN. Todos os microrganismos produziram ácido indol acético. No geral, os dados sugerem que a combinação de certas cepas bacterianas pode melhorar suas funções bioquímicas. A seleção de combinações específicas de bactérias pode ser estrategicamente adaptada para melhorar as atividades promotoras de crescimento das plantas.

Keywords: Rhizobacteria. Phosphorus solubilization. Indole-3-acetic acid (IAA) production. Nitrogen fixation. Biofilm production.

Palavras-chave: Rizobactérias. Solubilização de fósforo. Produção de ácido indol-acético. Assimilação de nitrogênio. Produção de biofilme.

Conflict of interest: The authors declare no conflict of interest related to the publication of this manuscript.



This work is licensed under a Creative Commons Attribution-CC-BY <https://creativecommons.org/licenses/by/4.0/>

Received for publication in: August 29, 2024.
Accepted in: December 8, 2025.

Editor in Chief: Aurélio Paes Barros Júnior
Section Editor: Flávio Pereira da Mota Silveira

Data Availability: The data that support the findings of this study can be made available, upon reasonable request, from the corresponding author.

***Corresponding author:**
galvesduarte1@gmail.com

INTRODUCTION

The United Nations (2022) estimates that the global population will reach 8.5 billion by 2030 and 9.7 billion by 2050. To keep up with this projected population growth, food production must increase substantially (BASSO; NEVES; SA, 2024), requiring the adoption of sustainable and eco-friendly agricultural practices. In this context, the use of biological inputs has emerged as a promising strategy to enhance agricultural efficiency while maintaining environmental sustainability.

However, to meet the growing demand for microbial applications in agriculture, a detailed characterization of the microorganisms involved is required. Comprehensive analyses of the genetic, biochemical, and physiological characteristics of these microorganisms enable a deeper understanding of their roles in enhancing plant growth, optimizing nutrient uptake, and improving resistance to pathogens. This knowledge can be applied to develop more efficient bioinoculants and management strategies, ultimately leading to higher crop yields while preserving long-term soil health (SINGH et al., 2024).

Plant growth-promoting bacteria interact with plants through direct and indirect mechanisms that stimulate plant growth. Direct mechanisms promote

plant growth through bacterial processes such as phytohormone production (auxins, cytokinins, and gibberellins), which enhance root development and nutrient uptake; biological nitrogen fixation; solubilization of nutrients such as phosphorus, potassium, and zinc; and biofilm formation (OLENSKA et al., 2020). Indirect mechanisms, in turn, refer to the ability of microorganisms to mitigate or suppress the harmful effects caused by phytopathogenic organisms. These indirect mechanisms include competition, induced systemic resistance, and the production of antibiotics, ACC deaminase, hydrogen cyanide (HCN), and siderophores (AJIJAH et al., 2023).

In this context, the development of bioinputs based on beneficial microorganisms and plant growth promoters emerges as a viable and sustainable alternative for cultivation systems, contributing to increased grain yield while aligning with responsible agricultural practices (CHERIF-SILINI et al., 2021). However, few studies have addressed the biochemical characterization of these microorganisms when used in combination. This study aimed to biochemically characterize bacterial strains with potential plant growth-promoting activity and to provide initial support for the

development of bioinput formulations.

MATERIAL AND METHODS

The study was conducted in 2024 at the Agricultural Microbiology Laboratory of Embrapa Rice and Beans, located in Santo Antônio de Goiás, Goiás State, Brazil. The microorganisms used in this study were originally isolated from radish and millet cover crops, as well as from maize. These bacterial isolates were previously screened under laboratory conditions and demonstrated plant growth-promoting potential, including IAA production and the solubilization of potassium, phosphorus, and zinc. The isolates are preserved and deposited under their respective identifications (Table 1) in the Collection of Multifunctional Microorganisms at Embrapa Rice and Beans.

A total of 29 treatments were evaluated, including individual bacterial strains (M01–M07), their pairwise combinations (M08–M28), and a non-inoculated control (M29).

Table 1. Strains used in this study.

Isolate	Species	Crop	Location
MIT3	<i>Leclercia adedecarboxylata</i>	Millet	Jataí – GO, Brazil
BRM69509	<i>Priestia megaterium</i>	Millet	Jataí – GO, Brazil
BRM69536	<i>Pseudomonas</i> sp.	Radish	Jataí – GO, Brazil
BRM69515	<i>Pseudomonas</i> sp.	Radish	Jataí – GO, Brazil
BRM69541	<i>Enterobacter hormaechei</i>	Maize	Jataí – GO, Brazil
BRM32110	<i>Bacillus toyonensis</i>	Rice	Dom Eliseu – PA, Brazil
BRM65920	<i>Serratia marcescens</i>	Rice	Santo Antonio – GO, Brazil

Before experimentation, each bacterial isolate was cultivated on solid nutrient agar medium in Petri dishes to prepare cell suspensions. These suspensions were subsequently transferred to Erlenmeyer flasks containing liquid medium 523 (KADO; HESKETT, 1970). The bacterial suspensions were placed on a shaker and incubated at 28 °C for 24 hours. Afterward, the bacterial concentration was standardized to 1×10^8 colony-forming units (CFU) mL^{-1} by adjusting the spectrophotometric reading to $A_{540} = 0.05$.

A microorganism compatibility test was conducted to determine whether any bacterial strain inhibited the growth of others. Each bacterial combination was inoculated onto nutrient agar plates at a distance of 1 cm between inoculation points, and compatibility was assessed by measuring colony growth using a caliper (HARSHITA et al., 2018). The Student's t-test ($p \leq 0.05$) was employed for the compatibility test. All statistical analyses were performed using the SAS statistical package (SAS INSTITUTE, 1999).

Nitrogen-fixing microorganisms were detected by observing color changes from green to yellow in a nitrogen-free semi-solid medium, as described by Felestrino et al. (2018). The medium is composed of: 5 g of mannitol, 5 g of sucrose, 0.4 g/L of K_2HPO_4 , 0.2 g/L of $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.02 g/L of CaCl_2 , 0.002 g/L of NaMoO_4 , H_2O , 0.01 g/L FeCl_3 , 0.075 g/L of bromothymol blue, and 2.3 g/L of agar, with a pH adjusted to 5. The medium was poured into 5 mL

tubes, and 50 μL of each isolate was pipetted into the tubes. After seven days, any color change noted would be due to bacterial interaction.

Phosphate solubilization: Phosphate solubilization by microorganisms was identified using modified NBRIP medium, according to the methodology adapted from Felestrino et al. (2018). The medium is composed of 10 g/L glucose, 5 g/L $\text{MgCl}_2 \cdot 7\text{H}_2\text{O}$, 0.25 g/L MgSO_4 , 0.2 g/L KCl, 0.1 g/L $(\text{NH}_4)_2\text{SO}_4$, 0.15 g/L phenol red, 15 g/L agar in 850 mL H_2O . Additionally, 100 mL of a 10% K_2HPO_4 solution (10 g) and 50 mL of 10% CaCl_2 solution (5 g) were added, and the pH was adjusted to 7 (SYLVESTER-BRADLEY et al., 1982). The medium was poured into plates, and 5 μL of bacterial suspension was pipetted onto each plate, which was then incubated at 28 °C for 7 days. The formation of a clear halo around the colony indicated phosphate solubilization (CATTELAN, 1999).

Zinc solubilization: the medium used comprised 2 g/L glucose, 0.2 g/L K_2HPO_4 (potassium phosphate dibasic), 1.5 g/L peptone, 0.2 g/L $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ (magnesium sulphate), 2 g/L yeast extract, 1.5 g/L glutamic acid, 1.2 g/L ZnO (zinc oxide), 15 g/L agar, 1 L distilled water, pH adjusted to 6.0. Evaluation involved measuring the diameter of a translucent halo formed around the colonies. (BERRAQUERO; BAYA; CORMENZANA, 1976).

Potassium solubilization: Pikovskaya's medium, with

modifications, was used to determine the potassium-solubilizing bacteria. The medium contains 0.5 g/L yeast extract, 10 g/L glucose, 5 g/L KNO₃, 0.5 g/L (NH₄)₂SO₄, 0.2 g/L KCl, 0.1 g/L MgSO₄, 0.0001 g/L FeSO₄, 0.001 g/L MnSO₄, 15 g/L agar-agar, and 0.026 g/L bromocresol green. Agar was added to the medium after the pH was adjusted to 7. Evaluation was conducted after 7 days of incubation. The formation of a translucent to yellow halo, caused by the acidification of the medium, indicated K solubilization (FERNANDES et al., 2020).

Biofilm production: The treatments were cultured on Congo Red Agar (CRA), modified by Freeman, Falkiner, and Keane (1989). The medium is composed of 28 g of nutrient agar, 50 g of sucrose, 0.8 g of Congo red dye, and 1 L of deionized water. The plates were incubated for 48 hours at 28 °C. Biofilm-producing strains formed rough and black colonies. According to Freeman, Falkiner, and Keane (1989), smooth and red colonies do not produce biofilms when the color of the adjacent medium changes to black.

Hydrogen cyanide (HCN) production: The medium for evaluating the production of HCN is composed of 10 g/L peptone, 5 g/L yeast extract, 5 g/L NaCl, supplemented with 4.4 g/L glycine and 0.081 g/L FeCl₃·6H₂O, stimulants of hydrogen cyanide production. The isolates were cultured for 7 days at 28 °C on separate plates. A filter paper soaked in a mixture of 5% w/v picric acid and 2% w/v Na₂CO₃ was placed on the lid of the petri dish. The change of color of the filter paper from yellow to brown indicated the production of HCN (FELESTRINO et al., 2018).

Indoleacetic acid (IAA) production: The bacterial isolates were cultured in diluted Tryptic Soy Broth (TSB) liquid medium (0.4 g in 100 mL of distilled water) supplemented with 10 g/L glucose, 5 g/L yeast extract, and 1 g/L tryptophan (TANG; BONNER, 1947). The experiment was conducted in a completely randomized design with ten treatments (bacterial isolates + control with TSB medium without bacteria) and three biological and three technical

replicates. The isolates were maintained at 28 °C under constant agitation (150 rpm) for 72 hours in the dark. Afterward, they were centrifuged at 10000 rpm for 10 minutes, and 1 mL of the supernatant was mixed with 5 mL of Salkowski reagent (EHMANN, 1977). The mixture was then transferred to wells of ELISA microplates, followed by absorbance reading at 540 nm using Gen5 software, after 30 minutes in the dark. A change in color from yellow to red-orange in the medium indicated the presence of IAA. The concentrations, in µg/mL, were calculated from a standard curve with known concentrations of the synthetic form of the hormone (0-100 µg/mL) and used to determine the IAA concentration in the samples (OLIVEIRA et al., 2009). An analysis of variance was performed for IAA, and the means were grouped using the Scott-Knott test ($\alpha \leq 0.05$).

RESULTS AND DISCUSSION

All bacterial cultures exhibited normal growth when co-cultured (Table 2). The strains *P. megaterium*, *Pseudomonas* sp.1, *Pseudomonas* sp.2, and *E. hormaechei* were the most compatible, as evidenced by increased colony growth when cultured in consortia with other bacteria. Notably, *E. hormaechei* showed particularly enhanced growth in combination with *L. adecarboxylata*, *P. megaterium*, and *Pseudomonas* sp.1. The only successful pairings for *S. nematodiphila* were with *P. megaterium*, *Pseudomonas* sp.2, and *Pseudomonas* sp.1; reduced growth was observed in combinations with the remaining strains. In contrast, *L. adecarboxylata* and *B. toyonensis* exhibited superior growth when cultured separately. Strain compatibility is crucial for the formulation of effective bioinoculants and must be considered during strain selection, as individual microorganisms may perform poorly when competing with diverse antagonistic microbial populations (SANTIAGO et al., 2017).

Table 2. Compatibility test among multifunctional microorganisms growing in pairs.

Control	M01	M02	M03	M04	M05	M06	M07
	100% a*	100% a	100% a	100% a	100% b	100% a	100% a
M01	-	98.80 b	99.65 a	103.36 a	126.69 a	78.56 b	88.46 b
M02	71.63 b	-	109.08 a	109.99 a	118.23 a	77.13 b	101.81a
M03	70.48 b	94.72 b	-	111.69 a	127.91 a	76.87 b	99.29 a
M04	80.90 b	93.08 b	103.39 a	-	106.81 b	74.54 b	105.67 a
M05	73.05 b	88.60 b	122.94 a	111.12 a	-	74.25 b	98.96 a
M06	70.40 b	80.50 b	104.02 a	117.28 a	105.25 b	-	98.80 b
M07	74.33 b	99.02 b	105.02 a	109.52 a	108.60 b	49.38 c	-

Values in the columns indicate the percentage of growth of the colonies of the microorganism listed when combined with the microorganism listed in the corresponding row; - indicates that no combination was made between the same microorganism.

*Means followed by the same letter in the row do not differ by the T test ($p \leq 0.05$); - means that no combination was made M01: *L. adecarboxylata*; M02: *P. megaterium*; M03: *Pseudomonas* sp.1; M04: *Pseudomonas* sp.2; M05: *E. hormaechei*; M06: *B. toyonensis*; M07: *S. nematodiphila*.

Except for M01, all the other treatments exhibited nitrogen assimilation (Table 3). Across different host plants, dominant genera recognized as nitrogen-fixing endophytic bacteria include *Acetobacter*, *Acinetobacter*, *Arthrobacter*,

Alcaligenes, *Azospirillum*, *Bacillus*, *Bradyrhizobium*, *Burkholderia*, *Enterobacter*, *Frankia*, *Klebsiella*, *Herbaspirillum*, *Rhizobium*, and *Pseudomonas* (RANA et al., 2023). Enhanced nitrogenase activity in the rice rhizosphere

under reduced nitrogen application, compared with higher nitrogen inputs, has been reported following co-inoculation with *Azospirillum brasilense* and *Pseudomonas fluorescens* (ZHANG et al., 2021). Bacteria that can fix nitrogen and

provide it to plants could help reduce nitrogen fertilization, as used in soybean crops, thereby reducing agricultural costs and greenhouse gas emissions, which could improve farmers' revenue.

Table 3. Biochemical characterization of isolate and combined microorganisms.

Treatment	Combination	N	K	Z	P	Biofilm	HCN	IAA ($\mu\text{g mL}^{-1}$)
M01	<i>Leclercia adecarboxylata</i>	-	-	+	+	+	-	7.62 f *
M02	<i>Priestia megaterium</i>	+	+	-	+	-	+	30.37 c
M03	<i>Pseudomonas</i> sp.1	+	+	-	+	-	+	4.16 f
M04	<i>Pseudomonas</i> sp.2	+	+	-	+	-	+	5.47 f
M05	<i>Enterobacter hormaechei</i>	+	-	-	+	+	+	36.63 b
M06	<i>Bacillus toyonensis</i>	+	+	+	+	-	-	1.16 f
M07	<i>Serratia nematodiphila</i>	+	-	-	+	+	+	4.23 f
M08	<i>L. adecarboxylata</i> + <i>P. megaterium</i>	+	+	+	+	+	+	18.23 d
M09	<i>L. adecarboxylata</i> + <i>Pseudomonas</i> sp.1	+	+	-	+	-	+	4.75 f
M10	<i>L. adecarboxylata</i> + <i>Pseudomonas</i> sp.2	+	+	-	+	-	+	7.77 f
M11	<i>L. adecarboxylata</i> + <i>E. hormaechei</i>	+	-	+	+	+	+	42.96 a
M12	<i>L. adecarboxylata</i> + <i>B. toyonensis</i>	+	+	+	+	-	-	9.33 f
M13	<i>L. adecarboxylata</i> + <i>S. nematodiphila</i>	+	-	-	+	+	-	4.09 f
M14	<i>P. megaterium</i> + <i>Pseudomonas</i> sp.1	+	+	-	+	-	+	3.74 f
M15	<i>P. megaterium</i> + <i>Pseudomonas</i> sp.2	+	+	-	+	-	+	22.78 d
M16	<i>P. megaterium</i> + <i>E. hormaechei</i>	+	-	-	+	+	+	49.08 a
M17	<i>P. megaterium</i> + <i>B. toyonensis</i>	+	+	+	+	-	+	3.67 f
M18	<i>P. megaterium</i> + <i>S. nematodiphila</i>	+	-	-	+	+	+	4.75 f
M19	<i>Pseudomonas</i> sp.1 + <i>Pseudomonas</i> sp.2	+	+	-	+	-	+	5.41 f
M20	<i>Pseudomonas</i> sp.1 + <i>E. hormaechei</i>	+	-	-	+	+	+	44.8 a
M21	<i>Pseudomonas</i> sp.1 + <i>B. toyonensis</i>	+	+	-	+	-	+	5.34 f
M22	<i>Pseudomonas</i> sp.1 + <i>S. nematodiphila</i>	+	-	-	+	+	+	7.55 f
M23	<i>Pseudomonas</i> sp.2 + <i>E. hormaechei</i>	+	-	-	+	+	+	46.73 a
M24	<i>Pseudomonas</i> sp.2 + <i>B. toyonensis</i>	+	-	-	+	-	+	13.33 d
M25	<i>Pseudomonas</i> sp.2 + <i>S. nematodiphila</i>	+	-	-	+	+	+	4.37 f
M26	<i>E. hormaechei</i> + <i>B. toyonensis</i>	+	-	+	+	+	+	43.57 a
M27	<i>E. hormaechei</i> + <i>S. nematodiphila</i>	+	-	-	+	+	+	9.45 f
M28	<i>B. toyonensis</i> + <i>S. nematodiphila</i>	+	-	-	+	+	+	3.94 f

N – nitrogen assimilation, K – potassium solubilization, Z – zinc solubilization, P – phosphorus solubilization, Biofilm – biofilm producer, HCN – cyanidric acid producer and IAA – indol acetic acid producer; +: the microorganism or the combination has the biochemical characteristic; -: the microorganism or the combination does not have the biochemical characteristic; * means followed by the same letter do not differ by the Scott-Knott test at $p < 0.05$.

All treatments were capable of phosphorus solubilization (Table 2). A study conducted by Rawat, Shankhdhar, and Shankhdhar (2022) revealed a significant increase in P uptake in upland rice treated with three effective bacterial strains with potent P-solubilizing activity (*Bacillus licheniformis*, *Pantoea dispersa*, and *Staphylococcus* sp.), individual or their consortia, compared to uninoculated control. *Serratia nematodiphila* and *Pseudomonas*

plecoglossicida also have the capacity to solubilize phosphate (JAGTAP et al., 2023). This characteristic is agronomically relevant, as it may reduce the need for phosphorus fertilization in agricultural systems.

Potassium solubilization was observed only in treatments M02, M03, M04, M06, M08, M09, M10, M12, M14, M15, M17, M19, and M21 (Table 2). *Bacillus* spp. and *Pseudomonas* spp. have been reported to be efficient

potassium solubilizers, increasing various parameters such as nutrient uptake, yield, growth, and biomass in different crops, including *Zea mays*, *Sorghum* sp., *Triticum* sp., *Oryza* sp., and *Glycine max* (KOUR et al., 2020). Bacteria that promote potassium solubilization can provide plants with more nutrients and reduce the need for potassium fertilization.

Zinc solubilization was less frequent and was primarily observed in treatments involving *L. adecarboxylata* and *B. toyonensis*, as well as in specific combinations of these species (M08, M11, M12, M17, and M26) (Table 2). Some of the most effective zinc-solubilizing bacterial strains include *Pseudomonas*, *Bacillus*, *Acinetobacter*, *Gluconacetobacter*, *Thiobacillus*, and *Rhizobium*, which can efficiently increase soluble zinc levels in the soil, benefiting plant growth and yield (RANI; RAJINDER; SUKHMINDERJIT, 2020). Zinc-solubilizing bacteria (ZSB) with plant growth-promoting traits can function as bioinoculants, supporting sustainable plant development through multiple mechanisms that are essential for maintaining plant health and productivity (SINGH et al., 2024).

A total of fourteen treatments were able to produce biofilm, including three single strains, (M01 (*L. adecarboxylata*), M05 (*E. hormaechei*), and M07 (*S. nematodiphila*)), and eleven bacterial combinations (M08, M11, M13, M16, M17, M20, M23, M25, M26, M27, and M28) (Table 2). Ajulo (2024) reported *E. hormaechei* and *S. nematodiphila* as biofilm-producing bacteria, which is consistent with our findings. *E. hormaechei* has also been reported to produce biofilm (LI et al., 2022). Biofilm is a crucial characteristic for biological control in plants, as it can enhance biocontrol by protecting colonization sites, increasing the production of antimicrobial compounds, and enhancing the tolerance of beneficial bacteria against biotic and abiotic stresses (AJIJAH et al., 2023).

HCN production was observed in all treatments except those involving *L. adecarboxylata* and *B. toyonensis* as single strains and in the combinations *L. adecarboxylata* + *B. toyonensis* and *L. adecarboxylata* + *S. nematodiphila* (Table 2). *Pseudomonas* sp. and *E. hormaechei* have been reported as producers of hydrogen cyanide by other authors (JAGTAP, 2023). HCN is another important characteristic for biological control in plants, as it acts as a broad-spectrum antimicrobial compound, inhibiting the growth of various plant pathogens.

Among the evaluated treatments, five showed high IAA production, ranging from 42.95 to 49.08 µg/mL (M05, M11, M16, M20, and M24) (Table 2). *E. hormaechei* and *P. megaterium* were the only bacteria to produce high values of IAA. Tomato seeds (*Solanum lycopersicum*) treated with *E. hormaechei* exhibited enhanced biomass and increased shoot length compared to the control. It not only enhanced plant growth but also modified the root architecture, leading to improved crop productivity (RANAWAT et al., 2021). In a study conducted by Zelaya-Molina et al. (2023), a strain of *P. megaterium* also stood out for its production of IAA. IAA is a crucial characteristic that enhances root development, as well-developed roots can explore a larger volume of soil, improving their ability to uptake water and nutrients, enhancing plant resilience, and reducing the need for chemical fertilizers, therefore supporting the development of sustainable agriculture (ETESAMI; GLICK, 2024).

Overall, the tested microorganisms, either individually or in pairs, exhibited key traits associated with plant growth promotion (Table 2). Phytohormone production (auxins,

cytokinins, and gibberellins) can improve root development, thereby enhancing nutrient absorption and increasing grain yield; fixation of nitrogen and solubilization of nutrients in the soil, such as phosphorus, potassium, and zinc, could reduce the need for nutrient fertilization; and production of biofilm and HCN can improve plant resistance to diseases and reduce the need for fungicides. Together, these results highlight both individual strains and microbial consortia with high potential for developing efficient and sustainable bioinoculants. The selection of specific bacterial combinations can be strategically tailored to improve nutrient uptake, plant resilience, and crop productivity.

CONCLUSION

Based on the biochemical characterization, *Bacillus toyonensis* (M06), *Serratia nematodiphila* (M07), *Enterobacter hormaechei* (M05), and *Priestia megaterium* (M02) demonstrated the greatest potential for future applications due to the combined expression of multiple plant growth-promoting traits, including nutrient solubilization, nitrogen fixation, biofilm formation, and indole-3-acetic acid (IAA) production. Among the evaluated treatments, M05, M11, M16, M20, and M24 exhibited the highest IAA production (42.95–49.08 µg/mL), suggesting synergistic effects that enhance auxin-mediated plant growth. Treatments M12, M17, and M28 exhibited strong multi-nutrient solubilization capacity.

REFERENCES

- AJIJAH, N. et al. Plant Growth-Promoting Bacteria (PGPB) with Biofilm-Forming Ability: A Multifaceted Agent for Sustainable Agriculture. **Diversity**, 15: 112, 2023.
- AJULO, A. A. et al. Efficiency of bacterial isolates on suppressing brown spot in rice plants. **Journal of Phytopathology**, 172: 1-13, 2024.
- BASSO, M. F.; NEVES, M. F.; SÁ, M. F. G. Agriculture evolution, sustainability and trends, focusing on Brazilian agribusiness: a review. **Frontiers in Sustainable Food Systems**, 7: 11, 2024.
- BERRAQUERO, F. R.; BAYA, A. M.; CORMENZANA, A. R. Establecimiento de índices para el estudio de la solubilización de fosfatos por bacterias del suelo. **Ars Pharmaceutica**, 17: 399-406, 1976.
- CATTELAN, A. J. **Modos qualitativos para determinação de características bioquímicas e fisiológicas associadas com bactérias promotoras de crescimento vegetal**. Embrapa Soja, 1999. Available at: <<http://www.infoteca.cnptia.embrapa.br/infoteca/handle/doc/461542>>. Access on: July 2, 2024.
- CHERIF-SILINI, H. et al. Tailoring Next Generation Plant Growth Promoting Microorganisms as Versatile Tools beyond Soil Desalinization: A Road Map towards Field Application. **Sustainability**, 13: 4422, 2021.

- EHMANN, A. The Van Urk – Salkowski reagent sensitive and specific chromogenic reagent for silica gel thin-layer chromatographic detection and identification of indole derivatives. **Journal of Chromatography**, 132: 267-276, 1977.
- ETESAMI, H.; GLICK, B. R. Bacterial indole-3-acetic acid: A key regulator for plant growth, plant-microbe interactions, and agricultural adaptive resilience. **Microbiological Research**, 281: 127602, 2024.
- FELESTRINO, E. B. et al. Biotechnological potential of plant growth-promoting bacteria from the roots and rhizospheres of endemic plants in ironstone vegetation in southeastern Brazil. **World Journal of Microbiology and Biotechnology**, 34: 156, 2018.
- FERNANDES, J. P. et al. Physio-agronomic characterization of upland rice inoculated with mix of multifunctional microorganisms. **Revista Caatinga**, 33: 679-689, 2020.
- FREEMAN, D. J.; FALKINER, F. R.; KEANE, C. T. New method for detecting slime production by coagulase negative staphylococci. **Journal of Clinical Pathology**, 42: 872-874, 1989.
- HARSHITA, S. A. et al. Compatibility of fungal and bacterial bio-agents and their antagonistic effect against *Fusarium oxysporum* f. sp. *Lycopersici*. **International Journal of Current Microbiology and Applied Sciences**, 7: 2305-2316, 2018.
- JAGTAP, R. R. et al. Impact of plant growth promoting rhizobacteria *Serratia nematodiphila* RGK and *Pseudomonas plecoglossicida* RGK on secondary metabolites of turmeric rhizome. **Biocatalysis and Agricultural Biotechnology**, 47: 102-622, 2023.
- KADO, C. I.; HESKETT, M. G. Selective media for isolation of *Agrobacterium*, *corynebacterium*, *Erwinia*, *Pseudomonas*, and *Xanthomonas*. **Phytopathology**, 60: 969-976, 1970.
- KOUR, D. et al. Potassium solubilizing and mobilizing microbes: Biodiversity, mechanisms of solubilization, and biotechnological implication for alleviations of abiotic stress. In: RASTEGARI, A. A.; YADAV, A. N.; YADAV, N.(Eds.). **New and Future Developments in Microbial Biotechnology and Bioengineering**. sl: Elsevier, 2020. cap. 11, p. 177–202.
- LI, H. et al. A manganese-oxidizing bacterium-*Enterobacter hormaechei* strain DS02Eh01: Capabilities of Mn (II) immobilization, plant growth promotion and biofilm formation. **Environmental Pollution**, 309: 119775, 2022.
- OLENSKA, E. et al. Beneficial features of plant growth-promoting rhizobacteria for improving plant growth and health in challenging conditions: A methodical review. **Science of the Total Environment**, 743:140682, 2020.
- OLIVEIRA, C. A. et al. Phosphate solubilizing microorganisms isolated from rhizosphere of maize cultivated in an oxisol of the Brazilian Cerrado Biome. **Soil Biology and Biochemistry**, 41: 1782–1787, 2009.
- RANA, K. L. et al. Endophytic nitrogen-fixing bacteria: Untapped treasurer for agricultural sustainability. **Journal of Applied Biology & Biotechnology**, 11: 75-93, 2023.
- RANAWAT, B. et al. *Enterobacter hormaechei* as Plant Growth-Promoting Bacteria for Improvement in *Lycopersicum esculentum*. **Current Microbiology**, 78: 1208-1217, 2021.
- RANI, N.; RAJINDER, K.; SUKHMINDERJIT, K. Zinc solubilizing bacteria to augment Soil Fertility-A Comprehensive Review. **International Journal of Agricultural Sciences and Veterinary Medicine**. 8: 75-79, 2020.
- RAWAT, P.; SHANKHDHAR, D.; SHANKHDHAR, S. C. Synergistic Impact of Phosphate Solubilizing Bacteria and Phosphorus Rates on Growth, Antioxidative Defence System, and Yield Characteristics of Upland Rice (*Oryza sativa* L.). **Journal of Plant Growth Regulation**, 41: 2449-2461, 2022.
- SANTIAGO, C. D. et al. Bacterial Compatibility in Combined Inoculations Enhances the Growth of Potato Seedlings. **Microbes and Environments**, 32: 14-23, 2017.
- SAS INSTITUTE. **Procedure guide for personal computers**. Version 5. Cary: SAS Institute, 1999.
- SINGH, S. et al. Harnessing the Power of Zinc-Solubilizing Bacteria: A Catalyst for a Sustainable Agrosystem. **Bacteria**, 3: 15-29, 2024.
- SYLVESTER-BRADLEY, R. et al. Levantamento quantitativo de microrganismos solubilizadores de fosfatos na rizosfera de gramíneas e leguminosas forrageiras na Amazônia. **Acta Amazonica**, 12: 15–22, 1982.
- TANG, Y. W.; BONNER, J. The enzymatic inactivation of indole-acetic acid. **Archives of Biochemistry and Biophysics**, 13: 11-25, 1947.
- United Nations (UN). **World Population Prospects 2022: Summary of Results**. New York: United Nations, Department of Economic and Social Affairs, Population Division, 2022. Available at: https://www.un.org/development/desa/pd/sites/www.un.org.development.desa.pd/files/wpp2022_summary_of_results.pdf. Accessed on: Aug. 1, 2024.
- ZELAYA-MOLINA, L. X. et al. Plant growth-promoting and heavy metal-resistant *Priestia* and *Bacillus* strains associated with pioneer plants from mine tailings. **Archives of Microbiology**, 205: 318, 2023.
- ZHANG, J. et al. Increased ammonification, nitrogenase, soil respiration and microbial biomass N in the rhizosphere of rice plants inoculated with rhizobacteria. **Journal of Integrative Agriculture**, 20: 2781–2796, 2021.