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Growth promotion of tomato plants through bacteria derived from spent substrate of edible mushrooms

Jessica Albach

albachjessica244@gmail.com

Universidade Estadual do Centro Oeste (UNICENTRO)

Fabiane Gelinski Dalla Rosa

Universidade Estadual do Centro Oeste (UNICENTRO)

Renata Moccellin

Universidade Federal de Pelotas (UFPel)

Cacilda Marcia Duarte Rios Faria

Universidade Estadual do Centro Oeste (UNICENTRO)

João Domingos Rodrigues

Universidade Estadual do Centro Oeste (UNICENTRO)

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Abstract

Tomato is one of the most widely cultivated vegetables worldwide, and Brazil is among its main producers. To meet increasing food demand, it is essential to enhance productivity sustainably. However, the excessive use of chemical fertilizers can cause environmental problems, including soil and water contamination. In this context, alternatives such as plant growth-promoting bacteria (PGPB) and organic residues, like spent mushroom substrate, have gained attention due to their agronomic and environmental benefits. Biochemical and antagonistic tests allow the identification of microorganisms capable of producing important metabolic compounds and contributing to plant development. This study aimed to evaluate, in vitro, the production of biochemical compounds by bacterial isolates obtained from mushroom substrate and to assess their potential to promote tomato growth under greenhouse conditions. Treatments were applied via foliar spraying, seed treatment, and their combination. Twenty bacterial isolates were tested for protease, cellulase, catalase, amylase, lipid hydrolysis, lipolytic activity, siderophore production, and indole-3-acetic acid (IAA) synthesis. Three isolates, BUD 5, BUD 7, and BUD 19, were selected for greenhouse experiments. Evaluated parameters included plant height, chlorophyll fluorescence (PAM), total chlorophyll content, shoot and root biomass, root length, and volume. BUD 5 showed the best performance, when applied spraying or seed treatment. In the first year, BUD 5 and BUD 19 increased root dry mass, while in the second year the control showed higher chlorophyll content after 24 hours, indicating annual variation. BUD 7 and BUD 19 also performed well in root development. These results highlight variable bacterial effects and potential to promote tomato growth under conditions.

Introduction

Tomato (*Solanum lycopersicum* L.), belonging to the family Solanaceae, is one of the most widely produced vegetable crops worldwide. Brazil ranks ninth among the world's largest tomato producers and leads production in South America (FAOSTAT, 2023). According to the Brazilian Institute of Geography and Statistics, the estimated national production for the 2024/25 growing season was approximately 4.7 million tons (IBGE, 2025).

Given population growth and the consequent demand for food, increasing agricultural productivity has become essential. However, this progress must occur in accordance with the principles of sustainable agriculture, considering environmental aspects such as the preservation of ecosystems and biodiversity, as well as economic viability for both producers and consumers (Carrara, 2023).

Although chemical fertilizers contribute to increased production, their continuous use may reduce microbial diversity in the soil and cause negative environmental impacts, such as phosphorus and nitrogen leaching into groundwater, resulting in soil and groundwater contamination (Rafi; Krishnaveni; Charyulu, 2019). In this context, the search for sustainable and economically viable solutions has intensified.

In this context, the use of efficient microorganisms, especially plant growth-promoting bacteria (PGPB), has gained prominence due to their role in nutrient mobilization and cycling. These microorganisms promote plant growth, reduce dependence on mineral fertilizers, contribute to the restoration of soil fertility and structure, and improve plant health in a sustainable manner (Cargnelutti et al., 2021; Neyser et al., 2016; Gamez et al., 2019).

In addition, PGPB can increase plant tolerance to water stress by stimulating antioxidant enzymes and preserving photosynthetic efficiency. Photosynthesis, which is essential for energy production, is highly sensitive to adverse conditions, especially in Photosystem II (PSII), located in the thylakoid membranes (Xu et al., 2019; Mihaljević et al., 2021; Lin; Chen; Zhuang, 2022; García-Sánchez et al., 2007).

PGPB also act by producing indolic compounds, solubilizing phosphate, synthesizing siderophores and enzymes, and colonizing the rhizosphere. These mechanisms contribute to increased plant growth and tolerance to abiotic stresses. Among these bacteria, the genus *Bacillus* stands out for being widely studied and frequently found in the rhizosphere and plant tissues. Species of this genus are capable of promoting plant growth even under adverse environmental conditions (Hashem et al., 2019; Miljaković et al., 2020; Narayanasamy et al., 2020; Manasa et al., 2021; Barrera-Ortiz et al., 2023; Zhu et al., 2023).

Thus, the search for new species and bioactive compounds is essential for the formulation and availability of new products, particularly for tomato disease management. The use of organic residues rich in organic matter has become a source of PGPB. One example is spent mushroom substrate, which is composed of materials such as husks, seeds, wood, and various agricultural by-products combined in specific proportions (Huang et al., 2020).

The objective of this study was to evaluate isolates from spent mushroom substrate that produce metabolic compounds capable of promoting the growth of tomato plants.

Materials and Methods

The *in vitro* biochemical tests were conducted at the Phytopathology Laboratory of the Midwestern State University (*Universidade Estadual do Centro-Oeste*), CEDETEG campus, in Guarapuava, Paraná, Brazil, and the *in vivo* experiment was carried out in a greenhouse at the same institution.

Bacterial isolate collection

Twenty bacterial isolates (BUD 1–BUD 20) belonging to the Phytopathology Laboratory were used in the experiments. These isolates were obtained from spent substrate of edible mushrooms cultivated at the CEDETEG campus.

In vitro experimental design

The biochemical characterization tests were conducted in a completely randomized design (CRD) with twenty bacterial isolates and four replications, over two years.

Biochemical characterization of bacterial isolates

All bacteria were subcultured on nutrient agar medium 24 h before use and maintained in a BOD incubator at 28°C.

Cellulase production

Cellulase production was determined using 1 L of agar medium supplemented with 100 mM carboxymethylcellulose (Kasana et al., 2008). The reagents were mixed, homogenized, and autoclaved for 20 min at 1 atm. The bacterial isolates were spot-inoculated onto Petri dishes containing the cellulose medium and incubated at 28°C for 24 h. Colonies showing a clear halo were considered positive.

Amylase

Amylase production was determined using 1 L of agar medium containing 10 g of starch, homogenized and autoclaved for 20 min. The bacterial isolates were spot-inoculated onto Petri dishes containing the amylase medium and incubated at 28°C for 24 h. Halo formation was considered a positive result (Mariano; Silveira, 2005).

Siderophores

The ability of the biocontrol isolates to compete for Fe^{3+} was evaluated using King B medium (King, 1954) supplemented with chrome azurol blue (Schwyn; Neilands, 1987). The biocontrol isolates were inoculated onto Petri dishes containing the siderophore medium and incubated in the dark at 28°C for seven days. The presence of a yellow halo was considered a positive result.

Indole-3-acetic acid

The isolates were grown in Luria-Bertani broth supplemented with 0.5 mg L^{-1} tryptophan in 50-mL tubes. After incubation, the cultures were centrifuged at 6000 × g for 10 min, and the supernatant was transferred to another 14-mL tube. One milliliter of supernatant was mixed with 4 mL of Salkowski reagent, incubated at room temperature for 30 min in the dark, and the development of a pink/red color was considered a positive result (Sawar and Kremer, 1995; Gordon and Weber, 1951).

Plant growth promotion

The experiment was conducted in a randomized complete block design with 10 treatments and four replications. The experiments were carried out over two growing seasons. The treatments consisted of: seed treatment (ST); foliar application (FA); and seed treatment + foliar application (ST + FA), using the three bacterial isolates that showed the best performance in the *in vitro* tests: BUD 5, BUD 7, and BUD 1, in addition to the absolute control (AC).

The bacterial suspensions (for ST and FA) were prepared in 0.8% NaCl saline solution and adjusted in a spectrophotometer to OD_{540} = 0.6 before treatment application.

The treatments involving seed application of the bacteria were performed by microbiolization. 'Santa Clara' tomato seeds were placed in Erlenmeyer flasks containing 50 mL of the bacterial suspension (BUD 5, BUD 7, and BUD 19) and maintained under agitation at 120 rpm for two hours at room temperature. After this period, the seeds were removed and immediately sown in 1-L pots containing autoclaved substrate, with three seeds per pot. After germination, thinning was performed, leaving only one plant per pot. For the absolute control and the foliar application treatment, the seeds were microbiolized only with sterile water.

The foliar treatments with BUD 5, BUD 7, and BUD 19 were applied 34 days after tomato sowing, both for the FA treatment alone and for the ST + FA treatments. The application was performed using 500-mL hand sprayers, applying 15 mL of the bacterial suspension per plant in a single application. In the control treatments, only sterile distilled water was sprayed.

During the experiment, irrigation was performed using a drip irrigation system three times a day for 10 min each.

The evaluations included plant height every three days starting 19 days after sowing, using a 30-cm graduated ruler and measuring the distance between the plant collar and the shoot apex. Chlorophyll fluorescence was also measured using a pulse-amplitude-modulated fluorometer (PAM) at 6 and 24 h after foliar application. Before the measurements, the leaves were dark-adapted for 10 min using appropriate clips. After dark adaptation, a high-intensity saturating light pulse was applied to determine the maximum photochemical efficiency of photosystem II (Fv/Fm). In addition, the following parameters were obtained: electron transport flux per cross-section (ETo/CSm); variable to initial fluorescence ratio (Fv/F_o); and fluorescence at 50 µs (F50 µs).

At the end of the experiment, morphological and biometric evaluations were performed. Root volume was determined using the water displacement method in a graduated cylinder with previously washed roots free of substrate. Root length was measured using a graduated ruler.

The fresh mass of shoots and roots was determined using an analytical balance immediately after harvest. Subsequently, the plant material was placed in a forced-air circulation oven at 60°C for 24 h and then weighed again to determine dry mass.

The data were subjected to analysis of variance, and the means were compared using the Scott-Knott test at the 95% probability level. Chlorophyll fluorescence (PAM) data were subjected to pairwise comparisons with Bonferroni adjustment at the 95% probability level (Ferreira, 2019).

Results

Biochemical characterization of the bacterial isolates

The production of enzymes and metabolites indicates that these microorganisms may act as biological control agents and/or plant growth-promoting bacteria (Table 1).

Table 1. Production of enzymes and metabolites by bacterial isolates obtained from spent substrate of edible mushrooms.

Bacterial isolates	Cellulase	Amylase	Siderophore	Indole-3-acetic acid (IAA)
BUD-1	+	+	+	-
BUD-2	+	+	+	-
BUD-3	+	+	-	-
BUD-4	+	-	+	-
BUD-5	+	-	+	+
BUD-6	+	+	+	-
BUD-7	+	+	-	+
BUD-8	+	+	+	-
BUD-9	+	+	+	-
BUD-10	+	+	+	-
BUD-11	+	+	-	-
BUD-12	+	+	+	-
BUD-13	+	+	+	-
BUD-14	+	+	+	-
BUD-15	+	+	-	-
BUD-16	+	-	+	-
BUD-17	+	+	+	-
BUD-18	+	+	-	-
BUD-19	+	+	+	-
BUD-20	+	+	-	-

(+) = Positive, compound production
(−) = Negative, no compound production

All isolates exhibited enzymatic and/or metabolic activity. Cellulase production was detected in all isolates, indicating cellulolytic activity and the ability to degrade cellulose. Regarding amylolytic activity,

most isolates tested positive, with the exception of BUD-4, BUD-5, and BUD-16. Siderophore production was observed in the majority of the isolates; however, isolates BUD-3, BUD-7, BUD-11, BUD-15, BUD-18, and BUD-20 did not exhibit the ability to chelate iron. Indole-3-acetic acid (IAA) production was detected only in isolates BUD-5 and BUD-7.

Plant growth promotion

The mean tomato plant height, expressed in centimeters, was evaluated at different assessment dates during the first experimental year. This temporal assessment allowed the monitoring of plant growth dynamics under the influence of the different treatments (Table 2.0).

Table 2.0. Mean tomato plant height recorded throughout the evaluation period under the influence of bacterial isolates obtained from spent substrate of edible mushrooms applied by different methods during the first experimental year.

Treatments	**Days after planting**				
	19	**22**	**25**	**28**	**31**
	Plant height (cm)				
Control	3.306 b	3.700 b	3.950^{ns}	4.481 b	5.375^{ns}
St BUD 5	3.656 b	4.031 a	4.006	4.681 b	6.093
St BUD 7	4.012 a	3.993 a	4.050	4.750 b	5.968
St BUD 19	3.587 b	3.987 a	4.143	4.750 b	5.593
Fa BUD 5	4.050 a	4.268 a	4.500	5.106 a	6.375
Fa BUD 7	3.437 b	3.843 b	4.237	4.525 b	5.406
Fa BUD 19	3.468 b	3.700 b	4.150	4.350 b	5.593
St + Fa BUD 5	3.950 a	4.275 a	4.525	5.206 a	6.437
St + Fa BUD 7	3.593 b	3.875 b	4.068	4.712 b	5.937
St + Fa BUD 19	3.643 b	3.762 b	4.062	4.893 a	6.031

ns = not significant. Means followed by the same letter do not differ significantly according to the Scott–Knott test ($p < 0.05$).ST = Seed treatment; FA = Foliar application; ST + FA = Seed treatment + Foliar application.

At the first evaluation, treatments FA BUD 5, ST BUD 7, and ST + FA BUD 5 showed increases of approximately 22.5%, 21.4%, and 19.5%, respectively, compared with the control.

At the second evaluation, treatments ST + FA BUD 5, FA BUD 5, ST BUD 5, ST BUD 7, and ST BUD 19 exhibited the greatest increases in plant height, approximately 15.54%, 15.35%, 8.95%, 7.92%, and 7.76%, respectively, compared with the control.

At 28 days after planting (DAP), treatments ST + FA BUD 5, FA BUD 5, and ST + FA BUD 19 showed increases of approximately 16.2%, 13.9%, and 9.2%, respectively, relative to the control, demonstrating the positive effect of the bacterial isolates on tomato plant growth.

At 25 DAP, no significant differences were observed among treatments, indicating a trend toward uniform plant growth at this stage of development. Likewise, from 31 DAP until the end of the experimental period, no significant differences were detected among treatments, indicating that the effects observed during the initial evaluations were not maintained throughout the crop cycle. In the second experimental year, no significant differences were observed among treatments in any of the evaluations, demonstrating that the bacterial isolates did not influence plant height during the evaluation period.

The bacterial isolate treatments influenced tomato root development, although the response varied according to the experimental year and the method of isolate application (Table 3.0). Overall, the treatments were effective in promoting plant growth, particularly with respect to root system development.

Table 3.0 Root development of tomato plants inoculated with bacterial isolates derived from spent substrate of edible mushrooms, subjected to different application methods, in the first and second year of the experiment.

Treatments	Root volume	Root length	Root volume	Root length
	(cm^3)	(cm)	(cm^3)	(cm)
	Year 1		Year 2	
Control	13.250 c	29.887^{ns}	11.000 b	28.593 b
St BUD 5	13.750 c	30.812	8.000 d	27.375 b
St BUD 7	11.625 d	28.875	13.500 a	31.531 a
St BUD 19	11.750 d	32.437	12.500 a	27.000 b
Fa BUD 5	12.625 d	33.937	9.500 c	25.406 b
Fa BUD 7	13.500 c	32.718	10.000 c	26.000 b
Fa BUD 19	14.750 c	30.906	7.500 d	31.093 a
St + Fa BUD 5	18.500 a	31.437	8.500 d	27.343 b
St + Fa BUD 7	17.000 b	28.206	10.000 c	28.843 b
St + Fa BUD 19	17.000 b	32.631	10.500 c	27.875 b

ns = not significant. Means followed by the same letter do not differ significantly according to the Scott–Knott test ($p < 0.05$).ST = Seed treatment; FA = Foliar application; ST + FA = Seed treatment + Foliar application.

In the first experimental year, the ST + FA BUD 5 treatment produced the greatest root volume, with an increase of approximately 39.6% compared with the control, whereas root length did not differ significantly among treatments.

In the second experimental year, treatments ST BUD 7 and ST BUD 19 showed increases of approximately 22.7% and 13.6%, respectively, in root volume compared with the control. Regarding root length, treatments ST BUD 7 and FA BUD 19 exhibited increases of approximately 10.3% and 8.7%, respectively, relative to the control, demonstrating the potential of these isolates to stimulate longitudinal root growth.

Fresh and dry root mass were evaluated to determine the effect of the treatments on tomato root development (Table 4.0).

Table 4.0. Mean root fresh and dry mass of tomato plants treated with bacterial isolates obtained from spent substrate of edible mushrooms, applied using different application methods, during the first and second experimental years.

Treatments	Fresh mass	Dry mass	Fresh mass	Dry mass
	(g)	(g)	(g)	(g)
	Year 1		Year 2	
Control	10.652 c	0.502 e	7.772 b	0.720 a
St BUD 5	11.210 c	0.597 d	6.352 c	0.472 b
St BUD 7	8.245 d	0.520 e	8.000 b	0.700 a
St BUD 19	10.130 c	0.532 e	9.112 a	0.725 a
Fa BUD 5	15.825 a	0.805 c	6.612 c	0.540 b
Fa BUD 7	11.772 c	0.610 d	7.340 b	0.630 a
Fa BUD 19	10.432 c	0.570 d	5.980 c	0.517 b
St + Fa BUD 5	14.852 b	0.925 b	8.112 b	0.687 a
St + Fa BUD 7	14.195 b	0.795 c	5.805 c	0.435 b
St + Fa BUD 19	16.005 a	1.290 a	6.180 c	0.540 b

Means followed by the same lowercase letter within a column do not differ significantly according to the Scott–Knott test ($p < 0.05$). ST = Seed treatment; FA = Foliar application; ST + FA = Seed treatment + Foliar application.

In the first experimental year, treatments ST + FA BUD 19 and FA BUD 5 increased root fresh mass by approximately 50.3% and 48.6%, respectively, compared with the control, indicating greater biomass accumulation in the roots. The ST + FA BUD 19 treatment produced the highest root dry mass, with an increase of approximately 157.0% relative to the control, demonstrating substantial dry matter accumulation in the root system.

In the second experimental year, treatment ST BUD 19 increased root fresh mass by approximately 17.2% compared with the control, indicating greater root biomass accumulation. Regarding root dry mass in the second year, the Control, ST BUD 7, ST BUD 19, FA BUD 7, and ST + FA BUD 5 treatments exhibited the highest root dry mass values and did not differ statistically from one another.

Table 5.0. Mean shoot fresh and dry mass of tomato plants treated with bacterial isolates obtained from spent substrate of edible mushrooms, applied using different application methods, during the first and second experimental years.

Treatments	Fresh mass	Dry mass	Fresh mass	Dry mass
	(g)	(g)	(g)	(g)
	Year 1		Year 2	
Control	15.747 b	1.750 c	19.860 b	2.487 b
St BUD 5	17.432 b	1.832 c	17.807 c	2.195 c
St BUD 7	16.902 b	1.922 c	24.490 a	2.930 a
St BUD 19	14.837 b	1.732 c	23.835 a	3.030 a
Fa BUD 5	22.822 a	2.635 a	26.572 a	3.102 a
Fa BUD 7	16.225 b	1.885 c	20.737 b	2.440 b
Fa BUD 19	14.910 b	1.660 c	20.460 b	2.465 b
St + Fa BUD 5	18.147 b	2.215 b	15.937 c	1.992 c
St + Fa BUD 7	17.222 b	1.995 c	19.832 b	2.337 b
St + Fa BUD 19	21.057 a	2.382 b	20.602 b	2.525 b

Means followed by the same lowercase letter within a column do not differ significantly according to the Scott–Knott test ($p < 0.05$). ST = Seed treatment; FA = Foliar application; ST + FA = Seed treatment + Foliar application.

In the first experimental year, treatments FA BUD 5 and ST + FA BUD 19 showed the highest shoot fresh mass values, corresponding to increases of approximately 44.9% and 33.7%, respectively, compared with the control, demonstrating the positive effect of these treatments on vegetative plant growth. Treatment FA BUD 5 increased shoot dry mass by approximately 50.6% relative to the control, indicating greater dry matter accumulation and enhanced vegetative development.

In the second experimental year, treatments ST BUD 7, ST BUD 19, and FA BUD 5 increased shoot fresh mass by approximately 23.3%, 20.0%, and 33.8%, respectively, compared with the control, demonstrating greater efficiency in promoting vegetative plant growth. Regarding shoot dry mass in the second year, treatments ST BUD 7, ST BUD 19, and FA BUD 5 exhibited increases of approximately 17.8%, 21.8%, and 24.7%, respectively, relative to the control, indicating greater dry matter accumulation and enhanced vegetative development.

Tomato plants were evaluated for photochemical efficiency, allowing the assessment of the influence of the different treatments on photochemical performance (Table 6.0).

Table 6.0. Mean photochemical efficiency parameters of the photosystem in tomato plants under the influence of bacterial isolates obtained from spent substrate of edible mushrooms applied by different methods.

Treatments	Fv/Fm	Fv/F0	ETo/CSm	F50µs
Control	0,779 a	3,780 a	0,874 ab	0,412 ab
St BUD 5	0,780 a	3,880 ab	0,878 ab	0,400 ab
St BUD 7	0,778 a	3,765 ab	0,770 ab	0,385 ab
St BUD 19	0,778 a	3,999 ab	0,796 ab	0,373 ab
Fa BUD 5	0,745 a	3,089 ab	0,574 ab	0,430 ab
Fa BUD 7	0,795 a	5,817 a	1,065 a	0,410 ab
Fa BUD 19	0,711 b	2,623 b	0,515 b	0,477 a
St + Fa BUD 5	0,799 a	4,301 ab	0,857 ab	0,341 b
St + Fa BUD 7	0,734 a	3,058 ab	0,601 ab	0,434 ab
St + Fa BUD 19	0,800 a	4,304 a	1,042 a	0,403 ab

Means followed by the same letter within a column do not differ significantly according to the Bonferroni-adjusted multiple comparison test ($p \leq 0.05$). ST = Seed treatment; FA = Foliar application; ST + FA = Seed treatment + Foliar application.

The chlorophyll fluorescence parameters demonstrated that the bacterial isolates obtained from spent substrate of edible mushrooms promoted distinct changes in the photochemical performance of tomato plants, varying according to the bacterial isolate and the application method used.

For the Fv/Fm variable, which represents the maximum photochemical efficiency of photosystem II, most treatments showed values similar to the control (0.779). However, treatment FA BUD 7 showed a significant reduction of 12.5% compared with FA BUD 19, which exhibited the highest mean value.

For Fv/F0, a variable related to the potential efficiency of photosystem II, a pronounced positive response was observed in treatment FA BUD 7, corresponding to an increase of approximately 53.9% relative to the control and 121.8% compared with FA BUD 19.

Regarding electron transport (ETo/CSm), treatments FA BUD 7 and ST + FA BUD 19 promoted increases of 106.8% and 102.3%, respectively, compared with treatment FA BUD 19, indicating greater electron transport efficiency and improved photochemical performance of photosystem II. Relative to the control, these treatments showed increases of 21.9% and 19.2%, respectively.

For the F50µs parameter, treatment FA BUD 19 exhibited values 39.9% higher than those observed for ST + FA BUD 5 and 15.8% higher than the control, indicating greater energy accumulation in the reaction centers of photosystem II during the initial stages of chlorophyll fluorescence. This increase may be associated with lower efficiency in the utilization of energy absorbed by the reaction centers, corroborating the lower values observed for Fv/Fm, Fv/F0, and ETo/CSm in this treatment.

Discussion

Seed treatment and foliar application constitute complementary strategies for promoting plant growth, acting at different stages of crop development. Seed treatment promotes physiological preconditioning during imbibition, favoring germination, early vigor, and the induction of a priming state, which increases the ability of plants to respond to stress during the early stages of development (Pasala et al., 2022; Huang et al., 2021; Ibrahim et al., 2022; Baltazar et al., 2021; Meyer et al., 2024; Taha et al., 2025; Mohamed et al., 2026). In contrast, foliar application provides more immediate physiological responses through the direct absorption of bioactive compounds, contributing to the maintenance of photosynthetic activity and plant metabolism during periods of greater physiological demand (Imran et al., 2022; Bakka et al., 2020 and 2022; Mohamed et al., 2026).

The results of this study confirm the complementarity between these strategies, since treatments combining seed treatment and foliar application promoted superior growth responses compared with the individual applications. These findings corroborate studies demonstrating that integrated programs increase nutrient use efficiency, preserve photosynthetic activity, and promote crop growth and productivity (Ceccarelli et al., 2021; Mohamed et al., 2026; Arikan-Algul et al., 2025).

Furthermore, the results highlight the potential of spent mushroom substrate (SMS) as a source of plant growth-promoting rhizobacteria. Due to the high availability of partially degraded organic matter and microbial metabolites, SMS favors the selection of functionally adapted microorganisms, a characteristic confirmed by the performance of isolates BUD 5, BUD 7, and BUD 19. These findings are consistent with Wang et al. (2022), who identified SMS as a promising source of microorganisms for the development of sustainable bioinputs.

Although the intensity of the responses varied between the experimental years, this variation is expected in studies involving PGPR, since factors such as edaphoclimatic conditions, plant physiological status, and soil microbiota composition influence inoculation efficiency (Nemeskéri et al., 2022). Nevertheless, the maintenance of the positive effects of the isolates across both growing seasons demonstrates their functional stability and reinforces their potential for use under different cultivation conditions, highlighting the importance of validation in multiple environments to consolidate their application as sustainable bioinputs.

The biochemical characterization of the isolates obtained from spent mushroom substrate revealed broad metabolic versatility, indicating that the effects observed on plant growth result from the integrated action of different growth-promoting mechanisms. Among these, the production of indole-3-acetic acid (IAA) by isolates BUD 5 and BUD 7 stands out. This phytohormone regulates cell division, elongation, and differentiation, in addition to stimulating the formation of lateral roots and root hairs, thereby increasing water and nutrient uptake (Long et al., 2008; Tiwari et al., 2018; Rubio et al., 2000; Ayyaz et al., 2016; Rina Sriwati et al., 2023; Kumar; Pater).

The results of the present study support this mechanism, as the IAA-producing isolates promoted significant increases in plant height at 19 days after planting, with foliar application of BUD 5 (22.5%) and seed treatment with BUD 7 (21.4%) showing the greatest effects. Similar results were reported by Kang et al. 2019 in tomato plants inoculated with *Leclercia adecarboxylata* MO1, whereas studies with *Providencia rettgeri* ST202 and *Leclercia adecarboxylata* MO1 also associated IAA production with increased plant biomass and greater tolerance to water stress (Taj; Kang).

Another important mechanism observed was siderophore production by isolates BUD 5 and BUD 19. These metabolites increase iron availability to plants while reducing its availability to phytopathogens, simultaneously contributing to plant nutrition and the biological control of diseases such as those caused by *Fusarium oxysporum* (Deb; Tatung, 2024. In addition, all isolates exhibited amylase and cellulase activity, enzymes that play fundamental roles in organic matter degradation and nutrient cycling in the rhizosphere (Sinsabaugh, 2010). According to Pintarič et al. 2024 and Qu et al. 2022, this enzymatic activity increases soil fertility, especially in systems that utilize organic residues.

The origin of the isolates from spent mushroom substrate enhances these mechanisms, since SMS is rich in partially degraded lignocellulose and stabilized organic matter, favoring the selection of microorganisms specialized in residue decomposition and nutrient recycling. According to Hong et al. (2022) and Isman, the incorporation of this residue improves microbial activity, soil fertility, and crop performance.

Taken together, the results indicate that the effects promoted by isolates BUD 5, BUD 7, and BUD 19 result from the combined action of phytohormones, siderophores, and hydrolytic enzymes, mechanisms that promote root development, increase nutrient uptake efficiency, and enhance plant adaptation to environmental variation. This multifunctionality reinforces the high biotechnological potential of these isolates for the development of bioinputs aimed at sustainable agriculture.

The bacterial isolates consistently promoted increases in the vegetative growth of tomato plants, demonstrating that inoculation enhanced plant physiological performance from the early stages of development. Although the magnitude of the responses varied between the experimental years, increases were observed in shoot biomass and root development, indicating that the benefits resulted from the integrated action of different plant growth-promoting mechanisms.

In the shoot, isolate BUD 5 stood out during the first experimental year, promoting increases of 44.9% in fresh mass and 50.6% in dry mass through foliar application. In the second year, the greatest gains were obtained with isolates BUD 7 and BUD 19 applied via seed treatment, which increased fresh mass by 23.3% and 20.0%, respectively. These results suggest that the greater biomass production results from the combined action of phytohormones, increased water and nutrient uptake, siderophore production, and greater photosynthetic efficiency, promoting the assimilation and allocation of photoassimilates for plant growth. Similar results were reported by Imran et al., who observed an increase of approximately 25% in the biomass of tomato plants treated with bioactive compounds, and by Lucas et al., who reported increased carbon assimilation in plants inoculated with *Pseudomonas* species.

The root system responded even more markedly to inoculation. In the first year, the combined seed treatment and foliar application with isolate BUD 5 promoted a 39.6% increase in root volume, whereas seed treatment with BUD 7 increased this variable by 22.7% in the second year. In addition, BUD 7 and BUD 19 increased root length by 10.3% and 8.7%, respectively. These results demonstrate that the isolates promoted not only root expansion but also the functional architecture of the root system, enhancing soil exploration and improving the efficiency of water and nutrient uptake. This behavior is consistent with the findings of Imran et al. 2022., Nemeskéri et al. 2022, and Taj et al. 2025, who associated PGPR inoculation with indole-3-acetic acid production, lateral root development, and increased resource acquisition capacity.

The most pronounced response was observed in root biomass. The Ts + Fa BUD 19 treatment promoted a 157.0% increase in root dry mass, whereas isolates BUD 19 and BUD 5 increased root fresh mass by 50.3% and 48.6%, respectively. The increase in root biomass has important physiological significance, as it enhances the plant's ability to explore the soil, improves water and nutrient uptake, and strengthens the supply of photoassimilates required for plant growth. Moreover, more robust root systems tend to provide greater stability to plant development, particularly under limiting environmental conditions.

The increases observed in this study exceed several reports available for PGPR in tomato crops. Kang et al. 2019 reported a 51.15% increase in root fresh weight following inoculation with *Leclercia adecarboxylata* MO1, whereas Wang et al. (2022) reported increases of up to 82.4% using spent mushroom substrate associated with PGPR. In comparison, the 157.0% increase in root dry mass promoted by BUD 19 demonstrates the high biotechnological potential of the evaluated isolates.

Overall, the results demonstrate that the growth of both the shoot and root system was promoted through the interaction of hormonal, nutritional, and physiological mechanisms induced by the bacterial isolates. This integrated response optimizes resource utilization, strengthens plant establishment, and reinforces the potential of isolates BUD 5, BUD 7, and BUD 19 as promising candidates for the development of bioinputs for sustainable tomato production.

The evaluation of chlorophyll fluorescence demonstrated that inoculation with the bacterial isolates improved the photosynthetic performance of tomato plants, indicating that the benefits of PGPR extend beyond growth promotion and include the maintenance of plant physiological efficiency. Among the evaluated parameters, the maximum photochemical efficiency of photosystem II (Fv/Fm) is an important indicator of the integrity of the reaction centers and the capacity to convert light energy into chemical energy (Lucas; Lobos).

The inoculated treatments maintained Fv/Fm values close to those of the control (0.77), indicating the preservation of photosystem II (PSII) functionality. This response suggests that the isolates contributed to reducing damage to the photosynthetic apparatus, possibly as a result of the integrated action of phytohormones, increased water and nutrient availability, and the activation of antioxidant mechanisms, thereby promoting ATP and NADPH production and carbon assimilation. Similar results were reported by Lucas et al. (2023), who observed greater PSII efficiency and increased biomass in plants inoculated

with PGPR, and by Taj et al. (2025), who demonstrated improvements in stomatal conductance, transpiration, and antioxidant capacity in plants subjected to bacterial inoculation.

Differences were also observed in other photochemical parameters. Isolate BUD 19 promoted a 19.2% increase in electron transport (ETo/CSm), indicating greater efficiency of the electron transport chain and a higher capacity to convert light energy into metabolic energy. This result is directly associated with the remarkable 157.0% increase in root dry mass observed for this treatment, highlighting the close relationship between enhanced photochemical activity and biomass accumulation.

Isolate BUD 7 was distinguished by a 53.9% increase in the Fv/F0 parameter, an indicator of the potential efficiency and structural integrity of PSII. This behavior suggests greater stability of the photosynthetic apparatus and may be associated with the physiological priming phenomenon induced by PGPR, which prepares plants to respond more efficiently to adverse environmental conditions (Taj et al., 2025).

Although environmental factors also influence photochemical efficiency, as demonstrated by Olvera-González et al. (2013) in studies using pulsed LED lighting, the results obtained here indicate that bacterial inoculation enhances the utilization of available light energy. Taken together, the maintenance of Fv/Fm values and the increases in ETo/CSm and Fv/F0 demonstrate that isolates BUD 7 and BUD 19 optimized the photochemical efficiency of tomato plants, providing physiological support for the greater biomass accumulation observed in this study and reinforcing the potential of these microorganisms as plant growth-promoting agents.

Conclusion

The bacterial isolates obtained from spent substrate of edible mushrooms showed potential to promote tomato plant growth, demonstrating the production of enzymes and metabolites associated with plant growth promotion. Among the evaluated isolates, BUD-5, BUD-7, and BUD-19 stood out by providing increases in important agronomic traits, including plant height, root development, biomass accumulation, and photochemical efficiency.

Declarations

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Author Contribution

J.A. conducted the study and wrote the manuscript. F.G. contributed to the practical work and reviewed the manuscript. R.M. contributed to the study and reviewed the manuscript. C.F. and J.D. reviewed the

manuscript.

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