

Article

Soil and Root Responses in Hazelnut Rhizosphere to Inoculate Rhizobacteria Immobilized via JetCutter Technology

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Abstract

Plant growth-promoting rhizobacteria (PGPR) have significant potential for enhancing soil quality and plant growth; however, their agricultural application is limited by challenges such as immobilization and desiccation vulnerability. Background: This study addressed PGPR solid formulation by applying JetCutter-assisted immobilization technology to PGPR strains isolated from the rhizosphere of hazelnut (*Corylus avellana*). Methods: Four immobilized PGPR strains were evaluated under controlled greenhouse conditions: *Serratia proteamaculans*, *Pseudomonas mohnii*, *Pseudomonas baetica*, and *Bacillus safensis*. Their effects on root development, gas exchange parameters, dissolved organic carbon (DOC), and soil enzymatic activities (phosphatase, urease, protease, and β -glucosidase) were assessed. Principal component analysis (PCA) was used to identify the top-performing strain. Results: Treatment with encapsulated bacteria resulted in a 27% increase in DOC compared to controls ($p < 0.05$), while phosphatase and urease activities increased by 35% and 28%, respectively. Root length and volume improved by 18% and 22%, respectively, with PCA identifying *P. baetica* as the most effective strain. Conclusions: Immobilized Gram-negative PGPR strains enhanced root development and soil biochemical activity in hazelnuts, whereas *B. safensis* enhanced photosynthesis but had minimal impact on soil properties. These results highlight functional differences and support the use of PGPR immobilization to promote early plant establishment.

Keywords: alginate encapsulation; rhizosphere microbiome; *Corylus avellana*; soil enzymes; biostimulant formulation



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1. Introduction

Plant growth-promoting rhizobacteria (PGPR) have been widely recognized as environmentally friendly tools to improve root development, stimulate soil enzymatic activity, and support early plant establishment [1,2]. PGPR influences plant growth promotion by

directly improving nutrient status through nitrogen fixation, solubilization of insoluble phosphates, and the production and/or regulation of various phytohormones, including auxins, cytokinins, gibberellins, ABA, SA, and JA [3,4]. At the physiological level, the beneficial effects of PGPR, including enhanced photosynthetic capacity [5–7] and improved yield and productivity [8–10], have been observed in numerous plant species. In recent years, PGPR–plant interactions under suboptimal conditions, such as extreme temperatures, drought, salinity, and nutrient limitations, have attracted attention due to their ability to enhance stress resistance [11]. Given the current challenges in agriculture from climate change and soil degradation, PGPR are increasingly seen as eco-friendly alternatives to chemical fertilizers, acting as key biostimulants and biocontrol agents to promote sustainable agriculture [12]. However, their application in perennial nut crops, such as hazelnuts (*C. avellana*), remains scarce, especially under non-stress conditions. Hazelnuts are agriculturally important and sensitive to drought, which affects photosynthesis and biomass production [13,14]. Although some studies have explored biostimulants for stress mitigation in hazelnut [15], research on Gram-negative strains and encapsulation technologies is still limited.

Several PGPR genera, including *Bacillus*, *Pseudomonas*, and *Serratia*, have demonstrated significant benefits in woody perennials and fruit crops by enhancing nutrient uptake [16–19]. Among PGPR, Gram-negative bacteria such as *S. proteamaculans*, *P. baetica*, and *P. mohnii* have shown promising attributes for root promotion and stress resilience. These bacteria utilize labile, plant-derived carbon sources, facilitating their activity in rhizosphere niches and contributing to nutrient cycling and soil organic matter turnover [20,21]. Moreover, their capacity to synthesize auxins, particularly indole-3-acetic acid (IAA), plays a central role in stimulating root elongation and lateral root formation—traits that are directly linked to enhanced water and nutrient acquisition in crops [22]. Global climate change is increasingly associated with prolonged droughts and reduced water availability, which impair gas exchange and limit plant growth and productivity due to stomatal closure [23–25]. PGPR can alleviate drought-induced limitations on photosynthesis by enhancing plant water use efficiency and supporting carbon assimilation through the sink–source relationship [26–28].

Despite their potential, the application of Gram-negative PGPR in agriculture remains limited. Liquid formulations, commonly used for field inoculation, often lead to decreased microbial viability due to desiccation and temperature fluctuations during storage and transport [29,30]. Gram-negative strains are particularly sensitive to these conditions, which has restricted their commercial development compared to more robust Gram-positive bacteria.

To overcome these limitations, JetCutter technology has emerged as a scalable and precise encapsulation method [31]. This system allows the production of uniform alginate-based microbeads with high bacterial density and viability, which are suitable for field applications as biostimulants. The pre-formed beads can be immersed in concentrated bacterial suspensions, ensuring effective entrapment without compromising metabolic activity [32]. This approach improves formulation stability and broadens the range of strains that can be delivered to the soil.

Although prior work by our group demonstrated the successful immobilization of *B. safensis* using JetCutter, with positive effects under water stress conditions [32], studies evaluating Gram-negative PGPR encapsulated by this method are still scarce. Previous research demonstrated that *Pseudomonas brassicacearum* improved photosynthesis, antioxidant responses, and hormonal balance in walnut (*Juglans regia*) seedlings under drought stress, with better performance than *Bacillus subtilis* [33,34]. Given the physiological similarities between walnuts and hazelnuts, this provides a relevant precedent. However,

the effect of PGPR on hazelnuts under non-stress conditions remains unexplored. In this study, we evaluated these effects using a novel JetCutter-based biostimulant formulation. Understanding their contributions to root development and soil functionality is crucial for designing new microbial formulations tailored to perennial crops.

This study aimed to evaluate the performance of three encapsulated Gram-negative PGPR strains—*S. proteamaculans*, *P. baetica*, and *P. mohnii*—on early root development and soil quality indicators in hazelnuts. The results were compared with those of the reference strain *B. safensis* to establish a functional profile and support the future application of these biostimulants in sustainable crop production.

2. Materials and Methods

2.1. Isolation and Selection of Bacterial Strains

Rhizosphere samples were collected from *C. avellana* orchards in Los Angeles, Chile (37°28'00" S, 72°21'00" W), and those from *C. avellana* cv. Barcelona and *C. giffoni* were grown under controlled conditions in a growth chamber in the laboratory. Rhizospheric soil (1 g) was collected from each sampling site and serial dilutions were performed for bacterial isolation. Morphotypes with typical characteristics of Gram-negative bacteria were selected based on Gram staining and macroscopic colony traits, such as iridescence. *B. safensis*, a strain from our collection, was included in the study as a reference, as it has been used previously [32,35]. Taxonomic identification was conducted via 16S rRNA gene sequencing. DNA was extracted (DNeasy PowerSoil Kit, Qiagen, Hilden, Germany), amplified with the primers 27F/1492R, and sequenced. The identities of the strains were confirmed using EzBioCloud. This process identified *P. mohnii*, *P. baetica*, and *S. proteamaculans*, which were immobilized for further evaluation.

2.2. Immobilization Process of Gram-Negative Bacteria and *Bacillus Safensis*

The immobilization methodology was adapted from Martín-Díaz et al. (2025), integrating modifications from our previous work using JetCutter technology for scale-up [32]. The optimized polymeric formulation, based on Benítez et al. (2024), consisted of 2.5% (*w/w*) medium-viscosity sodium alginate (MGSA) and 1.0% (*w/w*) soluble starch, supplemented with 0.2 g L⁻¹ L-tryptophan [35]. The immobilization process used JetCutter technology as previously reported by our group, with optimized parameters set at a nozzle diameter of 0.9 mm, cutting speed of 3540 rpm, and a flow rate of 10 m s⁻¹. These conditions ensured uniform bead morphology, mechanical stability, and high cell viability. Pre-formed microbeads were cross-linked in a 0.1 M CaCl₂ solution for 20 min, followed by washing with 0.85% (*w/w*) sterile NaCl solution [32].

For bacterial immobilization, microbeads were incubated in 800 mL of saline solution containing 1.34 g of bacterial biomass (*B. safensis*, *P. mohnii*, *S. proteamaculans*, and *P. baetica*). The suspension was agitated gently at 150 rpm for 20 min. Control microbeads were prepared under the same conditions but without bacterial immobilization.

2.3. Preparation and Quantification of Initial Bacterial Inoculum

The initial concentration of the bacterial inoculum in the biostimulant was determined prior to plant inoculation. Approximately 0.1 g of biostimulant was dissolved in 10 mL of disodium citrate buffer (55 mmol L⁻¹ disodium citrate, 30 mmol L⁻¹ EDTA dihydrate, and 150 mmol L⁻¹ NaCl, adjusted to pH 8), following the procedures described by Rojas-Padilla et al. (2022) and Benítez et al. (2024) [35,36]. The suspension was incubated at 28 °C for 24 h under agitation (150 rpm), then vortexed to ensure complete solubilization. Ten-fold serial dilutions (base 10) were prepared and plated on plate count agar to determine viable cell counts, expressed as colony-forming units per gram of product (CFU g⁻¹). These values

corresponded to the initial bacterial loads of the biostimulant and were quantified before inoculation and prior to any physiological or biochemical analyses.

2.4. Extraction and HPLC-Based Quantification of Auxins from JetCutter-Immobilized PGPR Strains

Auxin extraction from the immobilized strains was performed following a modified protocol based on Pan et al. (2010) and Prasad et al. (2010), as previously described in our earlier work [32,35]. In the present study, 0.1 g of biostimulant generated via JetCutter technology, containing each immobilized bacterial strain, was dissolved in 10 mL of sodium citrate buffer (disodium citrate dihydrate). Once fully solubilized, the solution was diluted 1:2 with citrate buffer. A volume of 5 mL from this solution was mixed with 5 mL of 0.4% (v/v) HCl in methanol (1:1, v:v), vortexed briefly, and then centrifuged at 7000 rpm for 10 min at 4 °C. The supernatant was filtered through 0.22 µm syringe filters, and auxin quantification was carried out by high-performance liquid chromatography (HPLC) using a Kromasil® C18 column under the same conditions as described in our previous work. The mobile phase consisted of formic acid in water and acetonitrile at a flow rate of 0.6 mL min⁻¹, with detection at 280 nm.

2.5. Plant Test Conditions

Hazelnut (cv. Barcelona) plantlets, propagated from suckers and maintained for 18 months, were acquired from Agrichile (Maule, Chile). Prior to the experiment, the plants were acclimated for three weeks in a growth chamber under controlled environmental conditions: 16 h light/8 h dark photoperiod, relative humidity of 60%, and photosynthetic photon flux density (PPFD) of 350 µmol m⁻² s⁻¹, consistent with shaded field conditions. Temperature was maintained between 24 and 26 °C during the day and between 14 and 17 °C at night. Air, soil moisture, and temperature were adjusted as required throughout the trial.

The experiment lasted 45 days. Plants were grown in 1.5 L pots and maintained under a 50% irrigation regime to simulate moderate water limitation. Four PGPR strains, *B. safensis*, *P. mohnii*, *P. baetica*, and *S. proteamaculans*, were applied via immobilized microbeads at a dose of 2 g/plant. One gram was placed in a sterile polyethylene tea bag on one side of the root zone and the remaining gram was evenly distributed on the opposite side to ensure symmetrical delivery. The baseline soil (Andisol) characteristics of the substrate used in the experiment were determined prior to inoculation. According to laboratory analysis (Soil and Plant Laboratory, Universidad de Concepción, Concepción, Chile), the soil had a pH of 6.11 (H₂O), an electrical conductivity (EC) of 0.10 dS/m, and an organic matter content of 6.94%. Available nitrogen was 12.4 mg/kg, Olsen phosphorus was 14.6 mg/kg, and exchangeable potassium was 0.21 cmol/kg. These values confirmed that the experimental soil presented moderate fertility and low salinity, suitable for hazelnut growth and microbial activity assessments. The experiment followed a completely randomized design with one factor: bacterial inoculation at six levels (including sterile bead inoculated and uninoculated controls), with five replicates per treatment, totaling 30 experimental units. Soil and plant samples were collected for biological, enzymatic, morphological, and physiological analyses of the plants.

2.6. Determination of Dry Biomass of Plants

For all treatments, plant tissues (leaf, stem, and roots) were dried in an oven at 100 °C for 48 h to remove moisture and determine dry weight. After incubation under these conditions, the dried samples were weighed using an analytical balance (AS220R2 Radwang®, Radom, Poland). Root length and volume measurements were performed

using WinRHIZO Reg software Pro V.2007d (Regent Instruments Inc., Québec, QC, Canada) to ensure accurate assessment of root system development.

2.7. Leaf Gas Exchange and Selected Plant Physiological Parameters

Photosynthetic gas exchange measurements were conducted on fully expanded, healthy leaves (third from the apex) of *C. avellana* var. Barcelona using a CIRAS-2 infrared gas analyzer system (PP Systems, Amesbury, MA, USA). Measurements were performed under a light intensity of $500 \mu\text{mol m}^{-2} \text{s}^{-1}$, and leaf temperature was set at 25°C . After a 10 min of equilibration period under a reference CO_2 concentration of 400 ppm and a leaf-to-air vapor pressure deficit (VPD) between 1.0 and 1.3 kPa, the following parameters were recorded: net photosynthetic rate (P_n , $\mu\text{mol CO}_2 \text{m}^{-2} \text{s}^{-1}$) and stomatal conductance (g_s , $\text{mol H}_2\text{O m}^{-2} \text{s}^{-1}$). Extrinsic water use efficiency (WUE_e) was calculated as the ratio between the net CO_2 assimilation rate (A , in $\mu\text{mol CO}_2 \text{m}^{-2} \text{s}^{-1}$) and the transpiration rate (E , in $\text{mmol H}_2\text{O m}^{-2} \text{s}^{-1}$), according to the formula $\text{WUE}_e = A/E$. Intrinsic efficiency (WUE_i) is determined as the ratio between A and the stomatal conductance to water vapor (g_s , in $\text{mol H}_2\text{O m}^{-2} \text{s}^{-1}$), expressed as $\text{WUE}_i = A/g_s$. Both parameters provide an approximation of the physiological efficiency of plants in relation to water use under water stress and non-water stress conditions. Measurements were obtained from five biological replicates per treatment in a completely randomized design, including uninoculated controls.

2.8. Soil Microbiological Analyses

Soil enzymatic activities were assessed to evaluate the microbiological response to bacterial inoculation. Fresh soil samples were used to determine the activities of dehydrogenase, urease, protease (N- α -benzoyl-L-argininamide [BAA] hydrolyzing), alkaline phosphatase, and β -glucosidase, following standardized colorimetric procedures adapted from Alguacil et al. (2012) [37].

Dehydrogenase activity is expressed as milligrams of iodonitrotetrazolium formazan (INTF) per gram of soil (mg INTF g^{-1}), while urease and protease activities are expressed as millimoles of ammonium released per gram of soil per hour ($\text{mmol N}(\text{NH}_4^+) \text{g}^{-1} \text{h}^{-1}$). Acid phosphatase and β -glucosidase activities are expressed as millimoles of p-nitrophenol (PNF) released per gram of soil per hour ($\text{mmol PNF g}^{-1} \text{h}^{-1}$). Acid phosphatase was measured because the soil samples presented acidic pH values. In addition, dissolved organic carbon (DOC) was measured from 1:5 ($w:v$) soil-to-water extracts using a total organic carbon analyzer (Shimadzu TOC-5050A, Kyoto, Japan), following standard non-purgeable organic carbon procedures (Alef and Nannipieri, 1995) [38]. Microbial biomass carbon (MBC) was quantified using two complementary approaches. The first was the chloroform fumigation–extraction method with 0.5 M K_2SO_4 as extractant, and values are expressed as mg C biomass per kg of dry soil. The second method followed the substrate-induced respiration (SIR) approach described by Anderson and Domsch (1978), using a μ -Trac 4200 infrared gas analyzer (SY-LAB GmbH, Austria) [39]. In this case, glucose was added to fresh soil (2 mg g^{-1}) and the initial respiratory response was used to estimate active microbial biomass. Basal soil respiration was measured in fresh soil samples maintained at 60% of their water-holding capacity and incubated at 22°C . CO_2 evolution was monitored using the same μ -Trac 4200 analyzer and is expressed as mg CO_2 per kg of soil per hour ($\text{mg CO}_2 \text{kg}^{-1} \text{h}^{-1} \text{soil}$), reflecting endogenous microbial activity.

2.9. Scanning Electron Microscopy (SEM) Analysis

Biostimulant particles from each treatment, including the control, were selected to observe the morphological microbeads and the presence of each bacterium immobilized before inoculating the plants. These biostimulants were preserved by immersion in 2.5% v/v glutaraldehyde solution in sodium cacodylate buffer (pH 7.2) for seven days at 4°C . Sub-

sequently, they were rinsed twice with sodium cacodylate (0.1 mol L^{-1}) for 10 min [35]. Stepwise dehydration was performed by immersing the biostimulant in ascending concentrations of ethanol (30, 40, 50, 70, 80, 90, and 100% *v/v*) for 10 min. The samples were then dried at the critical point of CO_2 and mounted on sample holders using a carbon tape (QUORUM K850). Finally, the biostimulants were coated with pure gold (24 carats) using an SPI-MODULE metallizer. The morphology of the biostimulants was analyzed using field emission at 3 kV and magnifications that varied between 50 and 800x. Micrographs of the exterior and cross section of the biostimulants were captured.

2.10. Statistical Analysis

One-way analysis of variance (ANOVA) was performed to evaluate the effects of each immobilized bacterial treatment on dry biomass, physiological parameters (gas exchange), and soil biological indicators. All variables were measured 45 days after inoculation. Significant differences among treatments were assessed using Duncan's post hoc test at a significance level of 0.05. Duncan's test was employed for multiple comparisons because it is particularly useful in pot-based experiments where biological variability among replicates can be greater, allowing clearer detection of treatment-induced differences. Mean values and standard deviations were calculated for each treatment group. Additionally, principal component analysis (PCA) was conducted to explore the multivariate relationships between soil and plant variables and to identify patterns associated with each bacterial strain. All statistical analyses were carried out using IBM SPSS® Statistics (version 25.0, IBM Corp., Armonk, NY, USA), RStudio®, (version 1.4.1106, RStudio, PBC, Boston, MA, USA), and GraphPad Prism® (version 8.0, GraphPad Software, San Diego, CA, USA).

3. Results

3.1. Microbial Viability and IAA Biosynthesis Efficiency After Immobilization

The analysis of colony-forming units (CFU g^{-1}) and auxin (IAA) production revealed notable differences among the immobilized strains. *S. proteamaculans* showed the highest microbial viability with an average of $1.3 \times 10^8 \text{ CFU g}^{-1} \pm 5.0 \times 10^7$, followed by *P. mohnii* ($9.5 \times 10^7 \pm 6.4 \times 10^7 \text{ CFU g}^{-1}$) and *B. safensis* ($3.8 \times 10^7 \pm 3.0 \times 10^7 \text{ CFU g}^{-1}$). In contrast, *P. baetica* displayed the lowest viability ($3.0 \times 10^6 \pm 1.2 \times 10^6 \text{ CFU g}^{-1}$). Interestingly, despite its lower viability, *P. baetica* produced the highest IAA concentration ($30.7 \pm 0.10 \text{ ppm g}^{-1}$), surpassing the reference strains *B. safensis* ($17.3 \pm 0.02 \text{ ppm g}^{-1}$), *P. mohnii* ($13.1 \pm 0.89 \text{ ppm g}^{-1}$), and *S. proteamaculans* ($10.0 \pm 0.94 \text{ ppm g}^{-1}$). These results indicate that *P. baetica* may exhibit high metabolic activity despite reduced cell recovery after immobilization (Figure 1).

3.2. Effect of Immobilized Bacterial Strains on Root Biomass and Volume in Hazelnut Plantlets

The evaluation of root biomass and volume in hazelnuts showed variable trends among treatments with immobilized PGPR. No significant differences were observed among the treatments in the leaf, stem, root, or total dry biomass of hazelnuts (Table 1). However, the highest average dry root biomass was observed in the treatment with *P. baetica* ($11.78 \pm 4.30 \text{ g}$), showing a tendency to increase compared to the control (no beads: $9.37 \pm 1.87 \text{ g}$), although differences were not statistically significant according to Duncan's test ($p > 0.05$) (Figure 2a). Significant differences in root volume were observed among treatments (Duncan's test, $p < 0.05$; Figure 2b). *B. safensis* exhibited the highest volume ($60.7 \pm 9.3 \text{ cm}^3$), significantly greater than *P. mohnii* ($23.3 \pm 3.7 \text{ cm}^3$). Intermediate values were recorded for *S. proteamaculans* ($47.9 \pm 25.9 \text{ cm}^3$), *P. baetica* ($39.6 \pm 8.5 \text{ cm}^3$), and the controls (no beads: $40.4 \pm 12.4 \text{ cm}^3$) and beads without bacteria ($33.0 \pm 18.4 \text{ cm}^3$), with no significant differences from either *B. safensis* or *P. mohnii* (Figure 2b). This trend

was especially notable in *B. safensis* and *P. baetica*, where both biomass and root volume values were consistently high, suggesting potential for enhanced root development under non-stress conditions (Figure 2c).

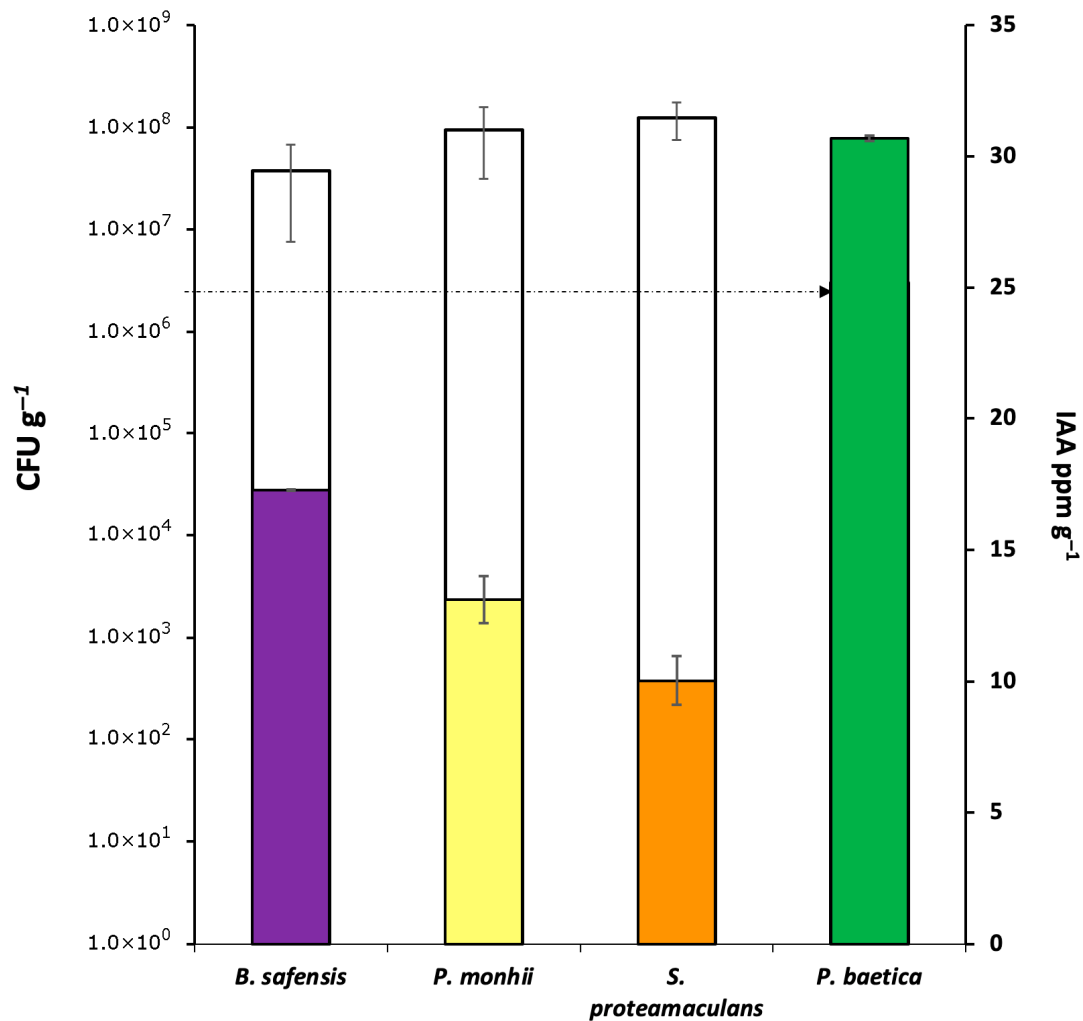


Figure 1. Microbial viability (CFU g⁻¹, white bars) and indole-3-acetic acid (IAA) production (ppm g⁻¹, color bars) of immobilized bacterial strains after 24 h of incubation. Bars represent the mean ± standard error ($n = 3$). No significant differences were detected according to Duncan's test ($p > 0.05$).

Table 1. Mean ($\bar{x}$) and standard error (SE) of dry biomass parameters (leaf, stem, root, and total) in *C. avellana* seedlings subjected to different PGPR treatments and bead formulations. No statistically significant differences were detected among treatments ($p > 0.05$).

	Dry Leaf Biomass (g)		Dry Stem Biomass (g)		Dry Root Biomass (g)		Dry Total Biomass (g)	
	$\bar{x}$	ES	$\bar{x}$	ES	$\bar{x}$	ES	$\bar{x}$	ES
Control (No beads)	7.6	2.45	32.1	16.10	9.3	1.87	49.1	18.99
Beads without Bacteria	11.3	3.50	43.3	11.10	10.6	3.47	65.3	14.14
<i>B. safensis</i>	8.4	1.23	37.8	9.87	10.5	4.10	56.8	10.60
<i>P. mohnii</i>	9.8	1.85	40.9	13.74	8.4	2.32	59.2	14.45
<i>S. proteamaculans</i>	10.0	3.78	40.0	14.10	10.4	3.47	60.5	18.73
<i>P. baetica</i>	7.9	2.12	40.5	10.39	11.7	4.30	60.3	12.85

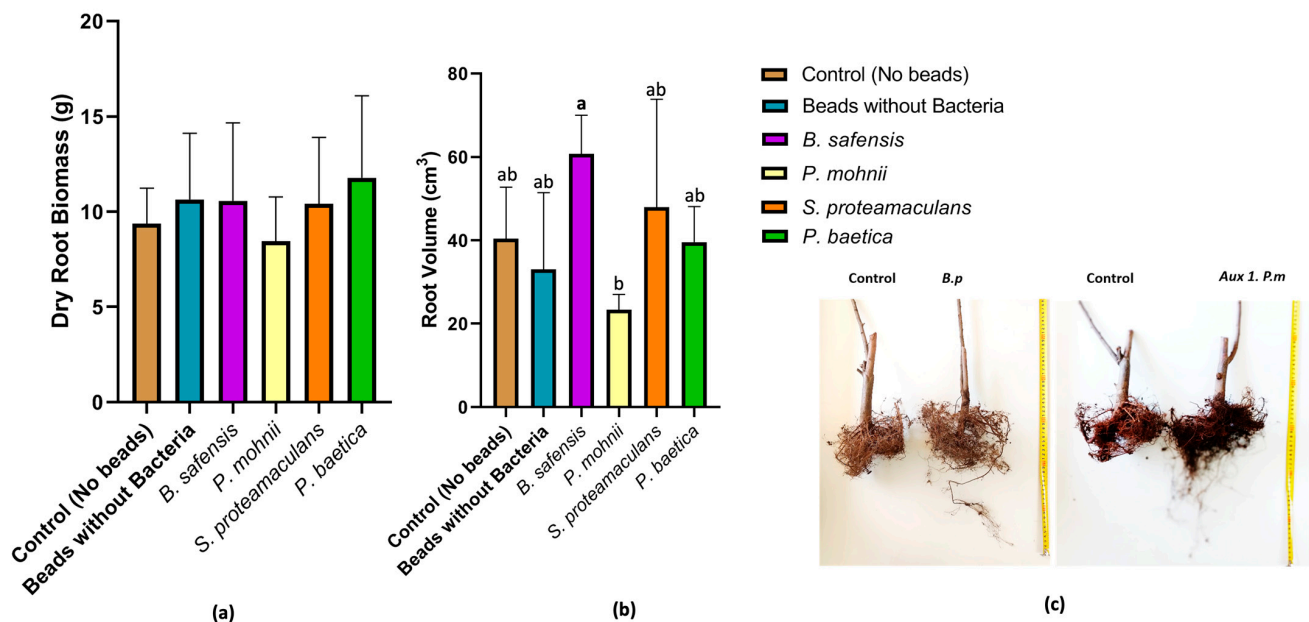


Figure 2. Effect of treatments on root development of *C. avellana* plantlets after 45 days. (a) Dry root biomass, (b) root volume, and (c) representative root systems from selected treatments. The treatments included control (no beads), beads without bacteria, and immobilized *B. safensis* (*B.s*), *P. mohnii* (*P.m*), *S. proteamaculans* (*S.p*), and *P. baetica* (*P.b*). Data represent the standard error of the mean ($n = 5$). Different letters indicate statistically significant differences among treatments ($p < 0.05$), and their absence denotes that no significant differences were detected according to Duncan's test ($p > 0.05$).

3.3. Trends in Photosynthesis, Stomatal Conductance, and Transpiration in Hazelnut Plantlets

The physiological parameters measured in *C. avellana* revealed no statistically significant differences among treatments with immobilized PGPR strains; however, interesting trends were observed. In terms of net photosynthesis, *B. safensis* exhibited the highest average rate ($5.34 \pm 1.52 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$), compared to the control (no beads: $3.76 \pm 1.33 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$) and other bacterial treatments, which ranged between 3.52 and $3.74 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ (Figure 3a).

For transpiration rate, values were consistent across all treatments, ranging from 0.62 to $0.70 \text{ mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$. *P. mohnii* and *P. baetica* both exhibited the highest transpiration values (0.70 ± 0.16 – 0.20), whereas *S. proteamaculans* showed slightly lower rates (0.62 ± 0.08) (Figure 3b). Stomatal conductance followed a similar pattern, with the control treatments (no beads: 65.2 ± 24.7 and beads without bacteria: $65.4 \pm 23.8 \text{ mol H}_2\text{O m}^{-2} \text{ s}^{-1}$) presenting slightly higher values than the inoculated treatments. *B. safensis* showed intermediate conductance ($61.2 \pm 9.7 \text{ mol H}_2\text{O m}^{-2} \text{ s}^{-1}$), whereas *P. mohnii*, *P. baetica*, and *S. proteamaculans* showed slightly lower conductance (55 – $56 \text{ mol H}_2\text{O m}^{-2} \text{ s}^{-1}$) (Figure 3c). Although not statistically significant, these results suggest a potential physiological modulation of hazelnut plantlets following PGPR inoculation under non-stress conditions.

Although photosynthesis, transpiration, and conductance showed no statistically significant differences among treatments, significant differences were observed in WUE_e (Duncan's test, $p < 0.05$) (Figure 3d), where *B. safensis* showed the highest extrinsic efficiency ($8.0 \pm 2.8 \text{ WUE}_e$). In contrast, no significant differences were detected in intrinsic WUE_i (Figure 3e), although *B. safensis* exhibited the highest value ($0.089 \pm 0.033 \text{ WUE}_i$).

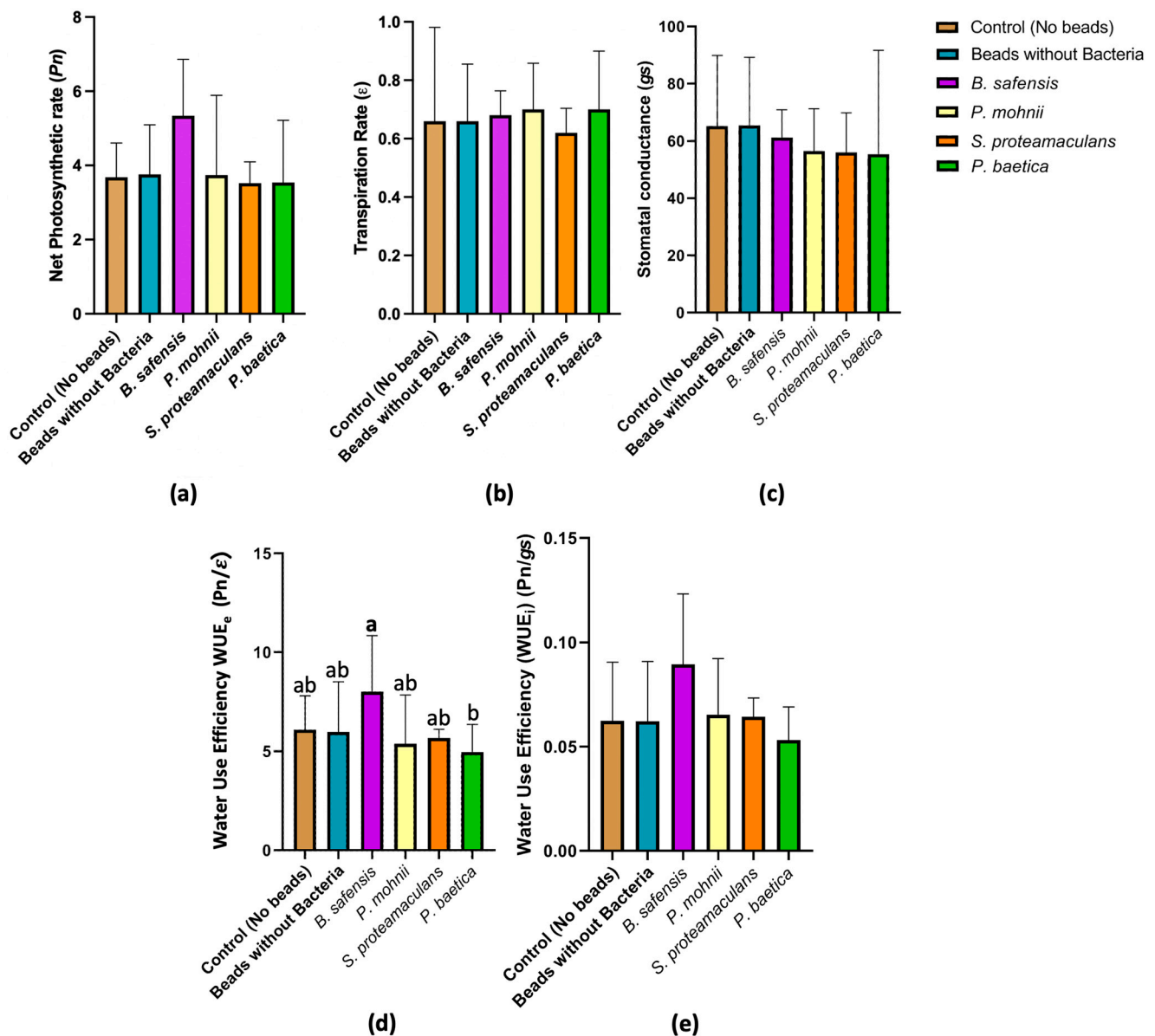


Figure 3. Gas exchange parameters in *Corylus avellana* plantlets after 45 days of treatment with immobilized PGPR strains. (a) Net photosynthesis, (b) transpiration rate, (c) stomatal conductance, (d) water use efficiency, and (e) intrinsic water use efficiency. The treatments included control (no beads), beads without bacteria, and immobilized *Bacillus safensis* (B.s), *Pseudomonas mohnii* (P.m), *Serratia proteamaculans* (S.p), and *Pseudomonas baetica* (P.b). Data are presented as mean $\pm$ standard error of the mean ($n = 5$). No statistically significant differences were detected among the treatments (Duncan's test, $p > 0.05$); in the absence of letter presentation, no statistically significant differences were observed among the treatments.

3.4. Trends in Soil Biochemical Activity

After 45 days of incubation, soil biochemical properties were assessed through dissolved organic carbon (DOC) content, urease activity, and alkaline phosphatase activity (Figure 4a–c). DOC levels exhibited consistent trends across treatments, with the highest value observed in *P. baetica* (2615.9 ± 395.9 g C kg⁻¹ soil), followed by *S. proteamaculans* (2429.1 ± 494.0 g C kg⁻¹) and *B. safensis* (2262.3 ± 446.0 g C kg⁻¹). The control beads displayed the lowest values, particularly in the treatment control (no beads: 1461.5 ± 207.6 g C kg⁻¹). Statistically significant differences were detected among treatments (Duncan's test, $p < 0.05$).

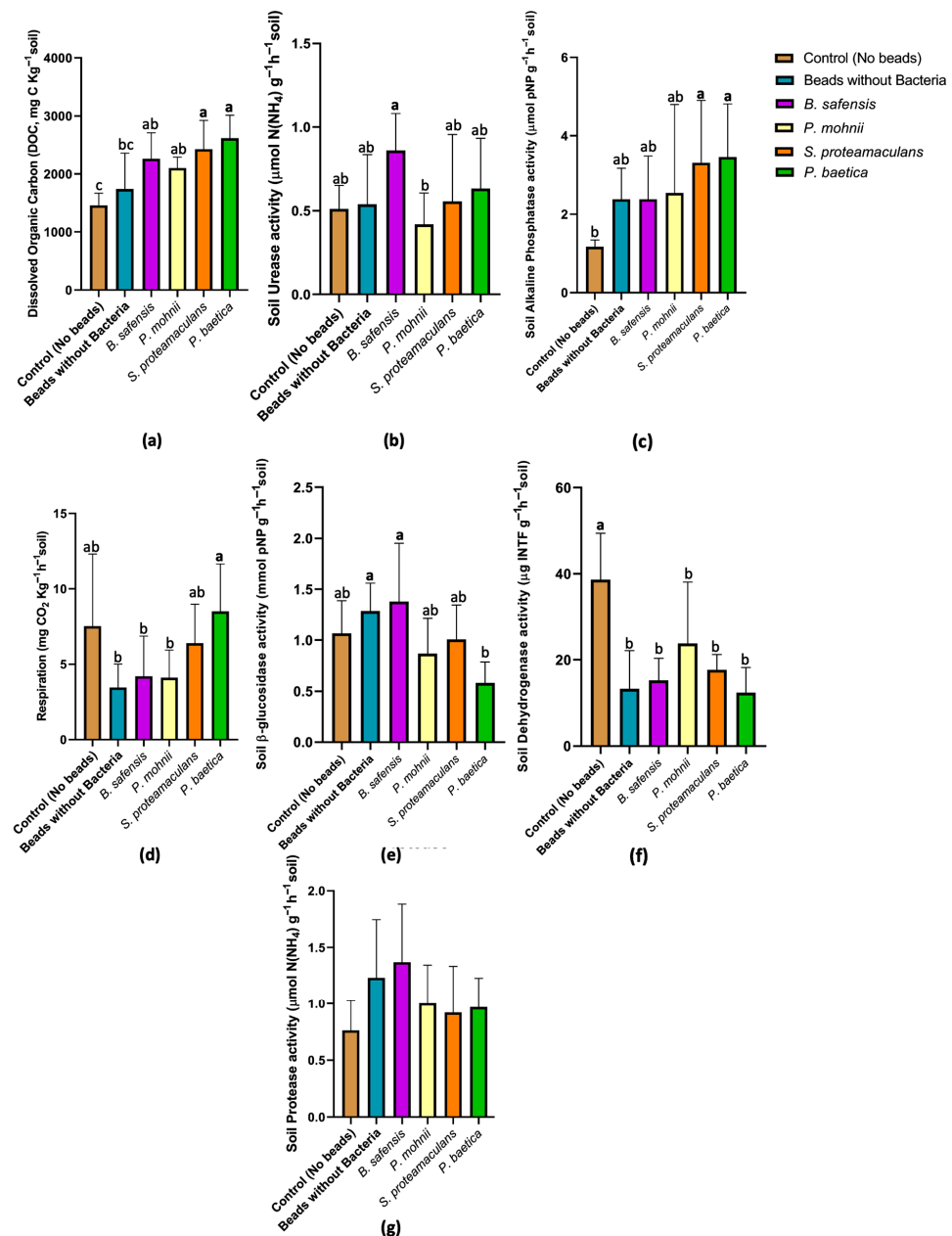


Figure 4. Soil biochemical responses after 45 days of treatment with immobilized PGPR. (a) Dissolved organic carbon, (b) urease activity, (c) alkaline phosphatase activity, (d) soil respiration, (e) β -glucosidase activity, (f) dehydrogenase activity, and (g) protease activity. Treatments included control (no beads), beads without bacteria, and immobilized *Bacillus safensis* (B.s), *Pseudomonas mohnii* (P.m), *Serratia proteamaculans* (S.p), and *Pseudomonas baetica* (P.b). Data are presented as mean $\pm$ standard error ($n = 5$). Different letters indicate statistically significant differences among treatments according to Duncan's test ($p < 0.05$). In the absence of letter presentation, no statistically significant differences were observed among the treatments.

Urease activity showed a similar pattern (Figure 4b). The highest activity was recorded in *B. safensis* ($0.86 \pm 0.22 \mu\text{mol NH}_4^+ \text{g}^{-1} \text{h}^{-1}$), followed by *P. baetica* (0.63 ± 0.30) and *S. proteamaculans* (0.56 ± 0.40). The remaining treatments exhibited lower urease activity, including *P. mohnii* (0.42 ± 0.19), control beads without bacteria (0.54 ± 0.30), and control (no beads: 0.51 ± 0.14).

For alkaline phosphatase (Figure 4c), the highest mean values were obtained in *P. baetica* ($3.46 \pm 1.36 \mu\text{mol pNP g}^{-1} \text{h}^{-1}$) and *S. proteamaculans* (3.32 ± 1.59), with moderate activity in *P. mohnii* (2.55 ± 2.25) and *B. safensis* (2.38 ± 1.10). The control treatments

exhibited the lowest enzymatic activity, particularly in the control (no beads: 1.17 ± 0.18). Despite the absence of statistically significant differences (Duncan's test, $p > 0.05$), the observed patterns suggest that some Gram-negative PGPR strains may enhance microbial activity and organic matter content under non-stress conditions. In contrast, protease and dehydrogenase activities did not exhibit significant differences or notable trends across treatments, indicating that these parameters were not strongly influenced by the inoculated strains under the conditions tested.

Soil respiration (Figure 4d) significantly increased in PGPR-treated soils, with the highest CO₂ emission observed in *P. baetica* (8.50 ± 3.14 mg CO₂ kg⁻¹ h⁻¹ soil). β -glucosidase activity (Figure 4e) was significantly enhanced in the treatment with *B. safensis* (1.37 ± 0.57 mmol PNF g⁻¹ h⁻¹) and beads without bacteria (1.29 ± 0.27), which both showed the highest enzymatic activities. Dehydrogenase activity (Figure 4f) was highest in the control (no beads: 38.65 ± 10.76 μ g INTF g⁻¹ h⁻¹), significantly exceeding the activity observed in all PGPR-treated soils, indicating a distinct microbial response to the different treatments. Protease activity (Figure 4g) did not show statistically significant differences among treatments, although beads without bacteria and *B. safensis* exhibited higher values.

3.5. Scanning Electron Microscopy Analysis

Scanning electron microscopy (SEM) revealed successful immobilization of all tested bacterial strains within the alginate-starch matrix. *P. mohnii* (Figure 5a–c) exhibited evenly distributed microcolonies. *S. proteamaculans* (Figure 5d) showed denser clusters with potential exopolysaccharide (EPS) networks, indicating strong surface attachment and matrix colonization. *P. baetica* (Figure 5e) formed compact aggregates, likely embedded in EPS, highlighting its potential for stable root colonization. In contrast, *B. safensis* (Figure 5f), the Gram-positive control, exhibited typical rod-shaped structures surrounded by a smoother alginate-starch layer, with less apparent EPS.

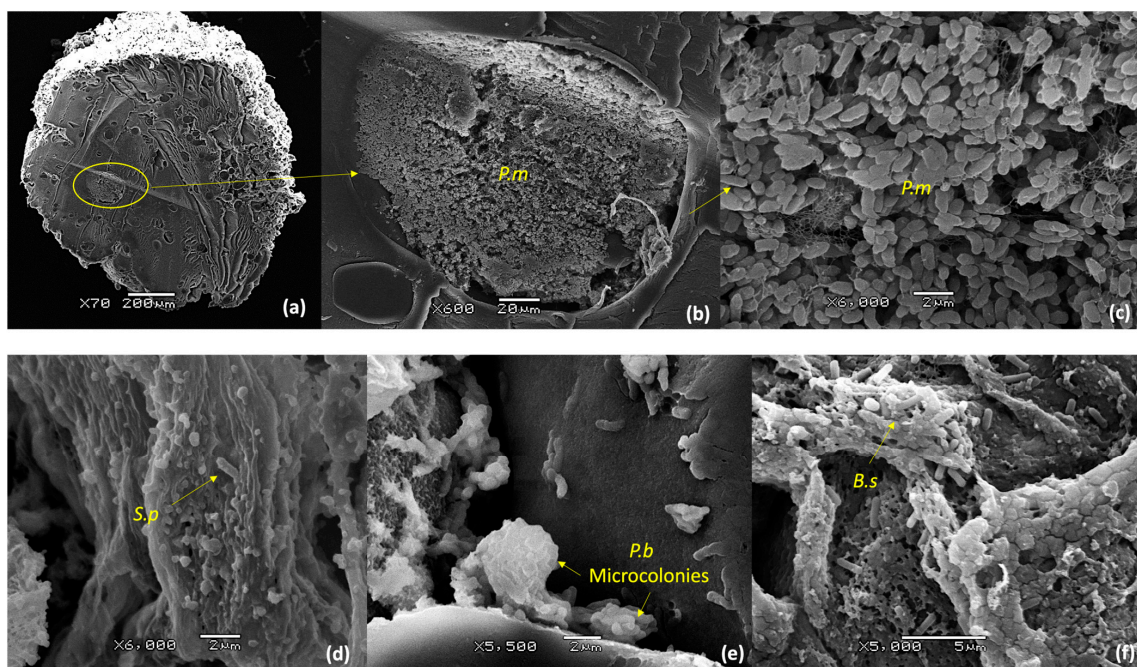


Figure 5. Scanning electron microscopy (SEM) micrographs showing bacterial immobilization within alginate-starch beads. (a–c) *P. mohnii* (*P.m*), (d) *S. proteamaculans* (*S.p*), (e) *P. baetica* (*P.b*), and (f) *B. safensis* (*B.s*). All images were captured 48 h after immobilization on the bead surface.

3.6. Multivariate Analysis of Plant Performance and Soil Responses

Principal component analysis (PCA) was conducted to explore the relationships among physiological performance, plant development, and soil biochemical indicators. The first five components accounted for 70.06% of the total variance (Table 2), with PC1 explaining 21.84%, PC2 14.72%, and PC3 12.57%. PC1 grouped variables related to morphometric and biomass accumulation, including root dry weight (0.690), stem dry weight (0.734), and dissolved organic carbon (DOC, 0.576), indicating a strong interdependence. PC2 was dominated by gas exchange traits such as transpiration (0.754), stomatal conductance (0.687), and photosynthesis (0.602). PC3 was moderately loaded by root length (0.534), while PC4 and PC5 captured variation associated with microbial and enzymatic soil activities.

Table 2. Principal component analysis (PCA) of plant growth, gas exchange, and soil biochemical parameters. The table presents the eigenvalues, percentage of explained variance, and cumulative variance for the first five extracted components. Components with eigenvalues greater than 1.0 (Kaiser criterion) were retained, cumulatively explaining 70.06% of the total variance.

	Components				
	1. Morphometric and Biomass Parameters				
	PC1	PC2	PC3	PC4	PC5
Root dry weight (g)	0.690	-	0.383	-0.150	-
Root volume (cm ³)	-0.208	-0.342	-0.225	-0.629	0.181
Root length (cm)	0.451	-	0.534	0.442	-0.321
Stem dry weight (g)	0.734	-0.339	0.184	-0.190	-
Leaf dry weight (g)	0.351	-	0.408	0.111	0.588
	2. Gas Exchange Parameters				
	PC1	PC2	PC3	PC4	PC5
	PC1	PC2	PC3	PC4	PC5
Photosynthesis (Pn)	-	0.602	-	-	-
Transpiration (ϵ)	-0.156	0.754	0.433	-	0.181
Stomatal conductance (gs)	-0.273	0.687	0.408	-	-
	3. Soil Microbial Activity and Biochemical Indicators				
	PC1	PC2	PC3	PC4	PC5
	PC1	PC2	PC3	PC4	PC5
Urease $\mu\text{mol NH}_4^+ \text{g}^{-1} \text{h}^{-1}$	0.656	0.324	-0.278	0.241	-
Alkaline phosphatase $\mu\text{mol pNP g}^{-1} \text{h}^{-1}$	0.467	0.363	-0.380	-0.284	-0.245
Protease $\mu\text{mol NH}_4^+ \text{g}^{-1} \text{h}^{-1}$	0.170	-	-0.506	0.430	0.530
β -glucosidase $\mu\text{mol pNP g}^{-1} \text{h}^{-1}$	0.384	0.137	-0.242	0.430	0.545
Dehydrogenase $\mu\text{g INTF g}^{-1} \text{soil}$	-0.285	-0.330	0.110	0.693	-0.399
DOC $\text{g C kg}^{-1} \text{soil}$	0.576	0.476	-0.355	-0.283	-0.206

These multivariate patterns suggest that plant biomass production is closely associated with soil carbon content and urease activity, while gas exchange variables are orthogonally distributed, highlighting their distinct contribution to the overall variance. A Pearson correlation matrix was constructed to further examine the inter-variable relationships (Figure 6a). The strongest positive correlations were observed between stem dry weight and total dry weight ($r = 0.98$) and between photosynthesis and stomatal conductance ($r = 0.81$). In contrast, root volume and root dry weight showed a weaker association.

The PCA biplot (Figure 6b) displays the distribution of variables across PC1 and PC2. Growth-related variables clustered together and pointed along PC1, whereas gas exchange parameters formed a separate cluster along PC2. Soil biochemical variables such as DOC and urease showed intermediate loading across both dimensions, illustrating their integrative role in linking plant and microbial responses.

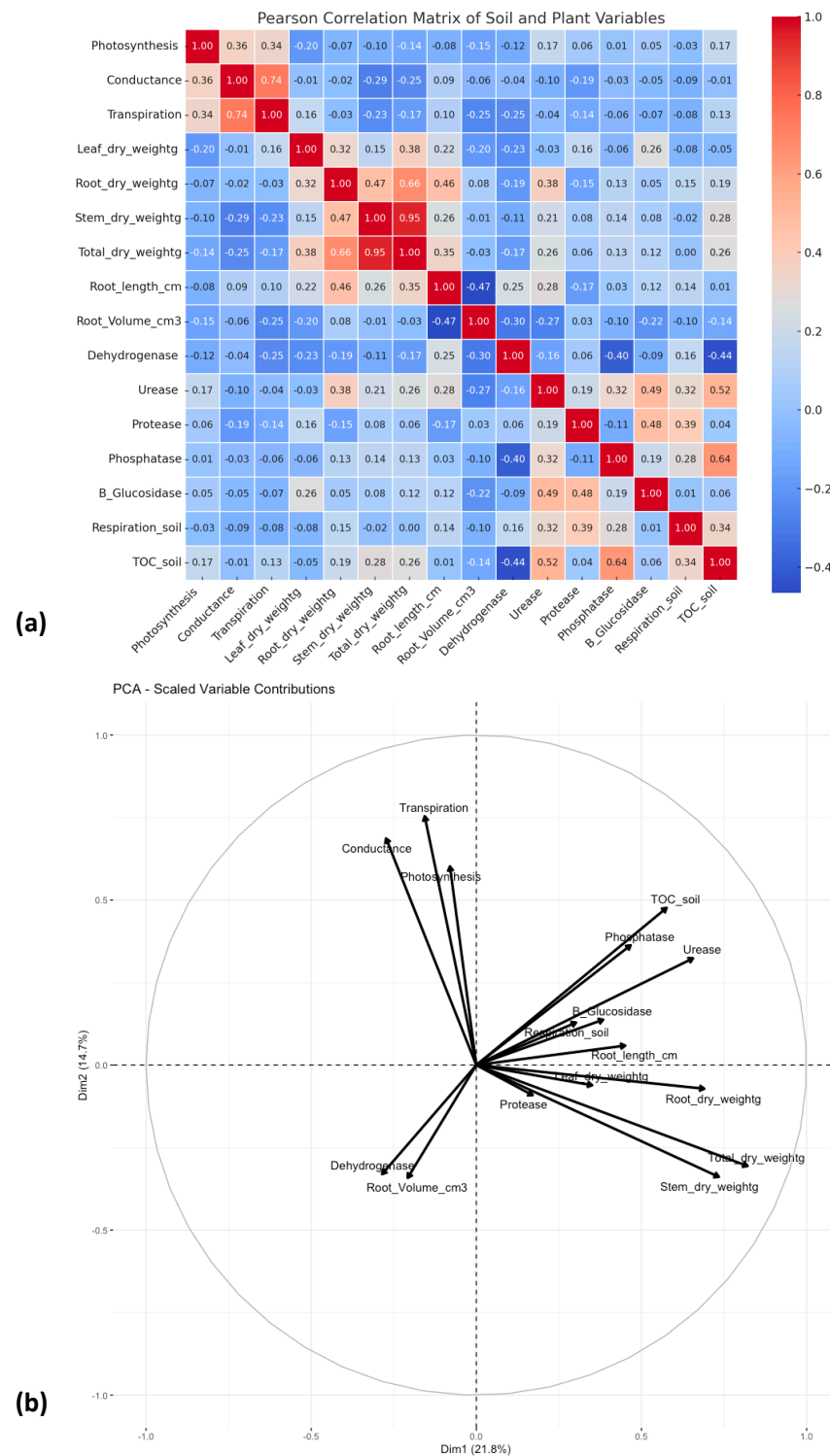


Figure 6. Multivariate analysis of morphophysiological and biochemical variables in *Corylus avellana* plantlets inoculated with immobilized PGPR strains. **(a)** Pearson correlation matrix of all measured variables, including plant growth (biomass, length, and volume), gas exchange parameters, and soil biochemical activities. Color intensity and size of the circles indicate the strength and direction of the correlations (positive in blue, negative in red). **(b)** Principal Component Analysis (PCA) biplot displaying the distribution of treatments based on the first two principal components (PC1 and PC2), which together explained 36.56% of the total variance. Arrows represent the contribution and direction of each variable, showing the association among treatments and key physiological and biochemical traits.

4. Discussion

4.1. Microbial Viability and IAA Production Efficiency

The immobilization process using JetCutter technology preserved microbial viability across most strains, with *S. proteamaculans* showing the highest cell recovery ($1.3 \times 10^8 \pm 5.0 \times 10^7$ CFU g⁻¹). However, the most remarkable result was observed in *P. baetica*, which, despite exhibiting the lowest CFU count ($3.0 \times 10^6 \pm 1.2 \times 10^6$ CFU g⁻¹), produced the highest concentration of indole-3-acetic acid (30.7 ± 0.10 ppm g⁻¹).

Our findings suggest that metabolic activity, particularly phytohormone biosynthesis, is not solely dependent on the number of viable cells but may reflect an inherent trait of the strain or a selective advantage within the alginate-starch matrix. Consistent with previous studies showing that secondary metabolite production is modulated by quorum sensing signals and specific symbiotic conditions rather than by the absolute number of viable cells [40]. Previous studies have associated this behavior with physiological strategies observed in bacteria during stationary phases, where cellular energy is reallocated to maintain key metabolic functions even without active proliferation [41,42]. Moreover, quorum sensing mechanisms may regulate the expression of genes involved in secondary metabolite production independently of cell density, especially under environmental constraints or in immobilized conditions [41,42]. The observed outcomes corroborate earlier studies indicating that immobilized PGPR can maintain or even enhance the production of secondary metabolites, such as auxins, due to the protective and stabilizing environment provided by the polymer matrix [35,43]. In this context, *P. baetica* emerges as a promising candidate for applications aimed at stimulating root development through hormonal modulation, even under non-stress conditions [44].

4.2. Root Development and Morphological Responses to Immobilized PGPR

The application of immobilized bacterial strains induced positive trends in root biomass and volume of *C. avellana* after 45 days of growth. Among the treatments, *B. safensis* showed the most consistent differences, increasing root volume (60.70 ± 9.29 cm³) and dry root biomass (10.56 ± 4.09 g) compared to the control (no beads: 40.37 ± 12.37 cm³ and 9.36 ± 1.86 g, respectively). *P. baetica* also exhibited a relatively high dry root biomass (11.78 ± 4.30 g), although its root volume (39.6 ± 8.5 cm³) was closer to that of the control (no beads). Although differences were not statistically significant, their biological relevance was evident.

Enhanced root development has been widely associated with PGPR-mediated mechanisms such as auxin production, improved nutrient availability, and biofilm formation [45,46]. In this context, the superior performance of *B. safensis* and *P. baetica* could be attributed to their high auxin synthesis, likely stimulating cell elongation and lateral root proliferation [44,47,48]. *B. safensis* also showed a positive trend in root volume, supporting its role as a reference PGPR under stress conditions [18,32].

In contrast, *P. mohnii*, despite moderate viability and auxin production, exhibited lower root biomass and volume. This response may be associated with extracellular xylanase activity, as previously reported by Paul et al. (2020). Although not evaluated in the present study, such enzymatic traits could affect root surface integrity under non-stress conditions, potentially limiting root expansion [49]. Therefore, the enzymatic profile of *P. mohnii* may explain its limited impact on early root development and soil enhancement compared to other Gram-negative strains.

4.3. Physiological Modulation Through PGPR Inoculation: Gas Exchange Parameters

Although no statistically significant differences were observed, gas exchange measurements revealed biologically relevant trends in *C. avellana* plantlets inoculated with immobilized

PGPR. Among the strains tested, *B. safensis* displayed the highest net photosynthetic rate ($5.34 \pm 1.52 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$), followed by the treatment controls and Gram-negative strains, which showed intermediate values between 3.5 and $3.8 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$.

The data support the hypothesis that *B. safensis*, previously reported as a resilient strain under abiotic stress, may stimulate chloroplast activity or enhance CO_2 assimilation efficiency under favorable conditions [50,51]. Notably, *P. mohnii* and *P. baetica* exhibited the highest transpiration rates ($0.70 \pm 0.20 \text{ mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$, in magnitude), potentially indicating increased stomatal aperture or water transport efficiency [52,53]. However, this pattern was not consistently associated with enhanced stomatal conductance, which remained higher in the controls ($65.2\text{--}65.4 \text{ mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$) compared to bacterial treatments ($55\text{--}61 \text{ mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$). This apparent dissociation suggests the presence of factors such as increased vapor pressure gradients, changes in leaf cuticle permeability, or internal water transport efficiency, rather than enhanced stomatal opening [54].

Water use efficiency measurements further supported these trends. Significant differences were observed in WUE_e (extrinsic water use efficiency) (Duncan's test, $p < 0.05$), with *B. safensis* showing the highest extrinsic efficiency (8.0 ± 2.8), suggesting improved carbon gain relative to transpiration water loss. In contrast, intrinsic WUE_i (intrinsic water use efficiency) showed no statistically significant differences among treatments ($p > 0.05$). However, *B. safensis* displayed the highest numerical value (0.089 ± 0.034), suggesting a consistent trend toward enhanced water use regulation and indicating potential variability in stomatal regulation efficiency. These observations suggest that *B. safensis* may enhance water–carbon dynamics through mechanisms favoring efficient water use under non-stress conditions [50]. *B. safensis* might additionally influence intrinsic physiological traits without significantly altering the overall gas exchange.

Although PGPR are primarily known for improving plant performance under stress, several reports indicate that their application can modulate photosynthetic and stomatal behavior even under optimal growth conditions [33]. The observed physiological shifts may reflect early priming effects or enhanced nutrient acquisition mediated by root-associated bacteria.

Overall, these trends reinforce the potential of PGPR to fine-tune physiological processes, supporting the hypothesis that immobilized inoculants may contribute to plant performance via subtle yet functionally relevant changes in gas exchange.

4.4. Soil Biochemical Responses to PGPR Immobilization

The application of immobilized PGPR strains induced notable changes in soil biochemical indicators, suggesting enhanced microbial activity and organic matter content, particularly under non-stress conditions [55]. *P. baetica* and *S. proteamaculans* consistently showed higher dissolved organic carbon (DOC), urease, and alkaline phosphatase activity than the treatment controls.

DOC levels were particularly elevated in *P. baetica* ($2615.9 \pm 395.9 \text{ g C kg soil}^{-1}$), followed by *S. proteamaculans* and *B. safensis*. These results are consistent with prior studies highlighting the potential of Gram-negative PGPR to stimulate rhizosphere carbon inputs through root–microbe interactions and exopolysaccharide production [56,57].

Urease activity, a key indicator of nitrogen turnover [58], was highest in *B. safensis* ($0.86 \pm 0.22 \mu\text{mol NH}_4^+ \text{ g}^{-1} \text{ h}^{-1}$), although *P. baetica* and *S. proteamaculans* also exhibited elevated levels compared to the treatment controls. This suggests that both Gram-positive and Gram-negative strains may enhance soil N cycling, possibly through differential metabolic capacities or biofilm-mediated stabilization within the beads [58–60].

Alkaline phosphatase activity followed a similar pattern, with *P. baetica* and *S. proteamaculans* displaying the highest activities (3.46 ± 1.36 and $3.32 \pm 1.59 \mu\text{mol pNP g}^{-1} \text{ h}^{-1}$,

respectively). Such results may indicate enhanced P mineralization and microbial demand for phosphorus in the rhizosphere, potentially benefiting early root establishment [48].

Soil enzymatic activity showed distinct responses to PGPR inoculation, depending on the metabolic function assessed. Soil respiration significantly increased in PGPR-treated soils, with *P. baetica* inducing the highest CO₂ emissions. This indicates an overall stimulation of microbial activity, likely driven by enhanced substrate availability or microbial proliferation, accelerating organic matter decomposition and carbon turnover [37,61].

The increased β -glucosidase activity observed in *B. safensis* and beads without bacteria may be partially attributed to the degradation of the alginate-starch matrix. Alginate lytic activity, whether from PGPR or native soil microbes [62,63], can release carbon-rich oligosaccharides, thereby enhancing β -glucosidase and cellulolytic activity. This suggests that alginate may act as an inert carrier and additionally as an active contributor to the soil carbon cycle [64].

Dehydrogenase activity was highest in the control (no beads), significantly exceeding that of all PGPR treatments. As an indicator of microbial oxidative metabolism, its reduced activity in inoculated soils may reflect changes in microbial dynamics, such as the probable competition between native and introduced bacteria. Moreover, dehydrogenase is sensitive to oxygen availability and tends to be more active under microaerophilic conditions, which may not be favored in the more aerated environments promoted by PGPR colonization [65,66].

Overall, these findings support the hypothesis that specific Gram-negative strains, particularly *P. baetica*, can effectively stimulate key soil biochemical functions, potentially surpassing the Gram-positive control strain (*B. safensis*) under non-stress conditions. Although protease activity did not show significant differences, the slightly higher values observed in *P. baetica* and *S. proteamaculans* suggest a potential for enhanced nitrogen mineralization, which may require prolonged incubation or specific environmental triggers to become evident [66]. Their influence on soil respiration, enzymatic activity, and carbon cycling highlights their potential roles in promoting soil health and plant productivity beyond stress mitigation.

4.5. Multivariate Integration of Plant Growth, Gas Exchange, and Soil Biochemical Activity

The multivariate analysis allowed for an integrated interpretation of plant and soil responses, highlighting functional relationships among physiological, biochemical, and morphometric variables in hazelnut plantlets treated with immobilized PGPR. Whereas many variables did not show statistically significant differences in univariate tests, Pearson correlations and PCA jointly revealed biologically relevant trends, particularly for Gram-negative strains such as *P. baetica* and *S. proteamaculans*.

The Pearson correlation matrix showed a strong association between stem and total dry weight ($r = 0.98$), suggesting coordinated biomass allocation. A similarly high correlation was found between photosynthesis and stomatal conductance ($r = 0.81$), indicating coherent physiological modulation in response to bacterial treatments under non-stress conditions.

Principal component analysis (PCA) explained 70.06% of the total variance, with PC1 and PC2 accounting for 36.56%. PC1 was dominated by root and shoot biomass parameters and dissolved organic carbon (DOC), revealing a tight coupling between belowground growth and soil organic matter accumulation [46]. Gas exchange variables, represented by PC2, were functionally independent of biomass and soil carbon (PC1), suggesting that PGPR inoculation mainly influenced below-ground processes. Such effects are likely attributable to localized rhizospheric interactions of certain strains, which enhance root-soil interactions without consistently affecting stomatal behavior or photosynthesis under non-stress conditions [54].

Additionally, distinct associations were observed between the microbial and morphometric variables. Root-related parameters were clustered with dehydrogenase activity, suggesting a potential relationship between aerobic microbial metabolism and early root development [67]. The DOC and urease activity appeared tightly coupled, reflecting the coordinated carbon input and nitrogen transformation in the rhizosphere following inoculation. In a separate component, phosphatase, β -glucosidase, and protease activities were grouped, indicating a shared role in nutrient mobilization through enzymatic degradation of organic matter [65]. These multivariate relationships emphasize that the functional impact of PGPR varies according to their capacity to modulate specific soil processes and plant traits.

4.6. Functional Potential of Immobilized PGPR Strains in the Rhizosphere

Functional evaluation of immobilized PGPR strains highlighted distinct but complementary roles in enhancing plant–soil interactions within the rhizosphere. Gram-negative bacteria, particularly *P. baetica* and *S. proteamaculans*, exhibited a more integrative contribution to the rhizosphere by enhancing soil microbial activity—evidenced by elevated urease and phosphatase levels—and promoting root biomass accumulation. The observed improvement in efficiency can be attributed to the intrinsic structural complexity of Gram-negative bacteria, which possess an outer membrane with specialized secretion systems [68]—such as type III and VI [69] secretion systems—and exopolysaccharide production [56]. These characteristics promote root colonization, biofilm formation, and the targeted release of bioactive compounds in the rhizosphere, enhancing nutrient solubilization and signaling interactions even at early developmental stages [68,70].

In contrast, the Gram-positive strain *B. safensis* exhibited a distinct physiological profile, improving photosynthetic performance and moderately increasing root biomass and urease activity. This balanced effect suggests a contribution to both below- and above-ground processes, although its impact on soil biochemical enrichment was less pronounced than that of Gram-negative strains. These responses align with previous findings showing the potential of *Bacillus* spp. to promote plant growth and suppress biotic stress under controlled conditions, similar to that of abiotic stress [71]. The high urease activity observed in *B. safensis* may be attributed to its intrinsic ureolytic capacity, as commonly described for *Bacillus* species, which hydrolyze urea into ammonia and carbon dioxide, increasing local pH and carbonate availability [72]. Additionally, the negative charge of the cell wall facilitates cation adsorption, such as Ca^{2+} , favoring calcite precipitation and altering the rhizospheric microenvironment [73]. Accordingly, such functional traits may reflect early metabolic activation in aerial tissues, supporting photosynthetic efficiency, although similar effects have been reported in other PGPR groups [18,50,51].

In summary, these results emphasize the importance of selecting PGPR strains based on their specific functional attributes. Gram-negative bacteria may be more effective in promoting root development and improving soil biochemical properties, while Gram-positive strains could contribute to enhancing photosynthetic efficiency and stress resilience. This functional complementarity supports the strategic design of microbial consortia aimed at optimizing plant performance through coordinated below- and above-ground benefits, according to defined agronomic objectives [74–76].

4.7. Advantages of JetCutter Technology for PGPR Immobilization

The implementation of JetCutter technology in this study demonstrated important advantages for the immobilization of Gram-negative PGPR strains. Compared to traditional droplet or extrusion methods, JetCutter-assisted immobilization produces highly uniform microbeads with better control over size, shape, and bacterial load [32]. This uniformity

is critical for ensuring reproducible field applications and consistent bacterial delivery to the rhizosphere.

Moreover, the coupling of JetCutter precision with a two-step strategy—bead pre-formation followed by immersion in high-density bacterial cultures—enabled high cell viability retention, particularly in *S. proteamaculans* and *P. mohnii* [32]. These results suggest that Gram-negative PGPR, which are typically more sensitive to desiccation and physical stress [77], can be successfully immobilized using this approach without compromising their metabolic functionality, such as IAA production and soil enzymatic activity.

In terms of scalability and formulation stability, JetCutter technology offers a cost-effective and environmentally friendly alternative to more complex or polymer-intensive methods [31]. It reduces the risk of bacterial loss during drying or storage and facilitates the inclusion of sensitive strains that are often excluded from commercial bioformulations [78]. Therefore, the use of JetCutter-based immobilization may represent a strategic tool to broaden the spectrum of PGPR strains applied in sustainable agriculture, especially those with proven plant growth-promoting and soil-enhancing properties under non-stressful or moderate environmental conditions.

5. Conclusions

Our findings confirm the efficacy of immobilized Gram-negative PGPR strains, particularly *P. baetica* and *S. proteamaculans*, in enhancing root development and stimulating soil biochemical activity in hazelnuts under non-stress conditions. These strains promoted higher root biomass, total organic carbon, and enzymatic activities (urease and alkaline phosphatase), suggesting their potential to improve belowground plant performance through microbiome–soil–root interactions.

In contrast, the Gram-positive strain *B. safensis* improved photosynthetic efficiency but showed limited effects on soil properties and root growth, highlighting functional differences between bacterial biotypes.

Multivariate analysis confirmed distinct patterns of association among plant and soil variables, supporting a model in which Gram-negative PGPR activate soil fertility pathways and contribute to early plant establishment. These findings underscore the relevance of microbial traits, such as IAA production, biofilm formation, and enzymatic activity, for sustainable plant growth promotion.

The use of immobilized Gram-negative strains via JetCutter technology has emerged as a promising tool for targeted rhizosphere applications in hazelnuts.

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Data Availability Statement: All data are presented in the paper.

Conflicts of Interest: The authors declare no conflicts of interest.

Abbreviations

The following abbreviations are used in this manuscript:

ACC	1-aminocyclopropane-1-carboxylic acid
ANOVA	Analysis of variance
APX	Ascorbate peroxidase
CAT	Catalase
CFU	Colony-forming units
CIRAS	Compact infrared gas analyzer system
CSIC	Consejo Superior de Investigaciones Científicas
DNA	Deoxyribonucleic acid
EPS	Exopolysaccharides
FONDEF	Fondo de Fomento al Desarrollo Científico y Tecnológico
GPX	Glutathione peroxidase
GR	Glutathione reductase
HPLC	High-performance liquid chromatography
IAA	Indole-3-acetic acid
IU	International Units
PGPR	Plant growth-promoting rhizobacteria
PCA	Principal component analysis
RH	Relative humidity
SOD	Superoxide dismutase
DOC	Dissolved organic carbon

References

1. Rovira, M. Advances in Hazelnut (*Corylus avellana* L.) Rootstocks Worldwide. *Horticulturae* **2021**, *7*, 267. [\[CrossRef\]](#)
2. James, S. Plant Growth-Promoting Rhizobacteria: Mechanisms and Agricultural Applications. *J. Plant Pathol. Microbiol.* **2024**, *15*, 729. [\[CrossRef\]](#)
3. Mhlongo, M.I.; Piater, L.A.; Madala, N.E.; Labuschagne, N.; Dubery, I.A. The Chemistry of Plant–Microbe Interactions in the Rhizosphere and the Potential for Metabolomics to Reveal Signaling Related to Defense Priming and Induced Systemic Resistance. *Front. Plant Sci.* **2018**, *9*, 112. [\[CrossRef\]](#) [\[PubMed\]](#)
4. Ryu, C.-M.; Farag, M.A.; Hu, C.-H.; Reddy, M.S.; Wei, H.-X.; Paré, P.W.; Kloepper, J.W. Bacterial Volatiles Promote Growth in Arabidopsis. *Proc. Natl. Acad. Sci. USA* **2003**, *100*, 4927–4932. [\[CrossRef\]](#)
5. Tahir, H.A.S.; Gu, Q.; Wu, H.; Raza, W.; Hanif, A.; Wu, L.; Colman, M.V.; Gao, X. Plant Growth Promotion by Volatile Organic Compounds Produced by *Bacillus Subtilis* SYST2. *Front. Microbiol.* **2017**, *8*, 171. [\[CrossRef\]](#)
6. Win, K.T.; Fukuyo, T.; Keiki, O.; Ohwaki, Y. The ACC Deaminase Expressing Endophyte *Pseudomonas* spp. Enhances NaCl Stress Tolerance by Reducing Stress-Related Ethylene Production, Resulting in Improved Growth, Photosynthetic Performance, and Ionic Balance in Tomato Plants. *Plant Physiol. Biochem.* **2018**, *127*, 599–607. [\[CrossRef\]](#)
7. Zaheer, M.S.; Raza, M.A.S.; Saleem, M.F.; Khan, I.H.; Ahmad, S.; Iqbal, R.; Manevski, K. Investigating the Effect of *Azospirillum brasilense* and *Rhizobium pisi* on Agronomic Traits of Wheat (*Triticum aestivum* L.). *Arch. Agron. Soil. Sci.* **2019**, *65*, 1554–1564. [\[CrossRef\]](#)
8. Sharma, K.; Sharma, S.; Prasad, S.R. PGPR: Renewable Tool for Sustainable Agriculture. *Int. J. Curr. Microbiol. Appl. Sci.* **2019**, *8*, 525–530. [\[CrossRef\]](#)
9. Nadeem, S.M.; Ahmad, M.; Zahir, Z.A.; Javaid, A.; Ashraf, M. The Role of Mycorrhizae and Plant Growth Promoting Rhizobacteria (PGPR) in Improving Crop Productivity under Stressful Environments. *Biotechnol. Adv.* **2014**, *32*, 429–448. [\[CrossRef\]](#)
10. Kenneth, O.C.; Nwadike, E.C.; Kalu, A.U.; Unah, U.V. Plant Growth Promoting Rhizobacteria (PGPR): A Novel Agent for Sustainable Food Production. *Am. J. Agric. Biol. Sci.* **2019**, *14*, 35–54. [\[CrossRef\]](#)
11. Yang, J.; Kloepper, J.W.; Ryu, C.M. Rhizosphere Bacteria Help Plants Tolerate Abiotic Stress. *Trends Plant Sci.* **2009**, *14*, 1–4. [\[CrossRef\]](#) [\[PubMed\]](#)
12. Bajracharya, A.M. Plant Growth Promoting Rhizobacteria (PGPR): Biofertiliser and Biocontrol Agent-Review Article. *J. Balkumari Coll.* **2019**, *8*, 42–45. [\[CrossRef\]](#)
13. Yeşil, Z.B.; Koç, İ. Effects of Drought and Biochar Treatments on Some Morphological and Physiological Parameters in Turkish Hazelnut Seedlings. *Membra Kastamonu Üniversitesi Ürünleri Fakültesi Derg.* **2024**, *10*, 211–222. [\[CrossRef\]](#)

14. Vinci, A.; Di Lena, B.; Portarena, S.; Farinelli, D. Trend Analysis of Different Climate Parameters and Watering Requirements for Hazelnut in Central Italy Related to Climate Change. *Horticulturae* **2023**, *9*, 593. [\[CrossRef\]](#)
15. Nicoletti, R.; Petriccione, M.; Curci, M.; Scortichini, M. Hazelnut-Associated Bacteria and Their Implications in Crop Management. *Horticulturae* **2022**, *8*, 1195. [\[CrossRef\]](#)
16. Soenens, A.; Imperial, J. Biocontrol Capabilities of the Genus *Serratia*. *Phytochem. Rev.* **2020**, *19*, 577–587. [\[CrossRef\]](#)
17. Amora-Lazcano, E.; Quiroz-González, H.J.; Osornio-Ortega, C.I.; Cruz-Maya, J.A.; Jan-Roblero, J. Plant Growth-Promoting Bacteria Belonging to the Genera *Pseudomonas* and *Bacillus* Improve the Growth of Sorghum Seedlings in a Low-Nutrient Soil. *Bot. Sci.* **2021**, *100*, 56–66. [\[CrossRef\]](#)
18. Chebotar, V.K.; Zaplatkin, A.N.; Chizhevskaya, E.P.; Gancheva, M.S.; Voshol, G.P.; Malfanova, N.V.; Baganova, M.E.; Khomyakov, Y.V.; Pishchik, V.N. Phytohormone Production by the Endophyte *Bacillus safensis* TS3 Increases Plant Yield and Alleviates Salt Stress. *Plants* **2024**, *13*, 75. [\[CrossRef\]](#)
19. Kshetri, L.; Naseem, F.; Pandey, P. Role of *Serratia* sp. as Biocontrol Agent and Plant Growth Stimulator, with Prospects of Biotic Stress Management in Plant. In *Microorganisms for Sustainability*; Springer: Berlin/Heidelberg, Germany, 2019; Volume 13, pp. 169–200.
20. Polyanskaya, L.M.; Ivanov, K.E.; Guzev, V.S.; Zvyagintsev, D.G. Estimation of Abundance Dynamics of Gram-Negative Bacteria in Soil. *Microbiology* **2008**, *77*, 760–764. [\[CrossRef\]](#)
21. Wu, W.; Dijkstra, P.; Hungate, B.A.; Shi, L.; Dippold, M.A. In Situ Diversity of Metabolism and Carbon Use Efficiency among Soil Bacteria. *Sci. Adv.* **2022**, *8*, eabq3958. [\[CrossRef\]](#)
22. Rostamikia, Y.; Kouchaksaraei, M.T.; Asgharzadeh, A.; Rahmani, A. The Effect of Plant Growth-Promoting Rhizobacteria on Growth and Physiological Characteristics of *Corylus avellana* Seedlings. *Ecopersia* **2016**, *4*, 1471–1479. [\[CrossRef\]](#)
23. Rosa, L.; Sangiorgio, M. Global Water Gaps under Future Warming Levels. *Nat. Commun.* **2025**, *16*, 1192. [\[CrossRef\]](#) [\[PubMed\]](#)
24. Qiao, M.; Hong, C.; Jiao, Y.; Hou, S.; Gao, H. Impacts of Drought on Photosynthesis in Major Food Crops and the Related Mechanisms of Plant Responses to Drought. *Plants* **2024**, *13*, 1808. [\[CrossRef\]](#)
25. Nour, M.M.; Aljabi, H.R.; AL-Huqail, A.A.; Horneburg, B.; Mohammed, A.E.; Alotaibi, M.O. Drought Responses and Adaptation in Plants Differing in Life-Form. *Front. Ecol. Evol.* **2024**, *12*, 1452427. [\[CrossRef\]](#)
26. Aranjuelo, I.; Cabrera-Bosquet, L.; Morcuende, R.; Avice, J.C.; Nogués, S.; Araus, J.L.; Martínez-Carrasco, R.; Pérez, P. Does Ear C Sink Strength Contribute to Overcoming Photosynthetic Acclimation of Wheat Plants Exposed to Elevated CO₂? *J. Exp. Bot.* **2011**, *62*, 3957–3969. [\[CrossRef\]](#)
27. White, A.C.; Rogers, A.; Rees, M.; Osborne, C.P. How Can We Make Plants Grow Faster? A Source-Sink Perspective on Growth Rate. *J. Exp. Bot.* **2016**, *67*, 31–45. [\[CrossRef\]](#)
28. Rolli, E.; Marasco, R.; Vigani, G.; Ettoumi, B.; Mapelli, F.; Deangelis, M.L.; Gandolfi, C.; Casati, E.; Previtali, F.; Gerbino, R.; et al. Improved Plant Resistance to Drought Is Promoted by the Root-Associated Microbiome as a Water Stress-Dependent Trait. *Environ. Microbiol.* **2015**, *17*, 316–331. [\[CrossRef\]](#)
29. Berninger, T.; González López, Ó.; Bejarano, A.; Preininger, C.; Sessitsch, A. Maintenance and Assessment of Cell Viability in Formulation of Non-Sporulating Bacterial Inoculants. *Microb. Biotechnol.* **2018**, *11*, 277–301. [\[CrossRef\]](#)
30. Chen, M.; Alexander, M. Survival of Soil Bacteria during Prolonged Desiccation. *Soil. Biol. Biochem.* **1973**, *5*, 213–221. [\[CrossRef\]](#)
31. Almeida, F.L.C.; Silveira, M.P.; Alvim, I.D.; da Costa, T.B.; da Silva, T.L.; Vieira, M.G.A.; Prata, A.S.; Forte, M.B.S. Jet Cutter Technique as a Tool to Achieve High Lipase Hydrolytic Activity. *Food Bioprod. Process.* **2023**, *137*, 189–199. [\[CrossRef\]](#)
32. Martín-Díaz, F.; Baeza-Aranzaez, J.; Giraldo, J.D.; Schoebitz, M.; Urrutia, H.; López-Belchí, M.D. Innovative JetCutter Technology to Scale the Production of a Solid *Bacillus pumilus* Biofertilizer to Transit to Sustainable Agriculture. *ACS Agric. Sci. Technol.* **2025**, *5*, 188–200. [\[CrossRef\]](#)
33. Jing, D.; Liu, B.; Ma, H.; Liu, F.; Liu, X.; Ren, L. Effects of Inoculation with Different Plant Growth-Promoting Rhizobacteria on the Eco-Physiological and Stomatal Characteristics of Walnut Seedlings under Drought Stress. *Agronomy* **2023**, *13*, 1486. [\[CrossRef\]](#)
34. Lotfi, N.; Soleimani, A.; Çakmakçı, R.; Vahdati, K.; Mohammadi, P. Characterization of Plant Growth-Promoting Rhizobacteria (PGPR) in Persian Walnut Associated with Drought Stress Tolerance. *Sci. Rep.* **2022**, *12*, 12725. [\[CrossRef\]](#) [\[PubMed\]](#)
35. Benítez, S.V.; Carrasco, R.; Giraldo, J.D.; Schoebitz, M. Microbeads as Carriers for *Bacillus Pumilus*: A Biofertilizer Focus on Auxin Production. *J. Microencapsul.* **2024**, *41*, 170–189. [\[CrossRef\]](#)
36. Rojas-Padilla, J.; De-Bashan, L.E.; Parra-Cota, F.I.; Rocha-Estrada, J.; de los Santos-Villalobos, S. Microencapsulation of *Bacillus* Strains for Improving Wheat (*Triticum turgidum* Subsp. *durum*) Growth and Development. *Plants* **2022**, *11*, 2920. [\[CrossRef\]](#)
37. Alguacil, M.d.M.; Torrecillas, E.; Torres, P.; García-Orenes, F.; Roldán, A. Long-Term Effects of Irrigation with Waste Water on Soil AM Fungi Diversity and Microbial Activities: The Implications for Agro-Ecosystem Resilience. *PLoS ONE* **2012**, *7*, e47680. [\[CrossRef\]](#)
38. Alef, K.; Nannipieri, P. *Methods in Applied Soil Microbiology and Biochemistry*; Academic Press: London, UK, 1995.
39. Anderson, J.; Domsch, K. A Physiological Method for the Quantitative Measurement of Microbial Biomass in Soils. *Soil. Biol. Biochem.* **1978**, *10*, 215–221. [\[CrossRef\]](#)

40. Shmuel, K. Urgencia y Mecanismo de Biosíntesis de Metabolitos Secundarios Microbianos Marinos. *Int. J. Sci. Soc.* **2022**, *4*, 138–147. [[CrossRef](#)]
41. Nev, O.A.; Nev, O.A.; van den Berg, H.A. Optimal Management of Nutrient Reserves in Microorganisms under Time-Varying Environmental Conditions. *J. Theor. Biol.* **2017**, *429*, 124–141. [[CrossRef](#)]
42. Zhang, Q.; Chen, Y.; Shi, H. Bacterial Strategies in Prolonged Stationary Phase: Tradeoff between Cell Growth, Maintenance, and Recycling. *Phys. Rev. Res.* **2023**, *5*, 013119. [[CrossRef](#)]
43. Bashan, Y.; de-Bashan, L.E.; Prabhu, S.R.; Hernandez, J.P. Advances in Plant Growth-Promoting Bacterial Inoculant Technology: Formulations and Practical Perspectives (1998–2013). *Plant Soil* **2014**, *378*, 1–33. [[CrossRef](#)]
44. González-Reguero, D.; Robas-Mora, M.; Probanza, A.; Jiménez, P.A. Evaluation of the Oxidative Stress Alleviation in *Lupinus albus* Var. Orden Dorado by the Inoculation of Four Plant Growth-Promoting Bacteria and Their Mixtures in Mercury-Polluted Soils. *Front. Microbiol.* **2022**, *13*, 907557. [[CrossRef](#)] [[PubMed](#)]
45. Glick, B.R. Plant Growth-Promoting Bacteria: Mechanisms and Applications. *Scientifica* **2012**, *1*, 963401. [[CrossRef](#)]
46. Grover, M.; Bodhankar, S.; Sharma, A.; Sharma, P.; Singh, J.; Nain, L. PGPR Mediated Alterations in Root Traits: Way Toward Sustainable Crop Production. *Front. Sustain. Food Syst.* **2021**, *4*, 618230. [[CrossRef](#)]
47. Neshat, M.; Chavan, D.D.; Shirmohammadi, E.; Pourbabaee, A.A.; Zamani, F.; Torkaman, Z. Canola Inoculation with *Pseudomonas Baetica* R27N3 under Salt Stress Condition Improved Antioxidant Defense and Increased Expression of Salt Resistance Elements. *Ind. Crops Prod.* **2023**, *206*, 117648. [[CrossRef](#)]
48. López-Hernández, J.; García-Cárdenas, E.; López-Bucio, J.S.; Jiménez-Vázquez, K.R.; de la Cruz, H.R.; Ferrera-Rodríguez, O.; Santos-Rodríguez, D.L.; Ortiz-Castro, R.; López-Bucio, J. Screening of Phosphate Solubilization Identifies Six *Pseudomonas* Species with Contrasting Phytostimulation Properties in Arabidopsis Seedlings. *Microb. Ecol.* **2023**, *86*, 431–445. [[CrossRef](#)]
49. Paul, M.; Nayak, D.P.; Thatoi, H. Optimization of Xylanase from *Pseudomonas Mohnii* Isolated from Simlipal Biosphere Reserve, Odisha, Using Response Surface Methodology. *J. Genet. Eng. Biotechnol.* **2020**, *18*, 81. [[CrossRef](#)]
50. Costa-Santos, M.; Mariz-Ponte, N.; Dias, M.C.; Moura, L.; Marques, G.; Santos, C. Effect of *Bacillus* spp. and *Brevibacillus* sp. on the Photosynthesis and Redox Status of *Solanum lycopersicum*. *Horticulturae* **2021**, *7*, 24. [[CrossRef](#)]
51. Mukhtar, T.; Ali, F.; Rafique, M.; Ali, J.; Afridi, M.S.; Smith, D.; Mehmood, S.; Amna; Souleimanov, A.; Jellani, G.; et al. Biochemical Characterization and Potential of *Bacillus Safensis* Strain SCAL1 to Mitigate Heat Stress in *Solanum lycopersicum* L. *J. Plant Growth Regul.* **2023**, *42*, 523–538. [[CrossRef](#)]
52. Wang, T.; Wang, K.; Wang, N.; Cui, D.; Li, S.; Lu, Q.; Zuo, Y. From Intercropping to Monocropping: The Effects of *Pseudomonas* Strain to Facilitate Nutrient Efficiency in Peanut and Soil. *Plant Physiol. Biochem.* **2025**, *219*, 109378. [[CrossRef](#)]
53. Arkhipova, T.; Sharipova, G.; Akhiyarova, G.; Kuzmina, L.; Galin, I.; Martynenko, E.; Seldimirova, O.; Nuzhnaya, T.; Feoktistova, A.; Timergalin, M.; et al. The Effects of Rhizosphere Inoculation with *Pseudomonas Mandelii* on Formation of Apoplast Barriers, HvPIP2 Aquaporins and Hydraulic Conductance of Barley. *Microorganisms* **2022**, *10*, 935. [[CrossRef](#)] [[PubMed](#)]
54. Gowtham, H.G.; Singh, S.B.; Shilpa, N.; Aiyaz, M.; Nataraj, K.; Udayashankar, A.C.; Amruthesh, K.N.; Murali, M.; Poczai, P.; Gafur, A.; et al. Insight into Recent Progress and Perspectives in Improvement of Antioxidant Machinery upon PGPR Augmentation in Plants under Drought Stress: A Review. *Antioxidants* **2022**, *11*, 1763. [[CrossRef](#)] [[PubMed](#)]
55. Jurys, A.; Feizienė, D. The Effect of Specific Soil Microorganisms on Soil Quality Parameters and Organic Matter Content for Cereal Production. *Plants* **2021**, *10*, 2000. [[CrossRef](#)] [[PubMed](#)]
56. Luo, Y.; Lopez, J.B.G.; van Veelen, H.P.J.; Kok, D.J.D.; Postma, R.; Thijssen, D.; Sechi, V.; ter Heijne, A.; Bezemer, T.M.; Buisman, C.J.N. Effects of Different Soil Organic Amendments (OAs) on Extracellular Polymeric Substances (EPS). *Eur. J. Soil. Biol.* **2024**, *121*, 103624. [[CrossRef](#)]
57. Ng, C.W.W.; Yan, W.H.; Tsim, K.W.K.; So, P.S.; Xia, Y.T.; To, C.T. Effects of *Bacillus subtilis* and *Pseudomonas fluorescens* as the Soil Amendment. *Heliyon* **2022**, *8*, e11674. [[CrossRef](#)]
58. Mekonnen, E.; Kebede, A.; Nigussie, A.; Kebede, G.; Tafesse, M. Isolation and Characterization of Urease-Producing Soil Bacteria. *Int. J. Microbiol.* **2021**, *2021*, 8888641. [[CrossRef](#)]
59. Cruz, D.; Cisneros, R.; Benítez, Á.; Zúñiga-Sarango, W.; Peña, J.; Fernández, H.; Jaramillo, A. Gram-Negative Bacteria from Organic and Conventional Agriculture in the Hydrographic Basin of Loja: Quality or Pathogen Reservoir? *Agronomy* **2021**, *11*, 2362. [[CrossRef](#)]
60. Gao, Y.; Zhang, H.; Zhang, R.; Huang, Z.; Yang, C. Impacts of *Pseudomonas* Fluorescent Bacterial Fertilizer Addition on the Soil Environment and Fruit Yield under Water Stress in Greenhouse Grape. *Front. Microbiol.* **2025**, *16*, 1540628. [[CrossRef](#)]
61. Burns, R.G.; DeForest, J.L.; Marxsen, J.; Sinsabaugh, R.L.; Stromberger, M.E.; Wallenstein, M.D.; Weintraub, M.N.; Zoppini, A. Soil Enzymes in a Changing Environment: Current Knowledge and Future Directions. *Soil. Biol. Biochem.* **2013**, *58*, 216–234. [[CrossRef](#)]
62. Zilda, D.S.; Yulianti, Y.; Sholihah, R.F.; Subaryono, S.; Fawzya, Y.N.; Irianto, H.E. A Novel *Bacillus* sp. Isolated from Rotten Seaweed: Identification and Characterization Alginate Lyase Its Produced. *Biodiversitas* **2019**, *20*, 1166–1172. [[CrossRef](#)]

63. Rhein-Knudsen, N.; Guan, C.; Mathiesen, G.; Horn, S.J. Expression and Production of Thermophilic Alginate Lyases in *Bacillus* and Direct Application of Culture Supernatant for Seaweed Saccharification. *Algal Res.* **2021**, *60*, 102512. [\[CrossRef\]](#)
64. Peng, C.; Wang, Q.; Lu, D.; Han, W.; Li, F. A Novel Bifunctional Endolytic Alginate Lyase with Variable Alginate-Degrading Modes and Versatile Monosaccharide-Producing Properties. *Front. Microbiol.* **2018**, *9*, 167. [\[CrossRef\]](#) [\[PubMed\]](#)
65. Piotrowska-Długosz, A.; Długosz, J.; Frąc, M.; Gryta, A.; Breza-Boruta, B. Enzymatic Activity and Functional Diversity of Soil Microorganisms along the Soil Profile—A Matter of Soil Depth and Soil-Forming Processes. *Geoderma* **2022**, *416*, 115779. [\[CrossRef\]](#)
66. Daunoras, J.; Kačergius, A.; Gudiukaitė, R. Role of Soil Microbiota Enzymes in Soil Health and Activity Changes Depending on Climate Change and the Type of Soil Ecosystem. *Biology* **2024**, *13*, 85. [\[CrossRef\]](#)
67. Kaur, J.; Kaur, G. Dehydrogenase Activity as a Biological Indicator of Soil Health Chemical Science Review and Letters Dehydrogenase Activity as a Biological Indicator of Soil Health. *Chem. Sci. Rev. Lett.* **2021**, *10*, 326–329.
68. Gupta, G.; Chauhan, P.S.; Jha, P.N.; Verma, R.K.; Singh, S.; Yadav, V.K.; Sahoo, D.K.; Patel, A. Secretory Molecules from Secretion Systems Fine-Tune the Host-Beneficial Bacteria (PGPRs) Interaction. *Front. Microbiol.* **2024**, *15*, 1355750. [\[CrossRef\]](#)
69. Bernal, P.; Llamas, M.A.; Filloux, A. Type VI Secretion Systems in Plant-Associated Bacteria. *Environ. Microbiol.* **2018**, *20*, 1–15. [\[CrossRef\]](#)
70. Lucke, M.; Correa, M.G.; Levy, A. The Role of Secretion Systems, Effectors, and Secondary Metabolites of Beneficial Rhizobacteria in Interactions With Plants and Microbes. *Front. Plant Sci.* **2020**, *11*, 589416. [\[CrossRef\]](#)
71. Benitez Hernández, S.V.; Aragón Rojas, S.; Novoa, S.; Madrigal, S.; Núñez, P.; Gómez, P.; Montenegro, M.; Ordoñez, E.; Lancheros, G.; Sánchez, L. Evaluation of a Fermented Millet Product of *Bacillus subtilis* ATCC 55033 as Potential in Biocontrol of Bacteriosis (*Xanthomonas axonopodis*) in Gulupa (*Passiflora edulis* Sims). *J. Agric. Sci. Technol. A* **2020**, *10*, 1473–1483. [\[CrossRef\]](#)
72. Xu, J.M.; Wang, W.J.; Chen, Z.T.; Zhou, Y.Y.; Pan, J.J.; Cheng, F.; Liu, Z.Q.; Zheng, Y.G. Exploring a High-Urease Activity *Bacillus Cereus* for Self-Healing Concrete via Induced CaCO₃ Precipitation. *Appl. Microbiol. Biotechnol.* **2023**, *107*, 6351–6362. [\[CrossRef\]](#)
73. Fitri, L.; Aulia, T.B.; Fauzi, A.; Kamil, G.A. Characterization and Screening of Urease Activity of Ureolytic Bacteria from Landfills Soil in Banda Aceh, Indonesia. *Biodiversitas* **2023**, *24*, 910–915. [\[CrossRef\]](#)
74. Song, H.; Ding, M.Z.; Jia, X.Q.; Ma, Q.; Yuan, Y.J. Synthetic Microbial Consortia: From Systematic Analysis to Construction and Applications. *Chem. Soc. Rev.* **2014**, *43*, 6954–6981. [\[CrossRef\]](#) [\[PubMed\]](#)
75. Kumar, N. Sustainable Agrobiotechnology: Design and Development of Microbial Consortia. Springer Nature: Berlin/Heidelberg, Germany, 2023; ISBN 9789811995699.
76. Vishwakarma, K.; Kumar, N.; Shandilya, C.; Mohapatra, S.; Bhayana, S.; Varma, A. Revisiting Plant–Microbe Interactions and Microbial Consortia Application for Enhancing Sustainable Agriculture: A Review. *Front. Microbiol.* **2020**, *11*, 560406. [\[CrossRef\]](#) [\[PubMed\]](#)
77. Kawakita, R.; Leveau, J.H.J.; Jeoh, T. Comparing Fluidized Bed Spray-Coating and Spray-Drying Encapsulation of Non-Spore-Forming Gram-Negative Bacteria. *Ind. Biotechnol.* **2021**, *17*, 283–289. [\[CrossRef\]](#)
78. de-Bashan, L.; Giraldo, J.D.; Cruz-Barrera, M.; Schoebitz, M. Enhancing the Survival Rate and Effectiveness of Plant Growth-Promoting Bacteria through Bioencapsulation Techniques. *Biol. Fertil. Soils* **2024**, *60*, 543–556. [\[CrossRef\]](#)

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