



Plant Growth-Promoting and Antifungal Effects of *Bacillus* spp. Against *Alternaria alternata* in Cherry Tomato

Murat Güler¹ · Fatma Kayaçetin²

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Abstract

Beneficial rhizobacteria, or PGPR, enhance agricultural productivity. The aim of this study was to isolate, identify, and evaluate indigenous rhizospheric *Bacillus* strains for their plant growth-promoting traits and biocontrol potential in cherry tomato (*Solanum lycopersicum* L.) cultivation. Three *Bacillus* strains (*Bacillus subtilis* E-30, *Bacillus thuringiensis* F1-32, and *Bacillus pumilus* G-33) were isolated from the cherry tomato rhizosphere and identified using morphological, biochemical, and 16S rRNA gene sequence analyses. The isolates were assessed for their plant growth-promoting attributes and antagonistic activity against the pathogenic fungus *Alternaria alternata*. Greenhouse pot experiments comprising five different treatments demonstrated that the multi-strain bacterial consortium (Treatment E) produced the most pronounced improvements in plant growth parameters. Compared with the uninoculated control, consortium application increased root and stem lengths by 14.3% and 20.5%, respectively, while root fresh weight and relative chlorophyll content (SPAD values) increased by 22.1% and 18.2%. Among the individual strains, *B. thuringiensis* F1-32 exhibited strong antagonistic activity, inhibiting *A. alternata* mycelial growth by 46.27% in vitro. Notably, the study highlights the superior performance of a locally derived multi-strain consortium compared to individual inoculations, providing new evidence for the synergistic potential of indigenous *Bacillus* strains in sustainable tomato production. Overall, the results indicate that these locally adapted *Bacillus* isolates particularly when applied as a consortium function as effective biostimulants and promising, eco-friendly alternatives to chemical fertilizers and fungicides for sustainable cherry tomato production.

Keywords *Bacillus* spp. · *Solanum lycopersicum* · *Alternaria alternata* · Beneficial rhizobacteria

Introduction

Cultivated globally, the tomato (*Solanum lycopersicum* L.) is a member of the Solanaceae family and represents the second most economically and nutritionally vital vegetable crop, surpassed only by the potato (Sahu et al. 2019). Its widespread cultivation in semi-arid regions highlights its remarkable adaptability to diverse environmental conditions. Beyond its commercial importance, the tomato serves as a pivotal model organism for molecular, cellular, and biochemical research. From a public health perspective, it is a dietary staple in many cultures due to its abundance of vitamins, minerals, dietary fiber, and essential amino acids.

High concentrations of antioxidants, such as ascorbic acid, phenolics, and β -carotene, have been linked to a reduced incidence of cardiovascular diseases and certain cancers (Wang et al. 2023).

The ongoing decline in arable land, coupled with climate-driven challenges like drought and global warming, has led to substantial yield losses in tomato production. Sustainable agriculture necessitates not only maintaining the soil's physical and chemical integrity but also revitalizing its biological vitality. Consequently, eco-friendly strategies that enhance soil microbial activity are paramount. The long-term environmental risks posed by the excessive use of synthetic fertilizers and pesticides have accelerated the global strategic shift toward greener production methods. Tomatoes are particularly vulnerable to various pathogens, notably the fungus *Alternaria alternata*, which can cause yield reductions of up to 79.00% (Sharma et al. 2021). To mitigate these losses sustainably, the study of the rhizosphere the micro-environment around roots rich in microbial life is essential. Root exudates in this zone

✉ Fatma Kayaçetin
kayacetin@ankara.edu.tr

¹ Ministry of National Education, 40100 Kırşehir, Turkey

² Faculty of Agriculture, Department of Field Crops, Ankara University, 06110 Ankara, Turkey

Fig. 1 Map of Türkiye showing the location of cherry tomato used in the experiment



attract microorganisms that facilitate nutrient decomposition and transform minerals into bioavailable forms for the plant. Rhizobacteria that actively stimulate plant development through either direct mechanisms (such as nitrogen fixation, phosphate solubilization, and hormone synthesis) or indirect pathways (like suppressing pathogens) are classified as Plant Growth-Promoting Rhizobacteria (PGPR) (Agustiyanı et al. 2022).

Although numerous studies have reported the plant growth-promoting and biocontrol potential of *Bacillus* spp. in tomato, most of these studies have focused on well-characterized reference strains or single inoculations under controlled conditions. Reports on the combined application of multiple *Bacillus* strains, particularly those derived from the same local rhizosphere, remain limited.

In this context, the present study provides a novel contribution by (i) isolating and characterizing indigenous *Bacillus* strains from the cherry tomato rhizosphere under local agro-ecological conditions, (ii) comparatively evaluating both individual strains and their multi-strain consortium, and (iii) demonstrating enhanced functional performance, including high indole-3-acetic acid (IAA) production and synergistic effects on plant growth parameters.

Furthermore, while *Bacillus thuringiensis* has been widely recognized for its antimicrobial properties, our findings highlight that its consortium-based application with other *Bacillus* strains leads to improved antifungal efficacy, particularly against *Alternaria alternata*.

Therefore, this study aimed to isolate, identify, and evaluate indigenous *Bacillus* strains from the cherry tomato rhizosphere and to investigate their plant growth-promoting traits as well as their biocontrol potential against *Alternaria alternata*.

Materials and Methods

Site Description and Sample Collection

Rhizosphere soil samples were collected from cherry tomato plants in Çayağzı (Cemele), Kırşehir, Türkiye (39°16'49.2"N, 34°04'42.9"E) in June 2024 (Fig. 1). Soil closely adhering to roots was sampled at 5–10 cm depth from three locations, labeled, and transferred to the lab under controlled conditions.

Physicochemical Characterization of the Collected Rhizosphere Soil

Key soil properties are summarized in Table 1. The soil had pH 4.8, EC 0.532 dS/m, 0.06% N, 0.70% organic matter, P 28.5 mg kg⁻¹, K 251.6 mg kg⁻¹, and exchangeable Ca, Mg, Fe of 3672, 178.4, and 19.45 mg kg⁻¹, respectively.

Table 1 Physicochemical characteristics of the collected cherry tomato rhizosphere soil samples.

Parameter	Value
Soil pH (1:2.5 w/v)	4.80
Electrical Conductivity (dS m ⁻¹)	0.53
Total Nitrogen (N, %)	0.06
Available Phosphorus (P, mg kg ⁻¹)	28.50
Exchangeable Potassium (K, mg kg ⁻¹)	251.60
Exchangeable Calcium (Ca, mg kg ⁻¹)	3672.00
Exchangeable Magnesium (Mg, mg kg ⁻¹)	178.40
Available Iron (Fe, mg kg ⁻¹)	19.45
Organic Matter Content (%)	0.70

Isolation and Cultivation of Rhizospheric Bacteria

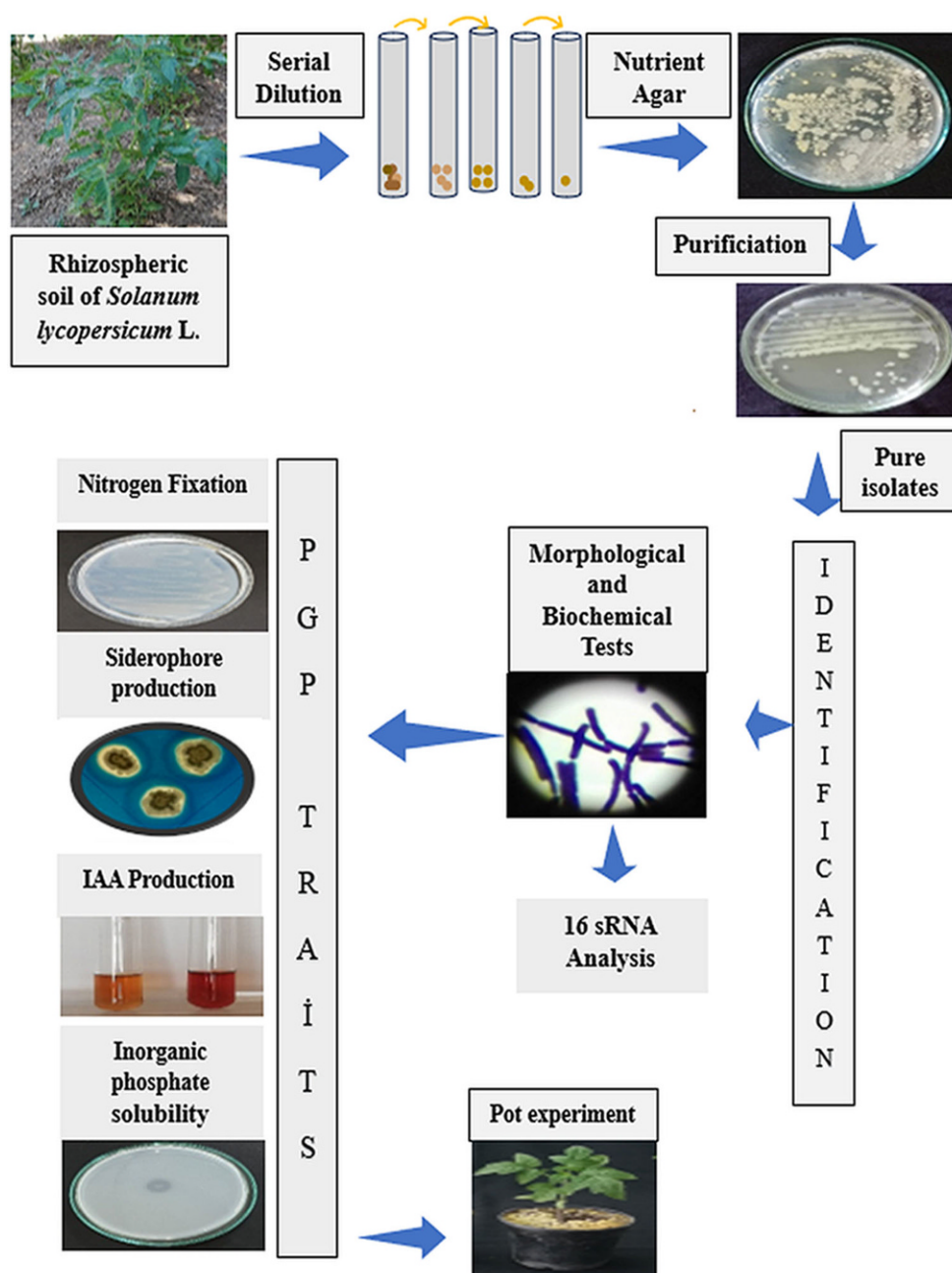
To isolate rhizospheric bacteria, 1 g of each soil sample was weighed and suspended in 9 mL of sterile physiological saline (0.85% NaCl). The suspension was homogenized on a mechanical shaker at 150 rpm for 10 min. Serial dilutions ranging from 10^{-1} to 10^{-6} were then prepared, and 100 μ L aliquots from appropriate dilutions were evenly spread onto Nutrient Agar (NA) plates. The plates were incubated at 28 °C for 24–48 h to allow colony development. Morphologically distinct colonies were selected and purified to obtain pure cultures. For long-term storage and future experi-

ments, the isolates were cryopreserved in a glycerol-based stock solution at -85°C . A schematic overview of the isolation and characterization workflow for the *Bacillus* strains from *S. lycopersicum* L. is visually summarized in the procedural flowchart (Fig. 2).

Morphological, Biochemical, and Molecular Identification of Isolates

Phenotypic characterization of the rhizobacterial isolates was conducted through Gram staining, endospore formation, and a series of physiological and biochemical tests,

Fig. 2 Schematic representation of the procedure used to isolate and characterize bacteria from the rhizosphere cherry tomato



following the protocols of Palleroni et al. (1984). For molecular identification, genomic DNA was extracted using the GeneMATRIX Bacterial & Yeast DNA Isolation Kit (EurX®, Poland) according to the manufacturer's instructions. DNA purity and concentration were assessed using a NanoDrop 2000 spectrophotometer (Thermo Scientific, USA). The 16S rRNA gene was amplified with the universal primers 27F (5'-AGAGTTTGATCMTGGCTCAG-3') and 1492R (5'-TACGGYTACCTTGTTACGACTT-3'). PCR reactions were prepared in a 25 µL volume using the FIREPol® DNA Polymerase Kit (Solis Biodyne, Estonia) following Rahmoune et al. (2017). The thermal cycling profile on a Kyrtec thermocycler included an initial denaturation at 95 °C for 5 min, 30 cycles of denaturation (95 °C, 45 s), annealing (57 °C, 45 s), and extension (72 °C, 60 s), followed by a final extension at 72 °C for 5 min. Amplified products (~1470 bp) were resolved on 1.5% agarose gels electrophoresis (100 V, 90 min) and visualized under UV light after ethidium bromide staining. Purified amplicons were sequenced using the Sanger method on an ABI 3730XL genetic analyzer. Sequence quality was evaluated with FinchTV v1.4.0, aligned using ClustalW, and phylogenetic relationships were inferred using MEGA v11.

Evaluation of Plant Growth-Promoting (PGP) Traits

Siderophore and IAA Production Siderophore synthesis was qualitatively assessed on Chrome Azurol S (CAS) agar medium as described by Schwyn and Neilands (1987). After 6 days of incubation at 28 °C, the Siderophore Production Index (SPI) was calculated by measuring the orange halos surrounding the colonies. For Indole-3-acetic acid (IAA) quantification, isolates were cultured in nutrient broth amended with 0.2% L-tryptophan at 30 °C and 200 rpm for three days. The supernatant obtained after centrifugation (10,000 rpm, 5 min) was reacted with Salkowski reagent (1.2% FeCl₃ in 42.8% H₂SO₄ and 57.2% nano-pure water) and the development of pink coloration was measured spectrophotometrically at 535 nm, following Gang et al. (2019).

Nitrogen Fixation and Phosphate Solubilization The potential for atmospheric nitrogen fixation was assessed on Burk's nitrogen-free solid medium (0.05 g L⁻¹ Na₂SO₄, 10 g L⁻¹ glucose, 0.2 g L⁻¹ CaCl₂, 0.41 g L⁻¹ KH₂PO₄, 0.52 g L⁻¹ K₂HPO₄, 0.0025 g L⁻¹ Na₂MoO₄·2H₂O, 0.1 g L⁻¹ MgSO₄·7H₂O, 0.005 g L⁻¹ FeSO₄·7H₂O, 1.8 g L⁻¹ agar and 1 L dsH₂O) following Park et al. (2005). Inorganic phosphate solubilization was examined on Pikovskaya (PKA) medium (0.2 g L⁻¹ NaCl, 10 g L⁻¹ glucose, 0.2 g L⁻¹ KCl, 5 g L⁻¹ Ca₃(PO₄)₂, 0.5 g L⁻¹ (NH₄)₂SO₄, 0.1 g L⁻¹ MgSO₄·7H₂O, 0.002 g L⁻¹ FeSO₄·7H₂O, 0.5 g L⁻¹ yeast ex-

tract, 0.002 g L⁻¹ MnSO₄·H₂O, and 1000 ml distilled water) as described by Pikovskaya (1948). After a 5-day incubation period, the Phosphate Solubilization Index (PSI) was determined by measuring the clear zones around the colonies.

Extracellular Enzyme and Antifungal Activity Assays

Enzyme Production Amylase and protease activities were detected using starch agar (yeast extract 5 g, NaCl 10 g, tryptone 10 g, starch 5 g, agar 15 g, pure water 1000 ml) and skim milk agar, respectively, according to Smibert and Krieg (1994). Pectinolytic potential was evaluated on M9 agar enriched with pectin as per Geetha et al. (2014), while chitinase activity was tested on colloidal chitin-supplemented minimal medium following Rodriguez-Kabana et al. (1983) at 28 °C. Cellulase production was confirmed on carboxymethylcellulose (2%) agar using the Congo Red staining technique as outlined by Moustaine et al. (2017).

Antifungal Activity The inhibitory effect of the isolates against the pathogen *Alternaria alternata* was tested using the dual culture technique on PDA-NAM (potato dextrose agar-nutrient agar medium). The percentage of Fungal Inhibition (FI %) was calculated according to Royse and Ries (1978) using the following equation:

$$FI (\%) = (C - T) / (C) \times 100$$

(Where C represents the mean radial diameter of the fungal mycelia in the control, and T represents the diameter in the treated plates).

Seed Preparation and Bacterial Inoculation

For this research, red cherry tomato seeds (*Solanum lycopersicum* L.) were provided by the Department of Horticulture, Faculty of Agriculture at Ankara University. To ensure surface sterility, the seeds were submerged in a 10% sodium hypochlorite (NaOCl) solution for 15 min, followed by five consecutive washes with sterile distilled water to remove residual disinfectant. The bacterial isolates, originally preserved at -85 °C, were reactivated on Nutrient Agar (NA) at 30 ± 2 °C for 24 h. Subsequently, pure colonies were transferred to Nutrient Broth (NB) enriched with 4% sucrose and cultivated under shaking conditions. The resulting bacterial density was standardized to a concentration of 10⁸ cfu mL⁻¹ using sterile distilled water. The sterilized seeds were then immersed in these bacterial suspensions for 3 h under aseptic conditions. Following the protocol of Heinonsalo et al. (2004), control seeds underwent the same sterilization process but were treated with sterile distilled water instead of bacterial inoculum.

Table 2 Physicochemical characteristics of the experimental soil substrate.

Characteristics	Sand (%)	Silt (%)	Clay (%)	P (mg kg ⁻¹)	K (mg kg ⁻¹)	Na (mg kg ⁻¹)	Ca (mg kg ⁻¹)	Cl (mg kg ⁻¹)
Physical	76.8	10.9	12.2	–	–	–	–	–
Chemical	–	–	–	24	14.075	186.44	27.25	134.35

Greenhouse Pot Trials

A greenhouse experiment (18–24 °C, with an 18 h/6 h light/dark period and 50–60% relative humidity) was conducted at the Department of Field Crops, Ankara University, to evaluate the growth-promoting influence of the rhizobacterial isolates. The pots (4.5 cm × 4.5 cm × 10 cm) were sterilized with potassium permanganate. The trial utilized a Completely Randomized Design (CRD) featuring three replicates for each treatment group.

Growth Medium and Soil Properties The plants were grown in a 1:1:1 mixture of garden soil, vermiculite, and perlite. Prior to mixing, the soil was air-dried and processed through a 2 mm sieve. The entire substrate was sterilized via autoclaving (twice at 121 °C for 60 min). The physicochemical profile of the soil, as detailed in Table 2, revealed a sandy texture (76.8% sand, 10.9% silt, and 12.2% clay) with the following nutrient concentrations: 24 mg kg⁻¹ Phosphorus (P), 14.075 mg kg⁻¹ Potassium (K), 186.44 mg kg⁻¹ Sodium (Na), 27.25 mg kg⁻¹ Calcium (Ca), and 134.35 mg kg⁻¹ Chloride (Cl).

Experimental Setup and Maintenance The study comprised five distinct groups: Treatment A (Uninoculated control), Treatment B, C, and D: (Individual inoculations with strains E-30, F1-32, and G-33, respectively), Treatment E (bacterial consortium consisting of E-30, F1-32, and G-33). Five seeds were planted in each of the nine pots per treatment. Each treatment was conducted with three independent replicates ($n=3$). The pots were arranged in a completely randomized manner within the greenhouse to minimize positional effects. Environmental conditions, including temperature (25 ± 2 °C), relative humidity, and photoperiod, were maintained as uniformly as possible throughout the experimental period. During planting, 20 mL of the respective bacterial suspension (10^8 CFU mL⁻¹) was applied to each

pot, while control pots received 20 mL of sterile broth. To ensure a stable microbial population in the rhizosphere, the inoculants were reapplied every five days. Plants were irrigated every three days.

Data Collection After a 30-day growth period, the tomato plants were harvested. The following parameters were documented to assess development: Stem and root lengths, Total fresh and dry biomass, Relative chlorophyll content (measured in SPAD units using a Minolta SPAD-502m, Japan).

Statistical Analysis

The experimental data were processed and analyzed using JMP® software (Version 13.2.1). Prior to statistical analysis, data normality was assessed using the Shapiro-Wilk test, and homogeneity of variances was evaluated using Levene's test. As these assumptions were satisfied, parametric analyses were applied.

For all variables demonstrating a normal distribution, results are expressed as mean ± standard deviation (SD). The biological parameters, including the Phosphate Solubilization Index (PSI), Siderophore Production Index (SPI), and Indole-3-acetic acid (IAA) concentrations, were evaluated using one-way analysis of variance (ANOVA).

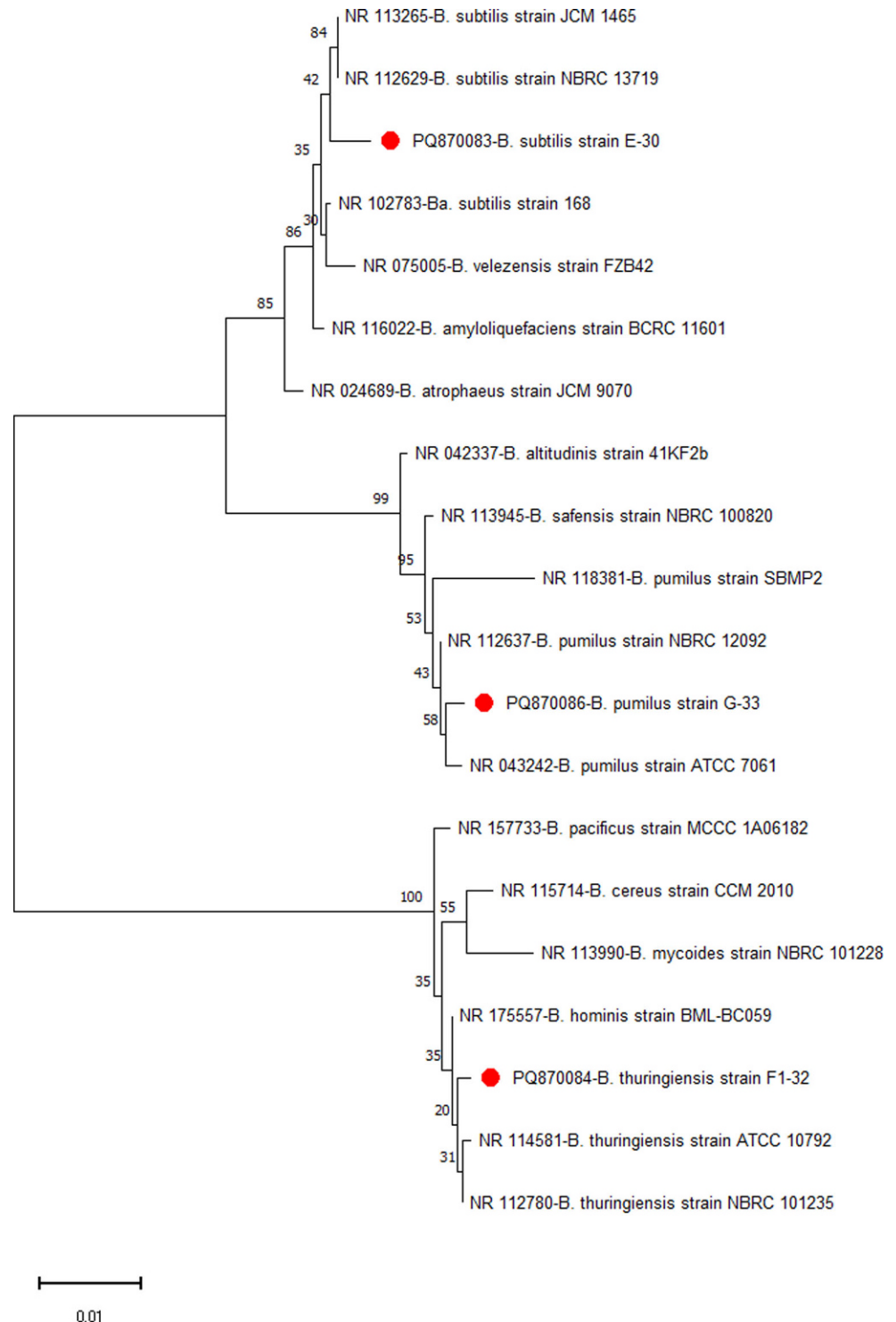
To determine significant differences among treatment groups, multiple comparisons were performed using Tukey's Honestly Significant Difference (HSD) test at a 5% significance level ($p \leq 0.05$). In addition, exact p -values are reported to ensure transparency and improve the interpretation of statistical significance.

Table 3 Morphological and biochemical characteristics of bacterial isolates from the cherry tomato rhizosphere.

Isolate	Identified Species	Gram Characteristic		Biochemical Characteristics			Endospore Forming
		Gram Reaction	KOH Test	Catalase	Oxidase	Motility	
E-30	<i>Bacillus subtilis</i>	+	–	+	+	+	+
F1-32	<i>Bacillus thuringiensis</i>	+	–	+	+	+	+
G-33	<i>Bacillus pumilus</i>	+	–	+	+	+	+

Note: + positive, – negative

Fig. 3 Neighbor-Joining phylogenetic tree of *Bacillus* isolates based on 16S rRNA sequences. Bootstrap values (1000 replicates) indicate clustering consistency. Analyses were conducted using MEGA11 software



Results and Discussion

Molecular Identification of *Bacillus* Strains

The taxonomic identity of bacterial isolates E-30, F1-32, and G-33 was confirmed through 16S rRNA gene sequencing. The sequences were submitted to NCBI GenBank

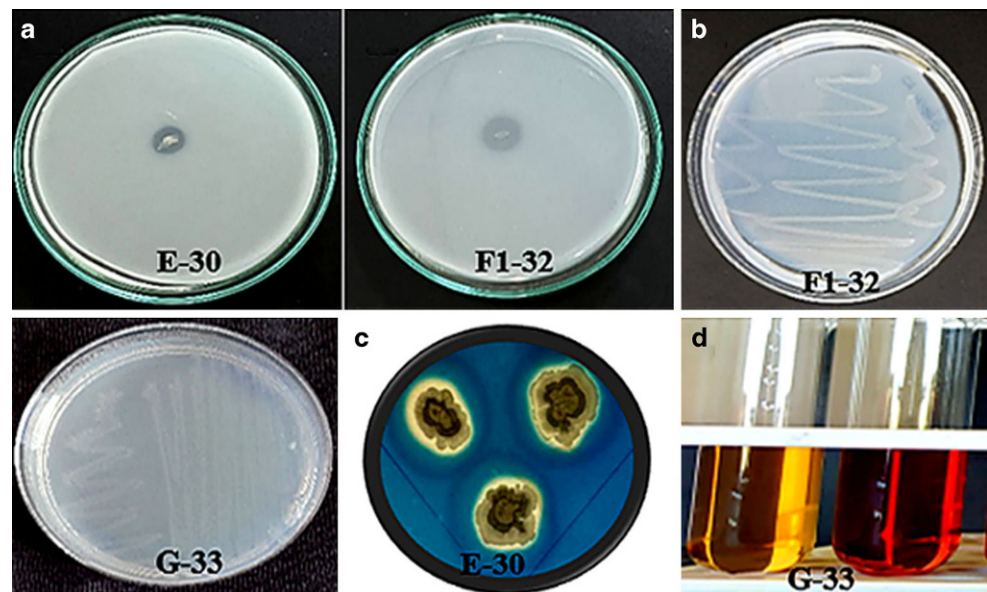
under accession numbers PQ870083 (E-30), PQ870084 (F1-32), and PQ870086 (G-33). Comparative analysis revealed 99.51% identity of E-30 with *Bacillus subtilis* (NR_113265, NR_112629), 99.72% identity of G-33 with *B. pumilus* (NR_112637), and 99.82% identity of F1-32 with *B. thuringiensis* strains (NR_114581, NR_043403, NR_112780) (Fig. 3). Phylogenetic analysis grouped the

Table 4 Plant growth-promoting (PGP) attributes of rhizobacterial isolates from the cherry tomato rhizosphere.

Isolate	Identified Species	PGP traits			
		Inorganic Phosphate Solubilization	Nitrogen Fixation	Siderophore Production	IAA Production
E-30	<i>Bacillus subtilis</i>	+	+	+	+
F1-32	<i>Bacillus thuringiensis</i>	+	–	+	+
G-33	<i>Bacillus pumilus</i>	–	+	+	+

Note: + positive, – negative

Fig. 4 PGP trait visualization: **a** phosphate solubilization, **b** nitrogen fixation, **c** siderophore production, **d** IAA synthesis



isolates into three main clades: *B. subtilis* group (E-30), *B. pumilus* group (G-33), and *B. cereus* group (F1-32, specifically *B. thuringiensis*).

Morphological and Biochemical Characterization

Isolates were phenotypically characterized using Bergey's Manual criteria and methods of Palleroni et al. (1984). All isolates were Gram-positive, endospore-forming *Bacillus* spp., with biochemical traits summarized in Table 3.

Plant Growth-Promoting (PGP) Traits

All isolates exhibited at least two PGP traits (Table 4). IAA refers to Indole-3-acetic acid, SPI to Siderophore Production Index, and PSI to Phosphate Solubilization Index.

- *B. subtilis* E-30: nitrogen fixation, phosphate solubilization, siderophore and IAA production.
- *B. thuringiensis* F1-32: phosphate solubilization, siderophore and IAA production.
- *B. pumilus* G-33: nitrogen fixation, siderophore and IAA production (Fig. 4).

Indole-3-Acetic Acid (IAA) Production

Indole-3-acetic acid (IAA) is a key phytohormone that regulates plant architecture by promoting cell division and elongation, enhancing root growth, lateral root proliferation, and root hair formation, which are critical for nutrient uptake (Lau et al. 2020). Identifying IAA-producing bacteria is therefore pivotal for sustainable agriculture, particularly for high-value crops like cherry tomato (Feng et al. 2024). All tested *Bacillus* strains synthesized IAA when cultured in Nutrient Broth (NB) supplemented with L-tryptophan, though concentrations varied considerably. *B. thuringiensis* F1-32 produced the highest IAA concentration ($144.2 \mu\text{g mL}^{-1}$) after 72 h, followed by *B. pumilus* G-33 ($83.2 \mu\text{g mL}^{-1}$) and *B. subtilis* E-30 ($45.1 \mu\text{g mL}^{-1}$). *B. pumilus* G-33 initially produced only $13.5 \mu\text{g mL}^{-1}$ on day 1 (Fig. 5). The strong sporulation and environmental resilience of *Bacillus* facilitate effective rhizosphere colonization and auxin-mediated plant growth promotion (Larekeng and Achmad 2020). Our findings for *B. subtilis* E-30 ($45.1 \mu\text{g mL}^{-1}$ at day 3) align with previous reports highlighting its consistent IAA production across diverse hosts (Sharma et al. 2020; Kalam et al. 2020). Precursor

supplementation significantly influences IAA biosynthesis; for instance, *B. subtilis* KM462851 produced $14.3\text{ }\mu\text{g mL}^{-1}$ without tryptophan, which increased to $107.4\text{ }\mu\text{g mL}^{-1}$ with 0.5 mg L^{-1} tryptophan (Dashti et al. 2021). Similarly, our *B. subtilis* E-30 isolate produced $45.1\text{ }\mu\text{g mL}^{-1}$ at day 3, corroborating strain- and condition-dependent IAA biosynthesis.

B. thuringiensis, typically known for entomopathogenic activity, also exhibits strong plant growth-promoting effects, including auxin production (Gomis-Cebolla and Berry 2023; Dehkian et al. 2024; Batista et al. 2021). Batista et al. (2021) reported robust IAA synthesis in rhizospheric *B. thuringiensis* isolates, which aligns with the high-performance trends observed in our study. Prior literature has documented significant fluctuations in the auxin-producing capacity of *B. thuringiensis* isolates from the cherry tomato rhizosphere, with reported levels ranging from $3.88\text{ }\mu\text{g mL}^{-1}$ (strain B9; Kouam et al. 2023) to $21.08\text{ }\mu\text{g mL}^{-1}$ (10NB47; Shah et al. 2020), and $4.59\text{ }\mu\text{g mL}^{-1}$ for RZ2MS9 under tryptophan-free conditions (Figueredo et al. 2023). In contrast, our isolate *B. thuringiensis* F1-32 demonstrated a remarkably high IAA production of

$144.2\text{ }\mu\text{g mL}^{-1}$, consistent with Hernández-Pacheco et al. (2021), who also reported substantial IAA synthesis by tomato-associated *B. thuringiensis* strains. Another prominent *Bacillus* species, *B. pumilus*, is widely recognized for its plant growth-promoting potential. The capacity of rhizospheric *B. pumilus* to secrete varying quantities of IAA has been well-documented (Kaushal et al. 2017; Bessai et al. 2022). Quantitative differences within the species are often attributed to growth stages, metabolic substrates, or culture conditions. For instance, *B. pumilus* ToIrMA isolated from tomato roots produced $29.7\text{ }\mu\text{g mL}^{-1}$ of IAA over 96 h (Heidarzadeh and Baghaee-Ravari 2015), whereas *B. pumilus* EU927414 from the wheat rhizosphere generated $149\text{ }\mu\text{g mL}^{-1}$ (Upadhyay et al. 2019). Robust production has also been observed in strains from diverse ecosystems, including SH-9 (Shaffique et al. 2023) and sabkha-derived isolates (Mahmoud et al. 2024). In the present study, *B. pumilus* G-33 exhibited a time-dependent increase in IAA, rising from $13.5\text{ }\mu\text{g mL}^{-1}$ on the first day to $83.2\text{ }\mu\text{g mL}^{-1}$ by the third day (Fig. 5).

Fig. 5 Quantitative IAA production by *Bacillus* isolates over 72 h. Error bars indicate SD ($n=3$). Different lowercase letters indicate statistically significant differences (one-way ANOVA, $p\leq 0.05$)

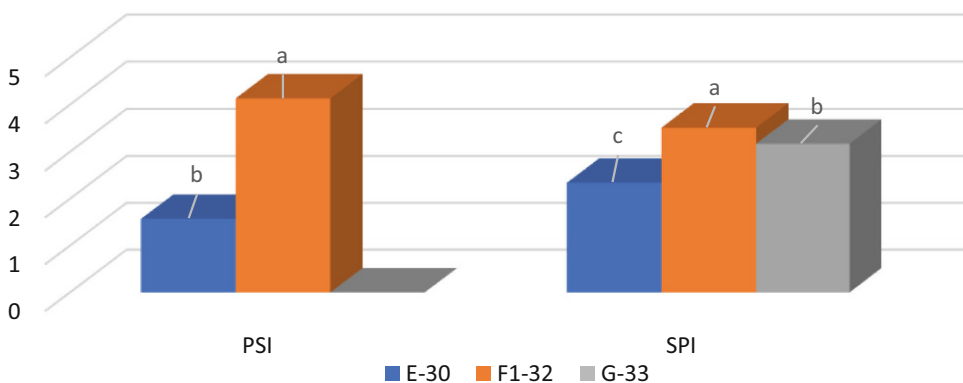
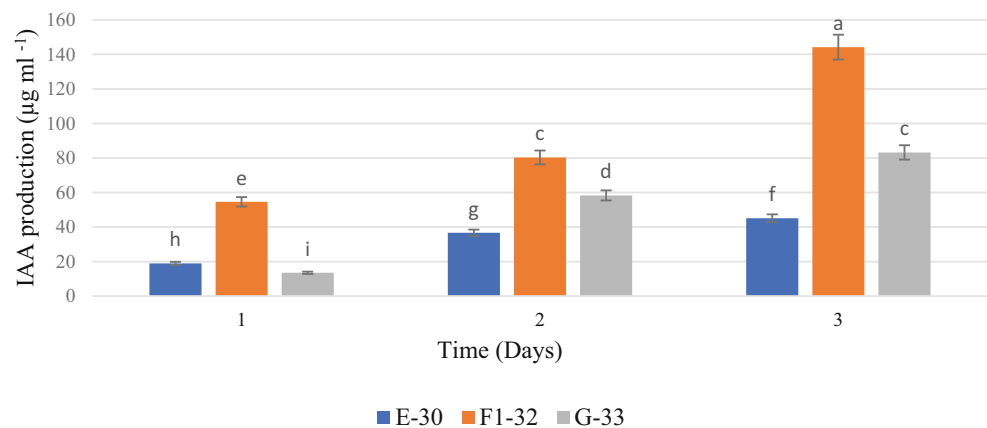


Fig. 6 Comparative assessment of phosphate solubilization index (PSI) and siderophore production index (SPI) by rhizobacterial isolates. PSI values were measured after 5 days of incubation on Pikovskaya's agar (PKA), while SPI values were recorded on day 6. Data represent the mean $\pm$ standard deviation ($n=3$). Bars labeled with different lowercase letters indicate statistically significant differences among isolates, as determined by one-way ANOVA ($p\leq 0.05$)

Table 5 Qualitative enzyme production by isolates.

Isolate	Identified Species	Pectinase	Chitinase	Cellulase
E-30	<i>Bacillus subtilis</i>	+	+	+
F1-32	<i>Bacillus thuringiensis</i>	+	+	–
G-33	<i>Bacillus pumilus</i>	–	+	–

Note: + positive, – negative

Phosphate Solubilization and Siderophore Production

Phosphorus limitation is a major constraint in agriculture, which phosphate-solubilizing bacteria (PSB) can alleviate by converting insoluble phosphorus into bioavailable forms (Silva et al. 2021). The Phosphate Solubilization Index (PSI) of isolates on Pikovskaya's agar ranged from 1.57 to 4.13. *B. thuringiensis* F1-32 exhibited the highest solubilization (PSI=4.13), followed by *B. subtilis* E-30 (PSI=1.57) after five days (Fig. 6). These results are consistent with previous findings highlighting *Bacillus* as a dominant phosphate-mobilizing rhizobacterium (ALKahtani et al. 2020; Kouam et al. 2024).

Siderophores chelate ferric iron, enhancing phytohormone biosynthesis and competing with pathogens for this essential micronutrient (Pahari et al. 2017). Our Siderophore Production Index (SPI) analysis showed that *B. thuringiensis* F1-32 reached the highest SPI (3.51) by day 6, while *B. subtilis* E-30 recorded 2.34 (Fig. 6). These results align with previous studies on siderophore-producing *Bacillus* (Nithyapriya et al. 2021; Shen et al. 2022; Roy et al. 2024).

Nitrogen Fixation

Nitrogen (N) is an essential mineral nutrient for plant growth, playing a central role in key physiological processes such as chlorophyll-mediated photosynthesis and protein biosynthesis. Although atmospheric dinitrogen is abundant, its chemically stable triple bond prevents direct plant assimilation. Certain *Bacillus* species act as diazotrophs, converting atmospheric nitrogen into forms accessible to plants (Vater et al. 2023). In this study, both *B. subtilis* E-30 and *B. pumilus* G-33 grew successfully on nitrogen-free media, indicating their potential for biological nitrogen fixation. Our findings for *B. subtilis* E-30 are consistent with previous reports identifying this species as a nitrogen-fixing inhabitant of the cherry tomato rhizosphere (Dashti et al. 2021; Cochard et al. 2022). Similarly, *B. pumilus* is recognized for its metabolic versatility and production of diverse growth-promoting phytohormones under abiotic stress (Dobrzyński et al. 2022). Nitrogen-fixing activity of *B. pumilus* has been documented in maize root isolates (Kuan et al. 2016) and cherry tomato rhizosphere strains (Masood et al. 2020), as confirmed by nitrogenase assays. The ability of these isolates to grow

without external nitrogen underscores their role as functional PGPR capable of enhancing nutrient availability in cherry tomato cultivation.

Enzymatic Profiles and Biocontrol Potential

The production of extracellular enzymes is a primary mechanism through which rhizobacteria contribute to nutrient cycling and suppress phytopathogens. In this study, the enzymatic profiles of the isolates varied: all three strains produced pectinase and chitinase, whereas cellulase activity was detected exclusively in *B. subtilis* E-30. Given their broad enzymatic capabilities, *B. subtilis* E-30 and *B. thuringiensis* F1-32 both exhibiting at least two positive activities were selected for subsequent antifungal screening. Qualitative results of these assays are presented in Table 5.

Mechanisms of Antagonism and Biocontrol Potential

The antifungal activity of *Bacillus* species is largely attributed to the secretion of lytic enzymes such as chitinases, glucanases, proteases, and cellulases, which degrade glycosidic bonds in fungal cell walls, leading to cellular lysis (Karačić et al. 2024). This enzymatic repertoire enables *Bacillus* strains to suppress a wide range of economically significant pathogens, including *Fusarium* sp. (Khedher et al. 2021), *Rhizoctonia solani* (Alfiky et al. 2022), and *Sclerotium rolfsii* (Ankitha et al. 2023). In the case of *Alternaria alternata*, Malik et al. (2022) reported that *B. subtilis* TD11, isolated from tomato rhizospheric soil, inhibited fungal growth by approximately 50%. Supporting this, Harish et al. (2023) demonstrated that lipopeptides and metabolites from *B. subtilis* strains (T3–T6) achieved inhibition rates up to 85.88%, highlighting the strong antagonistic potential of this species. Similarly, Ajuna et al. (2023) emphasized that the combined production of chitinase, protease, and cellulase is a key mechanism for suppressing phytopathogens and promoting plant health. Although *B. thuringiensis* is primarily recognized for its entomopathogenic properties, it also acts as a potent antimicrobial agent. Its antifungal effects are linked to diverse bioactive compounds, including zwittermycin, acylhomoserine lactone lactonases, and specific chitinase genes such as *chiA71* (Thamthiankul et al. 2001; Djenane et al. 2017). Azizoglu et al. (2023)

Fig. 7 Antifungal activity of selected rhizobacterial isolates against *Alternaria alternata*, expressed as percentage inhibition. Values represent the mean $\pm$ standard deviation ($n=3$). Different lowercase letters indicate statistically significant differences among treatments (one-way ANOVA, $p \leq 0.05$)

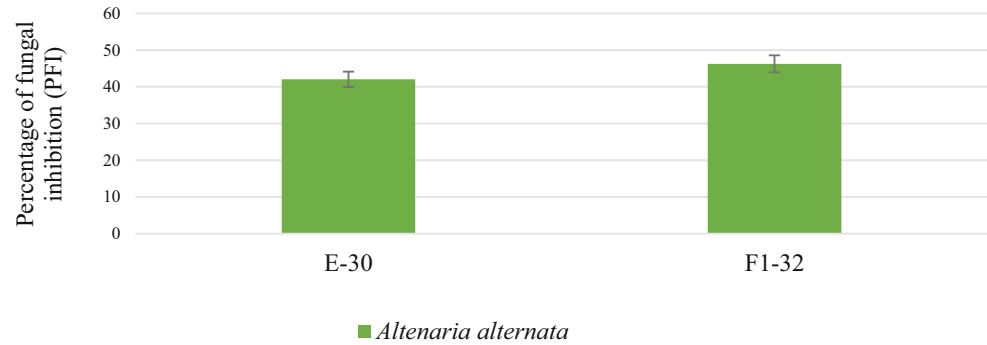
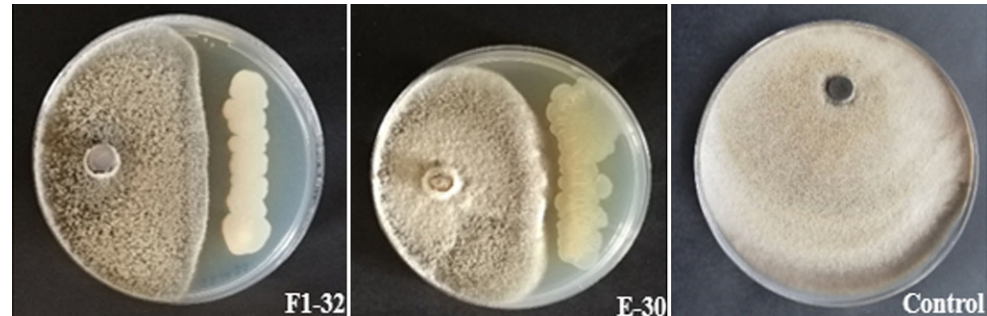


Fig. 8 Visual depiction of the antagonistic effects of rhizobacterial isolates against *Alternaria alternata* on Potato Dextrose Agar (PDA). Images show the zones of inhibition observed after 7 days of incubation at $28 \pm 2^\circ\text{C}$



further described these mechanisms as a synergistic effect of bacteriocins, autolysins, and plant growth-promoting traits such as hydrogen cyanide and IAA production. In addition, siderophore production serves a dual function by enhancing iron availability to the host plant and exerting competitive biocontrol by limiting iron access to pathogens (Hyder et al. 2020; Timofeeva et al. 2022). In this study, both *B. subtilis* E-30 and *B. thuringiensis* F1-32 active producers of siderophores and lytic enzymes effectively

inhibited *A. alternata* by 42.04% and 46.27%, respectively, consistent with the biocontrol models proposed by Harish et al. (2023) (Figs. 7 and 8).

Greenhouse Evaluation: Pot Trials

To validate the in vitro findings, three rhizobacterial isolates exhibiting superior plant growth-promoting activity *B. subtilis* E-30, *B. thuringiensis* F1-32, and *B. pumilus* G-33 were

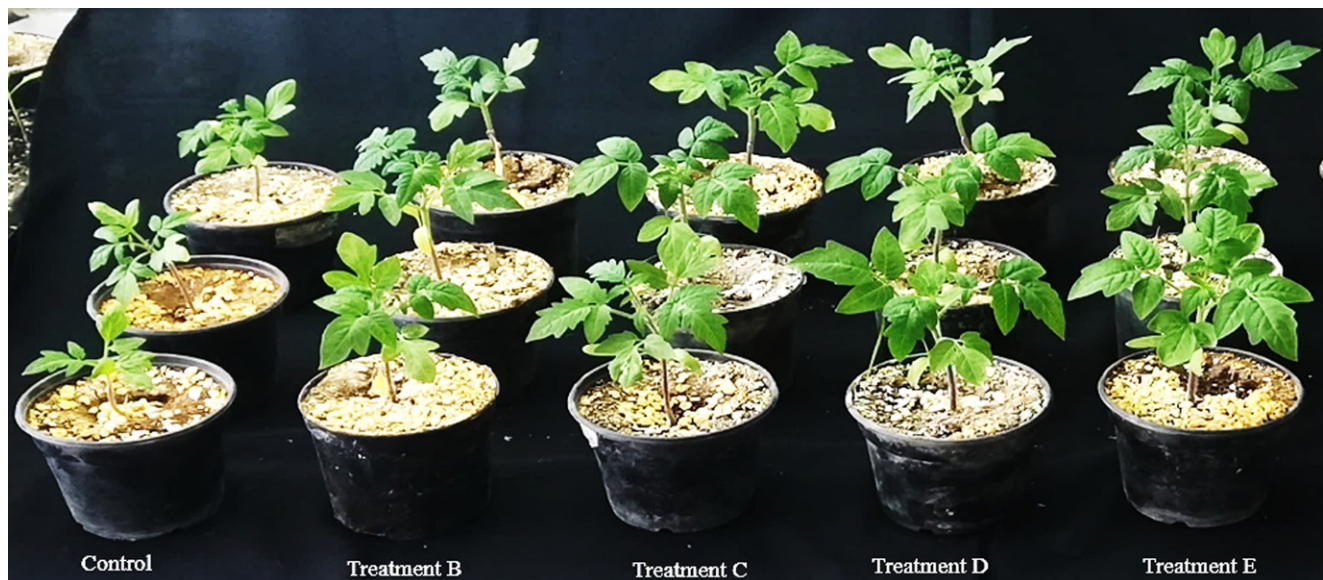


Fig. 9 Tomato seedling growth 30 days after inoculation with individual rhizobacterial isolates and a microbial consortium, compared with the uninoculated control

Table 6 Effects of rhizobacterial inoculation on tomato seedling growth parameters, including root and stem length, fresh and dry biomass, and chlorophyll content (SPAD units).

Treatment	Root Length (cm)	Stem Length (cm)	Fresh Weight—Root (g)	Fresh Weight—Stem (g)	Dry Weight—Root (g)	Dry Weight—Stem (g)	SPAD Units
<i>A (Control)</i>	6.07 ± 0.30	6.33 ± 0.36	2.80 ± 0.03 c	2.32 ± 0.04 b	0.32 ± 0.05 d	0.17 ± 0.01 c	35.0 ± 0.36 d
<i>B</i>	6.50 ± 0.30	5.90 ± 0.36	2.31 ± 0.03 e	1.61 ± 0.04 d	0.60 ± 0.05 c	0.22 ± 0.05 c	39.0 ± 0.31 b
<i>C</i>	5.93 ± 0.30	7.20 ± 0.36	2.62 ± 0.03 d	1.81 ± 0.04 c	0.42 ± 0.07 cd	0.25 ± 0.02 bc	38.5 ± 0.91 bc
<i>D</i>	6.73 ± 0.30	6.40 ± 0.36	3.14 ± 0.03 b	2.14 ± 0.04 b	0.84 ± 0.04 b	0.33 ± 0.02 b	36.6 ± 0.65 cd
<i>E (Consortium)</i>	6.93 ± 0.30	7.63 ± 0.36	3.42 ± 0.03 a	3.63 ± 0.04 a	1.23 ± 0.12 a	0.46 ± 0.03 a	41.4 ± 0.80 a
CV (%)	8.15	9.22	1.82	2.98	11.82	10.48	1.82
<i>p</i> (%)	0.1896	0.0477	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001

assessed in soil-based pot experiments. Overall, inoculated plants demonstrated significant improvements across morphological and physiological parameters compared to the uninoculated control.

Photosynthetic Pigments and Biomass Allocation

Chlorophyll content, as measured by SPAD values, was highest in Consortium E treatment, showing an 18.2% increase relative to the control. Other treatments exhibited moderate enhancements of 11.4% (B), 9.9% (C), and 4.4% (D). Root architecture responded differently depending on the isolate: G-33 (Treatment D) produced the longest roots (6.73 cm), while the shortest roots (5.93 cm) were observed in Treatment C (Table 6). The superior biomass accumulation and photosynthetic efficiency in G-33-treated plants are likely associated with its high IAA production (83.20 µg mL⁻¹) and nitrogen-fixing capability.

Seedling Development and Growth Kinetics

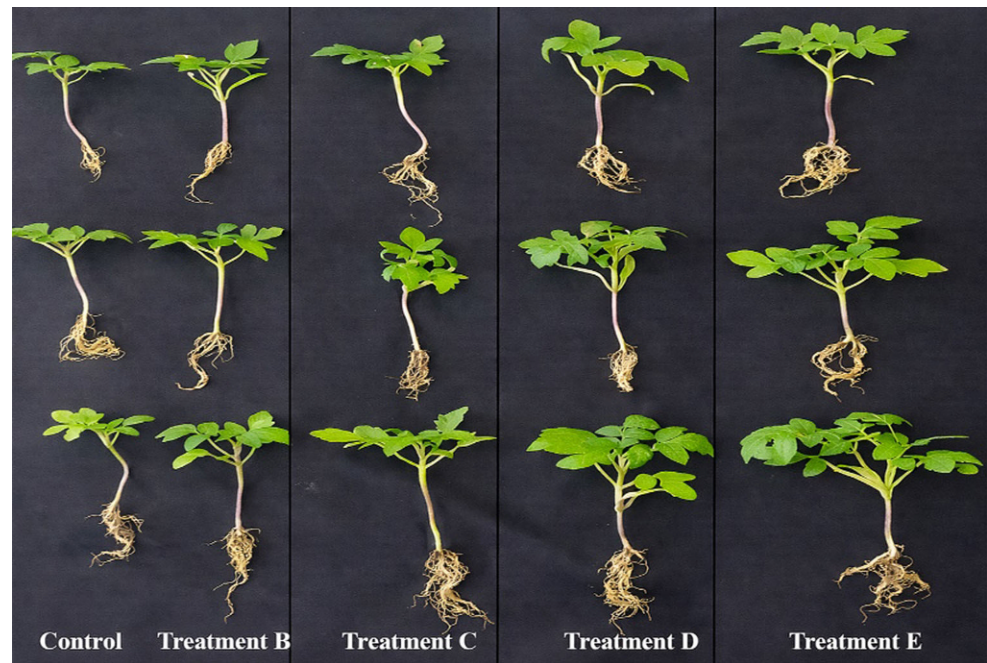
PGPR inoculation significantly enhanced vegetative growth of tomato seedlings. Stem elongation was higher in all bacterial treatments than in the uninoculated control, with Consortium E demonstrating the most pronounced effect, achieving the greatest stem length (7.63 cm) and fresh weight (3.63 g) (Figs. 9 and 10). Dry matter accumulation, a key indicator of nutrient-use efficiency, was also improved by microbial inoculation. Treatment E showed the highest root dry matter (1.23 g) and stem dry matter (0.46 g), followed by Treatments D, B, and C in descending order. These results confirm the robust plant growth-promoting potential of the selected isolates, emphasizing their role in accelerating seedling establishment and enhancing overall structural development of cherry tomato plants.

Values are presented as mean ± SD. Different letters (a–e) within a column indicate significant differences according to Tukey's HSD test ($p \leq 0.05$). Values in the same column sharing the same letter are not significantly different. Data satisfied normality (Shapiro-Wilk) and homogeneity of variances (Levene) assumptions ($p > 0.05$).

Synergistic Effects of Microbial Consortia Vs. Individual Inoculation

The rhizosphere harbors diverse microorganisms capable of providing essential nutrients and promoting plant growth through mechanisms such as IAA secretion, phosphate solubilization, and siderophore production (Jain et al. 2021). In our study, *B. subtilis* E-30, combining IAA production (45.1 µg mL⁻¹) with phosphate solubilization and nitrogen fixation, increased stem and root length by ~7% compared to the control. Similarly, *B. pumilus* G-33 achieved the

Fig. 10 Tomato seedling growth following inoculation with individual rhizobacterial isolates and a microbial consortium, compared with the uninoculated control



longest root length among single-strain treatments, likely due to its high nitrogen-fixing and IAA-synthetic activities, consistent with previous reports (Liu et al. 2023). Recent research emphasizes the benefits of microbial consortia over single-strain inoculants. While individual strains are effective, their performance can be limited by native rhizosphere competition and environmental variability. Co-inoculating multiple *Bacillus* strains can provide more reliable growth promotion (Shaffique et al. 2023; Narine et al. 2025). Unlike many previous studies that focus on single-strain inoculation, our findings clearly demonstrate that the combined application of indigenous *Bacillus* strains results in a more pronounced and consistent improvement in plant growth parameters. This suggests that synergistic interactions among strains may enhance their functional efficiency under greenhouse conditions. In our study, the three-strain consortium (Treatment E) produced the greatest improvements in stem/root length, total biomass, and leaf weight. These results align with He et al. (2019), who reported enhanced root-to-shoot ratios with a *Bacillus* mixture, and with Wang et al. (2025), who observed biomass increases of 180–500% in tomato plants treated with integrated PGPR consortia. Beyond growth promotion, consortia also confer disease suppression benefits, such as reducing *Verticillium* wilt incidence (Oulad Ziane et al. 2025).

Conclusion

This study highlights the functional significance of rhizospheric *Bacillus* strains isolated from cherry tomato

(*Solanum lycopersicum* L.) in fostering plant development and providing biological protection. Molecular screening and phylogenetic analysis successfully identified the isolates as *B. subtilis* E-30, *B. thuringiensis* F1-32, and *B. pumilus* G-33, each clustering within distinct, well-established taxonomic groups.

In vitro characterization revealed diverse plant growth-promoting (PGP) traits, including nitrogen fixation, inorganic phosphate solubilization, and the production of indole-3-acetic acid (IAA) and siderophores. Strain-specific variations were observed *B. thuringiensis* F1-32 showed the highest IAA and siderophore production, while *B. subtilis* E-30 excelled in phosphate solubilization. All isolates enhanced nutrient availability, and extracellular enzyme production by E-30 and F1-32 translated into significant antagonistic activity against *Alternaria alternata*, confirming their biocontrol potential. Greenhouse trials demonstrated the practical benefits of these strains. While individual inoculations improved seedling growth, the PGPR consortium (E-30, F1-32, G-33) achieved the greatest gains in root and stem development, biomass accumulation, and chlorophyll content, indicating that synergistic interactions can outperform single-strain treatments.

A limitation of this study is the lack of field validation. Future research should focus on multi-location trials to assess the stability, scalability, and efficacy of these microbial consortia under diverse environmental and soil conditions. Overall, these locally adapted rhizobacteria represent promising, eco-friendly alternatives to synthetic fertilizers and chemical pesticides, offering a sustainable strategy to

enhance cherry tomato productivity while reducing losses from soil-borne pathogens.

Author contributions Author contributions: Murat GÜLER (Conceptualization: Lead; Data curation: Lead; Formal analysis: Equal; Investigation: Lead; Methodology: Lead; Resources: Equal; Validation: Equal; Visualization: Lead; Writing – original draft: Equal; Writing – review & editing: Equal). Fatma KAYAÇETİN (Formal analysis: Equal; Resources: Equal; Validation: Equal; Writing – original draft: Equal; Writing – review & editing: Equal)

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Data Availability No datasets were generated or analysed during the current study.

Declarations

Competing interests The authors declare no competing interests.

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