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Endophytic and Rhizosphere Bacteria from *Opuntia dillenii* (Ker-Gawl.) Haw (Cactaceae) as Biological Agents against *Sclerotium rolfsii*

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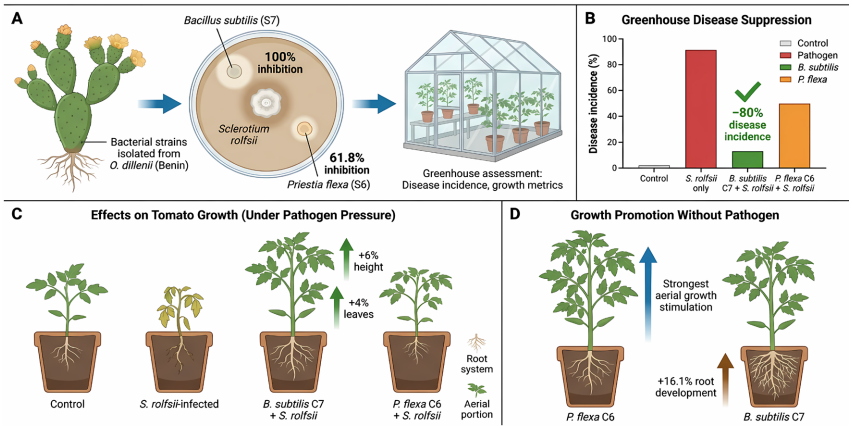
Abstract

Tomato is a crop of major agronomic importance, severely affected by soil-borne diseases such as collar rot caused by *Sclerotium rolfsii*. This study aimed to evaluate the biocontrol potential of bacterial strains isolated from *Opuntia dillenii* in Benin against this pathogen. An *in-vitro* screening identified the most effective antagonistic strains, which were subsequently assessed under greenhouse conditions for their effects on disease incidence and plant growth. Under pathogen pressure, strong variability was observed among the tested strains. *In-vitro*, *Bacillus subtilis* S7 and *Priestia flexa* S6 showed the highest inhibition potential, with inhibition rates of 100% and 61.77%, respectively. In greenhouse conditions, *B. subtilis* C7 was the most effective treatment, reducing disease incidence by 80% compared to the inoculated control while maintaining shoot growth (height: 6.0%; number of leaves: 4.0%). In the absence of pathogen pressure, strain-dependent growth-promoting effects were observed. *P. flexa* C6 exhibited the strongest stimulatory effects on aerial growth, while *B. subtilis* C7 significantly enhanced root development (16.1%). These results highlight the strong biocontrol potential of selected bacterial strains against *S. rolfsii*, while also demonstrating their capacity to promote tomato growth under non-stress conditions.

Graphical Abstract



Keywords

Tomato, *Sclerotium rolfsii*, Biocontrol, *Priestia flexa*, *Bacillus subtilis*, *Opuntia dillenii*

1. Introduction

Tomato (*Solanum lycopersicum* L.) is one of the world's most important vegetable crops. Annual tomato production is estimated at 180 million tons, with a market value exceeding $80 billion [1]. However, the production and marketing of fresh tomatoes are severely affected (or threatened) by numerous plant pathogens, particularly fungi and bacteria. These pathogens cause significant yield losses both during cultivation and during post-harvest preservation and storage [2]. Among the major diseases affecting tomatoes worldwide, collar rot caused by *Sclerotium rolfsii* arise among the most destructive one [3] [4]. *S. rolfsii* is considered one of the most aggressive soil-borne fungal pathogens affecting tomatoes. With an extremely broad host range comprising more than 500 species, it is capable of infecting the plant at all development stages of its. These infections can result in complete yield loss (100%), particularly in warm, humid environments conducive to the pathogen's development [5]. Over the past few decades, plant disease control has relied primarily on the use of chemical pesticides. Although these products offer, in most cases, immediate crop protection, their intensive and prolonged use can lead to numerous undesirable effects, including toxicity to non-target organisms, environmental pollution, loss of biodiversity, increased pathogen resistance due to selection pressure, higher economic costs, and even chronic or acute toxicity to humans [6].

In this context, the search for sustainable alternatives has led to growing interest in biological control based on the use of plant-growth-promoting bacteria (PGPB). The most frequently reported PGPB genera include *Bacillus*, *Azoarcus*, *Pseudomonas*, *Serratia*, *Enterobacter*, *Streptomyces*, *Herbaspirillum*, *Azospirillum*, *Azo-*

tobacter, *Klebsiella*, *Alcaligenes*, *Arthrobacter*, *Burkholderia*, *Microbacterium*, *Micrococcus*, *Pantoea*, and *Stenotrophomonas* [7]-[15]. These bacteria can exert an antagonistic effect against plant pathogens through various mechanisms, such as the production of antibiotic lipopeptides, the induction of systemic resistance, the release of hydrolytic enzymes and volatile organic compounds, as well as the modulation of the indigenous microbiota of the soil or the plants into which they are inoculated [16].

This study adopts an ecological and sustainable approach and aims to evaluate the antifungal potential of bacterial strains isolated from *Opuntia dillenii* against *S. rolfsii*. These strains had previously been isolated, identified, and characterized for their PGP traits, as well as various extracellular enzymatic activities, by Brun *et al.* [17].

2. Methodology

2.1. Origin of Bacterial Strains

The bacterial strains used in this study were isolated from the endophytic tissues and rhizosphere of *Opuntia dillenii*, collected from the coastal region of Benin. These isolates (31), characterized in our previous work [17], were stored at −20˚C in Nutrient Broth (HIMEDIA, India) supplemented with 30% glycerol (**Table 1**).

Table 1. Bacterial strains.

Origin/Source	Codes	GenBank accession no.	Identity
Cladode	C1	PZ149653	*Priestia flexa*
	C2	PZ149654	*Priestia flexa*
	C3	PZ102197	*Providencia rettgeri*
	C4	PZ149655	*Bacillus paranthracis*
	C5	PZ149656	*Priestia flexa*
	C6	PZ149657	*Priestia flexa*
	C7	PZ149658	*Bacillus subtilis*
	C8	PZ149659	*Bacillus amyloliquefaciens*
	C9	PZ102203	*Staphylococcus hominis*
	C10	PZ149660	*Priestia flexa*
	C11	PZ102205	*Micrococcus yunnanensis*
	C12	PZ149661	*Bacillus tropicus*
Root	R1	PZ102207	*Cronobacter sakazakii*
	R2	PZ149662	*Bacillus anthracis*
	R3	PZ149663	*Bacillus subtilis*

Continued

	R4	PZ149664	*Bacillus amyloliquefaciens*
	R5	PZ102211	*Cronobacter sakazakii*
	R6	PZ149665	*Bacillus subtilis*
	R7	PZ149666	*Priestia flexa*
	R8	PZ149667	*Bacillus cereus*
	R9	PZ149668	*Bacillus cereus*
	R10	PZ102216	*Pseudochrobactrum asaccharolyticum*
	R11	PZ102217	*Providencia rettgeri*
Soil	S1	PZ102218	*Microbacterium aborescens*
	S2	PZ102219	*Heyndrickxia oleronia*
	S3	PZ102220	*Alcaligenes faecalis*
	S4	PZ149669	*Priestia flexa*
	S5	PZ149670	*Priestia flexa*
	S6	PZ149671	*Priestia flexa*
	S7	PZ149672	*Bacillus subtilis*
	S8	PZ149673	*Priestia flexa*

2.2. Origin of Phytopathogenic Strains

S. rolfsii Sr01-T-0625 was obtained from the Laboratory of Analysis and Research for Crop Protection (LAR-DC) at the National Institute for Agricultural Research of Benin (INRAB).

2.3. *In Vitro* Screening of *S. rolfsii* Antagonistic Strains

The bacterial strains were tested for their ability to inhibit the mycelial growth of *S. rolfsii in vitro* using the direct confrontation method on Potato Dextrose Agar (PDA, HIMEDIA, India), following the method described by Bidima *et al.* [18]. For each bacterial strain, four streaks were made on PDA agar. They were arranged in a square, each at a radial distance of 3 cm from the center of the plate. After 48 hours of incubation at 30˚C, a *S. rolfsii* mycelial inoculum (5 mm in diameter, taken from a 5-day-old culture) was placed in the center of the plate. Observations were made after 72 hours of incubation at 28˚C ± 2˚C, at which point complete mycelial growth was observed in the control plates. Mycelial growth of the pathogen was measured individually in each Petri dish and expressed in millimeters (mm). The Percentage Inhibition Radius (PIR) [19] of the mycelial growth of *S. rolfsii*, induced by the various bacterial strains tested, was determined using Equation (1):

$$I = \frac{C - T}{T} \times 100 \quad (1)$$

where: C = radial growth of *S. rolfsii* on the control plate (mm), T = radial growth of *S. rolfsii* in the presence of the bacterial isolate (mm). All experiments were performed in triplicate.

2.4. *In Vivo* Evaluation of the Antagonistic Potential of the Selected Bacterial Strains

2.4.1. Experimental Conditions

The experiment was conducted in a greenhouse at LAR-DC. The ambient temperature in the greenhouse ranged between 25˚C and 29˚C. The experimental design was a completely randomized block design comprising four blocks. The experimental unit consisted of a pot containing a tomato plant, and five plants were assessed per treatment in each block, making a total of 20 plants per treatment.

Eight (08) treatments were evaluated: six antagonistic strains, one positive control and one negative control. A supplementary trial, conducted outside the main experiment without inoculation of the pathogen, was used to assess the pathogenicity of the strains. Tomato seeds (Akikonkouin variety), obtained from the authorized distributor "Jardin Pour Tous", were sown in trays and maintained for three weeks. The seedlings were then transplanted into pots containing 500 g of sterilized substrate (soil/compost, 2:1, v/v) and watered daily with 20 mL of sterile distilled water.

Monitoring was carried out for 35 days after transplanting. The entire trial was repeated twice in a greenhouse, using an identical experimental design, to ensure the robustness and reproducibility of the results.

2.4.2. Control of *S. rolfsii*

The *S. rolfsii* inoculum was prepared on PDA medium and incubated for seven days until a well-developed mycelium was obtained. Substrate inoculation was performed using four agar discs (2 mm in diameter) colonized by the fungus, buried at the four corners of each pot and covered with tomato leaf fragments to promote infection [20]. After 48 hours of watering with 20 mL of sterile distilled water, the three-week-old tomato seedlings were transplanted into the inoculated pots and then treated by applying 20 mL of an antagonistic bacterial suspension (10^8 CFU/mL). The incidence of collar rot was expressed as a percentage of the total number of affected seedlings and calculated using Equation (2):

$$\text{Incidence}(\%) = \frac{\text{Number of affected seedlings}}{\text{Total number of seedlings observed}} \times 100 \quad (2)$$

2.4.3. Growth Parameters

The effect of bacterial strains on tomato plant growth was assessed 35 days after transplanting. Plant height and root length were measured with a ruler. The number of leaves was counted. Root biomass was measured using a precision scale (G&G Electronic Scale, China) [3].

2.5. Statistical Analysis

The collected data were analyzed using R software (version 4.4.3). A one-way anal-

ysis of variance (ANOVA) was performed to assess significant differences between treatments. When significant differences were observed ($p < 0.05$), means were compared using the Student-Newman-Keuls (SNK) post hoc test. Additionally, a two-factor analysis of variance (treatment and replication) was performed to assess the stability of the treatment effect across replications and to test for the presence of a treatment × replication interaction.

3. Results

3.1. *In Vitro* Antagonistic Effects of Bacterial Strains against *S. rolfsii*

Analysis of the results presented in Table 2 reveals significant variability in the antagonistic activity of the bacterial strains against *S. rolfsii*. Of the 31 bacterial strains evaluated, the majority did not exhibit inhibitory activity against the pathogen (PIR = 0). However, some strains (19.35%) exhibited notable antagonistic activity. Strains derived from cladodes, particularly *P. flexa* C6 and *B. amyloliquefaciens* C8, induced weak inhibition of mycelial growth, with a PIR of 14.71%. The *B. subtilis* C7 strain stood out for its moderate inhibition, characterized by a PIR of 38.24%. Among the strains isolated from roots, only the *C. sakazakii* R5 strain exhibited moderate antagonistic activity, with a PIR also equal to 38.24%. In contrast, the strains from soil exhibited the highest levels of inhibition. The strains *P. flexa* S6 and *B. subtilis* S7 (Figure 1) recorded PIRs of 61.77% and 100%, respectively, indicating strong antagonistic activity against *S. rolfsii*.

Based on these results, the strains *P. flexa* S6, *B. subtilis* S7, *B. subtilis* C7, *C. sakazakii* R5, *P. flexa* C6, and *B. amyloliquefaciens* C8 appear to be the most promising candidates for further *in vivo* testing, due to their ability to effectively inhibit the growth of *S. rolfsii*.

Figure 1. Antifungal activity of *B. subtilis* S7 against *S. rolfsii*.

Table 2. Inhibitory effect of bacterial strains on *S. rolfsii.*

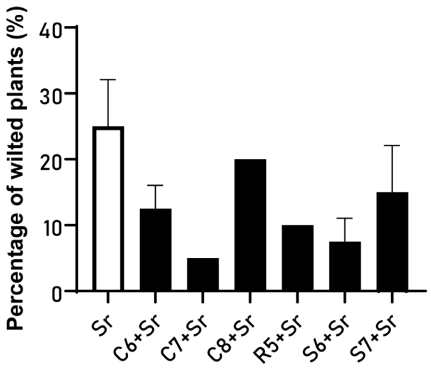
Origin/Source	Identity	Percentage Inhibition Radius (PIR) ($\bar{x} \pm \bar{\sigma}_x$)[1]
Cladode	*P. flexa* C1	0 ± 00^{d}
	P. flexa C2	0 ± 00^{d}
	P. rettgeri C3	0 ± 00^{d}
	B. paranthracis C4	0 ± 00^{d}
	P. flexa C5	0 ± 00^{d}
	P. flexa C6	14.71 ± 8.82^{c}
	B. subtilis C7	38.24 ± 2.94^{bc}
	B. amyloliquefaciens C8	14.71 ± 2.94^{c}
	S. hominis C9	0 ± 00^{d}
	P. flexa C10	0 ± 00^{d}
	M. yunnanensis C11	0 ± 00^{d}
	B. tropicus C12	0 ± 00^{d}
Root	*C. sakazakii* R1	0 ± 00^{d}
	B. anthracis R2	0 ± 00^{d}
	B. subtilis R3	0 ± 00^{d}
	B. amyloliquefaciens R4	0 ± 00^{d}
	C. sakazakii R5	35.29 ± 5.88^{bc}
	B. subtilis R6	0 ± 00^{d}
	P. flexa R7	0 ± 00^{d}
	B. cereus R8	0 ± 00^{d}
	B. cereus R9	0 ± 00^{d}
	P. asaccharolyticum R10	0 ± 00^{d}
	P. rettgeri R11	0 ± 00^{d}
Soil	*M. aborescens* S1	0 ± 00^{d}
	H. oleronia S2	0 ± 00^{d}
	A. faecalis S3	0 ± 00^{d}
	P. flexa S4	0 ± 00^{d}
	P. flexa S5	0 ± 00^{d}
	P. flexa S6	61.77 ± 2.94^{b}
	B. subtilis S7	100 ± 0.00^{a}
	P. flexa S8	0 ± 00^{d}

[1] $\bar{x}$: Mean; $\bar{\sigma}_x$: Standard Error; Within the same column, means marked with different letters are significantly different at the 5% level according to the Student-Newman-Keuls test.

3.2. Potential of Preselected Bacterial Strains for *in Vivo* Control *S. rolfsii*

3.2.1. Percentage of Wilting in Tomato Plants

In the presence of *S. rolfsii*, the wilting rate of tomato plants varies depending on the bacterial strains applied. The inoculated control (Sr) exhibits 25% wilting. Treatment with *B. subtilis* C7 shows the greatest efficacy, with only 5% wilting, representing an 80% reduction compared to the control. Significant reductions are also observed with *P. flexa* S6 and *C. sakazakii* R5, with respective decreases of 70% and 60% (Figure 2). No wilting symptoms were observed on plants not inoculated with *S. rolfsii*, confirming that the symptoms recorded in the other treatments are exclusively linked to the presence of the pathogen.



Sr: *S. rolfsii;* C6: *P. flexa* C6; C7: *B. subtilis* C7; C8: *B. amyloliquefaciens* C8; R5: *C. sakazakii* R5; S6: *P. flexa* S6; and S7: *B. subtilis* S7.

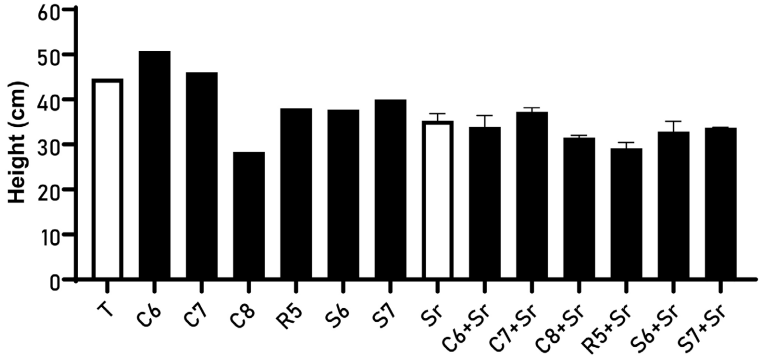
Figure 2. Percentage of wilted plants by treatment.

3.2.2. Effect of Treatments on Tomato Plant Height

The treatments significantly affected plant height ($p < 2 \times 10^{-16}$), whereas the replicate ($p = 0.700$) and the treatment × replicate interaction ($p = 0.986$) showed no significant effect. In the absence of *S. rolfsii*, treatment with the *P. flexa* C6 strain resulted in the greatest increase in plant height compared to the control (13.9%). In the presence of *S. rolfsii*, growth was generally reduced, and only treatment with the *B. subtilis* C7 strain resulted in a 6.0% improvement compared to the inoculated control (Sr) (Table 3, Figure 3).

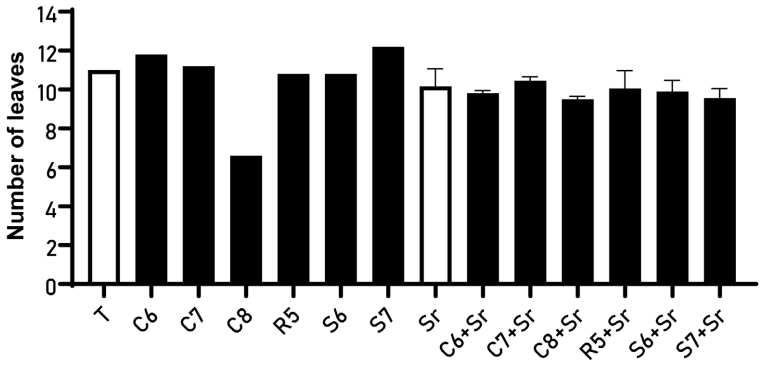
3.2.3. Effect of Treatments on the Number of Leaves

The treatments induced significant variations in the number of leaves. The two-factor ANOVA revealed a highly significant effect of the treatment factor ($p < 2 \times 10^{-16}$), while, neither the replication ($p = 0.114$) nor the treatment × replication interaction ($p = 0.903$) significantly influenced this parameter. Under conditions not inoculated with *S. rolfsii*, the number of leaves increased by 10.9% with *B. subtilis* S7 and by 7.3% with *P. flexa* C6 compared to the control. In contrast, in the presence of *S. rolfsii*, only a slight increase (4.0%) was observed with *B. subtilis* C7, as the other treatments failed to offset the negative effect of inoculation (Table 3, Figure 4).



Sr: *S. rolfsii*; C6: *P. flexa* C6; C7: *B. subtilis* C7; C8: *B. amyloliquefaciens* C8; R5: *C. sakazakii* R5; S6: *P. flexa* S6; and S7: *B. subtilis* S7.

Figure 3. Average plant height by treatment.



Sr: *S. rolfsii*; C6: *P. flexa* C6; C7: *B. subtilis* C7; C8: *B. amyloliquefaciens* C8; R5: *C. sakazakii* R5; S6: *P. flexa* S6; and S7: *B. subtilis* S7.

Figure 4. Average number of leaves per treatment.

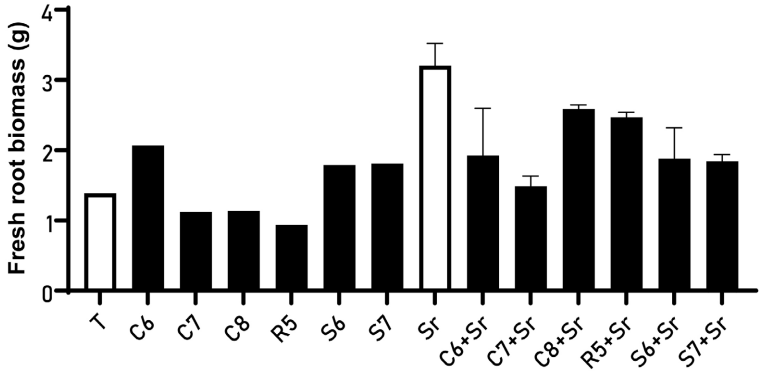
3.2.3. Effect of Treatments on Fresh Root Biomass

The treatments induced significant variations in fresh root biomass. The two-factor ANOVA highlights a highly significant effect of the treatments ($p < 2 \times 10^{-16}$), whereas the replication ($p = 0.259$) and the treatment × replication interaction ($p = 0.203$) are not significant. In the absence of *S. rolfsii* inoculation, the *P. flexa* C6, *B. subtilis* S7, and *P. flexa* S6 treatments increased fresh root biomass by 48.9%, 30.2%, and 28.8%, respectively, compared to the control. In contrast, in the presence of *S. rolfsii*, no improvement in fresh root biomass was observed regardless of the treatment, compared to the inoculated control (**Table 3**, **Figure 5**).

3.2.4. Effect of Treatments on Root Length

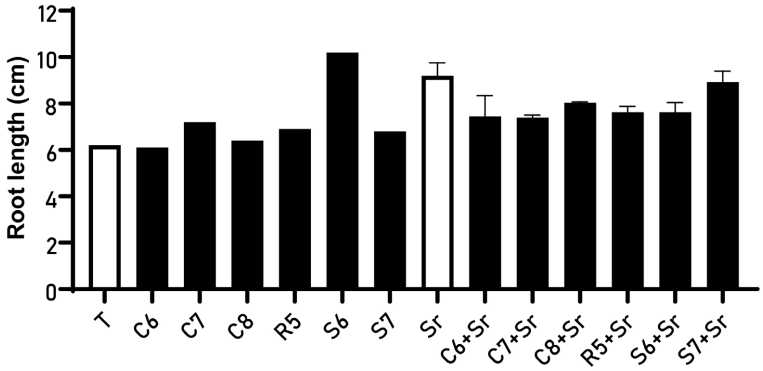
The treatments induced significant variations in the parameter studied. The two-factor ANOVA highlights a highly significant effect of the treatments ($p < 2 \times 10^{-16}$), while the replication ($p = 0.989$) and the treatment × replication interaction ($p = 0.934$) are not significant. In the absence of *S. rolfsii*, treatment with *P. flexa* S6 resulted in a significant increase in root length, corresponding to a 64.5% increase compared to the control group. Treatments with *B. subtilis* C7 and *C. sakazakii* R5 also induced increases of 16.1% and 11.3%, unlike *P. flexa* C6 and *B.*

amyloliquefaciens C8, which showed no notable improvement. In contrast, in the presence of *S. rolfsii*, no improvement in root length was observed regardless of the treatment, compared to the inoculated control (Table 3, Figure 6).



Sr: *S. rolfsii;* C6: *P. flexa* C6; C7: *B. subtilis* C7; C8: *B. amyloliquefaciens* C8; R5: *C. sakazakii* R5; S6: *P. flexa* S6; and S7: *B. subtilis* S7.

Figure 5. Average fresh root biomass by treatment.



Sr: *S. rolfsii;* C6: *P. flexa* C6; C7: *B. subtilis* C7; C8: *B. amyloliquefaciens* C8; R5: *C. sakazakii* R5; S6: *P. flexa* S6; and S7: *B. subtilis* S7.

Figure 6. Average root length by treatment.

Table 3. Effect of treatments on growth parameters of tomato seedlings.

Parameters	Plant height $\bar{x} \pm \bar{\sigma}_x$ (cm)		Number of leaves $\bar{x} \pm \bar{\sigma}_x$		Fresh root biomass $\bar{x} \pm \bar{\sigma}_x$ (g)		Root length $\bar{x} \pm \bar{\sigma}_x$ (cm)	
Treatments	**Replication 1**	**Replication 2**	**Replication 1**	**Replication 2**	**Replication 1**	**Replication 2**	**Replication 1**	**Replication 2**
T	44.6 ± 1.6^{ade}	44.6 ± 1.6^{ae}	11 ± 0.3^{abc}	11 ± 0.3^{abc}	1.39 ± 0.09^{cd}	1.39 ± 0.09^{ce}	6.2 ± 0.27^{a}	6.2 ± 0.27^{a}
C6	50.8 ± 3.4^{a}	50.8 ± 3.4^{a}	11.8 ± 0.5^{ab}	11.8 ± 0.5^{ab}	2.07 ± 0.25^{abc}	2.07 ± 0.25^{abc}	6.1 ± 0.17^{a}	6.1 ± 0.17^{a}
C7	46.0 ± 1.9^{ad}	46.0 ± 1.9^{ae}	11.2 ± 0.4^{abc}	11.2 ± 0.4^{abc}	1.12 ± 0.11^{d}	1.12 ± 0.11^{e}	7.2 ± 0.17^{ab}	7.2 ± 0.17^{ac}
C8	28.4 ± 2.1^{b}	28.4 ± 2.1^{b}	6.6 ± 0.7^{d}	6.6 ± 0.7^{d}	1.14 ± 0.09^{d}	1.14 ± 0.09^{e}	6.4 ± 0.18^{ab}	6.4 ± 0.18^{a}
R5	38.0 ± 1.3^{cde}	38.0 ± 1.3^{cde}	10.8 ± 0.3^{abc}	10.8 ± 0.3^{abc}	0.94 ± 0.07^{d}	0.94 ± 0.07^{e}	6.9 ± 0.11^{ab}	6.9 ± 0.11^{a}
S6	37.8 ± 1.2^{cde}	37.8 ± 1.2^{cde}	10.8 ± 0.3^{abc}	10.8 ± 0.3^{abc}	1.79 ± 0.04^{acd}	1.79 ± 0.04^{abce}	10.2 ± 1.06^{c}	10.2 ± 1.06^{d}

Continued

S7	40.0 ± 0.6^{cde}	40.0 ± 0.6^{de}	12.2 ± 0.2^{b}	12.2 ± 0.2^{b}	1.81 ± 0.11^{acd}	1.81 ± 0.11^{abce}	6.8 ± 0.34^{ab}	6.8 ± 0.34^{a}
SR	34.2 ± 1.1^{bc}	36.4 ± 1.8^{bcde}	9.5 ± 0.4^{ac}	10.8 ± 0.4^{abc}	2.98 ± 0.31^{b}	3.43 ± 0.39^{d}	8.8 ± 0.71^{bc}	9.59 ± 0.96^{bd}
C6 + SR	35.7 ± 1.6^{bc}	32.1 ± 2.1^{bcd}	9.7 ± 0.5^{ac}	9.9 ± 0.6^{ac}	2.40 ± 0.29^{ab}	1.45 ± 0.16^{bce}	8.08 ± 0.42^{abc}	6.81 ± 0.38^{ac}
C7 + SR	36.6 ± 2.2^{bce}	37.9 ± 2.9^{cde}	10.3 ± 0.5^{abc}	10.6 ± 0.3^{abc}	1.39 ± 0.17^{cd}	1.59 ± 0.10^{abce}	7.31 ± 0.44^{ab}	7.47 ± 0.35^{abc}
C8 + SR	31.9 ± 1.8^{bc}	31.1 ± 1.9^{bcd}	9.6 ± 0.5^{ac}	9.4 ± 0.8^{c}	2.63 ± 0.30^{ab}	2.55 ± 0.51^{ad}	8.06 ± 0.50^{abc}	8.00 ± 0.45^{abcd}
R5 + SR	28.2 ± 1.5^{b}	30.1 ± 1.2^{bc}	9.4 ± 0.5^{c}	10.7 ± 0.5^{abc}	2.52 ± 0.21^{ab}	2.42 ± 0.37^{abd}	7.45 ± 0.43^{ab}	7.80 ± 0.41^{abcd}
S6 + SR	31.3 ± 1.8^{bc}	34.5 ± 1.7^{bcd}	9.5 ± 0.7^{c}	10.3 ± 0.2^{abc}	2.19 ± 0.28^{abc}	1.57 ± 0.14^{abce}	7.92 ± 0.58^{ab}	7.32 ± 0.47^{abc}
S7 + SR	33.8 ± 1.7^{bc}	33.6 ± 1.3^{bcd}	9.2 ± 0.5^{c}	9.9 ± 0.3^{ac}	1.91 ± 0.24^{acd}	1.78 ± 0.16^{abce}	8.59 ± 0.49^{bc}	9.26 ± 0.42^{bcd}
P-value	***	***	***	***	***	***	***	***

$\bar{x}$: Mean; $\bar{\sigma}_x$: Standard Error; ° = $p > 0.05$ (not significant); * = $p < 0.05$ (significant); ** = $p < 0.01$ (highly significant); *** = $p < 0.001$ (very highly significant); Within the same column, means marked with different letters are significantly different at the 5% level according to the Student-Newman-Keuls test.

4. Discussion

Worldwide, tomato cultivation represents a crop of major nutritional, economic, and public health importance. However, its productivity is severely limited by soil-borne diseases such as collar rot caused by *S. rolfsii*. Given the limitations of conventional control methods, this study explored a biocontrol approach based on indigenous bacteria isolated from the rhizosphere and endosphere of *Opuntia dillenii*.

In this context, in vitro trials showed that the highest levels of inhibition were observed with *Bacillus subtilis* S7 (PIR = 100%) and *P. flexa* S6 (PIR = 61.77%), indicating strong antifungal activity. This efficacy is consistent with their high production of proteases and lipases previously described by Brun *et al.* [17]. Under *in vitro* conditions, direct enzymatic degradation of the mycelium is the central mechanism: proteases alter the protein structures of the fungal cell wall, whilst lipases disrupt membrane integrity, leading to the arrest of mycelial growth [21] [22]. Recent studies show that several strains of *B. subtilis*, *B. cereus,* or *B. velezensis* can induce inhibitions of over 60% - 100% of *S. rolfsii* mycelium, directly linked to the production of extracellular hydrolytic enzymes [23]-[25].

Beyond these in vitro observations, the results obtained under pathogen-associated conditions highlight an even greater potential for biocontrol, particularly for *Bacillus subtilis* C7, whose high efficacy suggests a particularly effective direct antifungal interaction. This performance is likely due to the simultaneous mobilizations of several functional traits, including strong enzymatic activity and the ability to compete for nutrients. This type of response is well documented in species of the genus *Bacillus*, which are known for their ability to produce a variety of antifungal metabolites, notably lipopeptides from the iturin, fengycin and surfactin families, as well as hydrolytic enzymes capable of damaging the cell wall,

mycelium and survival structures of *S. rolfsii* [26]. These compounds act by disrupting the cell membrane, inhibiting mycelial growth and limiting sclerotium germination, thereby leading to a significant reduction in the expression of symptoms at the collar and in the root system [27].

In addition to biocontrol effects, results obtained in the absence of the pathogen reveal growth-promoting effects in *P. flexa* C6, *P. flexa* S6 and *B. subtilis* S7, suggesting direct stimulation of plant development. This ability may be linked to their functional traits, notably the production of phytohormones such as IAA, phosphorus solubilization and the production of siderophores, which promote improved plant nutrition and metabolic activity [17]. Thus, inoculation with *Priestia megaterium* led to a significant increase in above-ground and root biomass, leaf length and weight, as well as chlorophyll content, after four months of cultivation under non-stressful conditions; these effects were directly correlated with AIA production and phosphorus solubilization (a significant increase in growth parameters compared with the non-inoculated control) [28]. Similarly, strains of *Bacillus subtilis* showed a marked improvement in phosphorus uptake in tomatoes, associated with stimulation of root growth and the elongation of root hairs, leading to a significant increase in plant biomass and P content in plants grown in uninfected soil [29].

In the presence of the pathogen, the marked reduction in plant growth observed indicates that fungal infection is the predominant factor driving the plant's physiological response, severely limiting the expression of growth-promoting traits. Under intense pressure from necrotrophic soil-borne pathogens, the plant engages in active trade-offs between growth and defence, during which metabolic resources are prioritized for immune responses. It has been shown that the activation of hormonal pathways associated with jasmonate, ethylene and salicylate leads to direct repression of processes related to cell elongation, division and carbon assimilation, resulting in a marked inhibition of vegetative growth [30] [31]. Furthermore, infection by aggressive soil pathogens is accompanied by a profound disruption of root function, including tissue damage, reduced water and mineral uptake, and qualitative and quantitative changes in root exudates. These changes have been identified as major factors in the restructuring of the rhizosphere, negatively affecting the recruitment and persistence of beneficial microorganisms and limiting the effective expression of their PGP traits, even when these are present at the genetic level [32] [33]. In this context of high biotic stress and rhizosphere dysfunction, *Bacillus subtilis* C7 stood out as the only strain capable of maintaining a measurable improvement in above-ground growth. This response suggests that the observed effect is primarily based on a sufficient reduction in pathogen pressure, allowing the plant's physiological functions to be partially preserved, rather than on direct stimulation of plant metabolism. This type of indirect effect, based on limiting the establishment and spread of the pathogen in the rhizosphere, is now recognized as a key characteristic of effective biocontrol agents [34]-[36].

The results also show that seedlings inoculated with the necrotrophic pathogen *Sclerotium rolfsii* Sacc. have an average root weight approximately twice that of the control seedlings inoculated with sterilised water. This may be explained by the fact that the infected plant develops a compensatory mechanism in the presence of S. rolfsii to ensure a continuous supply of nutrients. Indeed, *S. rolfsii* is a basidiomycete fungus that typically causes vascular necrosis, collar rot and significant yield losses, accompanied by an increase in root weight during the early or moderate stages of infection, notably through root overcompensation [37]. The theory of overcompensation suggests that pathogen pressure can paradoxically increase the biomass of the host or its organs. Preston *et al.* [38], in a study modelling structured host-pathogen interactions, reported that biomass increases proportionally with the prevalence of infection. In this study, it appears that the partial destruction of root tissues by the pathogen triggers an apical inhibition response on lateral root growth, thereby releasing compensatory growth potential. Rivera-Vega *et al.* [39], following their research into plant-fungus interactions, demonstrated that the host can redirect its resources towards compensatory vegetative growth in response to an attack. Thus, when *S. rolfsii* preferentially attacks the collar and proximal tissues, the plant can respond by stimulating the production of adventitious lateral roots in areas not yet colonized, thereby increasing root biomass.

The discrepancy observed between the *in vitro* and greenhouse performance of biocontrol strains is widely reported and is mainly explained by the complexity of plant-microorganism-environment interactions, which are not fully replicated under controlled laboratory conditions [40]. In particular, the efficacy of biocontrol agents *in vivo* depends largely on their ability to effectively colonise plant tissues and establish themselves within the microbial community of the rhizosphere, which constitutes a major limiting factor under real-world conditions [41] [42]. The superior performance of *B. subtilis* C7 under greenhouse conditions may therefore be linked to a greater ability to colonise roots, facilitated by high production of exopolysaccharides (EPS). EPS play a key role in bacterial adhesion to roots, the formation of protective biofilms, and the enhancement of the stability of plant-microorganism interactions [43] [44]. This ability, combined with better adaptation to biotic and abiotic soil conditions (microbial competition, water stress, fluctuating nutrient availability), would enable *B. subtilis* C7 to establish itself sustainably in the rhizosphere and effectively express its PGP traits in vivo. Conversely, although *B. subtilis* S7 performs well in vitro, its efficacy appears to be largely restricted to controlled laboratory conditions, suggesting a limited ability to maintain stable root colonisation and to mobilise its PGP traits particularly those related to physical and ecological interactions with the plant under real-world conditions.

5. Conclusions

This study revealed marked functional diversity among the indigenous bacteria

associated with *Opuntia dillenii*, both under pathogen pressure and in the absence of it. When exposed to *Sclerotium rolfsii*, the strains tested exhibited varying levels of efficacy, reflecting the specificity of plant-bacteria-pathogen interactions. Although some strains showed strong antagonistic potential in vitro, particularly via mechanisms linked to extracellular enzymatic activity, this performance was not consistently replicated under greenhouse conditions.

In this regard, *Bacillus subtilis* C7 stood out for its ability to significantly reduce disease incidence whilst maintaining the above-ground growth of tomato plants and promoting root development. In the absence of pathogen pressure, strain-dependent growth-promoting effects were also observed, highlighting the complementary nature of the mechanisms involved.

Overall, these results confirm the potential of indigenous bacteria as biocontrol agents and growth promoters, whilst highlighting the importance of selecting strains adapted to the targeted phytopathological context for effective agronomic application.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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