

Communication

# Compost-Derived Bacterial Communities Offer Promise as Biocontrol Agents against *Meloidogyne javanica* and Promote Plant Growth in Tomato

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**Abstract:** *Meloidogyne* nematodes, commonly known as root-knot nematodes, pose a considerable threat to crop yields, resulting in significant economic losses due to their intricate biology and limited control methods. In line with the European Union's focus on promoting organic farming and soil health to encourage sustainable agricultural practices, this study explores the efficacy of two characterized bacterial communities obtained from compost against *M. javanica* in tomato plants. Through pot experiments, it was demonstrated that both bacterial communities, namely SC1 and SC2, effectively suppressed nematode reproduction and root invasion, which was reflected by a reduction in the number of egg masses per root (by 63% and 28%, respectively) and a reduction in the total progeny population (by 68% and 28%, respectively), with various simultaneously enhanced growth parameters in tomatoes, i.e., aerial part fresh weight increased by 74% and 58%, aerial part dry weight increased by 90% and 55%, and plant height increased by 86% and 53%, respectively. These findings underscore the potential of compost bacterial communities as promising tools for organic or integrated pest management, thereby supporting sustainable agricultural practices and contributing to improved crop yields.



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**Keywords:** *Meloidogyne javanica*; bacterial communities; sustainable agriculture

## 1. Introduction

Root-knot nematodes (Nematoda: Heteroderidae: *Meloidogyne* spp., RKN for short) are clearly included among the most significant soil borne parasites that account for highly significant yield losses per year [1]. In particular, *M. javanica* (Treub) Chitwood is one of the major pests that infests tomato, as well as many other economically important plant species, has a worldwide distribution, and is the most abundant RKN species in Greece [2,3].

Control of these pests has always been an issue as they are considered extremely difficult to suppress due to their soil-inhabiting and endoparasitic nature, broad host range and lack of resistant varieties. Traditionally, RKN control is pursued with chemical nematicides [4], which, during the past few decades, have unequivocally been accused of having a negative environmental impact, leading to their almost total ban and the global scientific community being forced to study and develop more sustainable alternative solutions. These solutions, including biological control agents, should of course be incorporated into integrated pest management schemes [5].

Biological control research has revealed a number of microorganisms, such as bacterial and fungal species, that demonstrate potential control of RKN. For example, *Penicillium chrysogenum* and *Trichoderma* spp. caused 97.67 and 95% mortality of *M. javanica* juveniles in vitro, respectively [6]. Several soil-inhabiting fungal species that express a nematophagous function have been considered as efficient biocontrol agents, i.e., *Trichoderma harzianum*, *Verticillium chlamydosporium*, and *Purpureocillium lilacinum*, as well as species of

the *Pochonia*, *Penicillium*, and *Fusarium* genera. These microorganisms have been reported to effectively kill eggs, juveniles, or female RKN and significantly decrease the population of parasitic nematodes in the soil [7,8]. Other fungi are capable of trapping nematodes by capturing them with special hyphae forming rings or other adhesive structures where the nematodes are trapped and subsequently killed by released nematotoxic metabolites [9]. Additionally, many arbuscular mycorrhizal fungi species (AMF for short) have been shown to enhance growth and inhibit RKN activity when inoculated on the roots of plants growing in RKN infested soils [10]. On the other hand, several species of rhizobacteria including those of the genera *Pseudomonas*, *Bacillus*, and *Pasteuria* have been found to affect RKN species through antibiosis, direct parasitism, competition for food or space, the induction of systemic resistance in the host plant, and the reduction in root penetration [11]. Also, *Streptomyces*, *Bacillus*, *Pseudomonas*, and *Pasteuria* are reported to be nematicidal [11]. *Bacillus subtilis* has also been reported to suppress *M. incognita* egg hatching, and *B. subtilis* bioactive surfactin, lipopeptide, and other compounds have been reported as antagonistic to *M. incognita* [11].

Despite the promise of individual biocontrol agents, none have yet achieved the level of efficacy necessary to fully replace chemical nematicides. This raises an intriguing option that these agents, when working collaboratively within a well-structured community, with each contributing to RKN control, could collectively lead to more promising outcomes.

The plant rhizosphere, the soil zone influenced by root exudates, harbors a diverse microbial community known as the microbiome [12]. This microbiome plays a crucial role in plant health and can be influenced by the plant itself [13,14]. Root knot nematodes (RKN), however, disrupt the rhizosphere through their root invasion and feeding activities, potentially altering microbiome composition [15]. Understanding how RKN infection affects the rhizosphere microbiome and its impact on plant health is essential for developing strategies to manage this devastating pest. The rhizosphere microbiome offers a unique reservoir for engineering biocontrol solutions. By harnessing the diverse microbial communities naturally present around plant roots, we can design selected communities (SCs) with tailored functionalities. These SCs leverage the synergistic interactions between different beneficial microbes, potentially leading to more robust and effective biocontrol compared to single-agent approaches. The advantages of SCs include enhanced efficacy by combining multiple modes of action, improved stability due to their diverse composition, and the potential for plant growth promotion alongside pest suppression [15].

While several studies have focused on the combined control of RKN and soil borne fungi, especially *Fusarium* species, e.g., [16–19], our objective was to investigate the potential of selected bacterial communities (SComs) derived from compost to suppress RKN *M. javanica* infection in tomato plants. These SCs were previously successfully engaged in protecting tomato plants against *Fusarium oxysporum* f. sp. *lycopersici* and promoting plant growth [15]. The use of single biocontrol agents has shown some success, but exploring the effectiveness of the synergistic interactions of microbial communities may offer an interesting advancement in the knowledge of RKN control.

## 2. Materials and Methods

### 2.1. Preparation of *M. javanica* Inoculum

The *M. javanica* population employed for inoculation was reared at the Plant Protection Laboratory of the University of Patras, at Messolonghi, Greece, on tomato seedlings (*Solanum lycopersicum* L.) cv. Belladonna in a temperature-controlled environment ( $27 \pm 1$  °C; 60% humidity; 16 h light) for 4 weeks. The nematode population originated from the Laboratory of Nematology of the IOSV (Heraklion, Crete, Greece) [20]. Well-developed egg masses extracted from the source plant roots were placed on a Baermann funnel at room temperature until the emergence of second-stage juveniles (J2s). The J2s that emerged during the first three days were discarded because of age differences; then, the J2s collected on the fourth day were used as inoculum [21].

## 2.2. Preparation of Bacterial SCs

In this study, we employed two distinct bacterial communities with varying compositions. SC1 comprised a diverse array of bacterial genera and aimed to represent the diverse bacterial community naturally found in the rhizosphere of tomato plants grown in suppressive compost [15]. Conversely, SC2 consisted solely of *Bacillus* isolates, reflecting the dominance of the Firmicutes phylum in the rhizosphere of tomato plants grown in the same conditions [15] (Table 1).

**Table 1.** List of bacterial isolates composing the selected communities (SCs).

SC1	SC2
<i>Agrobacterium tumefaciens</i> 2Ba4	<i>B. chandigarhensis</i> 3Ba3
<i>Bacillus endophyticus</i> 1Ba2	<i>B. endophyticus</i> 1Ba2
<i>B. humi</i> 3Ba30	<i>B. endophyticus</i> 3Ba21
<i>B. licheniformis</i> 3Ba17	<i>B. humi</i> 1Ba28
<i>B. licheniformis</i> 3Ba9	<i>B. humi</i> 1Ba43
<i>B. firmus</i> 2Ba55	<i>B. humi</i> 3Ba30
<i>B. firmus</i> 2Ba2	<i>B. licheniformis</i> 1Ba18
<i>B. niacini</i> 1Ba10	<i>B. licheniformis</i> 3Ba16
<i>Bacillus</i> sp. 2Ba43	<i>B. licheniformis</i> 3Ba17
<i>Chryseobacterium</i> sp. 2Ba19	<i>B. licheniformis</i> 3Ba20
<i>C. wanjuese</i> 2Ba46s	<i>B. licheniformis</i> 3Ba28
<i>Enterobacter cloacae</i> 2Ba30	<i>B. licheniformis</i> 3Ba44
<i>Luteimonas mephitis</i> 1Ba33	<i>B. licheniformis</i> 3Ba45
<i>Microbacterium foliorum</i> 1Ba13	<i>B. licheniformis</i> 3Ba46
<i>M. foliorum</i> 2Ba37	<i>B. licheniformis</i> 3Ba9
<i>Microbacterium</i> sp. 1Ba51	<i>B. megaterium</i> 1Ba20
<i>M. esteraromaticum</i> 3Ba33	<i>B. subtilis</i> 1Ba19
<i>Ochrobactrum</i> sp. 2Ba42	<i>B. subtilis</i> 2Ba19s
<i>Ochrobactrum</i> sp. 2Ba51	<i>B. subtilis</i> CCS
<i>Pseudomonas anguilliseptica</i> 1Ba3	<i>B. circulans</i> 1Ba46
<i>P. putida</i> 3Ba4	<i>B. firmus</i> 2Ba55
<i>Stenotrophomonas maltophilia</i> 1Ba16	<i>B. foraminis</i> 1Ba49
<i>S. maltophilia</i> 2Ba27	<i>B. niacini</i> 1Ba10
<i>S. maltophilia</i> 2Ba50	<i>Bacillus</i> sp. 2Ba43
<i>S. maltophilia</i> 2Ba32	<i>Bacillus</i> sp. 3Ba35

The bacterial isolates within SCs were identified in a prior study by our group using 16S rRNA sequencing [22]. For the preparation of the SCs, each bacterial isolate underwent a 48 h growth period in tryptic soy broth (TSB) at 25 °C with agitation at 180 rpm. Following growth, the cultures were subjected to two rounds of rinsing with a sterile 10 mM MgCl<sub>2</sub> solution, followed by centrifugation at 2600 × g for 8 min. After the final rinse and centrifugation, the cells were resuspended in a 10 mM MgCl<sub>2</sub> solution. The optical density at 600 nm (OD<sub>600</sub>) of each suspension was adjusted to 0.5 (~2.75 × 10<sup>8</sup> cfu mL<sup>-1</sup>). To create selected communities (SCs), the bacterial isolates were mixed in equal proportions. For the tomato pot experiments, 2 mL of each SC suspension with an OD<sub>600</sub> of 0.5 was added to 50 mL of sterile 10 mM MgCl<sub>2</sub> and root-drenched in pots containing 200 g of sterilized potting substrate, resulting in an estimated density of approximately ~2.75 × 10<sup>6</sup> cells per gram of substrate. This mixture was prepared immediately before the surface-sterilized tomato seeds were sown [15], ensuring early colonization of the rhizosphere by the beneficial bacteria upon seed germination.

## 2.3. Pot Experiment

Seeds of the tomato (*Solanum lycopersicum*) cv. Ailsa Craig were surface sterilized following the procedure outlined by Chialva et al. (2016) [23]. Subsequently, the seeds were planted in square pots with dimensions of 9 cm × 9 cm × 8 cm (Pöppelmann TEKU VQB 9 × 9 × 8), each filled with 200 g of sterile peat-based potting substrate (Plantobalt substrate

2, Plantaflor, Vechta, Germany) [22]. The plants were cultivated in a controlled growth chamber (25 °C; 16 h light–8 h dark; 65–70% RH; light intensity 450  $\mu\text{mol m}^{-2} \text{s}^{-1}$  at pot height). The plants were watered every other day, with each receiving an equal volume of water.

At the 3-leaf stage, the plants were subjected to a second root-drenching with one of the SC suspensions, as previously described, or with water to further establish the presence of the bacterial communities in the root zone. Subsequently, they were placed in a growth chamber for acclimatization. After 48 h, the plants underwent manual inoculation with a 2000-J2 nematode inoculum, which was directly applied to the roots of each plant using a pipette. Two weeks later, the plants received a third root-drenching with the respective SC suspensions to reinforce their presence and activity after nematode challenge.

The effectiveness of SCs was compared with that of the commercial product Pochar. Pochar (MS BIOTECH, Roma, Italy) is a liquid formulation containing fungal antagonists *Pochonia* spp. and *Arthrobotrys* spp. and other microbial inocula, which serve as natural enemies against root-knot nematodes. The formulation was activated via mixing with water and Nutryaction (MS BIOTECH, Roma, Italy), an organic fertilizer containing yeast and brown algae extracts, using a ratio of 1:13:2 for formulation–H<sub>2</sub>O–Nutryaction, respectively. The mixture was prepared at least 8 h prior to application on the plants. Application of the formulation was conducted through root-drenching, with each plant receiving 10 mL during each application at the same time points as the application of SCs.

#### 2.4. Experimental Design

The effects of SC1, SC2, and Pochar on *M. javanica* nematode reproduction and tomato plant growth were assessed in a pot experiment performed in a growth room. The plants were arranged in rows and columns in a completely randomized design and rotated within the growth room every second day before watering. The experiment consisted of the following treatment groups:

- (a) SC1: Plants treated with SC1
- (b) SC2: Plants treated with SC2
- (c) Pochar: Plants treated with Pochar
- (d) H<sub>2</sub>O: Plants treated with water
- (e) SC1+nem: Plants treated with SC1 and then inoculated with nematodes
- (f) SC2+nem: Plants treated with SC2 and then inoculated with nematodes
- (g) Pochar+nem: Plants treated with Pochar and then inoculated with nematodes
- (h) H<sub>2</sub>O+nem: Plants treated with water and then inoculated with nematodes

Each treatment combination included ten (10) tomato plants, with one plant per pot, for a total of 80 plants per replicate (8 treatments  $\times$  10 plants/treatment). The experiment was repeated three (3) times with similar results. The data presented in the following sections represent the findings from a representative experiment.

#### 2.5. Plant and Nematode Variables Evaluated

Forty days following nematode inoculation, the plants were uprooted to assess various plant growth parameters. These included plant height, fresh and dry weights of the aerial parts, and fresh weight of the roots.

**Assessment of the total number of egg masses and eggs/egg mass:** The total number of egg masses per plant was counted under a stereomicroscope. Subsequently, for each plant, ten egg masses were randomly selected, placed in 1% sodium hypochlorite, and shaken for 10 min to disperse the eggs, and then, the number of eggs per egg mass was estimated under the stereomicroscope, using a nematode counting dish [21,24].

**Assessment of the nematode population in the soil:** The entire volume of soil extracted from each pot was processed following the conventional Baermann funnel technique [25] to estimate the RKN soil population.

**Assessment of the total number of progeny:** For each plant, the number of eggs per egg mass was multiplied by the total number of egg masses and added to the RKN soil population.

**Assessment of the fresh and dry weights of the aerial parts:** The aerial parts were separated from the roots, their fresh weight was weighed on a 2-digit scale, and then, they were placed in a paper bag and left in an oven at 50 °C until completely dry, and subsequently, their dry weight was recorded.

**Assessment of plant height:** Plant height, i.e., the height from the plant collar up to its top, was measured with a measuring tape in cm.

**Assessment of the fresh weight of roots:** The roots of each plant were separately washed thoroughly but carefully, under running tap water, excess water was removed with a paper towel, and their fresh weight was weighed on a 2-digit scale.

## 2.6. Statistical Analysis

All data were analyzed via one-way ANOVA (analysis of variance), and means were compared via Tukey's test ( $p \leq 0.05$ ) using GraphPad Prism 8 for Windows (GraphPad Software, Version 8.3.0, La Jolla, CA, USA).

## 3. Results

### 3.1. Nematode Population

Regarding the experiments on the impact of the treatments on the reproductive capacity of *M. javanica*, the results revealed that water-treated plants exhibited the highest number of egg masses and eggs per root, as well as total number of progeny, followed by plants treated with SC2 (Figure 1A,C,D). Conversely, plants treated with Pochar and SC1 displayed the lowest number of egg masses and eggs per root (Figure 1A,C), as well as the lowest total number of progeny (Figure 1D). As observed in the diagrams, all treatments (Pochar, SC1, and SC2) had a statistically significant negative impact on the extent of nematode reproduction. Specifically, plants treated with SC1 caused a 63% reduction in the number of egg masses per root and a 68% reduction in the total progeny population, for SC2, reductions of 28% and 28%, respectively, and for Pochar, reductions of 76% and 81%, respectively. Pochar and SC1 had a similar impact on nematode reproduction and the number of progeny, whereas SC2 resulted in a higher population but still lower as compared to the control (water-treated plants).

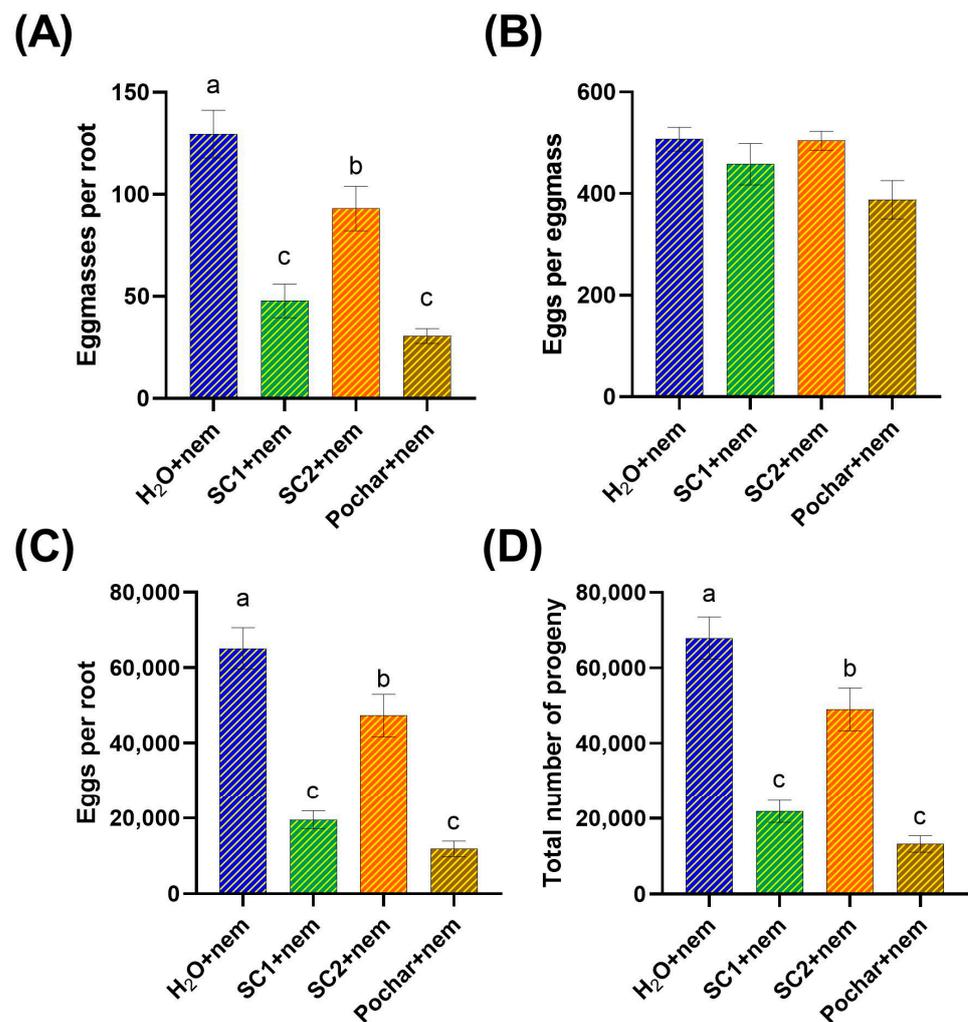
Regarding the number of eggs per egg mass presented in Figure 1B, no statistically significant differences were observed, suggesting that the various treatments did not have a significant impact on the fertility or reproductive success of *M. javanica* females.

Our findings suggest that Pochar, SC1, and SC2 have the potential to suppress nematode reproduction and reduce the overall nematode population in the plant's rhizosphere.

### 3.2. Morphological Parameters

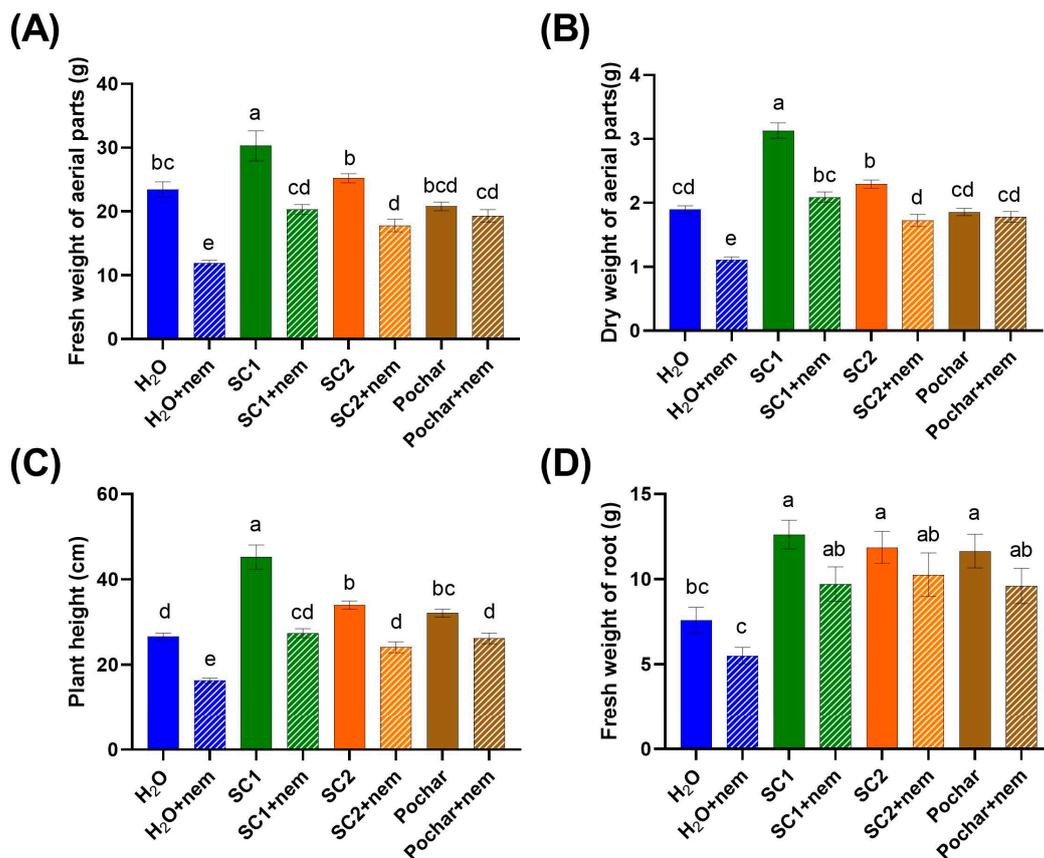
Regarding the experiments on the impact of the treatments on tomato plant growth, the results revealed notable differences in the fresh weight of plants among different treatments (Figure 2). Specifically, plants inoculated with nematodes exhibited a decreased fresh weight compared to their respective control plants, except for those treated with Pochar (Figure 2A).

SC1 treatment resulted in the highest shoot fresh weight, significantly distinct from the other treatments. SC2 and Pochar treatments did not significantly differ in fresh weight from water-treated plants. However, plants that were both inoculated with nematodes and treated with SC1, SC2, and Pochar exhibited a greater shoot fresh weight (74%, 58%, and 67%, respectively) compared to their counterparts treated solely with water and inoculated with nematodes (Figure 2A). The results suggest that SC1, SC2, and Pochar treatments might have a suppressive effect on nematode infestation and mitigate their negative effects on plant growth. In contrast, water-treated plants inoculated with nematodes exhibited a lower fresh weight compared to the control plants, emphasizing the negative impact of nematode infestation when no treatment was applied. These findings underscore the potential effectiveness of SC1, SC2, and Pochar in promoting healthier plant growth even in the presence of nematodes.



**Figure 1.** Impact of SCs and Pochar on nematode populations in tomato plants. (A) Egg masses per root: The average number of nematode egg masses found per root system. (B) Eggs per egg mass: The average number of eggs found within each individual egg mass. (C) Eggs per root: The average number of the number of eggs estimated for each individual egg mass multiplied by the number of egg masses per root system. (D) Total number of progeny: The RKN soil population plus the number of eggs per root. Vertical bars represent the standard error of the mean (n = 10 replicates). Different letters indicate statistically significant differences:  $p < 0.05$ ; one-way ANOVA, Tukey's test.

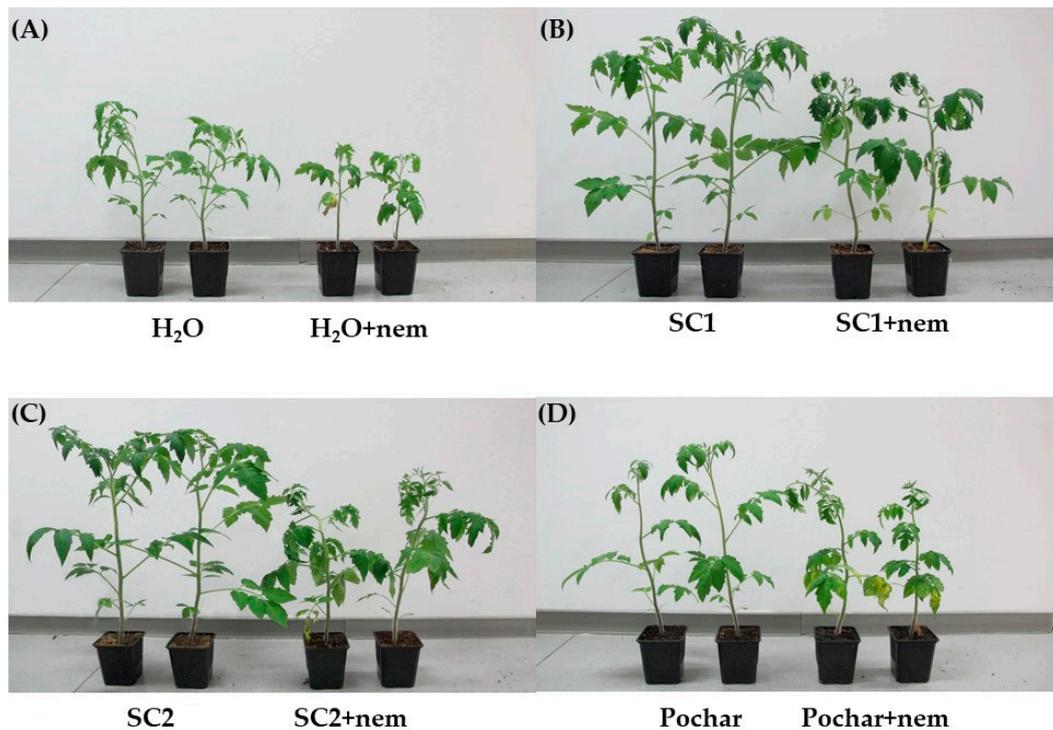
Similar trends were observed in the results for the dry weight of plants, mirroring the patterns seen in fresh weight (Figure 2B). Significant differences in dry weight among the various treatments were consistent with the findings for fresh weight. Notably, apart from SC1, SC2 treatment also exhibited greater dry weight compared to the water-treated plants (Figure 2B). These parallel outcomes between fresh and dry weight strengthen the implication that SC1, SC2, and Pochar treatments potentially mitigate the adverse effects of nematode infestation on plant growth. Conversely, water-treated plants inoculated with nematodes consistently exhibited a lower dry weight, highlighting the detrimental impact of nematode infestation in the absence of treatment.



**Figure 2.** Impact of SCs and Pochar on tomato growth and nematode control. (A) Fresh weight of aerial parts (g). (B) Dry weight of aerial parts (g). (C) Plant height (cm). (D) Fresh weight of roots (g). Vertical bars indicate the SE of the mean ( $n = 10$  replicates). Different letters indicate statistically significant differences among treatments:  $p < 0.05$ ; one-way ANOVA, Tukey's test.

Regarding plant height, significant differences were observed among the different treatments (Figures 2C and 3). Specifically, in each treatment group, plants inoculated with nematodes exhibited a reduction in height compared to their corresponding control plants, specifically 86% for SC1, 53% for SC2, and 73% for Pochar (Figure 3). SC1 treatment resulted in greater plant height compared to all treatments, while that of SC2- and Pochar-treated plants was significantly higher compared to that of the water-treated plants (Figures 2 and 3). Moreover, plants treated with SC1, SC2, and Pochar and inoculated with nematodes displayed increased height compared to their counterparts treated with water alone (Figures 2C and 3). These results align with data related to both fresh weight and dry weight, highlighting the potential of SC1, SC2, and Pochar in positively influencing plant height.

Root fresh weight was not significantly affected by nematode inoculation in any treatment compared to their respective controls (Figure 2D). Notably, water-treated plants (both control and nematode inoculated) had significantly lower root fresh weight compared to all other treatments (Figure 2D). More importantly, among the nematode-inoculated plants, those treated with SC1, SC2, and Pochar possessed roots with increased fresh weight of 50%, 67%, and 58%, respectively. These findings suggest that the treatments involving SC1, SC2, and Pochar may have benefits in mitigating the adverse effects on root fresh weight caused by nematode infestation, particularly when compared to untreated plants.



**Figure 3.** Effect of SCs and Pochar on tomato plant growth. The figure shows the visual impact of treatments on tomato plants 40 days after nematode inoculation (right side) compared to control plants without nematodes (left side). Treatments include: (A) plants treated with water (H<sub>2</sub>O); (B) plants treated with SC1; (C) plants treated with SC2; (D) plants treated with the commercial product Pochar (Pochar). Visual differences in plant growth parameters, such as plant height, might be observed between treatments with and without nematode inoculation.

#### 4. Discussion

The apparent success of SC1 and SC2 in controlling nematodes could be attributed to several factors. First, the selected communities might have introduced beneficial microbial consortia into the rhizosphere, fostering a microbiome that actively contributes to plant health and simultaneously interferes with nematode activity. The introduction of SC1 and SC2 might have contributed to an environment that is less hospitable for nematodes, disrupting their life cycle and reproductive success. This aligns with the findings of Zhou et al. [26], who demonstrated that soils in non-infested regions of fields under high root-knot nematode (RKN) pressure exhibit greater microbial diversity than infested areas and the inoculation of tomato roots with the microbiome from non-infested soils resulted in a reduction in the number of root galls. Consequently, this suggests that enhancing the diversity and abundance of specific microbial groups could be a viable strategy for managing RKN.

Second, the bacteria of the selected communities might produce bioactive compounds or exhibit antagonistic behaviors that directly or indirectly affect nematode viability. The direct mechanisms include the synthesis of lytic enzymes, antibiotics, toxins, and metabolites with inhibitory effects or parasitism [27]. Conversely, indirect mechanisms encompass the release of molecules that modulate nematode behavior, the competition for nutrients, the induction of systemic resistance (ISR), and the promotion of plant growth [28,29]. These indirect strategies involve niche exclusion, nitrogen metabolism, siderophore production, phosphate solubilization, and hormonal regulation [30].

Lytic enzymes produced by bacteria can cause damage to both the eggshells of nematodes, which consist of a protein matrix and a chitin layer [31], and the cuticle of the nematodes, which is composed of a proteinaceous membrane [32]. Chitinases, proteases, peptidyl-peptide hydrolases, and gelatinolytic proteins are enzymes capable of cleaving

these structures [27]. In our study, both SCs consisted of bacterial isolates, with some demonstrating the ability to produce a variety of hydrolytic enzymes including chitinases, proteases, cellulases, and pectinases. More specifically, as observed in Tsolakidou et al.'s study [15], 3 out of 25 bacterial isolates exhibited chitinase-producing capabilities within SC1; SC2 showed a higher proportion, with 10 out of 25 isolates demonstrating chitinase activity. Regarding proteases, a single isolate from SC1 exhibited protease activity, while SC2 displayed a higher activity level, with 6 out of 25 isolates showing protease activity. There is extensive literature on the pivotal role of lytic enzymes, especially chitinases and proteases, of microbial origin in combating nematodes by disrupting nematode structures, compromising their integrity, and contributing to their biocontrol in agricultural and ecological settings (reviewed in [33]).

In addition to the previously mentioned enzymes, various other lytic enzymes have been identified for their involvement in controlling the incidence of RKN infection. These include pectinases,  $\beta$ -glucanases, and cellulases [34]. In the study of Tsolakidou et al. [15] it was reported that, within SC1, 15 isolates demonstrated pectinase activity, and 2 isolates exhibited cellulase activity whereas, in SC2, 3 isolates showed pectinase activity, while 8 isolates demonstrated cellulase activity.

Phosphorus is an essential nutrient for plant growth and development, but it is often present in soil in insoluble forms, making it inaccessible to plants. Microorganisms capable of solubilizing phosphate play a crucial role in making this essential nutrient available to plants. Phosphate-solubilizing microorganisms also contribute to plant growth assistance by functioning as biocontrol agents against a variety of pathogenic organisms and nematodes. For example, El-Hadad et al. [35] noted that the application of *Bacillus megaterium*, an efficient phosphorus mobilizer, led to increased shoot length, plant biomass, NPK levels, and a reduction in the colonization of *M. incognita* in tomato plants. In the study by Tsolakidou et al. [15], it was reported that within SC1, one isolate demonstrated phosphate solubilization, while within SC2, eight isolates exhibited phosphate solubilization.

Beyond lytic enzymes, several studies suggest that rhizobacteria can reduce nematode severity by inducing systemic resistance (ISR) in plants. This involves reinforcing the cell wall and synthesizing defensive compounds (PR proteins, salicylic acid, etc.) [36–40]. Notably, our selected communities (SC1 and SC2) harbored genes linked to cyclic lipopeptide (CLP) biosynthesis, which have been shown to trigger ISR in specific plants [41–43].

Plant growth-promoting rhizobacteria (PGPR) can stimulate plant growth through phytohormone production (auxins and cytokinins) or enzymatic activities (ACC deaminase) [44,45]. Indole acetic acid (IAA) is a key phytohormone that promotes cell division, elongation, and root development [44,45]. Both SC1 and SC2 contain isolates capable of producing IAA (10/25 for SC1; 16/25 for SC2) [15].

ACC deaminase, another mechanism employed by PGPR, reduces stress hormone ethylene levels, benefiting plants under stress [46,47]. Studies using *Pseudomonas* strains as models demonstrate the effectiveness of ACC deaminase in controlling nematode-induced diseases [48]. Interestingly, SC1 contained isolates exhibiting ACC deaminase activity (11/25), whereas none were found in SC2 [15]. This difference may contribute to the observed variations in their efficiency against nematodes, with SC1 exhibiting a stronger effect.

## 5. Conclusions

The bacterial communities selected and evaluated in the present research demonstrate significant potential in mitigating nematode-induced crop losses. Our study highlights their capacity to promote plant growth while concurrently reducing nematode reproduction, positioning them as promising components in sustainable agricultural practices. While the current study did not directly investigate the specific mechanisms by which the selected bacterial communities (SC1 and SC2) affect nematodes, our findings provide valuable insights into their potential impact. A previous study by our group explored potential mechanisms underlying the activity of the isolates comprising the communities, demonstrating their ability to produce lytic enzymes, cyclic lipopeptides, IAA (indole-3-acetic

acid), and exhibit ACC deaminase activity. These mechanisms are well-established contributors to plant growth promotion and nematode suppression in other beneficial bacteria, and we hypothesize that they may similarly contribute to the observed effects on nematode susceptibility in this study. Moving forward, elucidating the specific mechanisms by which SC1 and SC2 influence nematode populations will be pivotal for refining and optimizing their application as biocontrol strategies. Future research should delve deeper into how these specific mechanisms translate to the observed effects of SC1 and SC2 on plant–nematode interactions in this study.

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

## References

1. Talavera-Rubia, M.; Vela-Delgado, M.D.; Verdejo-Lucas, S. A Cost-Benefit Analysis of Soil Disinfestation Methods against Root-Knot Nematodes in Mediterranean Intensive Horticulture. *Plants* **2022**, *11*, 2774. [\[CrossRef\]](#)
2. Gharabadiyan, F.; Jamali, S.; Komeili, R.H. Determining of root-knot nematode (*Meloidogyne javanica*) damage function for tomato cultivars. *J. Agric. Sci.* **2013**, *58*, 147–157. [\[CrossRef\]](#)
3. Tzortzakakis, E.A.; da Conceicao, I.L.P.M.; dos Santos, M.C.V.; Abrantes, I.M.D.O. Root-knot nematodes (*Meloidogyne* spp.) in Greece. *Hell. Plant Prot. J.* **2011**, *4*, 25–30.
4. Nyczepir, A.P.; Thomas, S.H. Current and future management strategies in intensive crop production systems. In *Root-Knot Nematodes*; CABI: Wallingford, UK, 2009; pp. 412–443.
5. Riyaz, M.; Mathew, P.; Zuber, S.M.; Rather, G.A. Botanical pesticides for an eco-friendly and sustainable agriculture: New challenges and prospects. In *Sustainable Agriculture*; Springer: Cham, Switzerland, 2022; pp. 69–96.
6. Ali, W.M.; Abdel-Mageed, M.A.; Hegazy, M.G.A.; Abou-Shlell, M.K.; Sultan, S.M.E.; Salama, E.A.A.; Yousef, A.F. Biocontrol agent of root-knot nematode *Meloidogyne javanica* and root-rot fungi, *Fusarium solani* in okra morphological, anatomical characteristics and productivity under greenhouse conditions. *Sci. Rep.* **2023**, *13*, 11103. [\[CrossRef\]](#)
7. Khalil, M.S.E.D.H.; Allam, A.F.G.; Barakat, A.S.T. Nematicidal activity of some biopesticide agents and microorganisms against root-knot nematode on tomato plants under greenhouse conditions. *J. Plant Prot. Res.* **2012**, *52*, 47–52. [\[CrossRef\]](#)
8. Khan, R.A.A.; Najeeb, S.; Mao, Z.; Ling, J.; Yang, Y.; Li, Y.; Xie, B. Bioactive secondary metabolites from *Trichoderma* spp. against phytopathogenic bacteria and root-knot nematode. *Microorganisms* **2020**, *8*, 401. [\[CrossRef\]](#)
9. Naz, I.; Khan, R.A.A.; Masood, T.; Baig, A.; Siddique, I.; Haq, S. Biological control of root knot nematode, *Meloidogyne incognita*, in vitro, greenhouse and field in cucumber. *Biol. Control* **2021**, *152*, 104429. [\[CrossRef\]](#)
10. Campos, M.A.S. Bioprotection by arbuscular mycorrhizal fungi in plants infected with *Meloidogyne* nematodes: A sustainable alternative. *Crop Prot.* **2020**, *135*, 105203. [\[CrossRef\]](#)
11. Ahmad, G.; Nishat, Y.; Ansari, M.; Khan, A.; Haris, M.; Khan, A.A. Eco-friendly approaches for the alleviation of root-knot nematodes. In *Plant Growth-Promoting Microbes for Sustainable Biotic and Abiotic Stress Management*; Springer: Cham, Switzerland, 2021; pp. 557–575.
12. Bais, H.P.; Weir, T.L.; Perry, L.G.; Gilroy, S.; Vivanco, J.M. The Role of root exudates in rhizosphere interactions with plants and other organisms. *Annu. Rev. Plant Biol.* **2006**, *57*, 233–266. [\[CrossRef\]](#)
13. Berendsen, R.L.; Vismans, G.; Yu, K.; Song, Y.; de Jonge, R.; Burgman, W.P.; Burmølle, M.; Herschend, J.; Bakker, P.A.H.M.; Pieterse, C.M.J. Disease-induced assemblage of a plant-beneficial bacterial consortium. *ISME J.* **2018**, *12*, 1496–1507. [\[CrossRef\]](#)
14. Stringlis, I.A.; Proietti, S.; Hickman, R.; Van Verk, M.C.; Zamioudis, C.; Pieterse, C.M.J. Root transcriptional dynamics induced by beneficial rhizobacteria and microbial immune elicitors reveal signatures of adaptation to mutualists. *Plant J.* **2018**, *93*, 166–180. [\[CrossRef\]](#)

15. Tsolakidou, M.-D.; Stringlis, I.A.; Fanega-Sleziak, N.; Papageorgiou, S.; Tsalakou, A.; Pantelides, I.S. Rhizosphere-enriched microbes as a pool to design synthetic communities for reproducible beneficial outputs. *FEMS Microbiol. Ecol.* **2019**, *95*, fiz138. [[CrossRef](#)]
16. El-Nagdi, W.M.A.; Abd-El-Khair, H. Biological control of *Meloidogyne incognita* and *Fusarium solani* in dry common bean in the field. *Arch. Phytopathol. Plant Prot.* **2014**, *47*, 388–397. [[CrossRef](#)]
17. Munawar, M.; Khan, S.A.; Javed, N.; Ul Haq, I.; Gondal, A.S. Bio-management of tomato wilt complex caused by *Meloidogyne incognita* and *Fusarium oxysporum* f. sp. *lycopersici*. *Nematology* **2015**, *17*, 479–485. [[CrossRef](#)]
18. Patil, J.A.; Yadav, S.; Kumar, A. Management of root-knot nematode, *Meloidogyne incognita* and soil borne fungus, *Fusarium oxysporum* in cucumber using three bioagents under polyhouse conditions. *Saudi J. Biol. Sci.* **2021**, *28*, 7006–7011. [[CrossRef](#)]
19. Shi, X.; Qiao, K.; Li, B.; Zhang, S. Integrated management of *Meloidogyne incognita* and *Fusarium oxysporum* in cucumber by combined application of abamectin and fludioxonil. *Crop Prot.* **2019**, *126*, 104922. [[CrossRef](#)]
20. Tsaniklidis, G.; Chatzistathis, T.; Fanourakis, D.; Nikoloudakis, N.; Kotsiras, A.; Delis, C.; Tzortzakakis, E.A. Leaf antioxidant machinery stimulation by *Meloidogyne javanica* infestation: A case study on *Cucumis melo* seedlings. *Plant Stress* **2021**, *1*, 100002. [[CrossRef](#)]
21. Hussey, R.S.; Barker, K.R. A Comparison of Methods of Collecting Inocula of *Meloidogyne* Species, Including a New Technique. *Plant Dis. Report.* **1973**, *57*, 1025–1028.
22. Antoniou, A.; Tsolakidou, M.-D.; Stringlis, I.A.; Pantelides, I.S. Rhizosphere microbiome recruited from a suppressive compost improves plant fitness and increases protection against vascular wilt pathogens of tomato. *Front. Plant Sci.* **2017**, *8*, 2022. [[CrossRef](#)]
23. Chialva, M.; Zouari, I.; Salvioli, A.; Novero, M.; Vrebalov, J.; Giovannoni, J.J.; Bonfante, P. Gr and hp-1 tomato mutants unveil unprecedented interactions between arbuscularmycorrhizal symbiosis and fruit ripening. *Planta* **2016**, *244*, 155–165. [[CrossRef](#)]
24. Baermann, G. Eine einfache Methode zur Auffindung von Ankllostomum (Nematoden) Larven in Erdproben. *Geneesk. Tijdschr. Ned. Indie* **1917**, *57*, 131–137.
25. Zhou, D.; Feng, H.; Schuelke, T.; De Santiago, A.; Zhang, Q.; Zhang, J.; Luo, C.; Wei, L. Rhizosphere Microbiomes from Root Knot Nematode Non-infested Plants Suppress Nematode Infection. *Microb. Ecol.* **2019**, *78*, 470–481. [[CrossRef](#)]
26. Gamalero, E.; Glick, B.R. The Use of Plant Growth-Promoting Bacteria to Prevent Nematode Damage to Plants. *Biology* **2020**, *9*, 381. [[CrossRef](#)] [[PubMed](#)] [[PubMed Central](#)]
27. Kamalanathan, V.; Sevugapperumal, N.; Nallusamy, S. Antagonistic Bacteria *Bacillus velezensis* VB7 Possess Nematicidal Action and Induce an Immune Response to Suppress the Infection of Root-Knot Nematode (RKN) in Tomato. *Genes* **2023**, *14*, 1335. [[CrossRef](#)] [[PubMed](#)] [[PubMed Central](#)]
28. Mhatre, P.H.; Karthik, C.; Kadirvelu, K.; Divya, K.; Venkatasalam, E.; Srinivasan, S.; Ramkumar, G.; Saranya, C.; Shanmuganathan, R. Plant growth promoting rhizobacteria (PGPR): A potential alternative tool for nematodes bio-control. *Biocatal. Agric. Biotechnol.* **2019**, *17*, 119–128. [[CrossRef](#)]
29. Sidhu, H.S. Potential of plant growth-promoting rhizobacteria in the management of nematodes: A review. *J. Entomol. Zool. Stud.* **2018**, *6*, 1536–1545.
30. Wharton, D. Nematode eggshells. *Parasitology* **1980**, *81*, 447–463. [[CrossRef](#)]
31. Ray, S.; Reddigarim, S.R.; Jansma, P.L.; Allen, R.; Hussey, R.S. Immunocytochemical analysis of the stage-specific distribution of collagen in the cuticle of *Meloidogyne incognita*. *Fundam. Appl. Nematol.* **1996**, *19*, 71–78.
32. Khanna, K.; Kohli, S.K.; Ohri, P.; Bhardwaj, R. Plants-nematodes-microbes crosstalk within soil: A trade-off among friends or foes. *Microbiol. Res.* **2021**, *248*, 126755. [[CrossRef](#)]
33. Krechel, A.; Faupel, A.; Hallmann, J.; Ulrich, A.; Berg, G. Potato-associated bacteria and their antagonistic potential towards plant-pathogenic fungi and the plant-parasitic nematode *Meloidogyne incognita* (Kofoid & White) Chitwood. *Can. J. Microbiol.* **2002**, *48*, 772–786.
34. El-Hadad, M.E.; Mustafa, M.I.; Selim, S.M.; El-Tayeb, T.S.; Mahgoob, A.E.A.; Aziz, N.H.A. The nematicidal effect of some bacterial biofertilizers on *Meloidogyne incognita* in sandy soil. *Braz. J. Microbiol.* **2011**, *42*, 105–113. [[CrossRef](#)]
35. Siddiqui, I.A.; Shaikat, S.S. Rhizobacteria-mediated induction of systemic resistance (ISR) in tomato against *Meloidogyne javanica*. *J. Phytopathol.* **2002**, *150*, 469–473. [[CrossRef](#)]
36. Sikora, R.A. Interrelationship between plant health promoting rhizobacteria, plant parasitic nematodes and soil microorganisms. *Meded. Fac. Landbouww. Rijksuniv. Gent* **1988**, *53*, 867–878.
37. Kloepper, J.W.; Ryu, C.-M.; Zhang, S. Induced systemic resistance and promotion of plant growth by *Bacillus* spp. *Phytopathology* **2004**, *94*, 1259–1266. [[CrossRef](#)]
38. Siddiqui, Z.A.; Mahmood, I. Role of bacteria in the management of plant parasitic nematodes: A review. *Bioresour. Technol.* **1999**, *69*, 167–179. [[CrossRef](#)]
39. Ramamoorthy, V.; Viswanathan, R.; Raguchander, T.; Prakasam, V.; Samiyappan, R. Induction by systemic resistance by plant growth promoting rhizobacteria in crop plants against pests and diseases. *Crop Prot.* **2001**, *20*, 1–11. [[CrossRef](#)]
40. Van Loon, L.C.; Geraats, B.P.J.; Linthorst, H.J.M. Ethylene as a modulator of disease resistance in plants. *Trends Plant Sci.* **2006**, *11*, 184–191. [[CrossRef](#)]
41. Raaijmakers, J.M.; De Bruijn, I.; Nybroe, O.; Ongena, M. Natural functions of lipopeptides from *Bacillus* and *Pseudomonas*: More than surfactants and antibiotics. *FEMS Microbiol. Rev.* **2010**, *34*, 1037–1062. [[CrossRef](#)]

42. Falardeau, J.; Wise, C.; Novitsky, L.; Avis, T.J. Ecological and mechanistic insights into the direct and indirect antimicrobial properties of *Bacillus subtilis* lipopeptides on plant pathogens. *J. Chem. Ecol.* **2013**, *39*, 869–878. [[CrossRef](#)]
43. Gopalakrishnan, S.; Sathya, A.; Vijayabharathi, R.; Varshney, R.K.; Gowda, C.L.L.; Krishnamurthy, L. Plant growth promoting rhizobia: Challenges and opportunities. *3 Biotech* **2015**, *5*, 355–377. [[CrossRef](#)]
44. Penrose, D.M.; Glick, B.R. Methods for isolating and characterizing ACC deaminase-containing plant growth-promoting rhizobacteria. *Physiol. Plant.* **2003**, *118*, 10–15. [[CrossRef](#)]
45. Zamioudis, C.; Mastranesti, P.; Dhonukshe, P.; Blilou, I.; Pieterse, C.M.J. Unraveling root developmental programs initiated by beneficial *Pseudomonas* spp. bacteria. *Plant Physiol.* **2013**, *162*, 304–318. [[CrossRef](#)]
46. Asari, S.; Tarkowská, D.; Rolčík, J.; Novák, O.; Palmero, D.V.; Bejai, S.; Meijer, J. Analysis of plant growth-promoting properties of *Bacillus amyloliquefaciens* UCMB5113 using *Arabidopsis thaliana* as host plant. *Planta* **2017**, *245*, 15–30. [[CrossRef](#)]
47. Van Loon, L.C.; Bakker, P.A.H.M.; Pieterse, C.M.J. Systemic resistance induced by rhizosphere bacteria. *Annu. Rev. Phytopathol.* **1988**, *36*, 453–483. [[CrossRef](#)]
48. Nascimento, F.X.; Vicente, C.S.; Barbosa, P.; Espada, M.; Glick, B.R.; Mota, M.; Oliveira, S. Evidence for the involvement of ACC deaminase from *Pseudomonas putida* UW4 in the biocontrol of pine wilt disease caused by *Bursaphelenchus xylophilus*. *BioControl* **2013**, *58*, 427–433. [[CrossRef](#)]

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