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Bacillus haynesii VWC18 Drives Growth and Flowering Improvements in *Euphorbia pulcherrima* and *Cyclamen persicum*: A Sustainable Approach for Ornamental Crop Performance

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Abstract

This study evaluated the microbial strain *Bacillus* VWC18, isolated from waste vegetable compost, as a plant growth-promoting rhizobacterium (PGPR) for two high-value ornamental crops: poinsettia (*Euphorbia pulcherrima*) and cyclamen (*Cyclamen persicum*). Identified as *Bacillus haynesii*, the strain VWC18 showed robust phosphate, potassium, and zinc solubilization, siderophore production, and indole-3-acetic acid synthesis, with γ-hemolytic biosafety confirmation. Greenhouse trials evaluated its biostimulant activity by comparing control plants (three cultivars of each species) under standard commercial protocols with those receiving *B. haynesii* VWC18 via sub-irrigation. Treated poinsettias exhibited 90% greater fresh foliar weight in white cultivars and over 50% increased root biomass, while red varieties produced twice as many pigmented bracts. Cyclamen nearly doubled fresh leaf and root weight. Photosynthetic pigments (chlorophyll-*a*, chlorophyll-*b*, and carotenoids) approximately doubled in both species. Anthocyanin content increased significantly in red and orange poinsettia bracts and red/violet cyclamen flowers. Quantitative PCR, targeting soil eubacteria, confirmed an increase of rhizosphere communities, with bacterial populations rising from ~5 × 10^5 to 1–2.6 × 10^7 CFU g^{-1} soil. *B. haynesii* VWC18 enhances vegetative growth and aesthetic quality through phytohormone production, nutrient mobilization and rhizosphere modulation, offering a strategy for sustainable input solutions in greenhouse floriculture while maintaining marketable quality.

Keywords: *Bacillus haynesii* VWC18; PGPR (plant growth-promoting rhizobacteria); biostimulant activity; *Euphorbia pulcherrima*; *Cyclamen persicum*; floriculture; sustainable horticulture

Check for updates

Received: 5 August 2026
Revised: 8 September 2026
Accepted: 12 September 2026
Published: 17 September 2026

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1. Introduction

Floriculture is a specialized branch of ornamental horticulture focused on the cultivation, management, and commercialization of potted plants, cut flowers, and dwarf shrubs for decorative and aesthetic purposes [1,2]. In winter, *Euphorbia pulcherrima* (Poinsettia) and *Cyclamen persicum* (Cyclamen) are among the most ornamental species cultivated in greenhouses, although both can be raised year-round; the poinsettia remains synonymous with Christmas, and it is renowned for its brightly colored bracts [3]. Poinsettia is a short-day perennial whose flowering and bract coloration depend on specific photoperiodic cues [4]. It is characterized by simple, ovate-lanceolate, membranous leaves (entire or toothed), and

by brightly colored apical bracts that enclose the inflorescences called "cyathia" [5]. As autumn days shorten, chlorophyll breaks down, and anthocyanin synthesis accelerates, turning the bracts from green to intense red, orange, violet, and white [6]. Poinsettia requires protected environments, well-drained substrates, and careful management of light and nutrition. Plants are maintained under relatively high nutrient regimes, with 200 ppm N being common; the strict standards for whitefly, *Botrytis*, and bract-edge burn lead to substantial chemical inputs. *Cyclamen persicum* is characterized by a protracted flowering window and cold tolerance that align it with winter greenhouse schedules. Its cultivation occurs predominantly in temperate zones, particularly in the Mediterranean regions [7]. Cyclamen species develop a tuber, triangular-shaped leaves, and flowers, ranging in color from white to pink to deep magenta. Flowering time responds to seasonal temperature variations; leaf development, and especially flowering, is influenced by winter temperature patterns more than in other cyclamen species, as it blooms after a cold period [7]. In addition, the cultivar (genotype) and the vegetative growth rate are extremely important [8]. A specific number of leaves (physiological age) is required before flower bud formation begins; continuous flower production is strictly dependent on leaf emergence [9]. As for poinsettia, cyclamen requires meticulous crop management characterized by high nutritional demands and intensive phytosanitary care [10]. Standard commercial protocols rely on peat-based, free-draining substrates, ebb-and-flow fertigation supplying 150 mg L^{-1} N, 30 mg L^{-1} P and 200 mg L^{-1} K, and preventive fungicide programs against *Fusarium oxysporum* f. sp. cyclaminis and *Phytonemus pallidus*.

Cultivation of ornamental plants requires a significant use of mineral fertilizers and plant protection products that generate high environmental impact and increase production costs [11]. Low-input strategies are more and more required to combine high aesthetic and productive standards with environmental sustainability; here's why biostimulants display a successful example for the floriculture industry. In this framework, the use of plant growth-promoting rhizobacteria (PGPR) is becoming a concrete tool in ornamental cultivation, following the huge evidence collected in horticulture and extensive crops [12,13]. PGPR application is known to enhance various growth parameters such as number of flowers per plant, diameter, and flower yield per plant, as well as physiological parameters such as chlorophyll, anthocyanin content, and shelf life of flowers [14]. Rhizobacteria function as fertilizers through a combination of direct and indirect mechanisms that boost plant performance, as thoroughly documented by Ruzzi and Aroca [12]. *Bacillus* spp. represent a wide group of PGPR frequently isolated from the rhizosphere and closely associated with root hairs. They synthesize key phytohormones (e.g., auxins, gibberellins, and cytokines), they are essential for mineral solubilization, nitrogen fixation and siderophores production. In addition, some *Bacillus* species may contribute to plant resilience by producing lipopeptide molecules and inducing systemic resistance mechanisms that power defense responses against pathogenic microorganisms and mitigate the impacts of abiotic stresses such as drought, salinity, and extreme temperature [12]. Collectively, these processes strengthen the entire plant system, from root to shoot, stems, buds, flowers, and fruits [15] and help reduce the use of chemical fertilizers and pesticides.

In this study, we investigated the potential of the plant growth-promoting activity of *Bacillus* spp. VWC18 in poinsettia and cyclamen; this is an innovative research work aimed at leading the way for a broader application of PGPR in the ornamental sector. *Bacillus* spp. VWC18 was isolated from waste vegetable compost, and it recently obtained patent granting n° 102023000006816, considering its documented activity [16,17]. Following 16S rRNA sequencing, the strain was identified as *Bacillus haynesii*, a thermophilic bacterial species first isolated by Dunlap et al. [18]; further, it was studied for in vitro PGPR activity (P, K, and Zn solubilization activity, siderophore production, indole-3-acetic

acid (IAA) synthesis, and hemolytic behavior). The plant growth-promoting performance was then evaluated in vivo in three commercial poinsettia and three cyclamen cultivars. *Bacillus haynesii* VWC18 was added by sub-irrigation together with a basic fertilization plan; the leaf/root biomass, photosynthetic pigments, and bract/flower aesthetic rating were improved compared to the control plants, and total soil eubacteria increased in the rhizosphere community. The obtained data provide additional experimental evidence supporting the plant growth-promoting activity of the patented strain and extend its evaluation to ornamental crops, a group that usually receives limited attention in this regard.

2. Materials and Methods

2.1. Bacterial Culture Conditions and Molecular Identification

Bacillus sp. VWC18 was grown on tryptic soy broth (TSA, Thermofisher; Milan, Italy) and incubated at 30 °C for 18–24 h under aerobic conditions. Genomic DNA extraction was performed by collecting cells from 10 mL of centrifuged suspension, using the Wizard® Genomic DNA Purification Kit (Promega, Madison, WI, USA). Two microliters of DNA (30 ng/µL) were used for 16S rRNA gene sequencing. Gene amplification was carried out with primers 8f (5′-AGAGTTTGATCCTGGCTCAG-3′) and 1520r (5′-AAGGGAGGTGATCCAGCCGCA-3′), according to Pagliarini et al. [19]. PCR products were purified using the NucleoSpin purification kit (Macherey-Nagel GmbH & Co. KG, Düren, Germany), and the sequencing was commissioned to Eurofins MWG Operon (Ebersberg, Germany). Chromatograms were elaborated with the software program Finch TV version 1.4, and the consensus sequence was assembled through DNAMAN software, version 6.0. Sequence assignment to bacterial species was investigated by matching available 16S rRNA sequences using Nucleotide BLAST (version 2.17.0) [20]; the final sequence was deposited in the GenBank database "https://www.ncbi.nlm.nih.gov/genbank (accessed on 10 March 2026)". Additionally, the strain was deposited in the Microbial Strain Collection of Latvia, University of Latvia (deposit number P 1649).

2.2. PGPR Characterization

2.2.1. Phosphate, Potassium, Zinc Solubilization and Siderophore Production

Phosphate solubilization was evaluated using Pikovskaya's agar medium [21]. Potassium and zinc solubilization were assessed using Aleksandrov medium [22] and Tris-minimal agar [23], respectively. With respect to siderophore production, the blue agar Chrome Azurol S (CAS) assay was (CAS) performed [24]. Briefly, 10 mL of an overnight culture of *Bacillus* sp. VWC18 was centrifuged and the cells pellet resuspended in 1 mL of TSB. For all assays, 50 µL was spotted on the plates (three spots per plate) and incubated at 30 °C for 7 days. For siderophore evaluation, the plates were incubated at 30 °C for 7–15 days. The assays were performed in triplicate. Solubilization was confirmed by the formation of clear decolorization halos surrounding the bacterial colonies. Halo diameters were categorized as follows: small (<0.5 cm), medium (0.5–1.0 cm) and large (>1.0 cm).

2.2.2. Indole-3-Acetic Acid (IAA) Synthesis

Indole-3-acetic acid (IAA) production was determined according to Gordon and Weber [25]. 10 mL of nutrient broth (NB, Thermofisher), supplemented with 0.1% (*w*/*v*) L-tryptophan (Sigma-Aldrich®, Merck KGaA, Burlington, MA, USA), was inoculated with an overnight bacterial culture and incubated in the dark under shaking conditions (120 rpm) at 28 °C for 72 h. IAA quantification was carried out after 24, 48, and 72 h. Liquid culture was centrifuged (5000× *g* for 10 min at 4 °C), and 1 mL of the resulting supernatant was mixed with 2 mL of Salkowski's reagent (2% 0.5 M $FeCl_3$ in 35% $HClO_4$ solution) and kept in the dark. After an incubation period of 30 min, the amount of IAA was determined

spectrophotometrically (550 nm). IAA production in the medium results in red-pink color formation, and the concentration was estimated using an IAA standard curve [25].

2.2.3. Hemolytic Test

The hemolytic activity was performed as described by Santini et al. [26], using the Columbia Blood agar (Sigma-Aldrich, Milano, Italy) supplemented with 5% defibrinated sheep blood. The strain was classified as hemolytic or non-hemolytic based on the production/absence of a blood lysis zone around the colonies (β-hemolysis/γ-hemolysis). Experiments were performed in triplicate.

2.3. Greenhouse Experimental Design

Fifteen-day-old seedlings of poinsettia (*Euphorbia pulcherrima* L.) and twenty-day-old seedlings of cyclamen (*Cyclamen persicum* L.) were purchased from Dunn Seeds (Boort, VIC 3537, Australia) and cultivated at "Floricoltura Paganotto Giorgio" (Boschi Sant'Anna, VR, Italy) within a temperate greenhouse facility. The experiment was designed as a randomized complete block design of 900 plants (Table 1).

Table 1. Greenhouse pot experiment: list of plant varieties, theses and codes.

Plant Species	Treatments	
	Control	***B. haynesii* VWC18**
Euphorbia pulcherrima var. Gran d'Italia	EP_R_CTR	EP_R_TR
Euphorbia pulcherrima var. Alaska	EP_W_CTR	EP_W_TR
Euphorbia pulcherrima var. Princettia Orange	EP_O_CTR	EP_O_TR
Cyclamen persicum var. Red Star	CP_R_CTR	CP_R_TR
Cyclamen persicum var. Halios White	CP_W_CTR	CP_W_TR
Cyclamen persicum var. Halios Violet	CP_V_CTR	CP_V_TR

Each plant species included three varieties, with 150 plants assigned to the control group and 150 to the treatment group, with three replicates each (50 pots per replicate). The selected poinsettia cultivars were: *Euphorbia pulcherrima* 'Gran d'Italia' (red bracts), *Euphorbia pulcherrima* 'Alaska' (white bracts) and *Euphorbia pulcherrima* 'Princettia Orange' (orange bracts). The three cyclamen cultivars were *Cyclamen persicum* 'Red Star' (red flowers), *Cyclamen persicum* 'Halios White' (white flowers) and *Cyclamen persicum* 'Halios Violet' (violet flowers). Poinsettia were grown in 4 L plastic pots previously disinfected with 80% commercial chlorine and 70% alcohol, as well as cyclamens, but in terracotta pots. The pots were filled with a peat based commercial substrate (Gramoflor gmbh & Co., Vechta, Germany) containing NP 23-14 mineral fertilizer (1g L^{-1}). Every two weeks, pots of poinsettia and cyclamen received 1g/L of NPK 20-15-8 (1 g L^{-1}) and NPK 12-10-12 (1 g L^{-1}), respectively. Fertilization was performed by sub-irrigation (30 min) every two weeks, as follows: NPK 20-15-8 (1 g L^{-1}), CaO (1 g L^{-1}), and NP 0-52 (0.5 g L^{-1}) for poinsettia; NPK 12-10-12 (1 g L^{-1}), CaO (0.5 g L^{-1}), and NP 0-52 (0.3 g L^{-1}) for cyclamen. Sixty days after transplanting, the poinsettia was treated with TEPPEKI® (Certis Belchim B.V., Saronno (VA), Italy) for *Trialeurodes vaporariorum* mitigation. A copper-based foliar treatment was applied to the cyclamen at 30, 60, and 90 days to prevent fungal diseases. A black shading net (93% shading) was placed over the top of the greenhouse from the first week of September until uniform pigmentation to protect bracts pigmentation and high temperature exposure. The net was opened daily at 9:00 a.m. and closed at 5:00 p.m. during this period (a total of 80 days).

2.4. Inoculum Preparation

Bacillus sp. VWC18 was grown in 50 mL of Tryptone Soy Broth (TSB, ThermoFisher) for 18–20 h at 28 °C under horizontal shaking. The microbial biomass was then inoculated into 2 L of TSB and grown at 28 °C until an OD of 1.0, corresponding to 2×10^9 colony forming unit (CFU) mL^{-1} [16]. Bacterial biomass was collected following centrifugation and resuspended in sterile deionized water. The inoculum was applied every two weeks, during the fertilization step, by adding the bacterial suspension to a tank containing 10.000 L of well water (estimated concentration: 2×10^5 CFU mL^{-1}). The control plants were irrigated using well water; the flooding period was 40 min. for *Euphorbia pulcherrima* and 30 min. for *Cyclamen persicum*.

2.5. Agronomical Analysis

2.5.1. Morphological and Destructive Analysis

A total of 120 plants (20 plants per replicate) were collected for the analyses after 180 and 150 days of growth for poinsettia and cyclamen, respectively. First, the number of clusters of pigmented leaves in poinsettia, and the number of expanded flowers in cyclamen was registered; then, plants were cut at the base of the main stem, and fresh weight (FW) of both roots and aerial parts was measured. Roots were previously placed on a grid and air-dried for 2 h to remove excess water, then weighed. Dry weight (DW) was determined after drying the material in an aluminum tray at 70 °C for 96 h; dry matter percentage (DM%) was calculated as DW/FW × 100.

2.5.2. Chlorophyll, Carotenoids, Anthocyanins Measurement

A total of 25 poinsettia and cyclamen plants were randomly selected from each treatment and transported to the laboratory. 3 fresh leaf/plant were collected for chlorophyll and carotenoid measurements following acetone extraction; absorbance was measured at 665 nm, 649 nm, and 470 nm. The content of anthocyanins was determined in the pigmented bracts of poinsettia and in the cyclamen flowers, using the same extraction method as for chlorophyll, but measurement was performed at 547 nm [27–29].

2.6. Soil Microbial Eubacteria Analysis

10 g of substrate were collected from 25 randomized plants for each treatment; total genomic DNA was extracted from 250 mg of rhizosphere topsoil using the DNeasy PowerSoil Pro Kit (Qiagen, Hilden, Germany), following the manufacturer's instructions. DNA concentration and purity were assessed by measuring the absorbance ratio at 260 and 280 nm (Infinite®200 PRO NanoQuant, Mannedorf, Switzerland). Total eubacteria were quantified through real-time PCR (StepOne™ Real-Time PCR System, Applied Biosystems) according to Pagliarini et al. [30]. PCR was performed in a 20 µL amplification mixture containing 10 µL of Fast SYBR® Green Master Mix (Applied Biosystems, Foster City, CA, USA), 10 µM of primers Eub338 (5′-ACT CCT ACG GGA GGC AGC AG-3′) and Eub518 (5′-ATT ACC GCG GCT GCT GG-3′), molecular-grade H_2O, and 2 µL of DNA (5 ng μL^{-1}). The PCR protocol started with an activation step at 95 °C for 30″, followed by 40 cycles at 95 °C for denaturation (3″) and 60 °C for annealing (30″). Standard curves were constructed using the 16S rRNA PCR product of the main microorganisms isolated from soil; the data were transformed to express the number of microorganisms as Log10 CFU g^{-1} on rRNA gene copy number [31].

3. Results

3.1. Bacterial Identification and PGPR Characterization

The 16S rRNA sequencing provided the taxonomic assignment of *Bacillus* VWC18 to the species *Bacillus haynesii* (similarity: 99.93%; accession number: PZ125164), following data processing and sequence analysis. Phylogenetic analysis confirmed its placement within the *Bacillus licheniformis* clade (Figure 1).

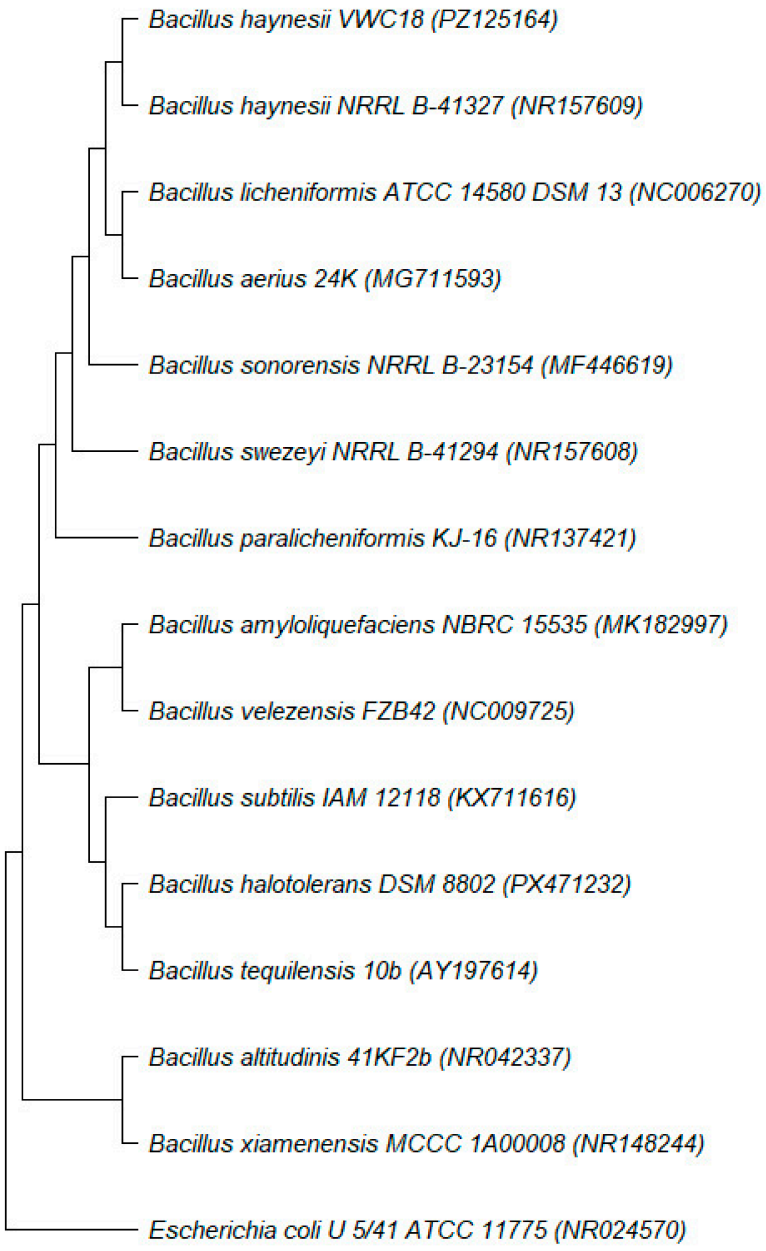


Figure 1. Phylogenetic tree showing the taxonomic position of *Bacillus haynesii* VWC18 strain based on 16S rRNA gene sequences, using the neighbor-joining (NJ) algorithm and MEGA version 5.0. The tree was rooted using Bacillaceae sequences from the NCBI database, and *Escherichia coli* U5/41 was used as an outgroup. The bar scale indicates phylogenetic distance. Branch numbers represent bootstrap values (bootstrap resampling of 1000).

Results also evidenced that *B. haynesii* VWC18 exhibits a multifaceted plant growth-promoting (PGP) phenotype (Table 2). The strain displayed significant phosphate-solubilizing activity on Pikovskaya medium, forming distinct medium-sized halos after seven days, representing the first documentation of in vitro phosphate solubilization by

this species. Additionally, *B. haynesii* VWC18 solubilized potassium and zinc, producing medium-sized transparent halos on potassium-mineral agar and small clear halos on zinc-amended media, respectively. The strain also exhibited γ-hemolytic activity (no erythrocyte lysis), thus showing a favorable biosafety profile. Quantification via the Salkowski method revealed time-dependent IAA accumulation, increasing from 28.6 ± 0.2 μg mL^{-1} at 24 h to 54.5 ± 0.3 μg mL^{-1} by 72 h. Concurrently, large orange halos on Chrome Azurol S (CAS) agar confirmed siderophore production, indicating the capacity to chelate ferric iron (Fe^{3+}) into soluble ferrous forms (Fe^{2+}) (Table 2).

Table 2. PGPR characterization of *B. haynesii* VWC18 (phosphate, zinc and potassium solubilization, siderophore production and indole-3-acetic acid after 24 h, 48 h and 72 h).

	Phosphate	**Zinc**	**Potassium**	**Siderophore**	**γ-Hemolytic**
B. haynesii VWC18	++	++	++	+++	−
		IAA production (μg mL^{-1})			
		24 h	48 h	72 h	
		28.6 ± 0.2	39.4 ± 0.3	54.5 ± 0.5	

The symbols indicate the following: −, no halos; ++, medium halos 0.5–1 cm; +++, large halos > 1.0 cm.

3.2. Bacillus haynesii VWC18 Treatment on Euphorbia pulcherrima and Cyclamen persicum: Agronomic and Microbiological Results

Agronomic parameters and flowering performance in three *E. pulcherrima* varieties were evaluated at the end of the growing period (180 days). Treated plants showed significantly higher fresh and dry above- and below-ground biomass compared to the control ($p \leq 0.01$; $p \leq 0.001$) (Table 3); in particular, the white variety exhibited a 90.3% increase in fresh foliar weight, from 74.46 ± 3.31 g (CTR) to 141.72 ± 3.12 g (TR). To confirm the results, Figure 2 highlights a control and a treated plant in their full appearance before selling.

Table 3. Fresh and dry weight of foliar and root in *Euphorbia pulcherrima* plants.

	Foliar Biomass (g $Plant^{-1}$)		**Root Biomass (g $Plant^{-1}$)**	
	Fresh weight	Dry weight	Fresh weight	Dry weight
EP_R_CTR	126.84 ± 3.34 ^{b}	8.47 ± 1.29 ^{b}	10.73 ± 1.79 ^{b}	3.63 ± 1.26 ^{b}
EP_R_TR	162.72 ± 2.63 ^{a}	9.92 ± 1.17 ^{a}	15.58 ± 1.15 ^{a}	5.69 ± 1.18 ^{a}
Significance	***	**	**	**
EP_W_CTR	74.46 ± 3.31 ^{b}	8.10 ± 1.11 ^{b}	9.92 ± 0.96 ^{b}	4.25 ± 1.16 ^{b}
EP_W_TR	141.72 ± 3.12 ^{a}	10.68 ± 1.15 ^{a}	13.63 ± 0.83 ^{a}	7.77 ± 1.14 ^{a}
Significance	***	***	***	***
EP_O_CTR	53.86 ± 3.01 ^{b}	6.18 ± 1.23 ^{b}	8.32 ± 0.97 ^{b}	5.08 ± 0.98 ^{b}
EP_O_TR	85.83 ± 2.86 ^{a}	8.15 ± 0.96 ^{a}	11.88 ± 0.92 ^{a}	9.95 ± 0.94 ^{a}
Significance	**	**	***	***

Mean values (±SD) of fresh weight and dry weight of foliar and root biomass. (a,b) Different letters within each column indicate significant differences according to the Tukey post hoc test ($p \leq 0.05$). ** and *** significant at $p \leq 0.01$ or $p \leq 0.001$.

Each variety showed a significantly higher average number of pigmented cluster bracts in treated plants compared to the control group (Figure 3). In particular, the red variety developed twice the number of red bracts (9.70 ± 1.17 vs. 4.84 ± 1.01). Table 4 reported the chlorophyll and carotenoid leaf concentrations, as well as the anthocyanin content in pigmented bracts. All pigments were significantly higher in the treated varieties

compared to the control plants; in particular, chlorophyll-*a*, chlorophyll-*b*, and carotenoids registered double values in all varieties ($p \leq 0.001$). Anthocyanin increase was particularly evident in red and orange varieties with significant values in treated plants; in the white variety, anthocyanin detection was weak due to the absence of red/orange pigmentation ($p \leq 0.01$).

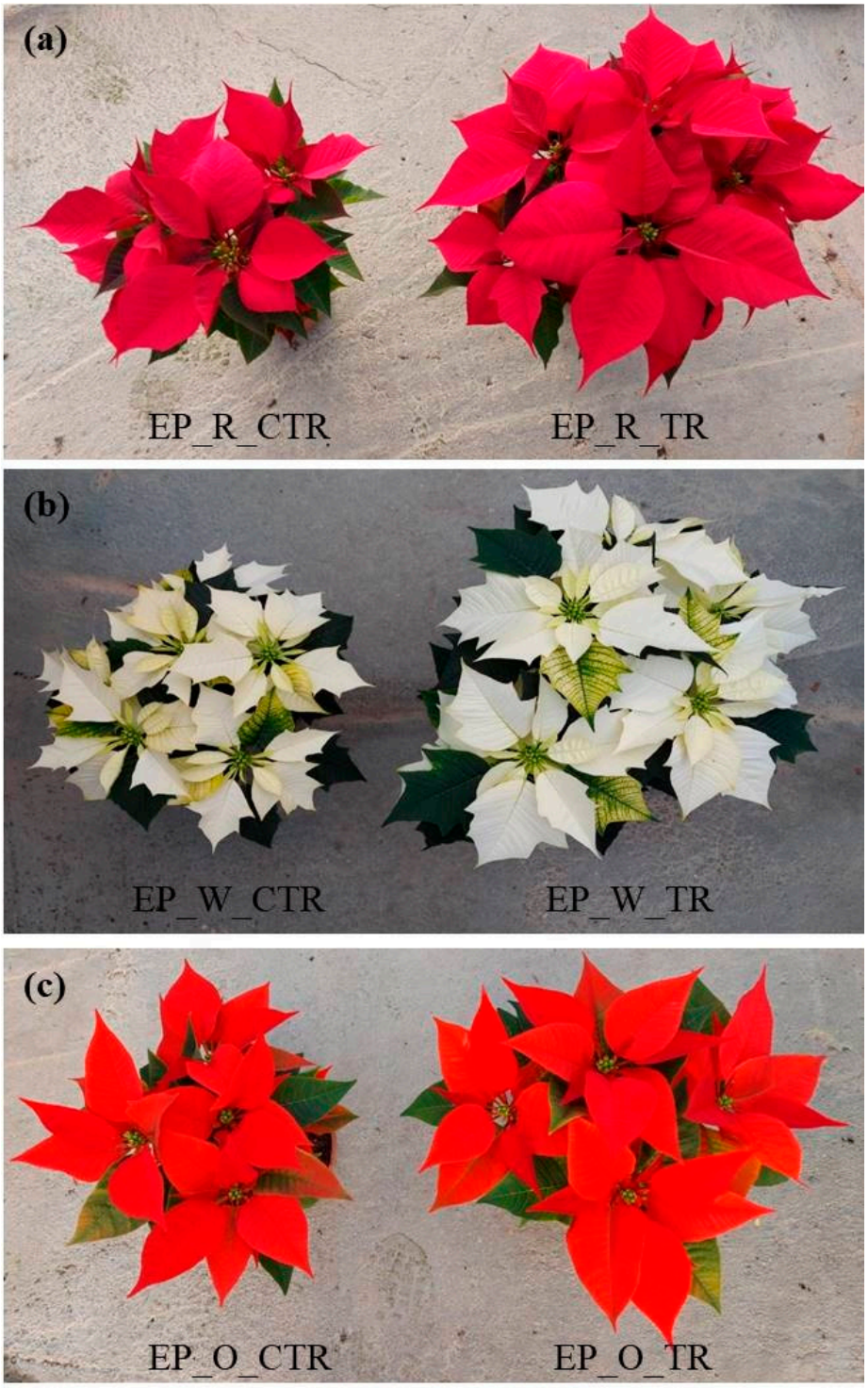


Figure 2. (**a**) *Euphorbia pulcherrima* var. Gran d'Italia (control: EP_R_CTR; *B. haynesii* VWC18 treatment: EP_R_TR); (**b**) *Euphorbia pulcherrima* var. Alaska (control: EP_W_CTR; *B. haynesii* VWC18 treatment: EP_W_TR); (**c**) *Euphorbia pulcherrima* var. Princettia Orange (control: EP_O_CTR; *B. haynesii* VWC18 treatment: EP_O_TR).

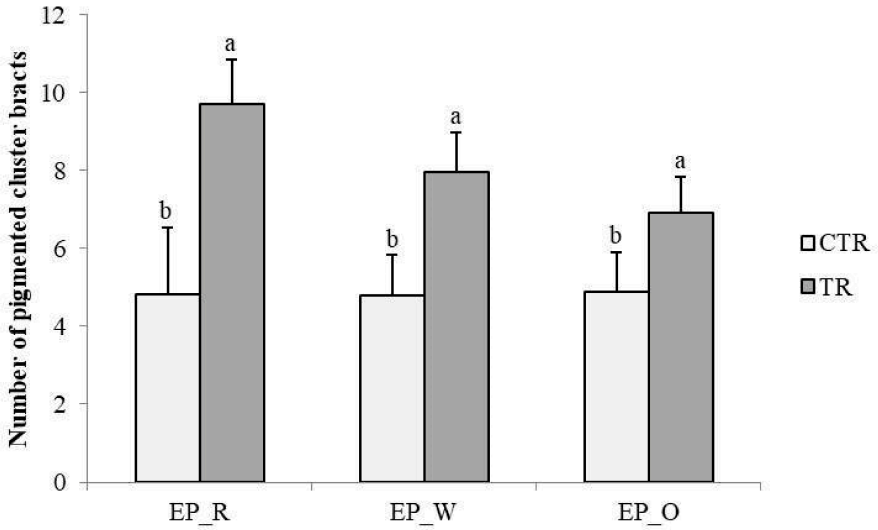


Figure 3. Mean values (±SD) of the number of pigmented cluster bracts in the control and treated *Euphorbia pulcherrima* plants. Different letters indicate significant differences for each variety according to the Tukey post hoc test ($p \leq 0.05$).

Table 4. Leaf chlorophyll-*a*, chlorophyll-*b*, carotenoids, and anthocyanins content in the *Euphorbia* experiment.

	Chlorophyll-*a*	**Chlorophyll-*b***	**Carotenoids**	**Anthocyanins**
		($\mu g\ g^{-1}$)		
EP_R_CTR	71.40 ± 3.64 ^{b}	116.95 ± 2.20 ^{b}	7729.07 ± 10.01 ^{b}	164.49 ± 5.42 ^{b}
EP_R_TR	152.84 ± 2.45 ^{a}	231.65 ± 2.34 ^{a}	17,326.74 ± 7.39 ^{a}	212.91 ± 3.05 ^{a}
Significance	***	***	***	**
EP_W_CTR	75.29 ± 2.41 ^{b}	130.74 ± 4.28 ^{b}	7835.05 ± 5.96 ^{b}	8.04 ± 3.02 ^{b}
EP_W_TR	148.98 ± 1.79 ^{a}	248.05 ± 3.22 ^{a}	14,660.61 ± 5.38 ^{a}	13.06 ± 3.38 ^{a}
Significance	***	***	***	**
EP_O_CTR	62.84 ± 3.27 ^{b}	104.73 ± 2.56 ^{b}	6873.36 ± 6.25 ^{b}	124.54 ± 5.82 ^{b}
EP_O_TR	131.30 ± 2.05 ^{a}	221.74 ± 2.51 ^{a}	15,721.12 ± 7.74 ^{a}	193.74 ± 2.25 ^{a}
Significance	***	**	***	***

Mean values (±SD) of chlorophyll-*a*, chlorophyll-*b*, carotenoids, and anthocyanins content. Different letters within each column indicate significant differences according to the Tukey post hoc test ($p \leq 0.05$). **, *** significant at $p \leq 0.01$ and $p \leq 0.001$, respectively.

The cyclamen varieties evidenced a significant impact of the microbial treatment (Figure 4); for what concern the number of flowers, all three species produced a significantly higher number in the treated varieties, reaching double the values (Figure 5). Following destructive analysis, the treated varieties evidenced significantly higher fresh biomass and dry weights (foliar and root; $p \leq 0.001$), compared to the untreated control (Table 5). Fresh leaf weight increased by over 50%, as well as dry weight, with the white variety being the most susceptible to treatment. Also, fresh and dry root weight almost doubled. With respect to Chlorophyll-*a* and -*b*; carotenoids, and anthocyanin content in flower petals, results are shown in Table 6. All leaf parameters were significantly higher compared to the control plants; except for chlorophyll-*b* in CP_W_TR and CP_V_TR, all values increased twofold. The anthocyanin pigment content in the flower petals reflected the same trend observed in poinsettia, with significantly higher values in all treated cyclamen species, in particular in the red and violet varieties.

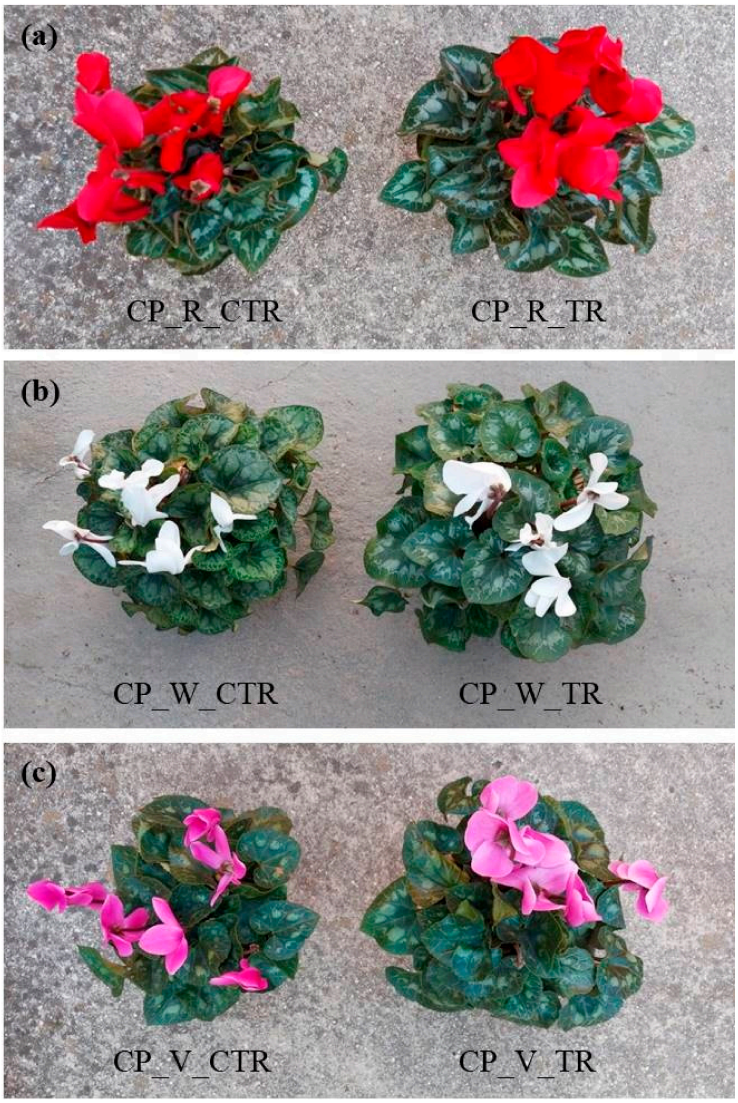


Figure 4. (**a**) *Cyclamen persicum* var. Red Star (control: CP_R_CTR; *B. haynesii* VWC18 treatment: CP_R_TR); (**b**) *Cyclamen persicum* var. Halios White (control: CP_W_CTR; *B. haynesii* VWC18 treatment: CP_W_TR); (**c**) *Cyclamen persicum* var. Halios Violet (control: CP_V_CTR; *B. haynesii* VWC18 treatment: CP_V_TR).

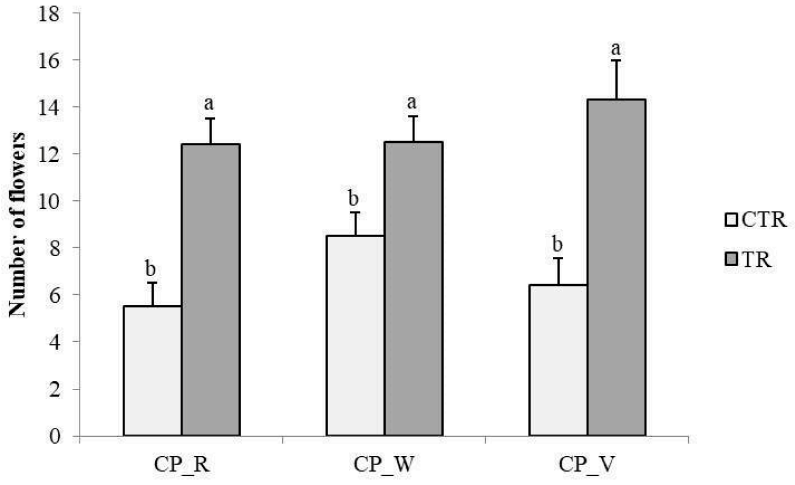


Figure 5. Mean values (±SD) of the number of flowers in the control and treated *Cyclamen persicum* plants. Different letters indicate significant differences for each variety according to Tukey's post hoc test ($p \leq 0.05$).

Table 5. Foliar and root fresh and dry weight of *Cyclamen persicum* plants.

	Foliar Biomass (g Plant^{-1})		**Root Biomass (g Plant^{-1})**	
	Fresh weight	Dry weight	Fresh weight	Dry weight
CP_R_CTR	69.65 ± 5.37 ^{b}	7.11 ± 1.33 ^{b}	6.93 ± 0.53 ^{b}	2.98 ± 0.17 ^{b}
CP_R_TR	109.90 ± 2.56 ^{a}	12.20 ± 1.29 ^{a}	13.36 ± 1.27 ^{a}	5.14 ± 0.58 ^{a}
Significance	***	***	***	***
CP_W_CTR	64.11 ± 4.57 ^{b}	6.68 ± 1.14 ^{b}	7.14 ± 0.72 ^{b}	3.21 ± 1.26 ^{b}
CP_W_TR	112.54 ± 1.77 ^{a}	11.41 ± 1.25 ^{a}	12.09 ± 0.39 ^{a}	7.64 ± 1.01 ^{a}
Significance	***	***	***	**
CP_V_CTR	59.09 ± 6.96 ^{b}	5.62 ± 2.34 ^{b}	6.98 ± 0.17 ^{b}	2.64 ± 1.78 ^{b}
CP_V_TR	97.68 ± 1.97 ^{a}	13.67 ± 2.51 ^{a}	11.21 ± 1.22 ^{a}	6.44 ± 0.34 ^{a}
Significance	**	***	***	**

Mean values (±SD) of fresh weight and dry weight of foliar and root biomass. Different letters within each column indicate significant differences according to Tukey's post hoc test ($p \leq 0.05$). ** and *** significant at $p \leq 0.01$ or $p \leq 0.001$.

Table 6. Leaf chlorophyll-*a*, chlorophyll-*b*, carotenoids, and anthocyanins content in the *Cyclamen* experiment.

	Chlorophyll-*a*	**Chlorophyll-*b***	**Carotenoids**	**Anthocyanins**
		(μg g^{-1})		
CP_R_CTR	64.01 ± 7.94 ^{b}	115.85 ± 6.66 ^{b}	2209.28 ± 67.51 ^{b}	188.01 ± 4.52 ^{b}
CP_R_TR	137.00 ± 6.81 ^{a}	206.14 ± 5.39 ^{a}	5848.14 ± 55.11 ^{a}	325.12 ± 2.65 ^{a}
Significance	***	***	***	***
CP_W_CTR	66.57 ± 7.46 ^{b}	127.85 ± 8.66 ^{b}	2311.85 ± 66.32 ^{b}	12.30 ± 2.06 ^{b}
CP_W_TR	134.71 ± 4.82 ^{a}	220.42 ± 6.24 ^{a}	7399.43 ± 51.76 ^{a}	24.06 ± 4.54 ^{a}
Significance	***	***	***	*
CP_V_CTR	52.43 ± 8.92 ^{b}	109.57 ± 7.97 ^{b}	2254.85 ± 49.21 ^{b}	65.80 ± 6.99 ^{b}
CP_V_TR	115.86 ± 4.74 ^{a}	188.85 ± 5.89 ^{a}	6344.57 ± 36.69 ^{a}	139.63 ± 4.54 ^{a}
Significance	***	**	***	***

Mean values (±SD) of chlorophyll-*a*, chlorophyll-*b*, carotenoids, and anthocyanins content. Different letters within each column indicate significant differences according to the Tukey post hoc test ($p \leq 0.05$). *, **, *** significant at $p \leq 0.05$, $p \leq 0.01$, and $p \leq 0.001$, respectively.

3.3. Rhizosphere Eubacteria Quantification

Soil bacteria quantification in the rhizosphere of potted plants is resumed in the text. Overall, all poinsettia varieties sub-irrigated with *B. haynesii* VWC18 exhibited a significant increase in total eubacteria, from 5.1 × 10^5 CFU g^{-1} to 1.2 × 10^7 CFU g^{-1} in the red genotype; from 5.6 × 10^5 CFU g^{-1} (CTR) to 1.4 × 10^7 CFU g^{-1} (TR) in the orange variety. The white variety also showed an increase, reaching 1.3 × 10^7 CFU g^{-1} in EP_W_TR. A similar trend was observed in the cyclamen trial. The control treatments reached a total bacterial count of 4.2 × 10^5 CFU g^{-1} in CP_R_CTR, 4.3 × 10^5 CFU g^{-1} in CP_V_CTR, and 4.2 × 10^5 CFU g^{-1} in CP_W_CTR. The respective treatments evidenced a significant increase of 2.6 × 10^7 CFU g^{-1} (CP_R_TR), 2.5 × 10^7 CFU g^{-1} (CP_V_TR), and 2.2 × 10^7 CFU g^{-1} (CP_W_TR).

4. Discussion

The scientific evidence associating PGPR with horticulture fully provides relevant successful assays, demonstrating that plants benefit from this association; floriculture, however, still lacks equally comprehensive and rigorous evidence, despite a few promising reports. In this context, *Bacillus haynesii* VWC18 is particularly relevant: in our previous

work, the strain was shown, through greenhouse trials, to improve plant growth and nutrient adsorption [16] and was subsequently used to support the granting of a patent; however, species-level identification was not achievable at that stage, and the isolate was therefore assigned only to the genus *Bacillus*. Sequencing accuracy and phylogenetic analysis have now allowed the strain to be reliably assigned to *Bacillus haynesii*. *Bacillus haynesii* is a recent species first isolated by Dunlap et al. [18] from desert soil. This strain clusters within the *Bacillus licheniformis* clade, which includes *B. licheniformis*, *B. paralicheniformis*, *B. glycinifermentans*, and *B. sonorensis*, all of which are widely recognized rhizosphere bacteria with putative PGPR activity. As with several *Bacillus* spp., the biostimulant activity of some *B. haynesii* strains is of recent discovery; it has also been studied for mitigation activity under drought and salt conditions in several crops [32–34]. *B. haynesii* was also isolated from fermented poultry manure, which is frequently used as an organic amendment in agriculture, and it is usually rich in PGPR [30]. However, most of the studies focused on the huge enzymatic activities, such as L-methioninase, acid phosphatase, and xylanase, relevant to the human, food, and biotechnology fields [35–37]. It is also known for its enzymatic synthesis to facilitate the breakdown of odor-causing substances [38]. This study evidenced that the strain VWC18 possesses relevant PGP activities, as already reported by Pagliarini et al. [16] and Parenti et al. [17] in horticulture and cereal crops. In lettuce and basil, the inoculation promoted plant growth, chlorophyll and mineral uptake in both crop species. Root weight doubled or tripled compared to control plants, and chlorophyll concentration reached even higher values. Interestingly, both parameters had a dose-dependent increase. In this study, a deeper investigation on the in vitro PGPR activities was carried out, in order to better understand the involved mechanisms; these activities (P, K and Zn solubilization) are well documented in *Bacillus* species and are usually referred to organic acid secretion, proton extrusion, and phosphatase production [39,40]; the secretion of low-molecular-weight organic acids (e.g., citric, oxalic, gluconic) lowers rhizosphere pH and forms soluble complexes with K^+ and Zn^{2+} ions [41–43], getting those minerals more available for plant adsorption. IAA quantification via the Salkowski method revealed time-dependent IAA accumulation, thus suggesting robust auxin-mediated root growth promotion [44,45]. Indeed, 3′-indole-acetic acid (auxin group) seemed to be the most common phytohormone acting as a promoter of lateral roots and root hair cells development. In Pagliarini et al. [16], the root weight increase was extremely evident in lettuce and basil weekly watered with *B. haynesii* VWC18. Concurrently, the Chrome Azurol S assay confirmed siderophore production, indicating the capacity of *B. haynesii* VWC18 to chelate ferric iron (Fe^{3+}) into soluble ferrous forms (Fe^{2+}). This could enhance plant iron acquisition by the plants while reducing pathogen competition [46]. From a biosafety perspective, *B. haynesii* VWC18 exhibited a γ-hemolytic phenotype, showing no erythrocyte lysis on blood agar. The absence of hemolytic activity indicates low virulence and supports its suitability for agricultural applications, including foliar treatment in food crops and medicinal ornamental plants, where microbial safety is paramount [47]. Collectively, these attributes further validate the use of *B. haynesii* VWC18 as a promising ingredient for biofertilizer formulations. Moreover, they may elucidate the previous findings reported by Pagliarini et al. [16] and Parenti et al. [17] (significant increases in foliar and root biomass and nutrient uptake in different crops) in which the PGP potential of *B. haynesii* VWC18 was hypothetical. Further, the greenhouse experiment was essential to assess the efficacy of the strain VWC18 in floriculture, a sector where the PGPR applications remain underexplored. The limited literature on poinsettia/cyclamen and PGPR application aligns with the findings of this study, although flower varieties and microorganisms often differ across studies. However, the few available pieces of evidence demonstrated that PGPR improve plant biomass (above and below) and flowering, thus confirming species- and/or genus-

specific modulation of plant physiology via bacterial inoculation. Martinetti et al. [48] observed a positive effect of *Rhodobacter capsulatus* in poinsettia cv. Peterstar and Maren; in particular, treatments with *R. capsulatus* improved the number of colored bracts and fresh and dry shoot weight. Rodriguez-Elizalde et al. [49] evaluated the use of different bacterial strains in combination with two doses (50% and 100%) of a commonly applied fertilizer; positive results on agronomic parameters were achieved with the mix of the tested PGPR with the 50% fertilization level. Noteworthy, Parlakova Karagoz [50] evidenced the beneficial role of *Bacillus megaterium* TV-91C and TV-6D and *Pantoea agglomerans* RK-79; this formulation enhanced poinsettia cutting propagation by increasing the number of rooted cuttings, root number, root length, number of newly formed leaves, and anthocyanin content, but not chlorophyll. With regard to cyclamen, several studies reported successful application of arbuscular mycorrhizal fungi and *Trichoderma harzianum*, improving both growth/flowering and disease susceptibility [51,52]; nevertheless, to our knowledge, the potential application and role of rhizobacteria in Cyclamen, and much less of *B. haynesii*, is uninvestigated. Although the evaluated PGP traits are limited and do not cover the full spectrum of potential mechanisms, and a basic fertilization plan was necessary to meet the plant grower's needs, *B. haynesii* VWC18 inoculation induced a significant physiological and growth improvement in both poinsettia and cyclamen, as the studies reported above. Moreover, the investigated microbial population on potted soil supports the hypothesis that the inoculated strain can survive and thrive in the rhizosphere. The establishment of a microorganism in the soil following repetitive inoculation is not a given, in particular under field conditions, since several factors may influence persistence and efficacy [53]. Although sybr-green chemistry did not allow the quantification of the target microorganisms, it is undoubtedly clear that the total eubacteria population increased after *B. haynesii* VWC18 inoculation. We can hypothesize that the Bacillus strain can potentially survive, colonize the rhizosphere, and act as a PGPR. *B. haynesii* VWC18 likely forms an intimate association with plant roots by synthesizing IAA and organic acids that solubilize minerals, which directly supports root and shoot development, as previously observed by Pagliarini et al. [16]. Indirectly, the increased rooting surface provides the basis for nutrient uptake and water retention, and consequently greater availability of elements fundamental for pigment synthesis and biomass accumulation. Root hair development, which is controlled by an intricate network of hormonal and molecular signals, represents one of the most effective morphological adaptations enhancing micronutrient acquisition [54]. Furthermore, the observed dry matter increase supports the hypothesis of enhanced nutrient uptake and structural development. Pagliarini et al. [16] well documented the adsorption improvement of key nutrients in lettuce and basil plants subjected to *B. haynesii* watering. The increase in chlorophyll, in particular, is a key indicator of enhanced N and Mg adsorption, which can translate into photosynthetic capacity and increase conversion of light energy into chemical energy. The observed increase in carotenoids is equally significant, as these pigments play a protective role against oxidative stress, contributing to the stability of photosynthetic complexes and preventing damage from intense light radiation [55]. Moreover, carotenoids can improve plant resistance to adverse environmental conditions and also directly influence the content of colored pigments in flowers [56]. The anthocyanin content in the poinsettia pigmented bracts as well as in the cyclamen flowers is noteworthy. The major pigments produced in Cyclamen flowers comprise flavonoids, carotenoids, chlorophylls, and betalains. Beyond aesthetic factors, the significant increase in anthocyanins in bracts/petals helps protect against environmental stress that can negatively affect flowering and the maintenance of open flowers [57]. Regarding flowering and flower number, it would be interesting to investigate plant gene expression–mediated mechanisms; however, it is well established that PGPR also influence flowering and the number of flowers per plant [58].

Nordstedt et al. [59] identified and applied in greenhouse *Pseudomonas poae* 29G9 and *Pseudomonas fluorescens* 90F12-2 that increased petunia flower number and plant biomass under drought and low-nutrient conditions. Collectively, the most pronounced PGP effect was shown on EP_W and EP_R poinsettia varieties, and CP_R for what concerns cyclamen. A possible inter-genotypic variability could result; however, how *B. haynesii* signals could specifically trigger plant-variety responses needs further investigation. Interaction between specific plant genotypes and microbial inoculants highlights the critical importance of selecting synergistic plant-microorganism combinations aimed at maximizing growth performance [60].

5. Conclusions

Bacillus haynesii VWC18 showed significant plant growth-promoting activity in poinsettia and cyclamen under commercial greenhouse conditions. *B. haynesii* is an emerging bacterial species of considerable interest because of its broad spectrum of biotechnological activities. The in vitro characterization revealed phosphate, potassium, and zinc solubilization, siderophore production, indole-3-acetic acid synthesis, and a γ-hemolytic phenotype. These multifaceted PGP features of *B. haynesii* VWC18 translated into measurable plant benefits. Its application by sub-irrigation in flower species increased foliar and root biomass in all tested cultivars. In poinsettia, fresh foliar biomass increased by approximately 50–90%, while root biomass also increased compared with the untreated controls. In cyclamen, both foliar and root biomass increased substantially following bacterial treatment. The inoculation also improved ornamental traits; treated poinsettia plants produced a greater number of pigmented bract clusters, and treated cyclamen plants produced more flowers. The concentrations of chlorophyll-*a*, chlorophyll-*b*, and carotenoids increased in both species. Anthocyanin content also increased in the pigmented bracts of poinsettia and in the flowers of cyclamen. Repeated application of *B. haynesii* VWC18 was associated with a marked increase in total eubacterial abundance in the rhizosphere, which may largely reflect the multiplication and persistence of the inoculated strain within the rhizosphere niche. These results support the potential use of this strain as a microbial fertilizer for ornamental crop production.

6. Patents

The authors declare that this research was conducted with the aim of supporting a patent granted in 2023: Title: "*Bacillus haynesii* promotore dello sviluppo fisiologico e qualitativo di vegetali nel settore orto-floricolo", patent n° 102023000006816; Inventors: Francesca Gaggìa, Elia Pagliarini, Diana Di Gioia.

Author Contributions: Conceptualization, E.P., F.G. and D.D.G.; methodology and software, E.P.; validation, E.P., F.G.; investigation, E.P., N.M.D.; data curation, E.P.; writing—original draft preparation, E.P. and N.M.D.; writing—review and editing, F.G. and D.D.G.; supervision, project administration and funding acquisition, F.G. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by AlmaValue for "RR" 2023 granted by the Ministry of Enterprises and Made in Italy and the NextGenerationEU (Prot. n. 0088324 del 30/03/2023).

Data Availability Statement: The data that support the findings of this study are available from the corresponding author upon request.

Acknowledgments: The authors would like to thank Giorgio Paganotto for hosting the Greenhouse experiment.

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

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