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Research Article

PLANT GROWTH-PROMOTING RHIZOBACTERIA (PGPR) AS A BIOSTIMULANT TO ENHANCE ECHEVERIA SEEDLING QUALITY AND OFFSET YIELD

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ABSTRACT. Echeveria, a genus within the *Crassulaceae* family, is a highly valued ornamental succulent known for its resilience, vibrant coloration, and attractive rosette morphology. In vegetative propagation of Echeveria, ensuring high-quality root development and increasing offset production are critical for commercial success. Plant Growth-Promoting Rhizobacteria (PGPR), known to synthesize auxins that stimulate root formation, have emerged as promising biostimulants in ornamental plant production. In this study, the effects of three different rhizobacterial formulations -*Bacillus pumilus* (with three identified active gene regions), *Stenotrophomonas* spp. (with five identified active gene regions), and a 1:1 mixture of both- were evaluated on seedling quality and offset yield in Echeveria. These formulations were applied through three methods: soil drench, foliar spray, and combined soil + foliar treatment. The results demonstrated that PGPR applications increased offset production by up to fourfold, while significantly enhancing vegetative growth and root development, achieving nearly twice the performance of the control plants. Additionally, protein isolation from leaves revealed notable differences in protein profiles associated with PGPR treatments. Overall, this study highlights the substantial positive effects of different PGPR species on rooting, seedling quality, and protein expression in ornamental succulents. The findings reinforce the growing recognition that rhizobacteria can play a transformative role in advancing more sustainable and environmentally friendly horticultural practices.

Keywords: *Echeveria, biostimulants, rhizobacteria*

INTRODUCTION

This Echeveria is a plant genus belonging to the *Crassulaceae* family. *Crassulaceae* is a large and diverse family that includes annual and perennial species, herbaceous plants, shrubs, and even small trees. The family comprises approximately 1410 species within 35 genera. Among these genera, Echeveria stands out as a highly valuable ornamental plant due to its durability, striking coloration, and aesthetically appealing rosette form [1]. Native to Mexico and the northwestern regions of South America, this succulent has a high water-storage capacity owing to its fleshy leaf structure [2].

The genus is named after the Mexican botanical illustrator Atanasio Echeverría y Godoy. Within this genus, which includes roughly 139 species, the species Echeveria secunda exhibits substantial varietal diversity. Among these varieties, 'Fleur Blanc' is especially noteworthy with its ornamental appearance, rosette form, and leaf margins displaying pink to light turquoise-blue hues [3]. Echeveria 'Fleur Blanc' is characterized by its ease of maintenance, drought tolerance, turquoise–blue foliage, and raceme-like inflorescences, all of which contribute to its high ornamental value [1, 2]. Its artichoke-like rosettes expand by forming offsets, creating a colony-like growth habit, and eventually developing into a low mound. It

blooms in racemes during spring. However, it is highly sensitive to low temperatures and cannot survive below -6.7 °C [4].

Echeveria species reproduce both sexually through pollination and asexually through offset formation. Their thick, fleshy leaves confer a high tolerance to drought, making them popular indoor ornamental plants. Following the completion of their chilling requirement, they begin flowering with rising temperatures in spring. Vegetative propagation can be performed easily during spring and summer, and the species is predominantly propagated via cuttings. Several factors influence propagation success, including the age and characteristics of the mother plant, the season and type of cutting, storage conditions before planting, chemical rooting enhancers, physical and chemical properties of the rooting medium, rooting temperature, sterilization treatments against fungal infections, and the chemical properties of misting water [5].

In species propagated by cuttings, various chemicals and hormones are used to accelerate root formation and promote strong root development. However, with growing interest in sustainable agriculture, the use of microorganisms in areas such as biocontrol, plant growth promotion, biofertilization, and rooting of cuttings has increased [6, 7]. Previous studies have shown that PGPR bacteria are among the most important beneficial microorganisms used in plant production [8, 9, 10]. Research has revealed that rhizobacteria belonging to Bacillus, Pseudomonas, Agrobacterium, Streptomyces, and Alcaligenes, as well as bacteria possessing genes involved in indole acetic acid (IAA) production, promote rooting in cuttings [10, 11].

Plant growth-promoting rhizobacteria (PGPR) adapt well to diverse environments, exhibit rapid growth, and readily establish themselves in soil ecosystems. Due to their natural genetic potential, they are considered valuable biological components for agricultural production [12]. Rhizobacteria colonize plant roots, promote plant growth, and simultaneously suppress potential pathogenic microorganisms. These bacteria play key physiological roles such as enhancing heavy metal solubility, lowering ethylene levels, fixing atmospheric nitrogen, and solubilizing phosphorus [13,14]. Additionally, they synthesize phytohormones such as auxins, cytokinins, and gibberellins, regulate microbial balance in the rhizosphere, facilitate nutrient uptake, and support overall plant development [15, 16]. Some PGPR species also enhance root development and improve plant stress tolerance through siderophore production and indole-3-acetic acid (IAA) synthesis [17,18]. Studies have shown that PGPR applied through inoculation colonize plant roots more effectively than free-living populations and enhance plant vitality. PGPR applications contribute to the conservation of soil and water resources, reduce the negative effects of chemical fertilizers and pesticides, improve nutrient uptake, mitigate biotic and abiotic stresses, and provide environmentally friendly and sustainable production opportunities [16]. Moreover, rhizobacteria offer advantages in promoting rooting and enabling faster production of high-quality seedlings [19].

In this study, the effects of three different rhizobacterial formulations prepared from *Bacillus pimulus* and *Stenotrophomonas* spp. (Formulation 1: *B. pumilus*; Formulation 2: *Stenotrophomonas* spp.; Formulation 3: a 1:1 mixture of *B. pumilus* and *Stenotrophomonas* spp.) were investigated with respect to seedling quality and offset production in Echeveria.

MATERIALS AND METHODS

Plant Materials

Commercial seedlings of the *Echeveria secunda* cultivar ‘Fleur Blanc’, belonging to the same genotype, propagated by cuttings, uniform in size, and grown under identical conditions, were obtained from commercial nurseries. The plants were cultivated in one liter pots containing an autoclaved and sterilized 1:1 peat:soil mixture in a heated greenhouse belonging to Erciyes University.

Preparation, Activation and Treatment of Rhizobacteria

PCR analysis confirmed that two rhizobacterial strains possessed the indole acetic acid (IAA) gene region, and bacterial solutions were prepared accordingly. Nutrient Agar (NA) and Nutrient Broth (NB) media were used for bacterial activation and culture [16, 20]. The rhizobacteria were inoculated onto NA plates and incubated at 35 °C for 24 hours. A single colony from the activated culture was transferred into NB medium and incubated overnight at 35 °C with shaking at 180 rpm [20]. The bacterial suspensions were adjusted to a concentration of 3×10^8 cells/ml. The experiment was conducted in three replicates, with two pots per replicate.

The rhizobacterial strains used in the study were identified at the species level based on 16S rRNA gene sequences and characterized using MALDI-TOF (Matrix-Assisted Laser Desorption Ionization–Time of Flight Mass Spectrometry), with the presence of 3 and 5 specific gene regions confirmed (Table 1). PGPR treatments were applied through three different methods:

1- Soil application via irrigation water,
2- Foliar application by spraying,
3- Combined soil (irrigation) + foliar (spraying) application.

Plant growth and development were monitored throughout the experiment following these treatments.

Table 1. *Bacterial strains used in the study, their code numbers, and gene presence status*

Bacterial code	Bacteria species	Determined gene regions
6-2-2-3EU	***Bacillus pimulus*	3 gen (siderefor, Fitaz, IAA)
EU5A21	**Stenotrophomonas* spp.	5 gen (Fosfataz, ACC deaminaz, siderefor, Fitaz, IAA)
Mix Rhizobacteria	Mix *Bacillus pimulus* and *Stenotrophomonas* spp. (v:v/1:1)	

*Species-level identification based on 16S rRNA Sanger sequencing (OL673802-accession number),
**Identification by the MALDI-TOF (Matrix-Assisted Laser Desorption Ionization–Time of Flight Mass Spectrometry) method

Morphological Measurements

To evaluate the effects of different rhizobacterial formulations on the quality characteristics and offshoot productivity of Echeveria seedlings, measurements and observations were recorded at the end of the 10th week. To assess the impact of PGPR applications, the following morphological and physiological parameters were measured: number of tubers, number of leaves, number of flower inflorescences, number of flowers per inflorescence, leaf diameter (mm), leaf length (mm), plant fresh weight (g), root fresh weight (g), total chlorophyll content, and root length (mm).

Protein Isolation

Proteins were extracted from Echeveria leaf samples using the TCA/acetone precipitation method [21]. Eighty grams of leaf tissue were pulverized in liquid nitrogen, and 1 g of the resulting powder was combined with 4 mL of 10% TCA/acetone. After centrifugation, the supernatant was discarded, and the pellet was suspended in 10% TCA/acetone containing 5 mM DTT. This procedure was repeated once, followed by a final wash in 80% acetone with 5 mM DTT and incubation at −20°C for 10 minutes. The pellet was then air-dried at 25°C.

Protein Quantification

Protein levels in the samples were determined using the Bio-Rad Protein Assay kit. Bovine serum albumin (BSA) was used as a standard to generate a calibration curve. Absorbance measurements were recorded at 660 nm for a series of BSA concentrations (0.125, 0.250, 0.500, 0.750, 1.0, 1.5, and 2.0 mg/mL), following the procedure described by Bradford (1976) [22].

SDS-PAGE (Sodium Dodecyl Sulphate–Polyacrylamide Gel Electrophoresis)

SDS-PAGE was employed to examine differences in protein expression profiles among the plant samples. Proteins were separated using polyacrylamide gel electrophoresis in the presence of SDS. Following electrophoresis, the gels were subjected to silver staining to visualize protein bands. The stained gels were then analyzed and interpreted according to the methodology of Walker (2002) [21].

Statistical Analyses

The experimental data were analyzed using analysis of variance (ANOVA) in SAS software (version 9.00). Mean comparisons were performed using Duncan's multiple range test at significance levels of 0.05 and 0.001.

RESULTS AND DISCUSSION

Morphological Parameters Results

Overall, PGPR treatments resulted in significantly higher seedling quality compared to the control group (Table 2; Figure 1). In this study, two different rhizobacterial species possessing distinct gene regions were used. According to previous studies in which the strains were screened with specific primers, *Bacillus pimulus* was found to carry three different gene regions, whereas *Stenotrophomonas* spp. possessed five distinct gene regions [23]. Overall, the results indicated that both rhizobacterial species significantly enhanced seedling quality, seedling growth, offset production, leaf number, root development, and root length in Echeveria compared to the control and medium treatments. When the two rhizobacterial species were compared directly, *Stenotrophomonas* spp. demonstrated superior performance in most morphological growth parameters. Furthermore, the comparison of application methods revealed that the combined soil and foliar treatment produced significantly higher values than either method applied individually.

Fig. 1. *a) Morphological comparison of seedlings treated with bacteria via soil application versus control and standard conditions; b) Seedling image for the second bacterial strain (S2) applied via foliar spraying; c) Seedling image for the second bacterial strain (S2) applied through combined soil and foliar treatment; d) Visual representation of the effect of soil-applied bacteria on root length.*

In particular, Echeveria plants were significantly affected by rhizobacterial applications in terms of offset formation. The highest number of offsets was observed in plants treated with *Stenotrophomonas* spp., with the combined foliar and soil application yielding the most successful results. Similarly, plants treated with *B. pimulus* produced a significantly higher number of offsets compared to the control, although this bacterial strain performed better when applied solely via soil (Figure 2).

Regarding leaf number, the statistically highest value was obtained from plants treated with *Stenotrophomonas* spp. via the combined foliar and soil application (75 leaves per plant), followed by the soil application of *B. pimulus* (68.66 leaves per plant). The interaction between rhizobacterial strain and application method was found to be statistically significant for both offset number and leaf count at $p < 0.01$ (Table 2).

In terms of fresh plant weight, the highest value was recorded in the treatment in which both rhizobacterial strains were applied as a mixture through combined foliar and soil application, reaching approximately 123 g, whereas the control group exhibited a mean weight of 85 g. Applications of *Stenotrophomonas* spp. led to a significant increase in shoot development, accompanied by notable enhancements in root length and root volume. Regarding root fresh weight, the combined foliar and soil application of the rhizobacteria produced the highest values among all treatments. Root length also increased significantly in the mixed-rhizobacteria soil application and in the soil application of *Stenotrophomonas* spp.. The interaction between rhizobacterial strain and application method was statistically significant ($p < 0.01$) for both fresh plant weight and root fresh weight (Table 2).

Overall, the findings of this study demonstrate that rhizobacterial strains possessing different gene regions significantly enhanced multiple plant growth parameters. Previous research similarly indicates that rhizobacterial applications can naturally promote rooting and plant development, offering a viable alternative to commercial rooting hormones. The effects of PGPR on ornamental species have been examined in several studies, which collectively report positive impacts on morphological traits. For example, beneficial outcomes have been documented in chrysanthemum, aster, zinnia, and geranium [24], as well as in iris and jasmine [25], petunia [26] and Kalanchoe [7]. In agreement with these earlier reports, the present study highlights the favorable influence of PGPR treatments on root formation in Echeveria. Larraburu et al. (2007) [27] also observed that combinations of rhizobacterial strains accelerated early rooting in *Schinus terebinthifolius*. Similarly, other investigations have shown the high rooting success of hardwood cuttings from ornamental plants treated with rhizobacteria [28,11]. Sezen et al. (2014) [29] found that among several bacterial species, Bacillus strains were particularly effective in promoting root development in *Ficus benjamina* cuttings. In another study, Alkaç et al. (2022) [30] reported significant improvements in both germination and morphological traits of aster flowers following rhizobacterial applications.

The growth-promoting capacity of rhizobacteria is often attributed to their ability to synthesize phytohormones such as indole-3-acetic acid (IAA), gibberellins, and cytokinins. In bacteria, the biosynthesis of IAA plays a central role in regulating root vascular differentiation, lateral root formation, and root gravitropism, thereby acting as a key determinant of overall root system architecture [31].

Table 2. *Effect of rhizobacterial treatments on morphological parameters of Echeveria*

Treatment	Number of offset	Number of leaves	Number of flower inflorescences	Number of flowers per inflorescence	Leaf diameter (mm)	Leaf length (mm)	Plant fresh weight (gr)	Root fresh weight (gr)	Total chlorophyll content	Root length (mm)
F1	3^{b}	$63^{a,b}$	2^{a}	$24,66^{ab}$	$24,32^{a}$	$44,18^{a}$	$94,07^{b}$	$2,7^{c}$	$277,396^{ab}$	$108,13^{d}$
F2	4^{a}	$67,33^{a}$	3^{a}	28^{a}	$24,73^{a}$	$43,15^{a}$	$95,16^{b}$	$3,31^{b}$	$281,658^{a}$	$141,53^{b}$
F-MİX	3^{b}	$64^{a,b}$	3^{a}	$23,33^{a}$	24,63a	$43,13^{a}$	$102,93^{a}$	$2,7^{c}$	$274,116^{ab}$	$151,94^{b}$
S1	$3,66^{b}$	$68,66^{a}$	$2,66^{a}$	$26,33^{a}$	$24,65^{a}$	$43,59^{a}$	$96,39^{b}$	$3,31^{b}$	$280,292^{a}$	$144,64^{b}$
S2	4^{a}	$68,33^{a}$	$2,66^{a}$	$25,66^{ab}$	$24,67^{a}$	$44,6^{a}$	$106,97^{a}$	$4,07^{a}$	$286,662^{a}$	$170,83^{a}$
S-MİX	3^{b}	$64,33^{ab}$	$2,33^{a}$	$19,33^{b}$	$23,29^{a}$	$44,38^{a}$	$114,83^{a}$	$3,71^{b}$	$276,894^{ab}$	$178,91^{a}$
F+S-1	$2,33^{c}$	59^{b}	$2,66^{a}$	$21,66^{b}$	$23,38^{a}$	$43,87^{a}$	$98,59^{b}$	$4,48^{a}$	$283,688^{a}$	$125,53^{c}$
F+S-2	$4,33^{a}$	75^{a}	3^{a}	$26,66^{a}$	$22,79^{a}$	$45,05^{a}$	$99,98^{b}$	$3,8^{a}$	$267,35^{ab}$	$125,18^{c}$
F+S-MİX	3^{b}	$65,33^{a}$	$2,66^{a}$	$21,33^{b}$	$23,73^{a}$	$43,15^{a}$	$122,73^{a}$	$3,58^{b}$	$188,564^{c}$	$143,84^{b}$
MEDİUM	$1,67^{d}$	46^{c}	$2,33^{a}$	24^{ab}	$21,65^{a}$	$41,15^{b}$	$90,43^{bc}$	$3,86^{a}$	$152,332^{c}$	$105,36^{d}$
CONTROL	1^{d}	$55,33^{b}$	2^{a}	$22,33^{b}$	$21,19^{a}$	$40,8^{c}$	$84,99^{c}$	$3,52^{b}$	$262,448^{ab}$	$87,05^{e}$
Interaction Bacteria	**	**	nsg	nsg	nsg	*	**	**	*	**
Treatment	**	*	nsg	nsg	nsg	*	**	**	nsg	*
B X T	**	**	nsg	nsg	nsg	*	**	**	*	*
cv	12,1	7,5	5,2	18,7	6,8	15,23	10,45	5,6	14,7	8,98

F: foliar treatment, S: soil treatment, MIX: combined treatment of bacterial strains, F+S: simultaneous foliar and soil treatment. *$p < 0.05$; **$p < 0.01$; nsg: not significant.

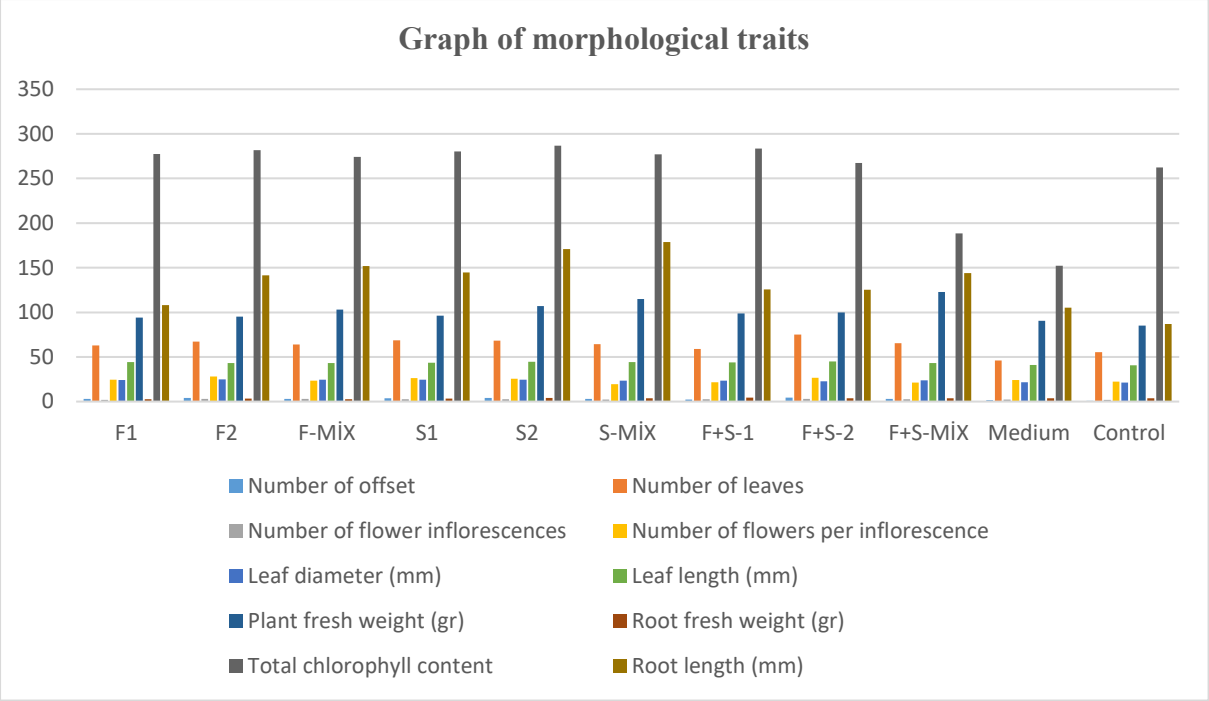

Fig. 2. *The Graph showing the effect of rhizobacteria treatments on the morphological parameters of Echeveria*

This study also aimed to evaluate the effects of rhizobacterial treatments on the protein profile of Echeveria plants. According to the SDS-PAGE analysis, prominent protein bands ranging from 10 to 130 kDa were detected. However, when compared with the control group, rhizobacterial applications did not result in major alterations in the overall protein profile. Minor differences were observed among application methods; foliar applications and combined foliar + soil applications produced slightly more intense protein bands than soil-only treatments.

Previous studies have reported that rhizobacterial inoculation can lead to noticeable changes in protein profiles, including increased band intensity in various ornamental plants [32]. In contrast, no such pronounced changes were observed in Echeveria in the present study. This outcome may be attributed to the succulent nature of Echeveria, which possesses a higher capacity for physiological adaptation under changing environmental conditions. Another possible explanation is the relatively short duration of the experiment; the plant's response to rhizobacterial stimulation may occur more slowly compared to herbaceous species. To more clearly elucidate the influence of rhizobacterial treatments on protein expression patterns, extending the experimental period is recommended.

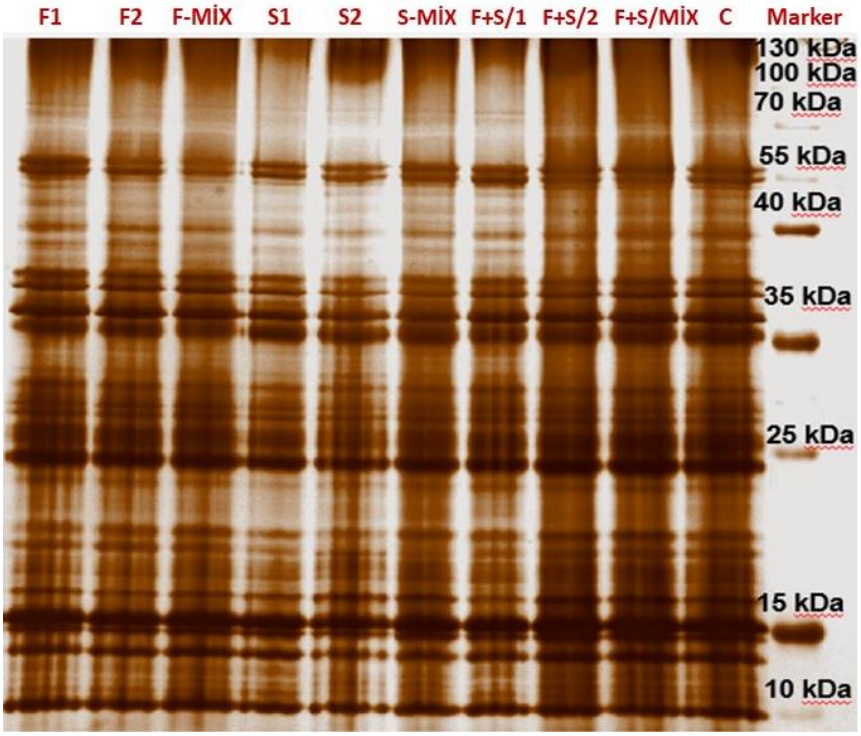

Fig. 3. *Gel image illustrating the effects of rhizobacteria treatments on the protein profile in Echeveria*

CONCLUSION

In this study, two rhizobacterial species possessing distinct gene regions were evaluated for their effects on offset yield and key morphological development parameters in Echeveria. The findings clearly show that both bacterial strains contributed to improvements in plant growth, highlighting their potential as effective biostimulants for succulent species.Beyond their direct growth-promoting effects, Plant Growth-Promoting Rhizobacteria (PGPR) offer a wide range of ecological and agronomic benefits. Their application supports soil and water conservation, reduces the environmental burdens associated with intensive pesticide and chemical fertilizer use, enhances plant nutrient acquisition, and mitigates both biotic and abiotic stresses. By promoting germination and stimulating robust root formation, PGPR facilitate the development of stronger, more resilient seedlings an advantage that is particularly valuable for horticultural production systems aiming to adapt to the impacts of climate change. The results of this study reinforce the growing recognition that biostimulants, including rhizobacteria, can play a transformative role in developing more sustainable, environmentally friendly, and climate-resilient agricultural practices. Future research that investigates the performance of these beneficial microorganisms across a wider range of plant species and environmental conditions will be critical for optimizing their use as biofertilizers. Expanding their commercial application and refining microbial-based technologies will contribute significantly to overcoming current ecological challenges and establishing more resilient and sustainable farming systems.

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