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Special Research Report #535 Part 2 Production Technology: Identifying Beneficial Bacteria from the Rhizosphere of Floriculture Plants That Can Enhance Production and Post-Production Quality

Part 2: Evaluating the Greenhouse Rhizospheric Bacteria Collection to Identify Beneficial Bacteria that Promote Plant Growth and Increase Stress Tolerance

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BACKGROUND

Greenhouse ornamental crops can be subjected to extreme environmental conditions throughout their production chain, which can result in exposure to unwanted stresses. Low-nutrient stress can occur throughout the production chain as a result of insufficient fertilizer rates, or lack of nutrient availability to crops even when adequate fertilizer rates are applied. Low-nutrient stress can result in stunted plant growth, increased production time, or low-quality plant appearance. In addition, water deficit stress can occur post-harvest and can increase the production of stress ethylene and permanently affect overall plant health and quality. The negative effects of water and low-nutrient stress can impact ornamental crop quality, increasing shrink for producers and retailers, while decreasing consumer success in the landscape. The use of microbe-containing biostimulant products has gained popularity in the horticulture industry as a sustainable solution to increase the tolerance and resiliency of plants subjected to stresses. However, many biostimulants currently labeled for use on greenhouse ornamentals are formulated with bacteria originating from agronomic crops, field soils, and other environments. The lack of efficacy of biostimulant products in greenhouse production may be due to the source of inoculum not being able to colonize and grow in soilless media production with high chemical inputs. In addition, there is still a lack of third-party trials that validate these commercial products for their use in ornamentals.

The goals of this study were to create a collection of bacteria isolated from the rhizosphere of greenhouse-produced plants (part one) and identify strains with the ability to increase the health and quality of ornamental crops subjected to water deficit stress or grown under low-nutrient conditions (part two).

Rhizosphere: Dynamic environment surrounding the plant root system that is directly influenced by compounds secreted from the plant and plays host to diverse microbial communities.

MATERIALS & METHODS

Preliminary greenhouse evaluation of lab-selected bacteria

Bacteria selected from the in-lab experiments (part one) were evaluated in both an initial water stress and a low-nutrient greenhouse trial **(Fig. 1)**. Selected bacteria were then independently evaluated in respective water stress and low-nutrient greenhouse validation trials.

Experiment 1: Water stress greenhouse validation trial

- *Petunia* ‘Picobella Blue’ and *Pelargonium* ‘Maverick Red’ **(Fig. 2)**
- Treated weekly with media drench of diluted culture of each bacterial strain in Luria Broth (LB) **(Fig. 3)**
- Irrigation withheld at flower bud initiation (five weeks post-transplant) until all plants had wilted, simulating post-harvest water stress
- Irrigation was resumed and plant health and quality measurements were taken

Water stress can permanently damage the photosynthetic health of plants. This long-term damage can lead to stunted growth, decreased flowering, and weakened performance in the landscape. Our study measured photosynthetic health of plants as an indicator of plant health after recovering from water stress. Our study also measured plant size and flower number of plants at the final marketable flowering stage after recovery from water stress as an indicator of plant quality.

Fig. 1: Bacterial drench application in preliminary greenhouse experiments.

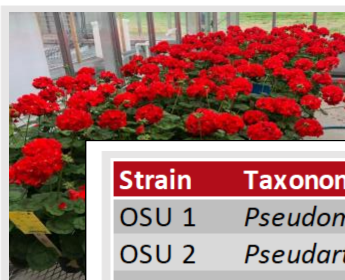

Fig. 2: ‘Maver… marke… stage.

Strain	Taxonomy
OSU 1	*Pseudomonas corrugata*
OSU 2	*Pseudarthrobacter* sp.
OSU 3	*Pseudomonas corrugata*
OSU 4	*Pseudomonas* sp.
OSU 5	*Herbaspirillum* sp.
OSU 6	*Leifsonia* sp.
OSU 7	*Herbaspirillum robiniae*
OSU 8	*Arthrobacter* sp.
OSU 9	*Pseudomonas* sp.
OSU 10	*Pseudomonas brassicacearum*

Fig. 3: Bacterial strains evaluated in the water stress greenhouse validation trial.

<u>Experimental controls</u>

- Negative (-) control: treated with uninoculated LB and subjected to water stress alongside bacteria treatments

- Well-watered: treated with uninoculated LB and not subjected to water stress
- UW4+: A bacterial strain well-documented to increase plant tolerance to abiotic stress, treated similar to other bacteria treatments. Added for comparison to our new bacterial strains.

Experiment 2: Low-nutrient greenhouse validation trial

- *Petunia* 'Picobella Blue'
- Treated weekly with media drench of diluted culture of each bacterial strain **(Fig. 4 and 5)**
- Control plants treated with uninoculated LB
- Bacteria-treated plants were fertilized with 25 ppm N 15-5-15 CaMg fertilizer (JR Peters) throughout the experiment
- Different sets of control (no bacteria) plants were fertilized with 25, 50, 75, 100, and 150 ppm N 15-5-15 CaMg fertilizer for comparison
- Plants were grown to the finished stage (fully flowering) and then harvested
- Multiple parameters of plant growth and quality were evaluated throughout the experiment and at harvest

Strain	Taxonomy
C7B12	*Caballeronia zhejiangensis*
C4E8	*Pseudarthrobacter equi*
C6G7	*Raoultella terrigena*
C4E12	*Pseudomonas putida*
C2H10	*Curtobacterium* sp.
C4B10	*Pseudomonas putida*
C7C8	*Herbaspirillum* sp.
C8D4	*Pseudomonas corrugata*
C9C3	*Herbaspirillum* sp.
C7C2	*Herbaspirillum* sp.
C2H3	*Pantoea dispersa*
C6C9	*Ochrobactrum* sp.
C5A9	*Microbacterium* sp.
C4H3	*Pseudomonas oryzihabitans*

Fig. 4: Bacterial strains evaluated in the low-nutrient greenhouse validation trial.

<u>Measurements of plant growth and quality</u>

At harvest/termination of experiment

- Number of open flowers per plant
- Total flower dry weight
- Number of buds per plant
- Total bud dry weight
- Canopy cover
- Plant architecture rating
- Shoot dry weight
- Color quality rating

Throughout experiment

- Time to first flower
- Weekly growth index
- Chlorophyll content (SPAD)

RESULTS

Fig. 5: Bacterial drench application in the low-nutrient greenhouse validation trial.

Preliminary greenhouse evaluation

The water stress and low-nutrient trials identified 10 and 14 bacteria, respectively, that increased parameters of plant growth and flowering. These bacteria were then independently evaluated in respective water stress and low-nutrient greenhouse validation trials.

Experiment 1: Water stress greenhouse validation trial

- All ten OSU bacterial treatments and UW4+ increased the size of *Petunia*
- With a few of the bacterial strains (OSU 1, 2, 7, and 8), treated *Petunia* that underwent water stress were larger than plants that had not experienced any water stress (i.e. the well-watered control plants)
- Eight of the ten OSU bacterial treatments and UW4+ increased the size of *Pelargonium*
- Seven of the OSU bacteria and UW4+ increased the average flower number of *Petunia*
- Plants treated with OSU 8 had an average of eight more flowers per plant than control plants that received no bacteria treatment
- Multiple bacteria treatments increased photosynthetic health of *Petunia* and *Pelargonium* after recovery from water stress

Additional results from this study may be found in:

Nordstedt NP and Jones ML (2020) Isolation of rhizosphere bacteria that improve quality and water stress tolerance in greenhouse ornamentals. Front. Plant Sci. 11, 1-16. doi:10.3389/fpls.2020.00826.

Experiment 2: Low-nutrient greenhouse validation trial

- Bacterial strain C7B12 was the top-performing strain in this study
- *Petunia* treated with C7B12 (fertilized with 25 ppm N) were larger plants with more flowers and covered more of the pot surface than control plants fertilized with 25 ppm N and not treated with bacteria
- Plants treated with C7B12 and grown with 25 ppm N fertilizer performed better than plants grown with 50 ppm N fertilizer that were not treated with bacteria **(Fig. 6)**
- Five additional bacterial strains were identified to increase different aspects of plant size, flowering, and quality

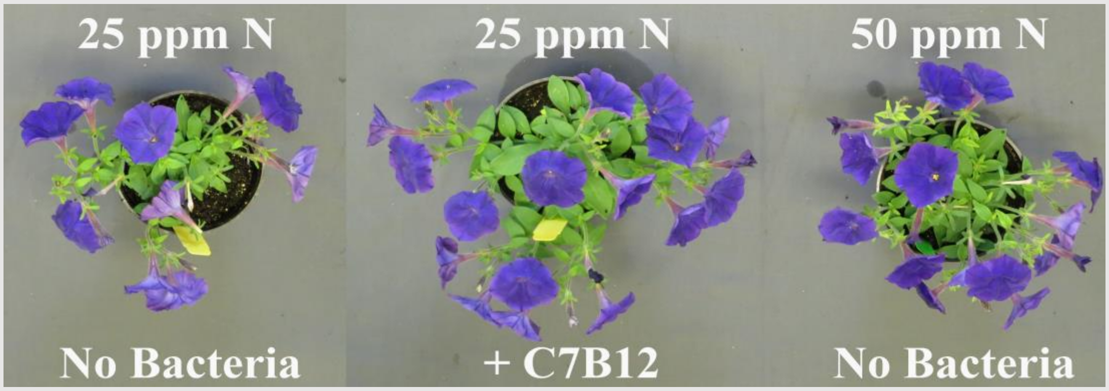

Fig. 6: Visual quality of Petunia 'Picobella Blue' treated with the bacterial strain C7B12 and grown with 25 ppm N fertilizer, compared to plants grown with 25 and 50 ppm N fertilizer that were not treated with bacteria.

Additional results from this study may be found in:

South KA, Nordstedt NP, and Jones ML (2021) Identification of beneficial bacteria that improve the performance of greenhouse-grown petunias under low fertility conditions. Front. Plant Sci. TBD. doi:TBD.

CONCLUSIONS

This work has isolated a diverse collection of bacteria from the rhizosphere of greenhouse-produced ornamental crops, now referred to as the greenhouse rhizospheric bacteria collection. Initial selection from in-lab and preliminary greenhouse experiments identified a subset of bacteria to be validated in production-scale greenhouse trials.

Many bacteria from the greenhouse rhizospheric bacteria collection increased the size of *Petunia* and *Pelargonium* plants and flower number in *Petunia* after recovery from water stress. In addition, multiple bacterial strains resulted in plants that performed as well or better compared to our treatment control UW4+ and when compared to well-watered controls in the water stress greenhouse trial. Application of bacteria also increased the photosynthetic health of plants recovering from water stress, which can contribute to increased resiliency during shipping, retail, and for consumers in the landscape.

Multiple bacteria also positively affected different aspects of *Petunia* plant growth, flowering, and quality when grown under low-nutrient conditions. Application of bacterial strain C7B12 increased *Petunia* plant growth to an equal or greater extent than plants grown at higher fertilizer levels that were not treated with bacteria, which may allow growers to reduce chemical fertilizer inputs without sacrificing crop growth and quality.

INDUSTRY IMPACT

This research provides industry partners with bacteria that can be formulated into products specifically for the greenhouse ornamental industry, providing a sustainable tool for growers to increase the stress tolerance, resiliency, and overall quality of ornamental crops during production and at retail. For additional information, contact jones.1968@osu.edu

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