Evaluation of Physiological Traits Expressed *in vitro* and Effects on Plant Growth by *Bacillus* Endophytes

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Introduction

- Synthetic chemicals, such as fertilizers and pesticides, reduce soil quality and often have detrimental effects on the environment, including increased greenhouse emissions.
- Plant growth-promoting rhizobacteria offer a potential sustainable alternative to enhance plant growth and protect against disease.
- These bacteria can increase growth by mobilizing nutrients, such as nitrogen and phosphorus, and inhibit pathogens by producing antimicrobial compounds.
- *Bacillus* species are an especially important group of rhizobacteria due to their spore-forming abilities.
- This study evaluated *B. atrophaeus* and *B. thuringiensis* for growth promotion on *Brassica rapa* as well as nitrogen fixation, siderophore production, and phosphate solubilization activities. Moreover, antagonistic effects against two soil-borne pathogens, *Fusarium oxysporum* and *Agrobacterium tumefaciens*, were evaluated for each species.

Objective

This study investigated the mechanisms used by *B. atrophaeus* and *B. thuringiensis* to promote plant growth and explored their potential as biofertilizers and biocontrol agents for application in agriculture.

Methods

Seeds of *Brassica rapa* were surface-sterilized, soaked in bacterial both, and grown under controlled greenhouse conditions in sterile soil. Dry mass and length were taken after four weeks

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ANOVA was used to compare the effects of each bacterial treatment

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Nitrogen fixation assay on GNFM agar, siderophore production assay on prepared O-CAS medium, phosphate solubilization assay on Pikovskaya's agar

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The nitrogenase subunit gene, *nifH*, was amplified and ran on agarose gel to verify nitrogen fixation activity

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Antagonistic activities against *F. oxysporum* and *A. tumefaciens* were evaluated on TSA

Results

Bacillus atrophaeus significantly increased the growth of *B. rapa* compared to the control. Moreover, *B. atrophaeus* was positive for the nitrogen fixation assay and inhibited both pathogens. *Bacillus thuringiensis* was only positive for siderophore production.

Figure 1A. *Brassica rapa* inoculated with *Bacillus atrophaeus;* more extensive growth is evident.

Figure 1B. *Brassica rapa* inoculated with *Bacillus thuringiensis.*

Figure 1C. *Brassica rapa* control treatment inoculated with Phosphate Buffered Saline.

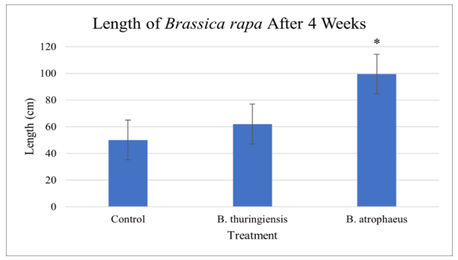

Figure 2. Average length of *Brassica rapa* control and treatment groups. Plants with *B. atrophaeus* showed a significant difference compared to control plants ($P = 0.012$), as indicated by asterisk (*).

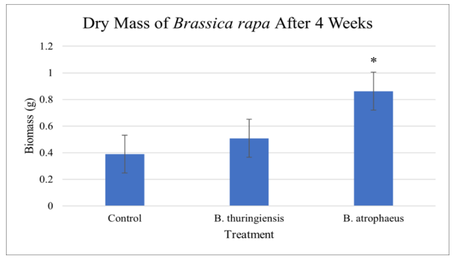

Figure 3. Average above ground dry biomass of *Brassica rapa* control and treatment groups. Plants with *B. atrophaeus* showed a significant difference compared to control plants ($P = 0.028$), as indicated by asterisk (*).

Figure 4. Pikovskaya plate showing no phosphate solubilization for *B. atrophaeus* or *B. thuringiensis*. *Serratia marcescens*, at right, was the positive control.

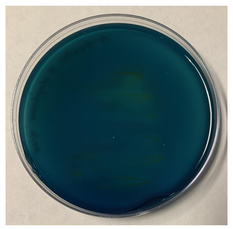

Figure 5. *Bacillus atrophaeus* cultured on GNFM agar. Dark blue indicates nitrogen fixation.

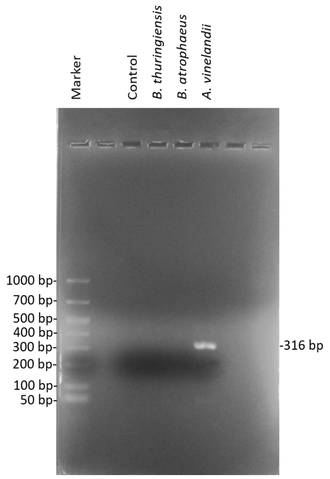

Figure 6. Agarose gel showing DNA fragment produced by PCR amplification of the *nifH* gene. Expected size of DNA fragment was 316 base pairs (bp). Sizes of the standard marker at left in bp.

Figure 7. *Bacillus atrophaeus* on O-CAS medium. This was recorded as negative for siderophore production.

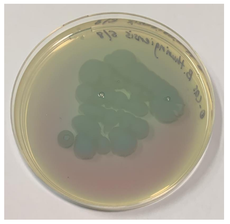

Figure 8. *Bacillus thuringiensis* on O-CAS medium. This was recorded as positive for siderophore production.

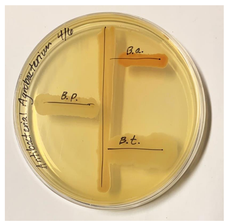

Figure 9. Inhibition of *Agrobacterium tumefaciens* by *B. atrophaeus*. *B. thuringiensis* did not inhibit the pathogen. *Bacillus pumilus* was a positive control.

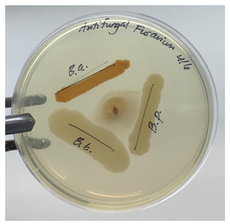

Figure 10. Inhibition of *Fusarium oxysporum* by *B. atrophaeus*. *B. thuringiensis* did not inhibit the pathogen. *Bacillus pumilus* was a negative control.

Discussion

- Results from the antimicrobial assays align with previous studies which identified *B. atrophaeus* as effectively inhibiting plant diseases caused by Fusarium species, such as root rot and Fusarium wilt.
- The nitrogen fixation assay did not clearly show whether *B. thuringiensis* had nitrogen fixation activity, but most closely resembled the negative control plate.
- *B. atrophaeus* was positive for nitrogen fixation but did not produce a *nifH* PCR product. This discrepancy may indicate that other genes are involved in nitrogen fixation.
- O-CAS medium indicated a positive result for *B. thuringiensis* while *B. atrophaeus* did not result in siderophore production.

Conclusions

- *Bacillus atrophaeus* has the potential to promote plant growth in *B. rapa*, possibly through nitrogen fixation.
- As *B. atrophaeus* also exhibited antimicrobial activity, it could potentially be useful in agriculture for biocontrol.
- *Bacillus thuringiensis* should be further studied for siderophore production and its effects on other plants.
- Further investigations in different ecological settings to evaluate plant-growth promotion potential are warranted.
- The combination of agrochemicals with these bacteria may reduce the degradative effects that synthetic chemicals often have on the environment.
- Inoculation of other endophytes along with *B. atrophaeus* could also be beneficial.

Literature Cited

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Acknowledgements

This project was funded by an Undergraduate Research Experience and Creative Activity (URECA) grant