Endobacteria May Increase Glucosinolate Production in Broccoli Sprouts

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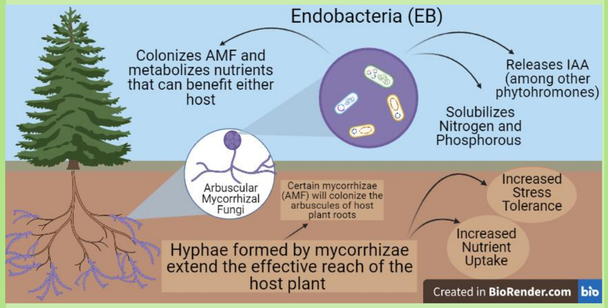
Introduction

In nature, most plants form symbiotic relationships with fungi, called mycorrhizae. Fungi help the host access and metabolize in exchange for nutrients and carbon. Mycorrhizae themselves act as hosts to many kinds of bacteria, including endobacteria (EB). Many EB have demonstrated potential agricultural applications, such as increasing plant growth and mycorrhizal stimulation [1]. This arises from their ability to cycle nutrients and produce useful phytohormones, such as Indole-3-acetic acid (IAA) [1].



Broccoli is one of few plants that cannot support mycorrhizae, and as such, are assumed not to support EB. Previous studies have explored methods of developing microbiota within broccoli, such as intercropping and using soil-based cultures [2].

Broccoli sprouts produce large amounts of glucosinolates (GLS), such as glucoraphanin. These are regarded by many to promote health [3]. Research into methods of increasing GLS production in broccoli sprouts have been explored, including treatment with UV light, plant hormones (such as IAA), and watering/harvest schedules [3]. Indeed, treatment with 0.1 mg/mL IAA increases GLS production in broccoli sprout root cultures [4].

We hypothesized that endobacteria would increase IAA levels within the soil, ultimately increasing broccoli sprout growth and GLS content.

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Isolation and Identification of Endobacteria

- Roots were obtained from White Pine trees in Charlottetown, P.E.I
- The roots were surface sterilized and cut into cross sections (~ 2 x 2 cm) then plated in the centre of tryptic soy agar plates.
- Cultures were isolated (dilution streak plate method) and visualised (gram stain)
- 5 cultures were selected and isolated for16s rRNA BLAST sequencing (UEB 1, UEB 3, UEB 4, UEB 14, UEB 15).
- IAA production was measured using Salkowski's Assay [1].

Broccoli Sprout Growth Trial

- Broccoli seeds (n=50 per treatment group) were sterilized and inserted into peat pellets, then left in dark conditions in a growth chamber to germinate for 2-3 days.
- After germination, the peat pellets were inoculated with 1 mL of each culture in tryptic soy broth or 1 mL of control broth (n=50 sprouts total per treatment in 2 separate trays) and left to grow for 12 days.
- After 12 days of growth, the plants were uprooted, and peat was sprayed off with water. The height of all viable sprouts was measured from the base. Sprouts were dried, combined by group, and weighed.

Extraction and Quantification of Glucosinolates

- Dry sprouts were crushed into a powder and then boiled in methanol (70°C for 30 min)
- Crude extract was purified using SPE on a DEAE column
- Sulfatase from *Helix Pomatia* was added to the column and allowed to drip overnight in order to desulphate glucosinolates (leaving desulfoglucosinolates [DSGs])
- DSGs were rinsed then eluted in 3 mL of di$H_2$0
- Samples were filtered then analyzed using an Aquity H-Class UPLC system equipped with a C18 reverse phase column detecting at 227 nm.
- The DSG desulfoglucoraphanin (DSGR) was detected using a standard curve (1-250 mg/L). The sample concentration was then used to determine concentration = amount of DSGR produced / g of plant matter for each treatment.

Some Endobacteria Illicit A Negative Growth Response

Interestingly, direct application of IAA (1 mg/ml) significantly reduced plantlet height and weight compared to untreated controls (Fig. 1A-B), suggesting that this dose was in the growth inhibitory range. Further, PP and UEB 4 also significantly reduced plant growth compared to controls.

Compared to direct IAA treatment, UEB 4 led to no significant increase in plant weight however all other UEB did (Fig 1B). UEB 4 was found to produce a similar amount of IAA (0.8 mg/mL) to that used in this study (Fig. 1C), suggesting that endobacteria-derived IAA may be responsible for growth inhibition observed.

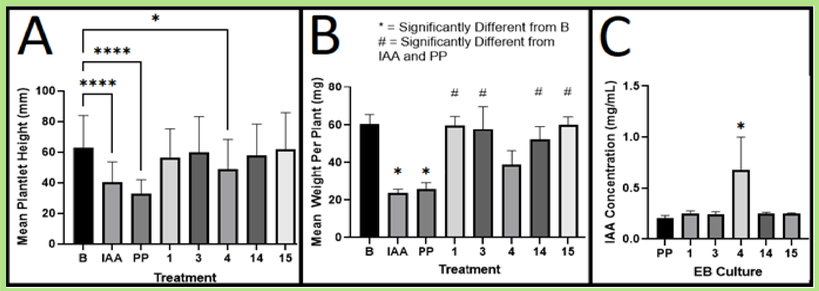

Figure 1 shows the height (A) and weight (B) of broccoli sprouts treated with the following: Tryptic Soy Broth as a negative control (B), 1 mg/mL Indole-3-acetic acid (IAA), *P. polymyxa* (PP), and unidentified EB (1-15). The sprouts were treated with 1 mL of each treatment, and only viable plantlets were measured (n= 37, 29, 33, 41, 34, 41, 41, 39, respectively). **A** shows the mean height of all viable plantlets. **B** shows the mean weight per plant (n=2; based on two overall growing tray measurements). **C** shows the IAA concentration of each culture as determined using the Salkowski's Assay (n=3).

Interestingly, no other cultures investigated caused a significant difference in plant size compared to B, despite producing similar amounts of IAA as PP (significantly less than the 1 mg/ml used in the IAA group). Together, these data suggest that multiple mechanisms may be responsible for affecting plant growth.

Glucosinolate Production Was Altered by Endobacteria

	DSG Conc. Of Sprouts (ug/g)	Standard Deviation
B	2.7	0.082
IAA	139.1	0.049
PP	128.5	0.140
1	30.5	0.028
3	31.9	0.019
4	86.2	0.110
14	38.8	0.011
15	36.2	0.041

Table 1. DSGR concentration averaged from 3 injections of each grouped sample.

The GLS desulfoglucoraphanin (DSGR) was measured (ug of DSGR/g of broccoli DW) using UPLC to perform mass balance calculations. All treatments led to an increase in concentration.

All treatments led to an increase in DSG concentration compared to the untreated control. Interestingly, IAA and PP treatment led to approximately triple the concentration of DSG compared to UEB groups. Since broccoli sprouts are reported to produce more GLS in response to stress [3], this may explain the increase, which is supported by the stunted growth in both groups.

UEB 4 treatment led to higher DSG concentrations compared to other UEB cultures investigated. This suggests that both IAA and EB may increase total GLS.

Conclusions

Our study shows that EB may potentially increase IAA concentration within the soil, increase GLS concentration within the sprouts, and affect their plant growth. Some cultures, when used at appropriate doses, may prove useful for growth supplements aiming to increase nutritional density in sprouts. Our study demonstrates that EB cultures may indirectly impact plant growth and development (despite the lack of mycorrhizal fungi), and that some EB could do so significantly. To determine the feasibility of using these cultures in sprout production, further studies should be performed to increase sample size, investigate different doses and species of endobacteria, and varying environmental conditions in trials emulating a production setting. Furthermore, total GLS content should be accurately determined, including identification of other GLS within the sample aside from DSGR.

References

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