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Methodology for Quantifying Antibiotic Production of Streptomyces Bacteria in Soil around Oat Plant Roots

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Introduction

- *Streptomyces* are soil bacteria that live around plant roots and can produce antibiotics that inhibit growth of other soil bacteria and fungi[1]
- May provide an economical, pesticide-free method of plant disease control in agricultural soils
- A pilot study was conducted to determine methods and timing for collecting *Streptomyces* from plant root zone soil

Methods

- Sterilized oat seeds were potted in agricultural field soil with perlite and vermiculite
- Soil was sampled after 6 weeks' growth
  - **Bulk soil:** soil that did not adhere to oat seedling roots
  - **Rhizosphere soil:** soil that closely adhered to roots
- Soil was suspended in sterile water, plated on starch-casein agar, and incubated for 4 days
- Colony-forming units (CFUs) on plates were counted before overlaying with water agar and a target bacterial strain - plates were incubated for another 3 days
- *Streptomyces* colonies that inhibited growth of target bacterial strain were counted

Results

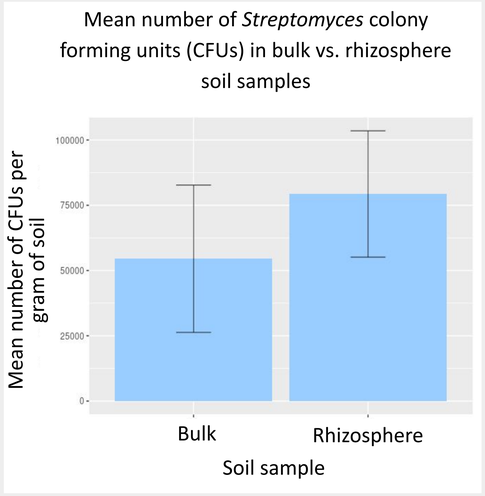

Figure 1. Mean number of CFUs/g soil from bulk and rhizosphere samples was determined by multiplying the number of colonies on soil dilution plates by 10^5. Error bars show standard error of the mean.

No significant difference was observed in mean number of *Streptomyces* colonies between bulk and rhizosphere soil samples.

No significant difference was observed in mean proportion of inhibitory *Streptomyces* colonies between bulk and rhizosphere soil samples.

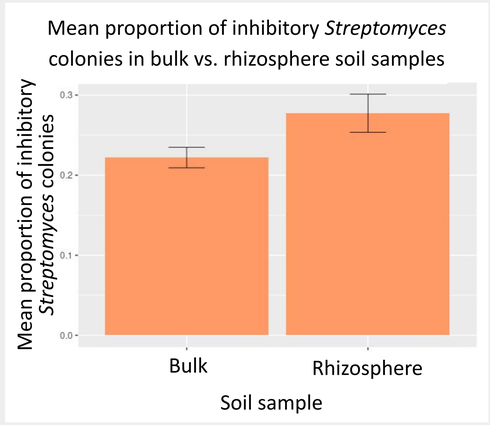

Figure 2. Mean proportion of inhibitory *Streptomyces* colonies in bulk and rhizosphere soil samples was determined by dividing the number of colonies with inhibition zones on soil dilution plates by the total number of inhibitory and non-inhibitory colonies. Error bars show standard error of the mean.

Discussion

- Similar studies report different numbers of antibiotic-producing soil bacteria[2] between bulk and rhizosphere samples
  - Different soil sampling methods
  - Larger scale with more replicates
- Perlite and vermiculite did not adhere to roots - may have inflated the number of *Streptomyces* colonies in bulk soil samples
- Roots could not be crushed and were not included in rhizosphere soil dilutions
- Further study is necessary to determine optimal soil conditions for inhibitory *Streptomyces*
  - Bulk and rhizosphere soil without perlite and vermiculite
  - Different ratios of sand:soil mix
- Results inform methods for summer 2023 study to measure effects of nitrogen fertilizer and pH on *Streptomyces* abundance and antibiotic production

References

[1] Gieske, M. F., Kinkel, L. L. (2020). Long-Term nitrogen addition in maize monocultures reduces in vitro inhibition of actinomycete standards by soil-borne actinomycetes. *FEMS Microbiol. Ecol.* 96: fiaa181. doi: 10.1093/femsec/fiaa181.

[2] Garbeva, P., van Elsas, J. D., van Veen, J. A. (2008). Rhizosphere microbial community and its response to plant species and soil history. *Plant Soil.* 302: 19-32. doi: 10.1007/s11104-007-9432-0.

[3] Header image from USDA Agricultural Research Service - "Plant Growth and Root Development"