Growth of Streptomycetes on Various Nutrients

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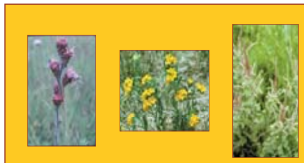
INTRODUCTION

Streptomyces, a genus of bacteria significant for their ability to produce antibiotics, are common in the soil, particularly in the rhizospheres of plants. Despite their importance, limited research has been conducted regarding the environmental factors that influence this antibiotic production. In a previous study, streptomycetes were isolated from the rhizospheres of three plants, *Amorpha canescens*, *Liatris aspera*, and *Lithospermum* sp. (commonly called Puccoon). A link between the species of plant and the antibiotic profiles of isolates from its rhizosphere was discovered. We hypothesize that differences between isolates may be due to the nutrients on which they thrive, and that different plants release different nutrients, contributing to streptomycete diversity. To test this hypothesis, we inoculated Biolog plates with streptomycete spore suspensions and measured their growth in the presence of specific nutrients. We found significant differences in the number of nutrients used and the efficiency of nutrient use between isolates from the three plants. This supports our hypothesis that variation in rhizosphere nutrients may contribute to streptomycete diversity.



Liatris Puccoon *Amorpha*

Fig 1. Plants From Whose Rhizospheres Isolates Were Collected

MATERIALS AND METHODS

Stock spore suspensions (made in a previous study) that had been stored at -80° C were spread on oatmeal agar plates amended with cycloheximide (100 µg/mL). These were incubated at 28°C and assessed for viability and purity. Pure, fast-growing isolates were chosen (31 from *Amorpha*, 26 from *Liatris*, and 25 from Puccoon) and grown on 25 g/L oatmeal agar plates amended with cycloheximide. Spores were harvested using a sterile cotton swab, suspended in 0.2% carrageenan solution, and vortexed. The suspensions were diluted with sterile 0.2% carrageenan to an optical density of 0.22 ± 0.02 (measured at 595 nm). The suspensions were diluted further by adding 1.5 mL of suspension to 13.5 mL sterile carrageenan. 100 µL of this suspension were pipetted into the wells of a Biolog (Biolog, Hayward, CA) plate (each of the 96 wells containing a different nutrient). The plates were incubated at 28° C and read in a plate reader after 24 and 72 hours. The absorbance in each well was recorded. Data were summarized quantitatively and qualitatively, using absorbances obtained in the 72-hour reading (1).

Fig 2. Biolog Plates After 72-Hour Incubation and Oatmeal Agar Plate With Streptomycete Growth. Biolog plates contain 95 discrete carbon sources, including various monosaccharides and polysaccharides, organic acids, amino acids and derivatives, lipids, nucleosides, and phosphorylated compounds (3).

RESULTS

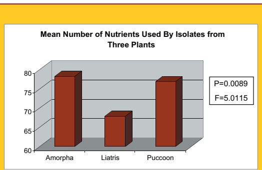


Fig 3. Mean Numbers of Nutrients Used

In mean numbers of nutrients used, isolates from *Liatris* ranked significantly lower than those from *Amorpha* and Puccoon (Fig 3). Nutrient use of isolates from *Amorpha* was not significantly different than that of isolates from Puccoon.

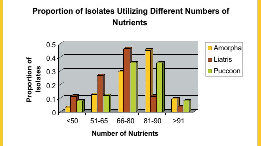


Fig 4. Proportion of Isolates Utilizing Numbers of Nutrients

Isolates from *Liatris* generally utilized fewer nutrients than isolates from *Amorpha* and Puccoon (Fig 4). This suggests that isolates from *Liatris* may be nutritional specialists, while those from *Amorpha* and Puccoon may be generalists.

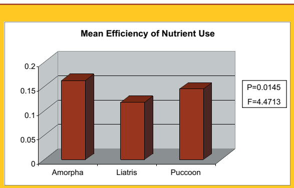


Fig 5. Efficiency of Nutrient Use

In quantitative nutrient efficiency (efficiency of nutrient use defined by optical density in the test well; higher optical density was considered equal to higher efficiency) , isolates from *Liatris* ranked lower than isolates from *Amorpha* and Puccoon (Fig 5). Isolates from *Liatris* did not use nutrients for growth as efficiently as isolates from the other plants. Nutrient efficiency of isolates from *Amorpha* was not significantly different than those from Puccoon.

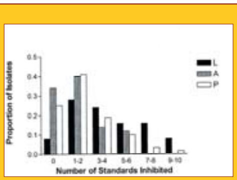


Fig 6. Proportion of streptomycetes from each plant soil that inhibit 10 streptomycete standards.

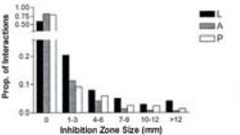


Fig 7. Proportion of Interactions within each category of inhibition zone size.

In a prior study of antibiotic inhibition activity, *Liatris* ranked highest in both number of standards inhibited and inhibition zone size (Fig 6, 7, [2]). In both aspects of antibiotic activity, isolates from *Amorpha* were not significantly different from those from Puccoon.

DISCUSSION

Of the three groups of isolates, those from *Liatris* ranked lowest in both number of nutrients used and efficiency of use. These isolates grew on fewer carbon sources, and used them more slowly or less efficiently. *Liatris* isolates also ranked highest in numbers of standards inhibited and size of inhibition zones. They produced more antibiotics than other isolates, and their antibiotics were more effective. This data suggests a possible link between nutrient use and antibiotic production. Because they utilize a smaller variety of food sources, *Liatris* isolates may need to be more protective of them, and thus they may be able to produce and use antibiotics more effectively to inhibit other microbes. No definite conclusions can be drawn, however, as nutrient analysis was performed on only a subset of the isolates from which inhibition data was gathered. Further study is necessary to determine the relationship between these traits. It is clear, however, that streptomycete isolates gathered from different rhizospheres have varying nutrient use profiles.

REFERENCES

Photos from http://wiscinfo.doit.wisc.edu/arboretum/virginiaklinepage and http://www.umn.edu/flora/pictures

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