Identifying Species of Bacteria that Assist in Survival in Environments with an Extremely High Salt Content or High Temperature

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Plant-growth promoting rhizobacteria assist plants in mitigating stress from not only pathogens but heat and salt stress [1,3], which is becoming more present in Earth's changing climate. High salinity levels directly impact biochemical reactions, limit photosynthesis capabilities, decrease transportable iron, as well as decreasing turgor[2] (the level of rigidity in the cells). High temperatures, leading to inadequate water supply for plants, can limit nutrient bioavailability in the soil.[1] To tackle the above concerns, identification of certain bacterial specimens from the selected environments have been collected and are currently being examined for evidence of their role in plant growth promotion. In our research, examining the resilience of selected bacteria on plated media as well as in the greenhouse can provide the information needed to possibly pursue new species identification of those same bacteria.

Samples for the salt water-based portion of the experiment were obtained in or near Mobile, AL, and the drought/heat tolerant varieties were found in New Mexico. Root sections around 1.0 cm in length are extracted using a sterile scalpel and stored in test tubes filled with sterile water. These sections would be vortexed and serially diluted, and plated onto tryptic soy agar. More dilutions would be made after the root tissue was macerated with a tissue grinder.

The resulting plates were incubated for 5 days at 28°C while colonies were selected based on morphology determined by a college at days 2 and 5. For storage, the samples were prepared with a tryptic soy broth amended with 30% glycerol and placed in cryopreservation.

Polymerase chain reaction (PCR) was run on all samples before plating to ensure bacterial presence and checked with gel electrophoresis. The potentially salt-tolerant bacteria were plated on a gradient of plates ranging from 2-10% NaCl in tryptic soy agar.[3]

Specimens from the drought-prone areas are currently being plated on a mixture of tryptic soy agar and concentrations of 3g, 5g, 7g, or 9g per liter of polyethylene glycol, testing osmotic resistance.[5]

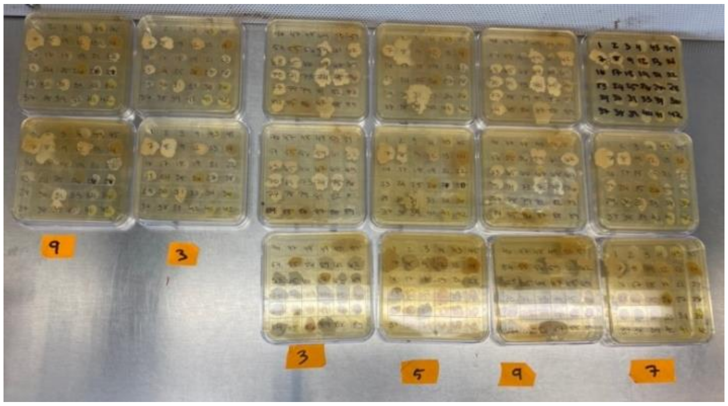

Fig. 1. The image above displays an example of incubated plates with varying concentrations of polyethylene glycol in the media.

Both the salt and drought-tolerant specimens were allowed to incubate at 30 °C for at least one day. Bacteria that grew through every gradient of salt concentration were sent to the MCLab for sequencing. The drought-tolerant varieties are currently undergoing greenhouse trials. The root systems of corn plants have been inoculated with a bacterial solution of a set turbidity standard in yellow 150cm^3 containers filled with pure sand. Inoculated plants will not be watered for 7 days, as watering will wash away the new bacteria and not create proper drought conditions.

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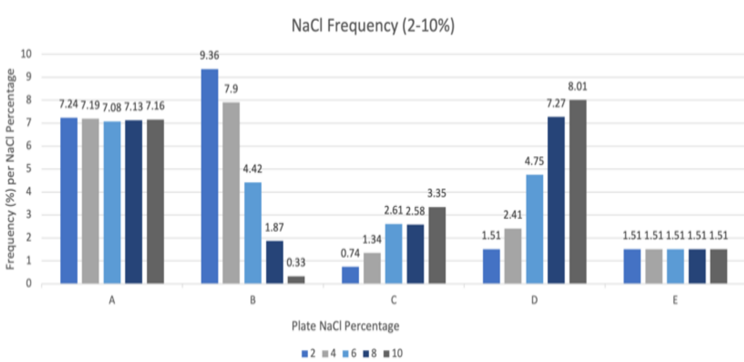
Fig. 2 & Fig. 3. Image 1 (left) shows plants that were inoculated with the bacterial solution after receiving no water for 7 days. The second image (right) shows five of the same plant species that did not receive any bacteria before not receiving water for 7 days. The figure on the right shows obvious wilt and stunted growth from water loss.

PCR was successful on most samples and helped to determine samples with sufficient DNA content for further examination, with only a few not producing bands in gel electrophoresis, even after DNA extraction. The salt-tolerant varieties that grew on every gradient and shipped off to MCLab for sequencing were analyzed using BioEdit, with the top 40 most promising strains currently starting assessment for new species identification. (See Figure 4 for more information) Plates with drought-tolerant varieties are finishing the gradient plating process with signs of resilient strains in the collection. The last batch of samples from the drought-tolerant specimens is being run through the greenhouse at this time. Previous tests show a distinct difference between the corn plants with the bacteria and without after 7 days of no watering. (See Figures 1 and 2) Due to this being an ongoing research project, the results are not yet complete.



A- Continuous Growth
B- Normal Growth
C- Light Growth
D- No Growth
E- Unable to Obtain Growth

Fig. 4 Above displays the percent occurrence of each category of growth (A-E) per NaCl % concentration. Differing concentrations of NaCl helped to identify the strongest strains collected.

Results from the greenhouse trials, although not complete, demonstrate that the collected species of bacteria serve as a plant growth-promoting agent when it comes to drought conditions. This information, however, does not try to claim that only a specific species or plant works with this growth-promoting relationship. Sequencing the most resilient strains from the salt-tolerant batch of bacteria shows enough genetic variation to investigate as a potentially new species. Continued plating on a TSA/polyethylene glycol gradient shows potential for bacteria that resists osmotic stress.

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Statement of Research Advisor

Beginning in the Fall of 2020, Walker has provided contributions to our studies of bacteria from high-salt and from arid environments. Her role, in addition to the upkeep and maintenance of bacterial cultures, was to conduct the Polymerase Chain Reaction (PCR) protocol on 500+ bacterial strains so that they could subsequently be identified by 16S rRNA gene sequencing. Before she sent her gene-products for sequencing, she had to confirm her results by gel electrophoresis.

-Dr. Kathy Lawrence, Entomology and Plant Pathology

Authors Biography

Walker R. Olive is a senior-year student pursuing a B.S. in Applied Biotechnology at Auburn University. She has contributed a year and a half to the project and has a passion for research and work with bacteria.

Dr. Kathy Lawrence is a professor and researcher in the department of Entomology and Plant Pathology. Areas of expertise are in soil borne and foliar fungal diseases, specifically plant parasitic nematodes and fungi attacking field crops, vegetables, and ornamentals, with emphasis on nematode and fungal pathogen interactions and host-pathogen relationships in the environment.