Customer Case Study

GENERAL AUTOMATION LAB TECHNOLOGIES

Rapid Generation of Isolate Libraries From Root Microbiome of Crop Plants Using Prospector™ System

Study conducted by:

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"Prospector allowed our scientists to spend time on research instead of tedious, repetitive tasks. Generating an isolate library in months versus years will greatly accelerate discovery of Actinobacterial strains that could improve fitness of an important crop such as Sorghum bicolor."

– Dr. Devin Coleman-Derr

INTRODUCTION

The intertwined phenomena of human population growth and global climate change are putting increased pressure on researchers to come up with solutions to protect crops from abiotic stresses such as drought. One important source of such solutions may be found in plant growth-promoting microorganisms (PGPMs) within the soil microbiome in which plants are rooted (Fig. 1). As with research into the various microbiomes inhabiting the human body, science has continued to reveal various ways that microbes can impact the health of their hosts.

Among the PGPMs important to agricultural science are endophytic bacteria, i.e., microbes that grow within plant root tissue. These bacteria are potentially able to exert even more influence on the fitness of their hosts due to their intimate position within the plant. However, as a result of this placement, endophytes can be more difficult to isolate than microbes living freely in the soil. Additionally, shotgun genomics of the root endosphere yields such a low percentage of microbial reads, isolating endophytes and sequencing individual isolates is arguably the best practice for understanding the functional potential of these organisms.

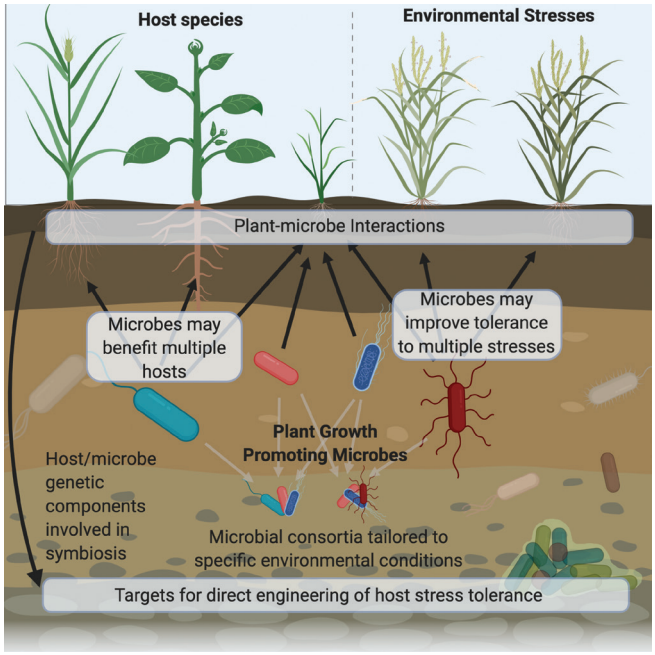


FIGURE 1. Mechanisms of plant growth promotion by microbial symbionts

Figure created using BioRender

The lab of Dr. Devin Coleman-Derr of the USDA and UC Berkeley is studying the interactions between PGPMs and Sorghum bicolor, a plant with worldwide importance as both a feed crop and a source of biofuel (Figure 2). Of interest to the lab is the response of the host plant and its associated PGPMs to drought, with a particular focus on the phylum Actinobacteria. Their ultimate goal is characterizing host/PGPM interactions at a molecular level, but such downstream research is completely dependent on first accurately identifying which strains are present, work that can be slow and tedious by classic methods of microbiology. Could the Prospector high-throughput microbial isolation and cultivation system[1] solve this problem by significantly accelerating library generation? The Coleman-Derr lab evaluated the system to assess the time to generate an Actinobacteria isolate library.

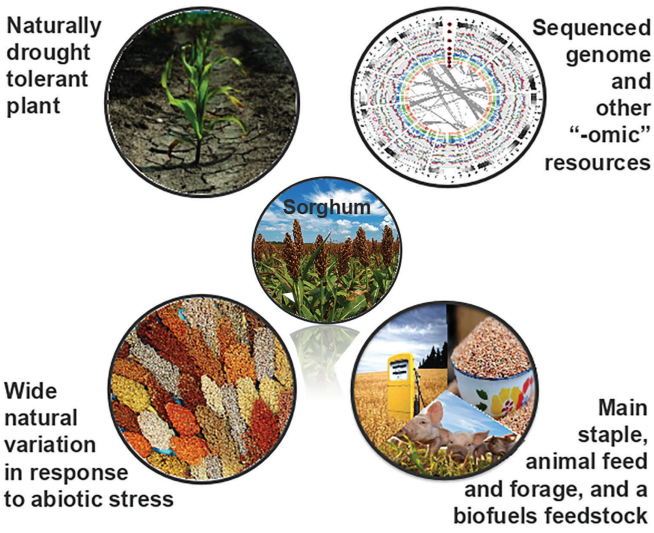


FIGURE 2. Sorghum as a model for drought tolerance research

Many challenges exist in untangling the means by which thousands of species of microbes interact with their hosts and each other under changing environmental circumstances, particularly when applying conventional microbial culture methods. Chief among these challenges is identifying less abundant and slower growing, but nevertheless important, microbial strains. Current Petri dish-based methods may cause such strains to be outcompeted by faster growing strains, thereby remaining unidentified. The Prospector system is particularly well suited to solve this problem. The 6000+ microwells of the Prospector array can capture individual bacteria and culture them in isolation from other strains enabling 1000s of microcolonies to be grown in parallel[1]. It is now possible to readily isolate rare, slow-growing bacteria as clonal cultures for identification and downstream characterization.

Prospector™ high-throughput microbial isolation and cultivation system

METHODS

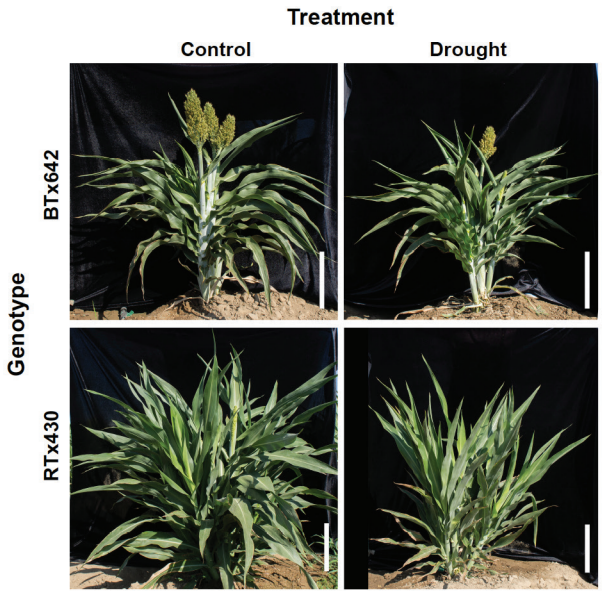
Root Collection

Ten-week-old plants subjected to drought conditions were harvested and their roots removed. Root pieces were placed into 50 mL polystyrene tubes containing 20 mL phosphate buffer and sonicated for 10 minutes to remove soil, then placed into clean 50 mL tubes and rinsed twice with sterile water. Next the roots were washed 60 seconds in 99% EtOH, then washed 6 minutes in 3% sodium hypochlorite, washed again for 30 seconds in 99% EtOH, and finally rinsed twice with sterile water. Roots were then cut into pieces approximately 1 cm in length with a sterile blade and placed loosely into 2 mL microtubes containing 1.5 mL filter-sterilized 25% aqueous glycerol. Root pieces were incubated in the glycerol solution for 20 minutes at room temperature, then stored at -80 °C.

Root Processing

Root samples were thawed and approximately 200 mg root material were placed with 5 mL PBS buffer into each of 2 stainless steel containers of a QIAGEN TissueLyser II and ground at 30 Hz for 3.5 minutes. Contents of the containers were combined into a 50 mL tube along with 20 mL additional PBS buffer. The ground root slurry was next filtered through sterile cheesecloth and then through a 10 µm pore size filter. The filtrate was centrifuged

at 4,000 rpm for 10 minutes, then the pellet was resuspended in 10 mL of culture medium (matching the volume that came out of the TissueLyser II). A number of different media were used, but the one that worked best for Actinobacteria was Tap Water Yeast Extract (TWYE).



Sorghum plants in the field

Prospector System

Each of these cell suspensions was mixed with growth indicator resazurin before loading onto the Prospector array. Resaurzin reacts with NADH to form resorufin, a compound that exhibits fluorescence upon excitation with green light[2]. Cell concentrations of the suspensions were assayed before loading to determine an appropriate dilution of suspension for the Prospector array[3]. (A target of 0.3 cells per well results in an optimal number of singly occupied wells.) Arrays were loaded with 3.0 mL of cell suspension in TWYE containing 0.1 mM resazurin, then sealed.

Prospector™ microbial isolation and cultivation array

Loading the Prospector array

Culturing

Microwell arrays were cultured for 7 to 10 days (depending on media used) at 30 °C until fluorescence imaging results indicated wells containing viable cultures. Contents of wells displaying growth were robotically transferred to 96-well microtiter plates with 200 µL media per well for scale-up. Scale-up cultures were incubated on a shaker at 30 °C for 7 to 14 days then stored at 4 °C.

Sequencing Prep

Sequencing preps were performed by PCR on 6 µL of scale-up cultures to which 10 µL of Buffer 1 (25mM NaOH + 0.2mM EDTA, pH 12) was added, followed by incubation at 95 °C for 30 mins. Next, 10 µL of Buffer 2 (40mM Tris-HCl, pH 7.5) was added to lower the pH; 1.5 µL of that solution was used as the template for a PCR reaction using universal primers 27F and 1492R for the full-length 16S rRNA gene.

Sequencing

The 16S rRNA PCR products were Sanger sequenced at MC Lab (South San Francisco). Sequences were input into NCBI's BLAST database by the Coleman-Derr lab to identify the taxon in the culture.

RESULTS

In the two years prior to using the Prospector system, the Coleman-Derr lab generated 400 total isolates as they built their Actinobacteria isolate library using classic microbiology techniques (Petri dishes). With the Prospector system the lab was able to generate a library of 1490 isolates in just 2.5-months – a 35-fold increase in generation rate (Table 1). The percentage of Actinobacteria in the Prospector-generated library based on IDs from a partial library was 76% which is comparable to the Petri dish-based method.

	Petri-dishes	Prospector
Total isolates	400	1490
Time to library generation	24 months	2.5 months
% Actinobacteria	75%	76%

Table 1. Results of isolate library generation using Petri dishes and Prospector system

SUMMARY

To discover how root microbiomes might be manipulated to improve fitness of crop plants, the Coleman-Derr lab investigates changes in populations of bacteria associated with the roots of Sorghum bicolor subjected to drought conditions. Earlier research had hinted at an association of members of the soil bacterial phylum Actinobacteria with sorghum plant drought resistance. Therefore, the lab sought to identify particular species of Actinobacteria that may be involved in providing such protective effects. However, Actinobacteria often grow more slowly that other bacteria associated with roots, highlighting a common problem in microbiology, specifically that any given set of culture conditions and media can favor more abundant and/or faster-growing bacterial phyla, potentially masking the presence of slow growers in samples. This was a problem that also affected the work of the Coleman-Derr lab in assembling an isolate library of Actinobacteria strains pertinent to Sorghum bicolor. The ability of the Prospector system to isolate and culture individual bacterial clonal cultures has provided a solution to identifying slow-growing and rare taxa. Additionally, the system's streamlined workflow automates the isolation and cultivation steps eliminating much of the tedium associated with classical microbiological techniques.

Results from the Prospector system versus the former use of traditional methods demonstrate how the system can significantly accelerate library generation. The previous 24 months using Petri dishes had yielded 400 isolates of which 75% were found to be Actinobacteria. In less than 3 months, the Prospector yielded 1490 isolates. 16S rRNA sequencing of a partial library showed 76% were Actinobacteria. Prospector enabled a ~90% reduction in time for library generation and a 35-fold increase in generation rate.

REFERENCES

1. GALT Product Bulletin: Prospector High-Throughput Microbial Isolation, Cultivation and Screening System
2. GALT Technical Note TN1: Applications of Resazurin in the Characterization of Bacterial Isolates in the Prospector System
3. GALT Technical Note TN3: Determination of Cell Dilution Factor for Loading a Prospector™ Array

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