Mapping Microbial Diversity of Culturable Strains in Oklahoma

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Abstract: Understanding the bacterial community that surrounds us is of utmost importance and is applicable to everyday life. By utilizing new bacterial species, we can increase sanitary measures, discover new way to produce biofuels, or even aid in antibiotic research; the applications are endless. In order to achieve a better understanding of the microbial community, we gathered an array of organic materials from regions such as Oklahoma and neighboring Midwest states, searching for novel microbial strains. Microbes in these organic samples were cultivated, identified as known or unknown, genetically sequenced, and the resulting data will be added to an online database system. Our results show that the majority of species that we gathered belong to the Genera Bacillus and Pseudomonas as well as fifteen unknown bacterial strains that we are further investigating.

Keywords: Microbial Diversity, Genetic Mapping, Bacteria

Introduction

Microbial diversity encompasses all living micro-organisms and their genetic differences. Breakthroughs in DNA sequencing technology are providing important opportunities for scientific research on microbial genome diversity. Many microorganisms remain undiscovered. As a result of the recent feasibility of identifying microbial species, new studies working in the microbial field have found efficient ways to identify microorganisms (Hebert *et al.* 2003; Moore *et al.* 2016). Once identified, the applications for novel microorganisms can be very beneficial in aspects such as public health safety, biofuel applications, and an overall understanding of the bacterial community (Keller and Zengler 2004). Since bacteria are abundant and can be found in almost every place on earth, the presence of harmful bacteria can be a major threat in high traffic areas such as water fountains (Kalmbach *et al.* 1997). Because of the harm that bacterial strains can cause, most of the bacteria in urban areas have been identified and treated to diminish health threats. Aside from the harmful nature of some bacteria, a few novel strains show promise in applications such as biofuel production and batteries (Randerson 2003). The applications of bacteria are endless. However, further identification and an understanding of unknown organisms is needed to lead to other scientific innovations. Our role in the research of microbial diversity is to discover new bacterial strains and to sequence their genomes. Genomes of closely related microbes are also valuable in create a pangenome for these organisms.

Here we report on the diversity of the microbial strains isolated thus far in this ongoing multi-semester project. This is the first report summarizing the phylogenetic properties of the strains isolated during the project. In addition to this analysis, we contributed to growing and extracting chromosomal DNA from 75 microbial strains isolated by undergraduate students at OSU.

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Methods

Once our organic materials were collected, the process of cultivating them on multiple agar plates was initiated, followed by chromosomal DNA extraction, and then genome sequencing in order to construct a database of genomic diversity of bacteria. The collected organic materials were diluted to a 10% concentrated saline solution. Approximately 100 µL of the remaining solution were placed onto an LB-Agar plate and streaked using a glass rod. The streaked plates were placed in an incubator for a day. After a day of growth, single bacterial colonies were selected from the plate and quad streaked onto a separate LB-Agar plate using a metal loop that is flamed before each streak for sterility. The new quad streaked plates were typically incubated for a day. After a day of growth, the plates were sent to mass spectrometry at the Oklahoma Animal Disease Diagnostics Laboratory for identification of previously studied bacteria. If the results yielded unknown or interesting bacterial strains, then these strains were chosen for DNA extraction/sequencing.

Table 1 - Strains From Bacterial Genera Found and Number of Samples Tested.

Genera	# of samples
Achromobacter	2
Acinetobacter	3
Arthrobacter	10
Bacillus	147
Curtobacterium	2
Erwinia	1
Exiguobacterium	2
Flavobacterium	1
Kocuria	1
Microbacterium	3
Micrococcus	5
Paenibacillus	3
Pantoea	2
Pseudomonas	46
Rhizobium	1
Rhodococcus	1
Staphylococcus	10
Stenotrophomonas	1
Unknown	15

Once the novel isolates were chosen, chromosomal DNA was extracted from the novel isolates using the Experienced User Protocol provided by MO BIO Laboratories, Inc. Cultures of bacteria of interest were added to power bead tubes provided in the DNA sequencing kit and vortexed . A separate solution, C1, was added to the existing bacterial solution in the power bead tubes and the created solution was vortexed briefly at first then vigorously for ten minutes. After the tubes are vortexed for ten minutes, they were transferred to the centrifuge for thirty seconds at room temperature. Once the tubes were removed from the centrifuge, the supernatant was transferred to a clean collection tube (provided in the DNA extraction kit.) A second solution, C2, was added to the existing solution in the clean collection tubes, vortexed for five seconds, then incubated at 4 degrees Celsius for approximately 5 minutes. After incubation, the tubes were centrifuged for one minute. Solution C3 was then added to the new collection tube containing the previous solution and was vortexed and incubated at four degrees Celsius for five minutes. After incubation, the tubes were centrifuged for one minute then the supernatant solution (careful to avoid the pellet) was transferred to a new collection tube. The C4 solution was then added to the supernatant and vortexed for approximately five seconds. Some of the supernatant was then loaded

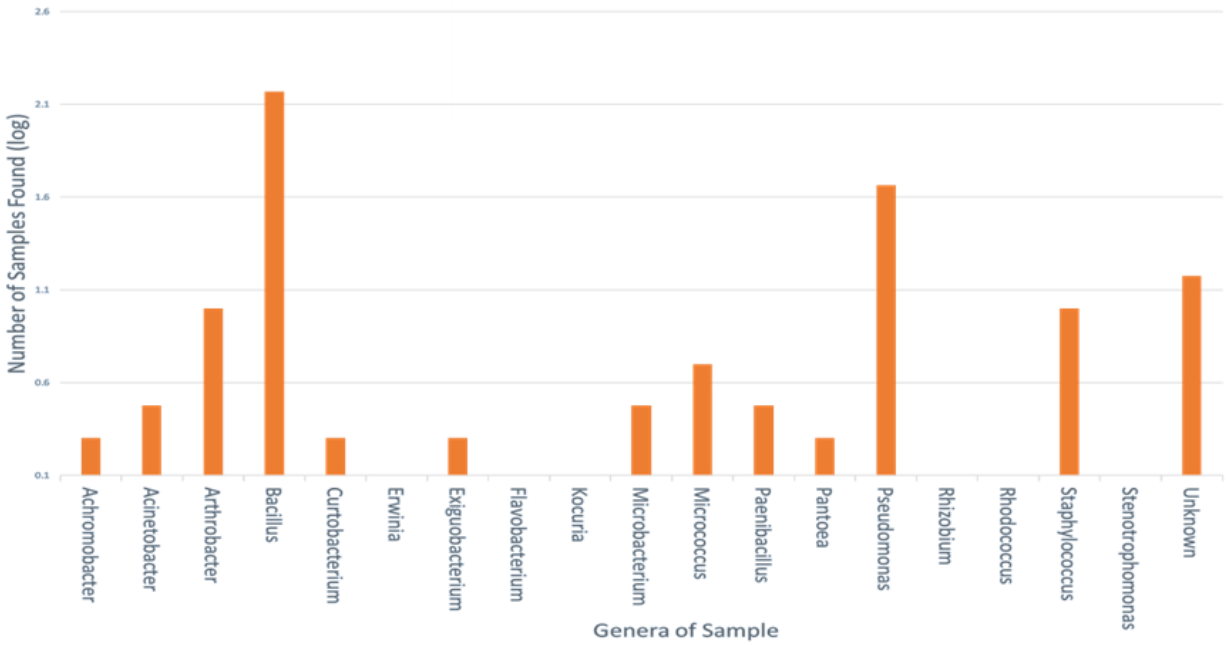

Figure 1 - Number of isolated strains for each of the indicated Genera plotted on a log scale.

into a spin filter and centrifuged for one minute. The supernatant was collected below the spin filter and was added back into the spin filter and the process of centrifuging was repeated (the process was repeated three times.) Solution C5 was then added and centrifuged for thirty seconds. After the time has elapsed, the excess solution below the spin filter was discarded. The spin filter was then centrifuged again for one minute. Then the spin filter was then placed into a new collection tube and solution C5 was added (avoiding splashing on the sides of tube.) The C6 solution was added after C5 and the new solution was centrifuged again. Once the centrifuge process was complete, the samples (collection tubes) were stored in a freezer.

The extracted DNA is then sent to a lab for sequencing. Data collected during the process of isolating and processing the microbial strains will be stored in the microbial database created by Dr. Hoff. The database will allow the microbial genomics team to compare the genes of many different bacterial strains for identification (Paul *et al.* 2003).

Results and Progress to Date

A large number of *Bacillus* was found from the environment (Figure 1). There were also 5 species in *Archaea* found (Figure 2). Approximately 75% of the samples were in the Genera of *Bacillus* or *Pseudomonas*. Fifteen samples were found inconclusive or unknown (Table 1).

Discussion

Our study has shown a large number of identified *Bacillus* bacteria. This large quantity could be due to the regions that we sampled in this experiment. Most of the bacterial cultures were gathered in Oklahoma and local Midwest states that have similar climates and geographic tendencies so this could explain the prevalence of common *Bacillus*

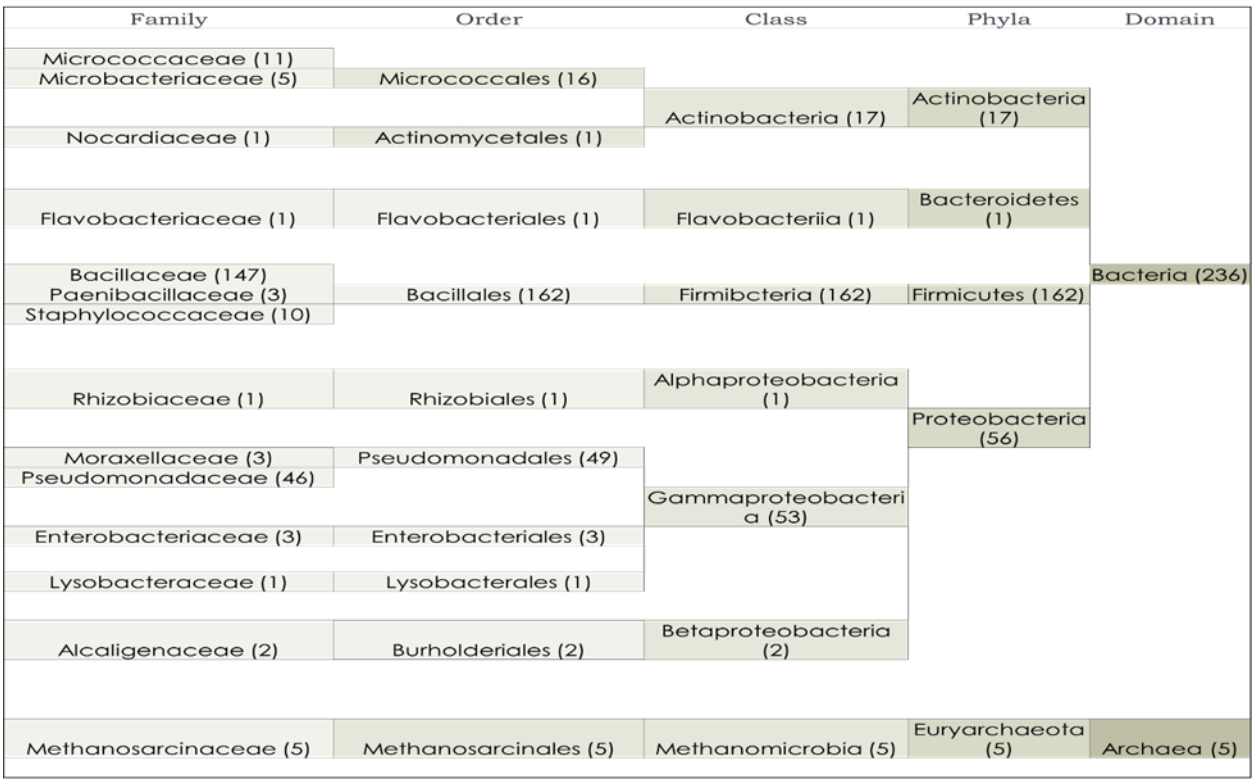

Figure 2 - Taxonomic tree of bacteria found with number of samples found from each.

microorganisms. Previous studies have identified *Bacillus* bacteria in oil reserves in Oklahoma (Simpson *et al.* 2011) but there are few studies that have depicted the magnitude of *Bacillus* bacteria in other geographic locations in the Midwest. That being said, our study provides an insight into the significant number of *Bacillus* species in Oklahoma, and particularly at Oklahoma State University where most of our samples were gathered. Another explanation for the outstanding number of *Bacillus* bacteria identified, is our use of LB Agar as a growth media. Glucose LB Agar is one of the most common forms of growth media used in the Microbiology field because it is easily metabolized by a variety of bacteria, with *Bacillus* being one of them.

By using LB Agar, we were able to grow many different bacterial cultures, including the second most prevalent bacteria *Pseudomonas*. Growing bacteria on LB Agar can be very beneficial and resourceful but can also limit the diversity of bacterial species since some bacteria, such as halophiles, do not grow on regular LB Agar. This use of a common media can explain why our results favored *Bacillus* and *Pseudomonas* bacteria over others, such as *Rhodococcus*.

In our future studies, we can broaden the bacterial diversity in our study by gathering samples from other regions of the United States as well as use different growth media to accommodate different bacterial species. By making these adjustments, we hope to find an increased number of novel bacteria, even more than the fifteen unknown species we obtained in this study.

Overall in this study, we gathered fifteen unknown bacterial cultures. We have sent out chromosomal DNA of these unknown species for DNA sequencing. In addition, a batch of 75 additional

chromosomal DNA samples are being prepared for submission for DNA sequencing. Once sequenced, we will have access to the genetic information and we will add it to our DNA database. This sequence information will allow us to fill in information that has yet to be discovered about the microbial community. The fifteen unknown bacterial cultures that we obtained in this study will help our microbial diversity team, as well as future studies, understand the diversity and vastness of the microbial world that surrounds us every day.

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