

Ouachita Parish vs. Tensas Parish: Analysis of Soil Microbes

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Abstract

Bacteria found within soil are diverse and abundant. Billions of bacteria can reside in only a gram of soil. The top 10 cm of soil is enriched with organic material, which provides an ideal habitat for most bacterial species. Bacteria populations in the soil are dynamic and can show fluctuating characteristics and amounts due to changes in the environment. Two parishes in Louisiana were chosen to demonstrate the diversity of microbes that can be found within the state. Soil was taken from Ouachita Parish and Tensas Parish during the fall of 2010 and winter of 2011. Four locations from each parish were chosen for a total of eight sites. Both fall and winter samples were taken from the soil. Winogradsky columns were created allowing us to sample organisms over the winter. Samples of bacteria were pulled from specific areas of the Winogradsky columns and cultured for purity. These individual selected microorganisms were then biochemically tested and classified to their genus taxonomic level. Ouachita Parish and Tensas Parish microbial biodiversity of the soil was then compared.

Background

Isolating, culturing, and identifying bacteria from soil is a very difficult task. Less than 5% of microorganisms in soil have been cultured in a laboratory (5). Knowing that we would not be able to isolate all organisms from the soil, we chose to focus on a subset of organisms which produce red, yellow, and orange bacterial colonies. These bacteria were chosen because they are easily differentiated from others.

Bacteria play an important role in the soil by nitrification, carbon cycling, and decomposing. Through these processes, microbes enhance the quality of the soil. In contrast, the overall health of soil may have an impact on the type of bacteria that can be found in the soil. Pesticides and fertilizers used in agricultural areas affect the soil pH, which can narrow the range of microbial life that can survive. However, some bacteria thrive in these areas and help the breakdown of pesticides in the soil, while nitrogen fixing bacteria aggregate in areas where ammonium fertilizers are used (4). Tensas parish's economy is highly dependent on agriculture in which pesticides and fertilizers are commonly used. We hypothesize that these agricultural methods may have an impact on the diversity of microbial life in soil. Thus, we may see a difference between the selected sites from Tensas Parish, when compared to the Ouachita Parish sites which are not near agricultural land.

A biofilm is an aggregation of bacteria that adheres to a surface or to one another within a polymeric extracellular matrix. Biofilming organisms, which are common in soil, can survive harsh environments because of the ability to biofilm (6). We hypothesize that some of our isolated organisms will be good biofilers.

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Methods and Materials

Collection of Soil

Microbial soil biodiversity was tested in Ouachita Parish and Tensas Parish. Soil was collected on August 30, 2010, September 6, 2010, February 8, 2011, and February 11, 2011. Ouachita Parish sites 001 and 002 contained dry soil while Sites 003 and 004 contained moist soil. Site 004 is located near Bayou Desiard. Tensas Parish sites 005, 006, and 007 contained dry soil, while 008 contained moist soil. Site 008 is located near Lake Bruin. Each sample was taken into the laboratory where debris was removed.

Winogradsky Column

Winogradsky columns create an artificial ecosystem that allows the study of microbial succession (1).

Different types of bacteria form colored layers within the column depending on their requirements. The soil samples in the column were contained in a 20 oz plastic bottle. The soil was then enriched with nutrients, such as sulfur (egg yolk) and cellulose (shredded paper). The Winogradsky columns were left to incubate for 6 weeks in the University of Louisiana Monroe greenhouse.



Figure 1: Winogradsky column

Isolation of Bacteria

A standard amount of sample from different layers of the Winogradsky columns was placed onto standard (LB) plates for the growth of soil bacteria. Pigmented colonies were then chosen from the plates and quadrant streaked for purity. An effort was made to choose colonies that were not similar to each other.



The morphologic characteristics of the colonies, such as color, form, surface, edge, and elevation were observed and recorded. Gram stains and endospore stains were performed. Biochemical testing was then used to further characterize the soil bacteria.

Serial Dilution

To estimate the amount of bacteria found in the soil, a serial dilution was performed. One gram of dirt was collected from each of the 8 sites and brought to the laboratory. The soil was suspended in broth and then diluted in a series. The CFU/ml was then determined. For example, the spring average CFU of the soil sample in Ouachita parish was 3.42×10^8 CFU/ml. The spring average CFU of the soil sample in Tensas parish was 1.74×10^8 CFU/ml. Recognizing the abundance of bacteria, we chose to focus on only a subset.

Programs Used for Identification

Identification of bacteria isolates was done by the Abis 6 (http://www.tgw1916.net/bacteria_logare.html) identification program. Bacterial identification program PIBWin (<http://www.som.soton.ac.uk/research/sites/pibwin/download/>) was used to confirm the results.

Location of Sites

Ouachita Parish



001 002 003 004

Tensas Parish



005 006 007 008

Biochemical Test Results

Isolate Name	Colony color	Shape	Gram stain	Endospore	MacConkey	Nitrate	VP	Motility	Starch Plates	Glucose	Sucrose	Mannitol	Indole	Anerobic	Bactracin	Lactose	Milk Agar
Su10-001-001-001	L	C	+	-	-	+	-	-	-	-	+	-	-	-	-	S	
Su10-002-001-001-001	Y	R	+	-	-	+	-	+	-	-	+	-	-	-	-		+
Su10-002-001-001-002	O	R	+	-	-	+	-	-	-	-	-	-	-	-	-		-
Su10-003-001-001	L	C	+	-	-	+	+	+	+	+	+	+	-	-	-	S	
Su10-003-001-002	L	R	+	-	-	+	+	+	+	+	+	+	-	-	-		+
Su10-004-001-001	Y	R	-	-	-	+	-	-	-	-	+	+	-	-	-		-
Su10-004-001-001-002	Y	R	+	-	-	+	-	+	-	-	+	+	-	-	-		+
Su10-004-002-001-001	L	R	+	-	-	+	-	+	-	-	+	+	-	-	-		+
Tsu10-005-001-001	Y	C	+	-	-	+	-	-	+	+	+	+	-	-	-	S	
Tsu10-005-001-002	L	C	+	-	-	+	+	+	+	+	+	+	-	-	-		R
Tsu10-006-001-001	L	C	+	-	-	+	+	+	+	+	+	+	-	-	-		R
Tsu10-006-002-001	O	R	+	-	-	+	+	+	+	+	+	+	-	-	-		-
Tsu10-007-002-001	R	R	-	-	-	+	+	+	+	+	+	+	-	-	-		-
Tsu10-008-001-001	Y	C	+	-	-	+	+	+	+	+	+	+	-	-	-	S	
Tsu10-008-001-002-001	O	R	+	-	-	+	+	+	+	+	+	+	-	-	-		+
Tsu10-008-002-001	Y	C	+	-	-	+	+	+	+	+	+	+	-	-	-	S	

Chart 1: Results of staining techniques and biochemical testing



Figure 3: MacConkey Agar



Figure 4: Inoculated Glucose Durham Fermentation Tubes

MacConkey agar is used for the identification of gram negative bacteria and to test the ability to utilize lactose. Enterobacteria can be differentiated by this agar.

The glucose biochemical test determines if the organism can utilize glucose. Yellow and orange indicates a positive results for the fermentation of glucose.

Identification Results

Isolate	Type of Organism	% Identity
Su10-001-001-001	<i>Staphylococcus muscae</i>	82%
Su10-002-001-001-001	<i>Bacillus firmus</i>	92%
Su10-002-001-001-002	<i>Bacillus freudenreichii</i>	83%
Su10-003-001-001	<i>Staphylococcus scleri</i>	72%
Su10-003-001-002	<i>Bacillus thuringiensis</i>	95%
Su10-004-001-001	<i>Proteus myofasciens</i>	89%
Su10-004-001-001-002	<i>Bacillus pumilus</i>	91%
Su10-004-002-001-001	<i>Syribacillus sphaericus</i>	90%
Tsu10-005-001-001	<i>Staphylococcus aureus</i>	99%
Tsu10-005-001-002	<i>Neisseria or Vellonella</i>	
Tsu10-006-001-001	<i>Micrococcus</i>	
Tsu10-006-002-001	<i>Serratia marcescens</i>	98%
Tsu10-007-002-001	<i>Serratia marcescens</i>	98%
Tsu10-008-001-001	<i>Staphylococcus aureus</i>	99%
Tsu10-008-001-002-001	<i>Brevibacillus brevis</i>	99%
Tsu10-008-002-001	<i>Staphylococcus aureus</i>	99%

Chart 2: Identification of bacteria isolates by the Abis 6 identification program and PIBWin

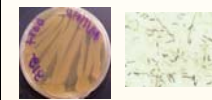


Figure 5: *Bacillus thuringiensis* and its gram stain

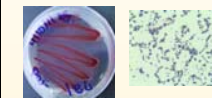


Figure 6: *Serratia marcescens* and its gram stain

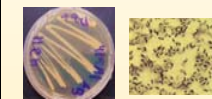


Figure 7: *Lysinibacillus sphaericus* and its gram stain

Conclusion

From our year long study, we isolated 16 different types of bacteria. Eight isolates were from Ouachita sites and 8 isolates were from Tensas sites. While we only focused on colonies of one color range, our isolates were very different between Ouachita and Tensas. We have determined the following from the 16 isolates: 6 *Bacillus* sp. isolates, 3 *Enterobacteria* sp. isolates, 1 *Micrococcus* sp. isolate, 5 *Staphylococcus* sp. isolates, and 1 possible *Neisseria* sp. or *Vellonella* sp. isolate. There are no similarities in bacterial isolates between Ouachita Parish and Tensas Parish. Five isolates out of the total 8 from Ouachita parish are *Bacillus* sp. isolates. *Serratia marcescens* and *Staphylococcus aureus*, both of which are biofilers, were found at multiple sites in Tensas Parish. This could be an indicator of soil health. The CFU for Tensas parish could possibly demonstrate that the agricultural practices in Tensas parish may have affected the diversity of bacteria. Although our study is a very brief survey of soil microbe diversity, we believe that further studies are warranted due to the difference in diversity.

Future Directions

- > Test soil for precursors such as pH, temperature, etc.
- > Identify other organisms that can form a biofilm.
- > Investigate competitive relationships with other microbes as putative biocontrol agents for plant infection.

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