BIOFILMING CAPABILITIES OF LOUISIANA SOIL MICROORGANISMS

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ABSTRACT

Soil is a very dynamic component of the ecosystem; just a teaspoon of soil may contain more than 10 billion bacteria of thousands of different species. Some microbes found in this type of environment can form biofilms, which in turn may play a significant role in either the protection or infection of plants. Biofilms can adhere to a surface and consist of an agglomeration of many species of bacteria living within an extracellular polymeric matrix. The first step in determining the extent of the symbiotic relationship between a plant and bacteria is to identify the types of soil microbiota. To analyze the diversity of microbes of the soil, selected locations in Northeastern Louisiana were sampled during each of the four seasons. The microorganisms were characterized to the genus level through biochemical analysis. Recent studies have begun looking for microbes that can be used as control agents against plant diseases. The identified bacteria were then tested for their biofilm capabilities both as isolates and in combinations. We began this study in an effort to characterize possible biocontrol agents for *Agrobacterium tumefaciens*, which causes crown gall disease. A putative biocontrol agent would inhibit the biofilm formed by *Agrobacterium tumefaciens.*

INTRODUCTION

Soil is a highly heterogenic environment, but there is limited understanding of soil microorganisms because many species have not been described. Less than 5% of the total countable cells of soil microbiota have been cultured in a laboratory (4). A large incongruity occurs between the number of bacterial colonies that are able to form on agar when compared to the amount found in soil.

The absence of pure cultures found in soil creates difficulty in fully understanding the role that each organism has in its community. Bacteria play an important role in soil because their diverse capabilities enable them to exploit many sources of energy and carbon in the soil (1). Soil bacteria are known to have a significant impact in the prevention of plant disease because they are able to displace each other through the competition for nutrients (1). For example, *Bacillus licheniformis* has already been determined to protect against *Agrobacterium tumefaciens* through production of antibiotics that are detrimental to pathogens (1).

The identified bacteria will be used to determine if the same can be done through the use of a physical barrier and not a chemical one. A physical barrier may be achieved with the production of a biofilm. A biofilm is an aggregate of microorganisms in an extra cellular polymeric substance that adhere to each other and/or a surface. Louisiana soil microorganisms may possess the capability to form a strong biofilm to become a physical barrier against pathogens.

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METHODS AND MATERIALS

Soil sampling

Soil diversity was surveyed through collection from four locations in Northeastern Louisiana on the University of Louisiana at Monroe campus. Soil samples were collected on November 17, 2009, January 22, 2010, March 12, 2010, and July 29, 2010 and each sample transported and processed within 1 hour of collection. Large roots and stones were discarded. Site #1 and site #2 contain dry and loose topsoil from a greenhouse area. Moist soil at site #3 was collected near a stream. Site #4 was moist and located near Bayou Desiard.

Fig. 1: Panel illustrates the collection sites. (A) Site #1 (B) Site #2 (C) Site #3 (D) Site #4

Winogradsky column

The Winogradsky column is a miniature ecosystem that illustrates microbial succession as an array of microbes. Distinctive layers of microbial growth appear where specific organism types occur. For example, the red regions are indicative of non-sulfur bacteria.

One gram of soil sample was taken from the red layer of growth in the winogradsky column. This sample was then placed into 8 ml of LB Broth and placed in incubation at 37 degrees for 24 hours. The broth was then used to perform a quadrant streak plate. Colony formation was monitored by examining the plates after 24 hours and 48 hours. To begin isolation, individual colonies were removed and subcultured with the colonies of carotenoid pigment given preference. An effort was made to select different colony types. Overall, 22 isolates were successfully cultured, with representatives from each of the four locations.

Fig. 2: Winogradsky column - Day 1.

Absorbance assay for biofilm formation

The formation of biofilms can be tested by inocululating bacterial cultures into a plastic well, allowing them to incubate and then analyzing the material that has attached to each well.

We used overnight cultures of each identified organism and read absorbance values with a spectrophotometer at 546 nm. A higher absorbance reading indicates a larger biofilm.

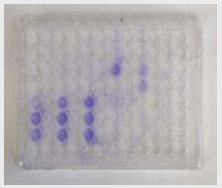

Fig. 3: Microtiter plate used in biofilm absorbance assays. A stain of purple is indicative of biofilm formation.

RESULTS

Figure 4

	Colony Color	Gram Stain	Bergey's Manual	Accuracy	PIB	Biofilm Abundance	Glucose	Lactose	Mannitol	Catalase	Citrate	Sheep Blood	Sucrose	Motility	MSA	Bacitracin	Novobiocin	Nitrate	Catalase	MR	40 Degrees	15 Degrees	Sucrose	Mannose	Fructose
W10-002-001-003B *M. sedentarius*	O	+C	100%	38%		.0212	-	-	-	+	-	G	+	-	+	S	S	+	-						
W10-002-001-004A *Staphylococcus cohnii*	Y	+C	91%	34%		.0193	+	-	-	+	-	G	+	-	+	R		-	-	+	-	-	+	+	+
BS#1 *Micrococcus sedentarius*	S	+C	100%	38%		.0088	+	-	-	+	-	B	+	+	-	R	R	+	-	+	+	+	-	-	-
Site C #2A Taxon 28	D	+R			54%	.0261	-	-	-	+			+	+				-		+					
BS #13A Enterotube: *Shigella* subgroup	D		-	-		.0239	-	-	-	+	-		+	+				-							
TFBS 5C,B *M. sedentarius*	Y	+C	100%	38%		.0165	-	-	-	+	-	B	+			S		-							
TFBS #5C,A Enterotube: *Acinetobacter lwoffii*	S	-R	-	-		.0028	-	-	-	+	-		+	-	+			-							
TFBS #1 *M. sedentarius*	S	+C	100%	38%		.0242	-	-	-	+	-	G	+	+	-	S	R	+	-	+	+				
Site C #1 *M. sedentarius*	S	+C	100%	38%		.0052	-	-	-	+	-	G	+	-	+	R	R	+	-		+	+	-	-	-
W10-003-001 *Serratia marcescens*	R	- R	100%	100%		.0155	+	-	+	+	-		+	+					+		+				
W10-003-002 *M. sedentarius*	B	+C	100%	38%	-	.0239	-	-	-	+	-	B	+			S	R	-							

Fig. 4 Colony Color: S - Salmon, B.Y. - Bright Yellow, D.Y. - Dark Yellow, O - Orange, Y - Yellow; **Gram Stain**: +R = Gram + Rod, +C = Gram + Cocci, -R = Gram – Rod; **Bacitracin/Novobiocin** – R = Resistant, S = Susceptible

Figure 5

	Gram	Abis 6	Accuracy	PIB	Biofilm	Glucose	Lactose	Mannitol	Citrate	Milk	Motility	Gelatin	MR	VP	Acid	Nitrate	Endospo	6.5 %	45	55	75 NaCl	5.7 Ph
BS 4CA Abis: *B. coagulans* PIB: *B. carotarum*	+ R	84%	62%	99%	.0120	+	-	+	+	+	+	+	+	+		+	+	+	+	+	-	-
SP10-001-001A Abis: *B. anthracis* PIB: *B. subtilis*	+R	82%	65%	61%	.0123	+	-	+	-	-/+	-			+		+	+	-	+	+	+	+
SU10-004A Abis: *B. coagulans* PIB: *B. coagulans*	+R	96%	51%	99%	.0240	+	+	+	+	-/+	+	+		+		+	-	+	+	+		-
SU10-004B Abis: *B. anthracis* PIB: *B. subtilis*	+R	92%	58%	84%	.0074	+	-	+	-	+	-	+	+	+	-	+	+	+	+	- /+	+	-
SP10-002B Abis: *B. subtilis* PIB: *B. carotarum*	+R	88%	62%	97%	.0286	+	+	+	-	+	-	+	+	+	-	+	+	+	+	- /+	+	-
W10-001-001-002A PIB: Taxon 27	+R			83%	.0129	+	-	+	-	-	+	-	+	+		-	+	+	+	-		-
BS #6A Abis. *B. mycoides* PIB: *B. carotarum*	+R	98%	51%	52%	.0930	+	+	+	-	+	+	-	+	+	-	-	+	+	-	-		
BS #3A Abis. *B. anthracis* PIB: *B. subtilis*	+R	93%	62%	83%	.0071	+	-	+	-	+	+	+	+	+		+	+	+	+	- /+	+	+/
BS 2A Enterotube: *ACINETOBACTER LWOFFII*	+R				.0303	-	-	-	-	-	-	-		-		+	-	+	+	-		-
SU10-001-001 PIB: Taxon 41	+R			94%	.0641	-	+	-	-	-	-	+	-	-		-	+		+	-		
W10-001-001-002A PIB: Taxon 27	+R			83%	.0129	+	-		-		+					-						
SITE 3B-001 *Enterotube: Acinetobacter lwoffii*	+R				.0258		-	-	-	-	-	+		+		+	+		+	-		
SP10-002A PIB: *Bacillus badius*	+R			74%	.0355	+	-	+	-		+	-	+	-		-	+					
W10-004-001-002B Abis: *B. anthracis* PIB: *B. subtilis*	+R	93%	62%	61%	.0377	+	-	-	-	+	-	+	+	+		+	+		+	-		
TFBS 3CA Abis: *B. feundenreichii* PIB: *Aneurinibacillus*		98%	58%	48%	.0372	+	+	-	+	-	+	-		-		-	-		+	-	-	-

Fig. 5 Abis 6 and PIB is the percentage of tests that matched with and Accuracy is the percentage of tests done out of total suggested.

CONCLUSIONS

Figure 6

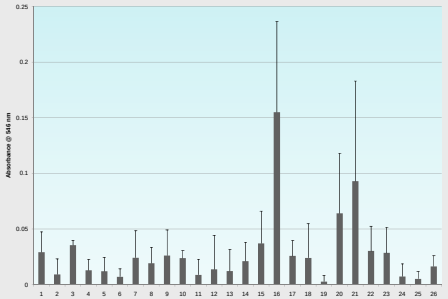

Fig. 6 This graph details the results of the data collected from the absorbance assays for each organism. The grey bar is indicative of the absorbance averages for each organism with a standard deviation line above.

Our newly identified and isolated bacteria were analyzed to determine if any were significant biofilmers. We hypothesize that a good biofilming organism might be able to be a deterrent to *Agrobacterium tumefaciens* infection through a physical, not a chemical barrier.

The results indicate that organism #16, *Serratia marcescens* is the leading biofilm-former of the identified isolates with an average absorbance of .155. Organism # 21, *Bacillus mycoides*, follows with an absorbance of .093. Organism #20, from *Bacillus* sp., was found to have the third highest absorbance at .064.

FUTURE DIRECTIONS

- Determine if identified bacteria may inhibit *Agrobacterium tumefaciens* through a chemical process
- Use top biofilm-former that does not exude chemical inhibition to test against *Agrobacterium tumefaciens* infection in *Arabidopsis thaliana*

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