Saint Xavier University

Comparison of the bacterial populations in commercial topsoil with populations in nutrient media of deep water hydroponic culture of lettuce (*Lactuca sativa*)

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Mentors
Joseph R. Dertien, Ph. D.
David Elmendorf, Ph. D.

Abstract

Hydroponics is the method of growing plants in water where nutrients, pH, and light are controlled in a closed-loop system. One important benefit hydroponic farming provides is greater conservation of water than traditional farming methods due to recycling the same aqueous solution and not producing agricultural effluents. However, by reusing the same treated water, hydroponic systems provide an ideal environment for microorganisms to grow and proliferate. While research has been conducted on the microbial populations of hydroponic reservoirs, little research has been done to directly compare the microbial populations of hydroponic systems with those of soil-based systems. Therefore, the objective of this study is to isolate, determine, and compare the bacterial populations found in a deep water culture hydroponic system in comparison with soil bacteria. We hypothesized that there would be different microbial populations present in both treatments due to the different type of growing substrates. Bacterial isolates from both treatments were examined for their morphological, physiological, and biochemical properties which were used to determine their identities. Results of the classification tests indicated a close similarity in the bacterial genera isolated from both treatments.

Introduction

History of Hydroponics

Hydroponics is a method of growing plants suspended in water treated with nutrient solution in place of soil. In these systems, plants receive constant access to water, nutrients, dissolved oxygen, and additional hours of light vital for growth and development (Vallance et al. 2011). These idealized and controlled growing conditions enable the use of indoor farming which reduces the need for the use of herbicides, or pesticides. Hydroponic farming is theorized to have originated with the Hanging Gardens of Babylon built along the Euphrates River by King

Nebuchadnezzar (604-562 BC); however, the precise origin of the practice of hydroponic farming is not clear (Hershey, 1994). In 1699, John Woodward and Robert Boyle became the first scientists recorded to grow plants suspended in aqueous culture (Hershey, 1994). Additionally, in 1887, Sachs and Knop developed the first nutrient solution recipes for creating a standard soilless method of growing plants. The use of soilless cultures developed in the late 17th century was predominantly used for botanical experiments under controlled conditions, but in 1929, William Frederick Gericke reported that soilless cultures could be used for the commercial production of crops and coined the term "hydroponics" (Hershey, 1994).

Table 1: History of Hydroponics (Hershey, 1994)

Year	Achievement
604-562 BC	Hanging Gardens of Babylon
1699	Woodward and Boyle first to grow plants in aqueous culture
1887	Sachs and Knop develop first nutrient solutions
1929	William Frederick Gericke coined the term "hydroponics"

Global Demand for Produce

Currently, the worldwide human population exceeds 7 billion, and it is projected that the population will increase to 8.5 billion people by 2030, and 9.7 billion people by 2050 (Shaha et al. 2016). Additionally, by 2050, 70% of the worldwide population is projected to live in urban areas creating an increase in food consumption and demand for produce without a corresponding increase in available land for agriculture (Al-Chalabi, 2015) (Oliano de Carvalho, 2015). Without sufficient importation of produce in urban areas, cities often lack access to locally grown fresh produce creating "food desserts" which can be detrimental to nutrition and public health (Tomlinson, 2015). Furthermore, the increased demand on food production and produce has led to a significant increase in global water shortages (Ren et al. 2017). These fresh water

shortages have historically led to government efforts to tax fresh water in arid countries resulting in political uprisings, police riots and deaths in countries such as Bolivia, Ghana, and South Africa (Johnson et al. 2016). To ameliorate these issues, there is a vested interest to create efficient food production systems which require less land, soil, and water resources that can produce a high agricultural output as a supplement to traditional farming (Saha et al. 2016). Therefore, hydroponic farming functions as an alternative supplement to traditional farming (Oliano de Carvalho, 2015, Al-Chalabi, 2015, and Han et al. 2016).

Improvements to Quality and Yield of Produce

Hydroponics has evolved as an efficient form of improving crop quality and yields in comparison to traditional farming particularly in urban centers. The ability to efficiently and sustainably provide fresh produce is a growing concern in society, and hydroponic farming serves as a suitable method of meeting demands (Al-Chalabi, 2015).The use of deep water hydroponic culture in which plants are grown directly in treated water, enables produce to be grown in a controlled indoor setting where water, nutrient supply, pH, and light can be controlled and manipulated (Vallance et al. 2017). Indoor hydroponic farming protects plants from harsh outdoor environments and changing conditions, allowing for plants to be grown all year round and in a variety of climates (Xydis et al. 2017). The ability to grow produce throughout the entire year in a controlled setting also enables faster plant growth and an increased frequency of harvests relative to traditional farming (Haberman et al. 2014, Vallance et al. 2017, and Sheridan et al. 2017). Additionally, hydroponic farming can reduce the effects of pollution of chemical residues into soil which could persist for years and affect crop quality and yields (Vallance et al. 2011, Xydis et al. 2017). Hydroponics also prevents soil-born plant diseases, pests, weeds, and reduces the use of herbicides and pesticides that could be harmful to the environment and quality

of produce (Saijai et al. 2016). Han et al. (2016) projects that an individual family utilizing hydroponic farming can save 7.5 kg of chemical fertilizer and 450 g of pesticides every year versus traditional farming. Overall, hydroponic farming promotes multiple quality harvests of produce and enables control of growing variables compared to traditional farming methods.

Spatial and Economic Constraints of Hydroponic Farming

With the promise of increased yields and quality of produce, available green space still poses a challenge to the viability of hydroponic farming. Supplying heat, electricity, and sustaining an appropriate constant indoor environment increases operating costs for hydroponic farming in comparison with traditional farming (Xydis et al. 2016). Due to spatial constraints in heavily populated urban areas, vertical farming is the preferred method of hydroponic farming to maximize productive output (Al-Chalabi, 2015). However, the size of useable space is restricted with respect to operating costs for electricity and heating which can be as high as 1400-1700 kWh/yr per flood table (Xydis et al. 2016). Therefore, a building designated for hydroponic farming not only needs to provide sufficient space to maximize growing area, the building also must be located in an area that meets suitable energy requirements at an affordable rate in order to be economically feasible to operate.

Solutions to Spatial and Economic Constraints

Research has been conducted to provide feasible solutions to the challenges to infrastructure and energy costs of hydroponic farming. For example, Haberman et al. (2015) identified that unutilized vacant space which includes industrial rooftops, abandoned buildings, and specified residential space in each borough of Montreal could satisfy the entire city's vegetable demand and be economically feasible. In another qualitative assessment, Han et al. (2016) describes that the Courtyard Integrated Ecological System (CIES) in the Hainan rural area

of China which utilizes hydroponic farming could create an increase of 75 m^2 of green land, 40 m^3 of green volume, and produce an additional 2400 kg of vegetables. To combat energy demands and costs in urban settings, describe that alternative sources of energy such as solar or wind power can be used to meet electricity demands of indoor hydroponic systems (Xydis et al. 2016) (Al-Chalabi, 2015). Therefore, sustainable methods can be utilized to reduce the costs attributed to infrastructure and energy demands of hydroponic farming.

Water Conservation and Hydroponic Farming

In addition to benefits regarding quality, yields, and space, the perhaps most important benefit of hydroponic farming is its ability to conserve more water than traditional farming methods. The water conservation in hydroponic systems stems from recycling the same aqueous solution in a closed loop cycle and not producing agricultural effluents (Khalil et al. 2001). In a closed loop system, the treated water containing nutrients which includes nitrates, ammonia, and phosphorus is supplied to the plant roots which absorb the nutrients while cleaning the water and maintaining a constant flow of minerals to the plant (Saha et al. 2016). As a result, water initially supplied to the system is maintained within the system with constant circulation. Tomlinson (2015) reports hydroponic-based farms use ninety percent less water than traditional soil-based farming despite these systems using water in place of soil. Specifically, a survey conducted by Han et al. (2016) reports that an average closed-loop hydroponic farm can annually save 70,870 kg of fresh water compared to traditional farming. By recycling the same water, hydroponic systems are capable of being utilized in arid climates where water is scarce.

Microbiome of Hydroponic Systems

Additionally, in reusing the same treated water, hydroponic systems provide an ideal environment for microorganisms and viruses to grow and proliferate. Microorganisms rapidly

colonize the substrate, nutrient solution, and rhizosphere in hydroponic solution soon after a soilless culture is planted (Vallance et al. 2011). The retention of water and organic compounds in closed-loop deep water culture hydroponic systems facilitates the colonization and growth of various different types of microorganisms (Kalil et al. 2001). Additionally, the indoor setting of hydroponic farms provides favorable and constant conditions that enable bacterial populations to increase in the absence of seasonal change (Kalil et al. 2001). Rather, the type of nutrient solution, substrate material, rhizosphere composition, and age of the plant species shape the diversity and density of bacteria in hydroponic solution (Vallance et al. 2011).

To determine the microbial composition of hydroponic systems, Schwarz et al. (2005) conducted an analysis of the water quality of two water sources used for hydroponic systems (peat ditch and a natural lake) to evaluate them in terms of nutrient concentration, diversity of bacterial populations, and algal development in the water. After collecting bacterial samples every three weeks for one year, results of the experiment indicated that *Bacillus* and *Pseudomonas* were the most abundant genera isolated of the over 58 species of bacteria isolated. Other genera included *Janthinobacterium, Paenibacillus, Bacillus, Micrococcus, Pseudomonas, and Acinetobacter*. Schwarz et al. (2005) continues that microorganisms can be potentially detrimental to hydroponic systems by releasing toxic chemicals into the water as well as forming biofilms that can clog drains and pipes.

Importance of Microbial Populations in Hydroponic Systems

While microorganisms can pose harmful effects to hydroponic systems, bacterial species perform vital functions necessary for nutrient cycling in hydroponic systems. There is a class of both eukaryotic and prokaryotic microorganisms called plant growth promoting organisms (PGPMs) which promote plant growth through nitrogen fixation, facilitation of nutrient access,

and direct plant growth stimulation (Sheridan et al. 2017). The organisms play a key role in the plant rhizosphere by decreasing the amount of nutrients needed to sustain plentiful harvests (Sheridan et al. 2017). Furthermore, certain bacterial species found in soil and hydroponic systems participate in the utilization of nitrogen compounds such as ammonia, nitrate, and nitrite (Sajai et al. 2017). Nitrogen is a key component of the chlorophyll biomolecule, and under low light conditions, plants utilize nitrogen to synthesize chlorophyll to improve photosynthetic efficiency (Fu et al. 2017). Studies have been conducted to analyze the microorganisms in hydroponic systems capable of nitrification and the generation of ammonia in hydroponic solution (Saijai et al. 2017). These findings have shown that *Bacillus* and *Pseudomonas* species are largely involved in the formation of ammonia, and *Nitrobacter* species typically participate in the nitrification process in hydroponic solution (Saijai et al. 2017). Additionally, report that *Bacillus* and *Proteobacillus* species are primarily selected as PGPMs in promoting plant growth in hydroponic systems (Sheridan et al. 2017). However, despite the important roles that microorganisms provide, hydroponic systems typically lack microbial communities capable of degrading nitrogen compounds to nitrate ions and performing nitrogen fixation (Saijai et al. 2017).

Objective and Hypothesis

While research has been conducted on the microbial populations of hydroponic reservoirs, little research has been done to directly compare the microbial populations of hydroponic systems with soil bacteria (Schwarz et al. 2005, Sajai et al. 2017, Sheridan et al. 2017). Chemical analyses are more frequently performed on hydroponic systems to determine their nutrient and chemical content are rather than microbial content (Schwarz et al. 2005). Furthermore, while traditional culturing methods have been used to characterize hydroponic

microflora, experiments in recent years depend on other methods based on sole-carbon-source utilization (Vallance et al. 2011). While sole-carbon-source utilization techniques have proven useful in the identification of unknown organisms, an in-depth examination of the physiological, morphological, and biochemical properties of bacterial species could not only be used to identify unknown organisms, but possibly determine their role in the hydroponic "ecosystem." Furthermore, while the bacterial composition of hydroponic systems and soil bacteria have been examined in depth, studies that directly compare the microorganisms found in hydroponic systems with the microorganisms found in soil under the same experimental conditions are very rare. Therefore, the objective of this study is to isolate, determine, and compare the bacterial populations found in deep water culture hydroponic systems in comparison with soil bacteria. After isolation, the collected bacterial isolates were examined for their morphological, physiological, and biochemical properties. These properties were then used to determine their identities and possible roles played in the rhizosphere of both treatments. It was hypothesized that there would be different microbial populations presents in both treatments due to the different type of growing substrates.

Materials and Methods

Plants and Growing Conditions

Lettuce seeds (*Lactuca sativa*) were germinated in rockwool cubes. Following germination, the lettuce seedlings were planted with eight plants per system (**Figure 1**). The water in both systems was maintained to a pH of 6.0, using commercial hydroponic buffer solutions (*pH Up* and *pH Down*, Botanicare). Additionally, the water in both treatments received 24 hour aeration from an aquarium air pump. Plants in both treatments received 14 hours of artificial light per day.

Hydroponic Treatment Design

The hydroponic system was a deep water culture (DWC) setup in which eight lettuce plants were grown in a hydroponic reservoir (68 x 68 x 16cm, ~80L). Plants were suspended in 35-40L of water treated with 100ml of commercial nutrient solution containing nitrogen (N) 3.0%, calcium (Ca) 1.0%, magnesium (Mg) 0.5%, phosphate (P_2O_5) 2.0%, soluble potash (K_2O) 4.0%, and humic acid 0.20% (*Pure Blend Pro Grow*, Botanicare) once a week over the course of five weeks. Additionally, plants in the hydroponic treatment received 24 hour aeration.

Soil Treatment Design

The soil treatment consisted of plants grown in commercial topsoil (New Plant Life) in modified net pots lined with coffee filters. The soil treatment was designed with an irrigation system that dispensed water from a holding reservoir into each pot for five minutes per day. Water dispensed into the pots was filtered and collected in a basin below the pots. Afterward, overflow water from the basin cycled back down into the reservoir below creating a constant level of water in the holding reservoir from which samples were taken.

Figure 1: Design of both Growing Treatments. The Deep Water Culture (DWC) treatment is shown to the **left**, and the Soil Treatment is shown on the **right.**

Water Sampling and Bacterial Isolation

Water samples were collected at weeks 2, 3, and 5. Water samples of approximately 100 ml were collected directly from the DWC reservoir, and 100 ml of water runoff was collected from the holding basin from the soil treatment. Serial dilutions were prepared in 10mL of sterile saline set to pH 6.0 with dilution factors ranging from 10^{-1} to 10^{-6}. The prepared dilutions were then spread on sterile tryptic soy agar (TSA) and low nutrient media (LN) plates using the spread plate technique of isolation. After 48 hours of incubation at room temperature, microbial colonies were counted as colony forming units (CFUs), and the colonial density of the dilution was calculated in CFU/ml. The colonies were counted on plates that exhibited between 30-300 colonies. Plates with over 300 colonies were considered "too numerous to count" (TNTC). Additionally, as standard protocol, plates with fewer than 30 colonies were not counted and reported as "<30". Individual colonies were selected for isolation and identification based on their distinct morphological characteristics on each type of medium. Each individual colony was

then streaked for isolation on new sterile media plates using the streak plate method to achieve a pure culture for each bacterial isolate. Once pure cultures were achieved for each isolate, the collected cultures were transferred to slant media in tubes for the identification procedures.

Physiological and Morphological Classification Methods

A series of physiological, morphological, and biochemical tests were conducted on the isolates from both treatments to determine the identity and possible ecological roles of the bacterial samples. The physiological tests included the fluid thioglycollate test to determine the aerotolerance of the microorganisms and the motility test to determine if the microorganisms are motile or non-motile. The morphological tests performed include the Gram stain, the Schaeffer-Fulton endospore Stain, and the capsule Stain. The Gram stain technique was utilized to whether the collected bacteria could be classified as Gram-positive or Gram-negative and to determine the cell shape and arrangement of the bacterial cells in each sample. The Schaeffer-Fulton endospore stain technique was utilized to differentiate bacterial isolates based on their ability to produce endospores. Additionally, the capsule stain procedure was utilized to differentiate bacterial cells based on their ability to produce extracellular capsules for protection in harsh environments.

Biochemical Classification Test Methods

Biochemical tests used in this study included a series of experiments designed to test the metabolic capabilities and hydrolytic abilities of the unknown organisms from each treatment (Table 2). The metabolic tests included the oxidation-fermentation test, phenol red broth test, nitrate reduction test, and the catalase test. The oxidation-fermentation test and phenol red broth test were used to differentiate the microorganisms based on their ability to oxidize or ferment different sugars to pyruvic acid used in cellular respiration. Additionally, the nitrate reduction

test was used to differentiate the microorganisms based on their ability to reduce nitrate to nitrite (nitrate reduction) or reduce nitrate to nitrogen gas (denitrification). The nitrate reduction test was also utilized to possibly indicate the contribution each microorganism makes to the microenvironment of the growing treatments. The catalase test was used to differentiate microorganisms based on their ability to produce the enzyme catalase that degrades hydrogen peroxide which is toxic to the cell. The IMViC series of biochemical tests was also applied in this study which includes the indole test, methyl red-vogues proskaur test, and the citrate test to differentiate microorganisms of the group *Enterobacteriaceae* based on further metabolic capabilities. The tests used to examine the hydrolytic abilities of the unknown organisms included the DNA hydrolysis test, starch hydrolysis test, casein hydrolysis test, urea hydrolysis test, gelatin hydrolysis test, and the bile esculin test. These tests were utilized to differentiate unknown organisms based on their ability to degrade different chemical compounds.

Table 2: Summary of Classification Test Objectives

Test Type	Purpose
Morphological Tests	
Gram Stain	Determines Gram+ or Gram- and the shape and arrangement of cells
Endospore Stain	Determines ability to produce endospores
Capsule Stain	Determines ability to secrete an extracellular capsule
Physiological Tests	
Fluid Thioglycollate Test	Distinguishes aerobic or anaerobic properties
Motility Test	Differentiates between motile and nonmotile genera
Biochemical Tests	
Indole Test	Differentiates bacteria based on ability to degrade tryptophan to indole
Methyl Red Test	Differentiates bacteria based on ability to perform mixed-acid fermentation
Vogues Proskauer Test	Differentiates bacteria based on ability to convert pyruvic acid to acetoin and 2,3-butanediol
Citrate Test	Differentiates bacteria based on ability to use citrate as a sole-carbon source
Nitrate Reduction	Differentiates bacteria based on ability to reduce nitrate to nitrite (reduction) or nitrogen gas (denitrification).
Catalase Test	Differentiates bacteria based on ability to produce catalase
DNA Hydrolysis Test	The following tests differentiate bacterial species based on their ability to hydrolyze/breakdown each compound to various end products
Starch Hydrolysis Test	
Casein Hydrolysis Test	
Urea Hydrolysis Test	
Gelatin Hydrolysis Test	
Bile Esculin Test	
Phenylalanine Deaminase Test	Differentiates bacteril based on ability to deaminate phenylalanine
Oxidation Fermentation Test	Differentiates bacteria based on ability to degrade glucose to pyruvic acid in aerobic (oxidation) or anaerobic (fermentation) conditions
Phenol Red Broth Test	Differentiates bacteria based on ability to ferment different sugars to pyruvic acid to be used in cellular respiration

Identification of Unknown Microorganisms

From the results of the morphological, physiological, and biochemical tests, the unknown microorganisms from both treatments were identified using the *Bergey's Manual of Systematic Bacteriology*. From each of their characteristics, a presumptive identification was achieved to illustrate whether or not there is a difference in the types of microorganisms present in either treatment.

Results

Spread Plate Count Results

Results of the spread plate procedure indicated a similarity in the CFUs and CFUs/ml calculated for both treatments **(Tables 3 and 4)**. However, the plate count (CFU) and colonial density (CFUs/mL) values varied between weeks for both treatments. Full results for the spread plate procedure are shown in **Tables A1-A3** in **Appendix A**.

Table 3: Spread plate count results using tryptic soy agar (TSA), CFUs/mL estimated from 10^{-1} dilution factor

	Deep Water Culture			Soil Treatment		
	Week 2	Week 3	Week 5	Week 2	Week 3	Week 5
Replicate 1	$4.8x10^{3}$	$1.52x10^{4}$	$2.14x10^{4}$	$9.9x10^{3}$	$2.5x10^{3}$	$5.8x10^{3}$
Replicate 2	$7.0x10^{3}$	$9.5x10^{3}$	$1.86x10^{4}$	$9.7x10^{3}$	$2.4x10^{3}$	$1.51x10^{4}$
Replicate 3	$3.4x10^{3}$	$8.2x10^{3}$	$1.91x10^{4}$	$4.3x10^{3}$	$3.9x10^{3}$	$1.08x10^{4}$
Average	$5.7x10^{3}$	$1.10x10^{4}$	$1.97x10^{4}$	$8.06x10^{3}$	$2.9x10^{3}$	$1.06x10^{4}$

Table 4: Spread plate count results using low nutrient agar (LN), CFUs/mL estimated from 10^{-1} dilution factor unless otherwise noted

	Deep Water Culture			Soil Treatment		
	Week 2	Week 3	Week 5*	Week 2	Week 3	Week 5
Replicate 1	$1.1x10^{4}$	$1.0x10^{4}$	$8.0x10^{4}$	$2.2x10^{3}$	$1.5x10^{3}$	$2.37x10^{4}$
Replicate 2	$4.0x10^{3}$	$1.38x10^{4}$	$1.07x10^{5}$	$9.6\ x10^{3}$	$4.4x10^{3}$	$2.00x10^{4}$
Replicate 3	$6.8x10^{3}$	$9.3x10^{3}$	$2.37xx10^{4}$	$9.6x10^{3}$	$6.3x10^{3}$	$2.14x10^{4}$
Average	$7.2x10^{3}$	$1.10x10^{4}$	$9.4x10^{4}$	$7.1x10^{3}$	$4.1x10^{3}$	$2.17x10^{4}$

* Indicates CFUs/mL estimated from 10^{-2} dilution

Total Count Results

A total of 74 bacterial isolates were collected from both systems with 34 isolates collected from the deep water culture hydroponic treatment and 40 isolates collected from the soil treatment **(Table 5)**. However, the number of bacterial isolates varied between weeks.

Table 5: Total bacterial isolates collected

Week	Deep Water Culture		Soil	
	TSA	LN	TSA	LN
2	6	2	2	3
3	5	10	11	7
5	5	6	8	6
Total Isolates Per Medium	16	18	21	19
Total Isolates Per Treatment	34		40	
Total Isolates	74			

Morphological and Physiological Test Results

Results of the morphological and physiological tests indicated similar characteristics of bacterial isolates in both treatments. The results for the morphological and physiological tests were calculated as percentages of the total bacterial population isolated from each week from both treatments (**Table 6**). From these results, the average percentages of characteristics for all weeks were calculated for both treatments and are shown in **Figures 4 and 5**., The predominant

type of bacteria isolated from both treatments were Gram-Positive (95.5% DWC, 95.7% soil) with bacillus cellular shapes and arrangements (84.8% DWC, 79.3% soil). Over half of the isolates from each treatment were positive for the production of endospores (51.5% DWC, 57.0% soil). Bacteria isolated from both treatments exhibited physiological similarities with respect to motility (73.9% DWC, 72.1% soil) and aerotolerance. More obligate aerobic bacteria were isolated from the DWC treatment (15.8%) than the soil treatment (10.9%), while the soil treatment contained more facultative anaerobic bacteria (49.6%) compared to the DWC (40.1%). The morphological and physiological characteristics of all isolates are listed in **Appendix B**, **B1-B3**.

Table 6: Percentage calculations for morphological and physiological test results

	Deep Water Culture				Soil Treatment			
	Week 2	Week 3	Week 5	**Avg.**	Week 2	Week 3	Week 5	**Avg.**
Bacillus Shape	100%	73.3%	81.1%	**84.8%**	80.0%	72.2%	85.7%	**79.3%**
Coccus Shape	0%	26.7%	18.1%	**14.9%**	20.0%	22.2%	14.2%	
Spirillum Shape	0%	0%	0%	**0%**	0%	5.55%	0%	**1.85%**
Gram Positive	100%	86.6%	100%	**95.5%**	100%	94.4%	92.8%	**95.7%**
Gram Negative	0%	13.3%	0%	**4.43%**	0%	0%	0%	**0%**
Gram Variable	0%	0%	0%	**0%**	0%	5.55%	0%	**1.85%**
Endospore Forming	33.3%	66.6%	54.5%	**51.5%**	40.0%	66.6%	64.3%	**57.0%**
Capsule Producing	75%	53.3%	90.9%	**73.1%**	40.0%	77.8%	78.5%	**65.4%**
Motile	37.5%	93.3%	90.9%	**73.9%**	60.0%	77.8%	78.6%	**72.1%**
Obligate Aerobes	25.0%	13.3%	9.09%	**15.8%**	20.0%	5.55%	7.14%	**10.9%**
Microaerophiles	25.5%	40.0%	54.5%	**40.0%**	20.0%	55.5%	42.8%	**39.4%**
Faculative Anaerobes	37.5%	46.6%	36.3%	**40.1%**	60.0%	38.8%	50.0%	**49.6%**
Number of Isolates Per Week	8	15	11		5	18	14	

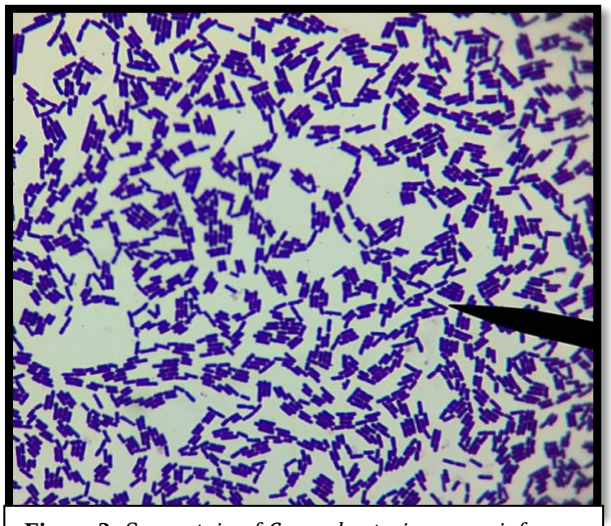

Figure 2: Gram stain of *Corynebacterium xerosis* from the week 2 deep water culture hydroponic treatment. This image depicts a bacteria that is Gram Positive with a bacillus shape and single bacillus arrangement

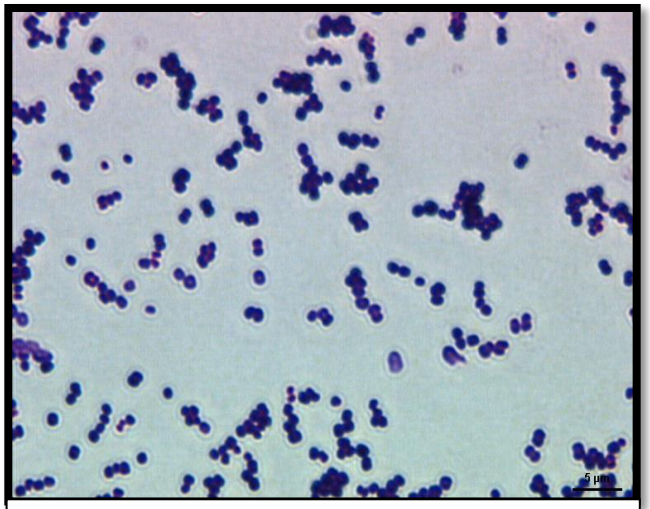

Figure 3: Gram stain of *Sporosarina ureae* from the week 3 soil treatment. This image depicts a Gram Positive bacterium with a coccus shape and diplococcus arrangement.

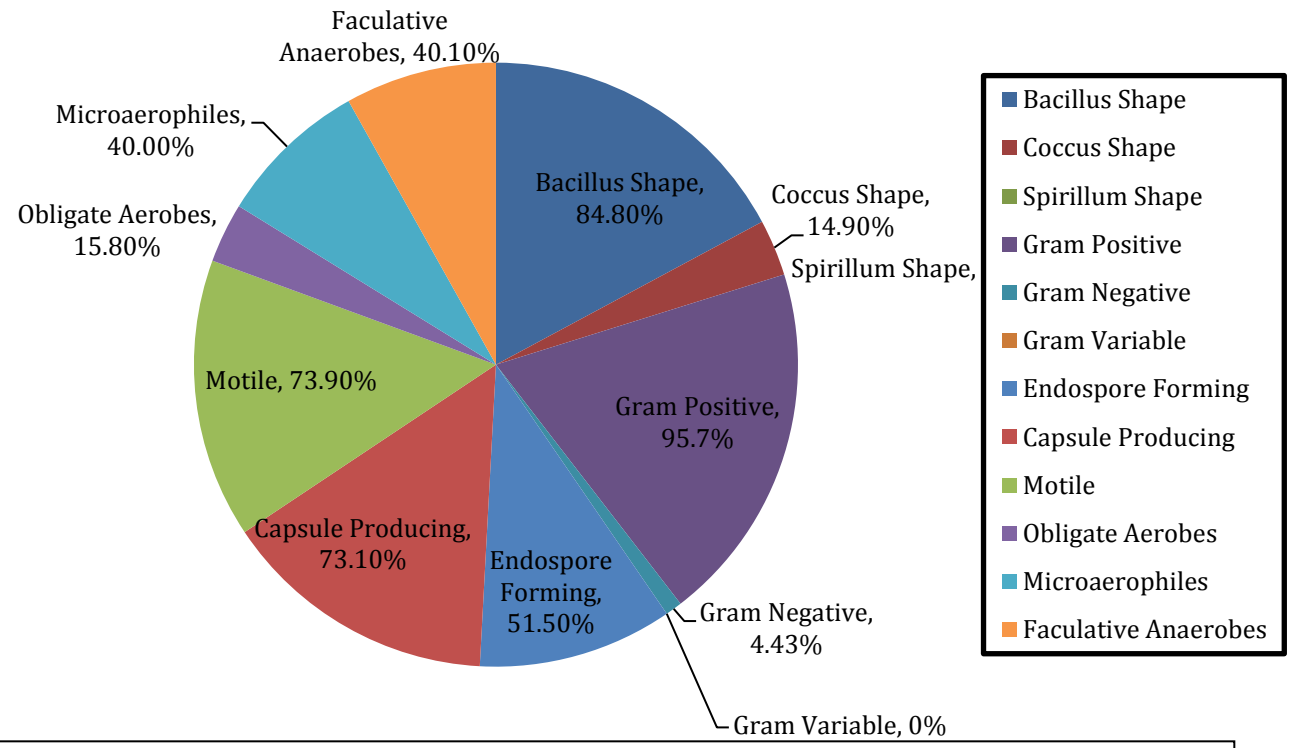

Figure 4: Average percentages of morphological and physiological test results for deep water culture (DWC) bacteria

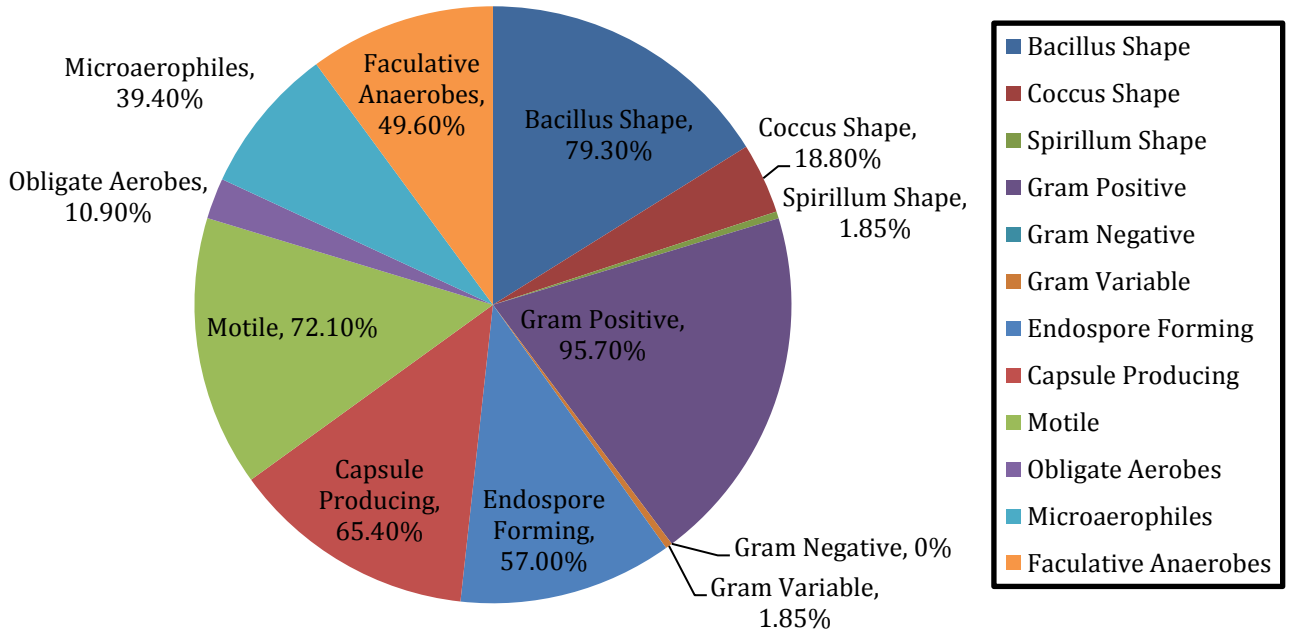

Figure 5: Average percentages of morphological and physiological test results for soil treatment bacteria

Biochemical Test Results

Additionally, the data from the biochemical analysis indicated a similarity in the biochemical test results for the bacteria present in both treatments. The results for the biochemical tests were calculated as percentages of the total bacterial population isolated from each week from both treatments (**Table 7**). From these results, the average percentages of characteristics for all weeks were calculated for both treatments and are shown in **Figures 6 and 7**. Additionally, the biochemical characteristics of each individual isolate from both treatments for each week are shown in **Appendix C**, **C1**-C**6**. Overall, while both treatments exhibited microbial diversity, results of the morphological, physiological, and biochemical tests indicated a close similarity in the bacteria genera as well as species found in each treatment.

Table 7: Percentage calculations of biochemical test results

Biochemical Test	**Deep Water Culture**				**Soil**			
	Week 2	**Week 3**	**Week 5**	**Avg.**	**Week 2**	**Week 3**	**Week 5**	**Avg.**
Indole	0%	0%	0%	**0%**	0%	0%	0%	**0%**
Methyl Red	100%	46.7%	36.3%	**61.0%**	0%	33.3%	50.0%	**27.8%**
Vogues Proskauer	62.5%	53.3%	54.5%	**56.7%**	40.0%	38.9%	50.0%	**43.0%**
Citrate Utilization	25.0%	26.7%	36.7%	**29.5%**	80.0%	55.6%	50.0%	**61.9%**
Nitrate Reduction	75.0%	26.7%	36.4%	**46.0%**	60.0%	27.8%	21.4%	**36.4%**
Denitrification	25.0%	6.71%	9.00%	**13.6%**	40.0%	0%	14.2%	**18.0%**
Catalase Production	87.5%	66.7%	72.7%	**75.5%**	80.0%	55.6%	50.0%	**61.9%**
DNA Hydrolysis	62.5%	26.7%	63.6%	**50.9%**	40.0%	44.4%	42.8%	**42.4%**
Starch Hydrolysis	62.5%	20.0%	18.1%	**33.5%**	0%	11.1%	28.6%	**13.2%**
Casein Hydrolysis	40.0%	26.7%	54.5%	**40.4%**	60.0%	55.6%	57.1%	**50.4%**
Urea Hydrolysis	37.5%	33.3%	63.6%	**44.8%**	40.0%	61.1%	50.0%	**13.2%**
Gelatin Hydrolysis	37.5%	6.67%	9.09%	**17.8%**	0%	11.1%	28.6%	**45.3%**
Bile Esculin Test	0%	26.7%	45.5%	**24.1%**	40.0%	38.8%	57.1%	**0%**
Phenylalanine Deaminase	0%	0%	0%	**0%**	0%	0%	0%	**57.6%**
Glucose Fermentation	100%	80.0%	54.5%	**78.1%**	40.0%	94.4%	78.6%	**71.1%**
Lactose Fermentation	37.5%	0%	0%	**12.5%**	0%	22.2%	7.14%	**9.78%**
Sucrose Fermentation	50.0%	66.7%	45.5%	**54.1%**	60.0%	55.5%	50.0%	**55.2%**
Number of Isolates Per Week	8	15	11	**8**	5	18	14	**12**

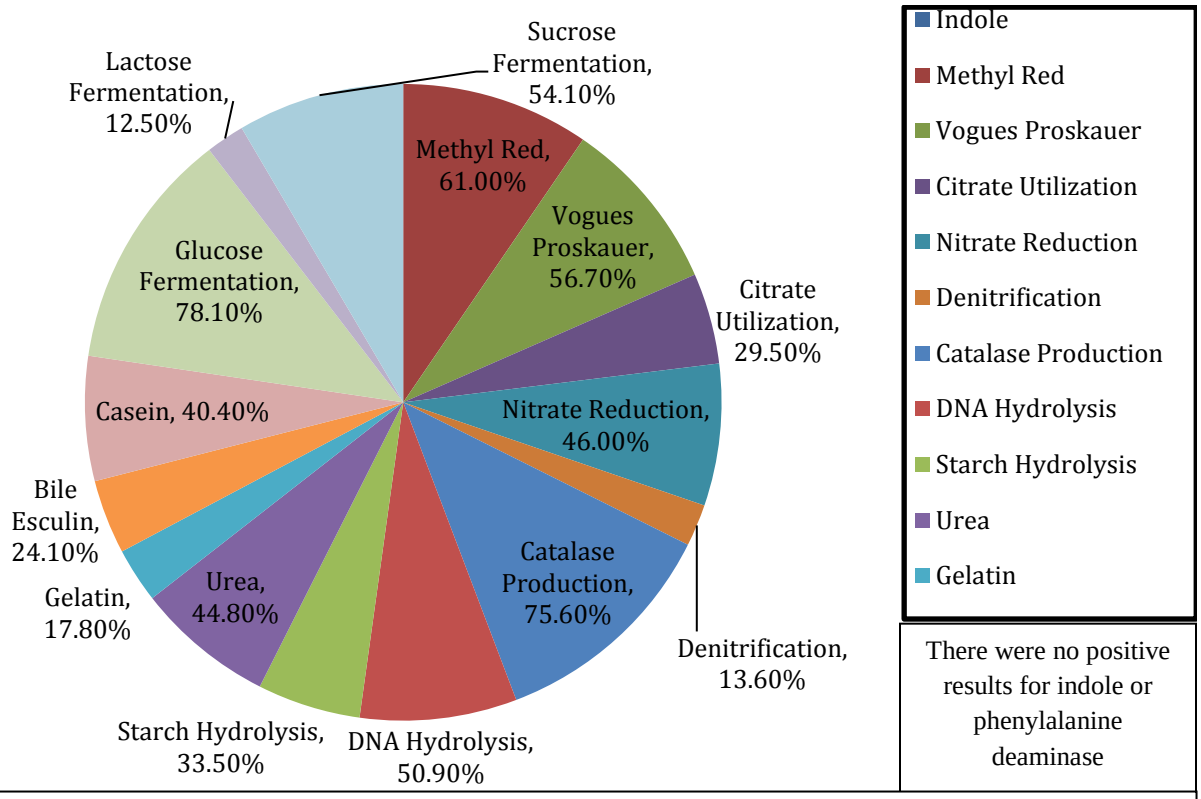

Figure 6: Average Percentage of positive biochemical test results for deep water culture (DWC) bacteria

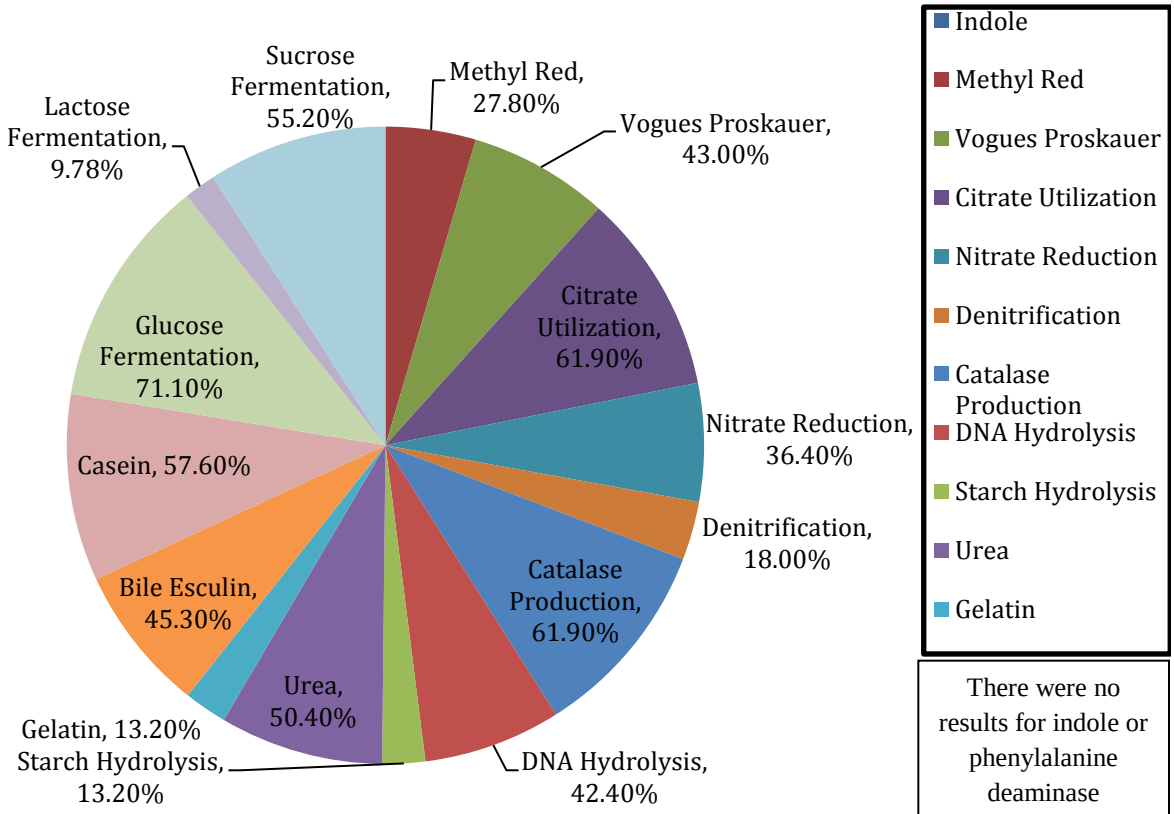

Figure 7: Average Percentage of positive biochemical test results for soil treatment bacteria

Presumptive Identification Results

Results of the identification tests indicated a similarity in bacterial species isolated from both treatments in addition to certain species confined to each treatment **(Table 6)**. The only genus presumptively unique to the DWC treatment was *Brochothrix* isolated from the Week 2 water sample. In contrast, three bacterial genera were presumptively unique to the soil treatment which included *Arthrobacter*, *Streptococcus*, and *Spirochaeta*. Only one individual isolate from each of these confined genera was isolated from each treatment. Therefore, comparison of the presumptive identifications indicated that the majority of the bacterial species were common to both treatments. These common genera include species belonging to the Gram-Positive genera of *Corynebacterium*, *Bacillus*, *Lactobacillus*, *Sporolactobacillus*, *Sporosarcina*, *Listeria*, *Planococcus* and *Cellulomonas* and the Gram-Negative genus *Enterobacter*. However, there were also certain species belonging to these common genera that were confined to either treatment. A comparison of the bacterial species isolated from both treatments from each week is shown in **Figure 6.** The specific species identified for each week are shown in **Appendix D, D1-D6**.

Table 6: Total count of bacterial species present in both treatments

Bacterial Species	Deep Water Culture	Soil Treatment
	Total	Total
Arthrobacter agili	0	1
Bacillus brevis	**2**	**5**
Bacillus coagulans	**4**	**3**
Bacillus megaterium	0	2
Bacillus subtilis	**2**	**1**
Bacillus thuringiensis	**3**	**4**
Brochothrix thermosphacta	1	0
Cellulomonas biazotea	**1**	**4**
Cellulomonas flavigena	0	0
Corynebacterium pseudodiphtheriticum	**1**	**1**
Corynebacterium xerosis	**2**	**2**
Enterobacter agglomerans	**1**	**1**
Enterobacter gergoviae	1	0
Lactobacillus alimentarius	1	0
Lactobacillus delbreuckii	**1**	**1**
Lactobacillus plantarum	0	1
Lactobacillus homohiochii	0	1
Listeria denitrificans	**5**	**1**
Listeria grayi	1	0
Planococcus citreus	**1**	**1**
Renibacterium salmoninarium	1	0
Spirochaeta halophila	0	1
Sporolactobacillus inulinus	**4**	**1**
Sporosarcina halophila	1	0
Sporosarcina ureae	**1**	**3**
Streptococcus agalactiae	0	1

*__Bolded__ names indicate bacterial species common to both treatments

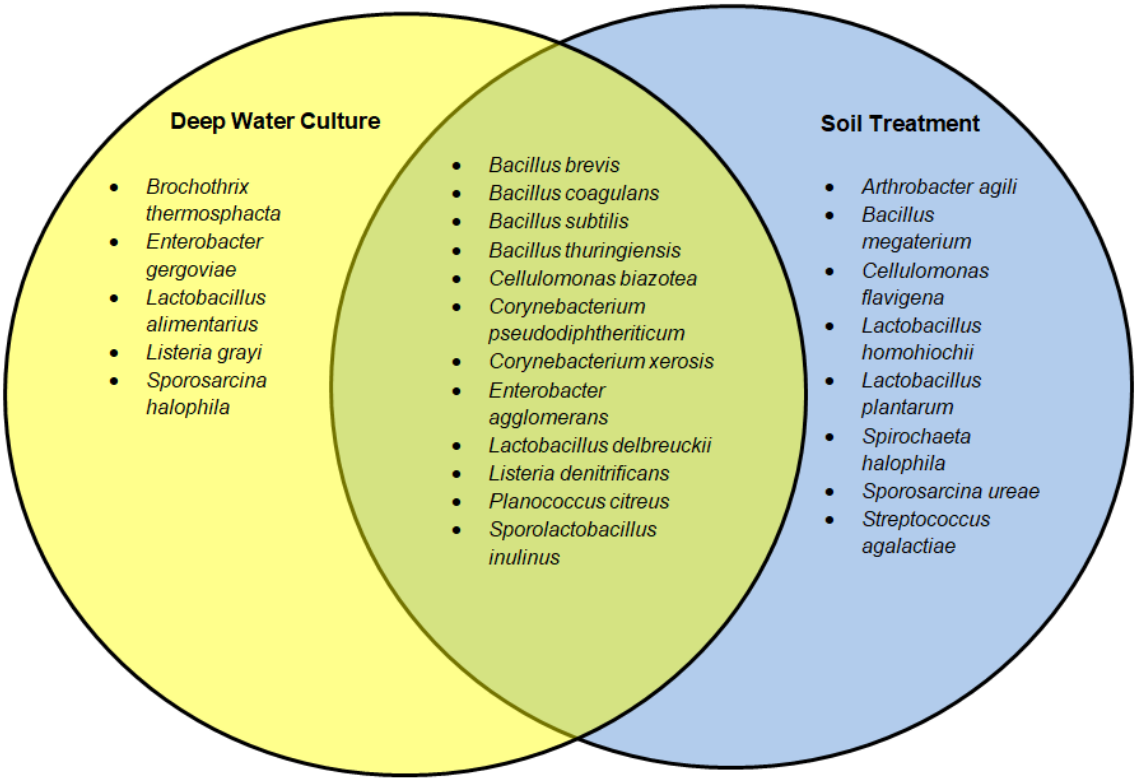

Figure 8: Bacterial Species present in growing treatments identified by the morphological, physiological, and biochemical test results

Discussion

The objective of this study was to identify the bacterial populations that live in hydroponic systems in comparison with the bacteria found in soil under the same growing conditions over the course of five weeks. It was hypothesized that there would be a difference in the bacterial populations seen in both treatments due to the different type of growing substrate. This prediction was based on the different material properties of soil versus water.

Instead, the results of the morphological and physiological tests suggest that the bacteria isolated from both treatments exhibit similar characteristics and physiological processes. The majority of the bacteria isolated from both treatments were Gram-Positive with bacillus cellular shapes and arrangements. This observation confirms the work of Schwarz et al. (2005), Shara et al. (2017), and Gardner et al. (2011) who report that these types of bacteria are predominately

found in hydroponic and soil growing treatments. The higher percentage of obligate aerobic bacteria found in the DWC treatment than the soil treatment could suggest that bacteria in hydroponic solution may require higher levels of oxygen to survive versus soil. Similarly, the higher average percentage of facultative anaerobic bacteria seen in the soil treatment could suggest that soil bacteria are better adapted to live at lower levels of oxygen possibly due to the compaction or saturation of the soil.

Results of the biochemical tests indicate that the bacteria in both treatments are capable of metabolically performing similar biochemical functions suggesting a similarity in the chemical environment of both treatments. While many of the biochemical tests conducted to solely differentiate bacteria on the basis of metabolic and hydrolytic properties, a number of the tests illustrate how the bacteria in both growing treatments are capable of supporting plant development. For example, the nitrate reduction test examined the capability of the bacteria in both treatments at reducing nitrate to nitrite, a nitrogen compound useful to plant growth (Fu et. al. 2017). Many of the bacterial isolates identified in both treatments were positive for the reduction and denitrification of nitrate. Additionally, in accordance with the work of Saijai et al. (2017) and Sheridan et al. (2017), the majority of the bacterial isolates collected from both treatments capable of these processes were of the genus *Bacillus*. Saijai et al. (2017) additionally reported that hydroponic systems typically exhibit less nitrogen fixing and reducing bacteria than soil treatments. However, in this study, the DWC treatment exhibited a higher average percentage of nitrate reduction (46.0%) than the soil treatment (36.4%) **(Table 5) (Figures 5 and 6)**. However, there was a lower average percentage of denitrification seen in the DWC treatment (13.6%) than the soil treatment (18.0%). Nevertheless, these results show that microorganisms

that utilize nitrogen in the manner that may be beneficial to the lettuce plants are present in both treatments.

Additionally, the catalase and vogues-proskauer biochemical tests suggest bacteria in both treatments could further support plant development. The catalase test examined bacteria for their ability to degrade hydrogen peroxide, a byproduct of cellular respiration that is toxic to the cell. Since the majority of the bacteria isolated in both treatments were capable of producing catalase, this result may suggest the ability of the bacteria to degrade hydrogen peroxide in the plant rhizosphere, thereby protecting the plant. The vogues-proskauer test (VP) differentiates bacterial species based on their ability to convert pyruvic acid, an important compound used in cellular respiration, to acetoin and 2,3-butanediol. Fincheira et al. (2018) and Rojas-solìs et al. (2018) reported that acetoin and 2,3-butanediol are volatile organic compounds (VOCs) that serve as plant growth inducers. In this study, about half of the bacteria isolated from both treatments were VP positive. Specifically, 56.7% of the bacteria in the DWC treatment were VP positive while only 43.0% of the bacteria in the soil treatment were VP positive. Additionally, since the majority of the VP positive organisms isolated were *Bacillus* species, these results are consistent with the work of Finchiera et. al. (2018) who reported that *Bacillus* species are predominate Gram-Positive bacteria that produce VOCs to induce growth of lettuce. Additionally, this finding is also consistent with Sheridan et. al. (2017) who reported that *Bacillus* species are commonly used as growth promoting organisms (PGPMs) which promote plant growth through nitrogen fixation, facilitation of nutrient access, and direct plant growth stimulation. Overall, the biochemical results from the nitrate reduction test, catalase test, and vogues proskaur test illustrate that the bacteria in both treatments are capable of performing the same biochemical processes that promote plant development. Additionally, the similarity in the

results of all of the biochemical tests suggests that a similar microbiome can be established using either growing substrate.

The majority of the bacteria isolated from both treatments were belonged to nonpathogenic taxa. The nonpathogenic bacteria isolated from both treatments include species belonging to the genera of *Bacillus*, *Lactobacillus*, *Sporolactobacillus*, *Sporosarcina*, *Planococcus*, *Cellulomonas*, *Arthobacter*, and *Corynebacterium*. Of the genera identified from both treatments, the most prominent genus was *Bacillus*. *Bacillus* species are Gram-Positive, endospore-forming, and produce the enzyme catalase (Krieg, 1984). Bacillus is a diverse genus with species capable of surviving at across a wide range of pH ranges and nutrient levels (Krieg, 1984), allowing them to live in diverse habitats including soil and hydroponic treatments. Additionally, *Bacillus* species are chemoorganotrophs, which degrade organic matter for energy enabling them to utilize organic materials such as starch and glucose produced by the plants (Krieg, 1984). Like *Bacillus*, *Lactobacillus* is a diverse genus comprised of various species isolated from wide assortment of environments including grain, dairy, meat, and fish products as well as in beer, wine, fruit juice, sauerkraut, sewage, and soil runoff (Krieg, 1984). Additionally, *Lactobacillus* species are acidophilic and optimally live within an ideal pH range from 6.4 to 4.5; which is consistent the parameters used in both growing treatments (Krieg, 1984). *Sporolactobacillus*, *Sporosarcina*, *Planococcus*, *Cellulomonas*, *Arthrobacter*, and *Corynebacterium* are soil bacteria commonly isolated from the rhizosphere of wild plants (Krieg, 1984).

The bacteria isolated were not tested for pathogenicity and were not suspected to be harmful to human health; however they include taxa that have the potential to be pathogenic. While Bacillus was the predominant bacterial genus identified in this study, many of the *Bacillus* species identified in this study are also potentially pathogenic. For example, B. *thuringiensis* is

an insect pathogen, and B. *brevis* and B. *subtilis*, are associated with producing exotoxins that cause food poisoning (Krieg, 1984). Other potentially pathogenic bacteria presumptively identified in this study include bacteria belonging to the genera of *Listeria*, *Brochothrix*, and *Streptococcus*. *Listeria gravi* and *Listeria denitrificans* are generally nonpathogenic. However, *Listeria ivanovii* has been identified as a potential pathogen that can cause listeriosis in adults (Guillet et al. 2010). *Brochothrix thermosphacta* isolated from the deep water culture treatment in Week 2 is a known respiratory tract pathogen in adults (Krieg, 1984). Additionally, *Streptococcus agalactiae* isolated from the deep water culture treatment in Week 5 is a member of the Group B *Streptococci* subgroup which includes pathogenic *Streptococcus* species that are foremost cause of life-threatening bacterial infections in newborns (Rajagopal, 2010). However, while these bacteria have been identified as potential pathogens, we are unable to determine whether or not the bacteria isolated in this study are pathogenic to either growing treatment. Further tests would have to be conducted on the bacterial isolates beyond the scope of the materials readily available to us to determine any pathogenic properties. Nevertheless, this research could lead to further research of pathogenic bacteria and its potential application in human health and food safety.

Overall, the presumptive identifications obtained in this study are relatively consistent with past research identifying bacteria found in hydroponic and soil treatments. Our results are consistent with Schwarz et. al. (2005) who identified Bacillus as the most prominent genus present in hydroponic treatments with species including B. *subtilis,* B. *thuringiensis,* and B. *megaterium*. However, unlike Schwarz et. al. (2005), species belonging to the genera of Pseudomonas, *Janthinobacterium, Paenibacillus, Micrococcus, and Acinetobacter* were not identified. As possible reason for this difference could be due to the different sources of water

used by Schwarz et. al. (2005) (natural lake and a peat ditch) and this study (tap water). Additionally, our results were consistent with Sajai et al. (2016) who reported species belonging to the genera of *Bacillus*, *Arthrobacter*, *Cellulomonas*, and *Enterobacter* in hydroponic solution. Finally, our findings are consistent with Gardner et al. (2011) with respect to the frequency of Gram-positive phyla isolated from soil treatment. Similar to Gardner et al. (2011), the predominant Gram-positive organisms isolated in this study belonged to the phyla of Bacilli and Actinobacter. The phylum Bacilli includes *Bacillus*, *Listeria*, *Lactobacillus*, *Sporolactobacillus*, *Planococcus*, and *Sporosarcina* while the phylum Actinobacter includes *Corynebacterium*, *Cellulomonas*, and *Arthrobacter*. However, in contrast to the results of Gardner et. al. (2011), the majority of the bacteria isolated from the soil treatment were Gram-positive, and only one isolate was identified under the Gram-negative phylum *Proteobacteria*.

This research contributes to further the understanding of the similarities and differences between hydroponic farming and traditional farming methods at the microbial level. Unlike we initially hypothesized, the majority of the bacteria isolated from both treatments exhibited similar microbial diversity. Additionally, the biochemical results suggest that there is a similarity in the metabolic capabilities of the bacteria isolated in both treatments as well as a resultant similar chemical environment of the plant rhizosphere of both treatments. Therefore, while both treatments exhibited some differences in microbial diversity, results of the morphological, physiological, and biochemical tests suggest that a similar microbiome can be established using either soil-based growing or hydroponics.

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Appendix

Appendix A: Spread Plate Count Results

A1: Week 2 spread plate results

Set		**Deep Water Culture**				**Soil**			
		TSA		**LN**		**TSA**		**LN**	
1	**Dilution Factor**	**Plate Count**	**CFUs/mL**	**Plate Count**	**CFUs/mL**	**Plate Count**	**CFUs/mL**	**Plate Count**	**CFUs/mL**
	10^{-1}	48	$4.8x10^{3}$	109	$1.09x10^{4}$	99	$9.9x10^{3}$	22	$2.2x10^{3}$
	10^{-2}	<30	-	30	-	<30	-	<30	-
	10^{-3}	<30	-	<30	-	<30	-	<30	-
	10^{-4}	<30	-	<30	-	<30	-	<30	-
	10^{-5}	<30	-	<30	-	<30	-	<30	-
	10^{-6}	<30	-	<30	-	<30	-	<30	-
2	10^{-1}	70	$7.0x10^{3}$	40	$4.0x10^{3}$	97	$9.7x10^{3}$	96	$9.6x10^{3}$
	10^{-2}	<30	-	30		<30	-	<30	-
	10^{-3}	<30	-	<30	-	<30	-	<30	-
	10^{-4}	<30	-	<30	-	<30	-	<30	-
	10^{-5}	<30	-	<30	-	<30	-	<30	-
	10^{-6}	<30	-	<30	-	<30	-	<30	-
3	10^{-1}	34	$3.4x10^{-3}$	68	$6.8x10^{-3}$	43	$4.3x10^{-3}$	96	$9.6x10^{-3}$
	10^{-2}	<30	-	<30	-	<30	-	<30	-
	10^{-3}	<30	-	<30	-	<30	-	<30	-
	10^{-4}	<30	-	<30	-	<30	-	<30	-
	10^{-5}	<30	-	<30	-	<30	-	<30	-
	10^{-6}	<30	-	<30	-	<30	-	<30	-

A2: Week 3 spread plate results

Set		**Deep Water Culture**				**Soil**			
		TSA		**LN**		**TSA**		**LN**	
1	**Dilution Factor**	**Plate Count**	**CFUs/mL**	**Plate Count**	**CFUs/mL**	**Plate Count**	**CFUs/mL**	**Plate Count**	**CFUs/mL**
	10^{-1}	152	$1.52x10^{4}$	100	$1.00x10^{4}$	<30 (25)	$2.5x10^{3}$	<30 (15)	$1.5x10^{3}$
	10^{-2}	<30	-	<30	-	<30	-	<30	-
	10^{-3}	<30	-	<30	-	<30	-	<30	-
	10^{-4}	<30	-	<30	-	<30	-	<30	-
	10^{-5}	<30	-	<30	-	<30	-	<30	-
	10^{-6}	<30	-	<30	-	<30	-	<30	-
2	10^{-1}	95	$9.5x10^{3}$	138	$1.38x10^{4}$	<30 (24)	$2.4x10^{3}$	44	$4.4x10^{3}$
	10^{-2}	<30	-	<30	-	<30	-	<30	-
	10^{-3}	<30	-	<30	-	<30	-	<30	-
	10^{-4}	<30	-	<30	-	<30	-	<30	-
	10^{-5}	<30	-	<30	-	<30	-	<30	-
	10^{-6}	<30	-	<30	-	<30	-	<30	-
3	10^{-1}	82	$8.3x10^{3}$	93	$9.3x10^{3}$	39	$3.9x10^{3}$	63	$6.3x10^{3}$
	10^{-2}	<30	-	<30	-	<30	-	<30	-
	10^{-3}	<30	-	<30	-	<30	-	<30	-
	10^{-4}	<30	-	<30	-	<30	-	<30	-
	10^{-5}	<30	-	<30	-	<30	-	<30	-
	10^{-6}	<30	-	<30	-	<30	-	<30	-

A3: Week 5 spread plate results

Set		Deep Water Culture				Soil			
1		TSA		LN		TSA		LN	
	Dilution Factor	Plate Count	CFUs/mL	Plate Count	CFUs/mL	Plate Count	CFUs/mL	Plate Count	CFUs/mL
	10^{-1}	214	$2.14x10^{4}$	TNTC	-	58	$5.8x10^{3}$	237	$2.37x10^{4}$
	10^{-2}	<30	-	80	$8.0x10^{4}$	<30	-	<30	-
	10^{-3}	<30	-	31	$3.1x10^{5}$	<30	-	<30	-
	10^{-4}	<30	-	<30	-	<30	-	<30	-
	10^{-5}	<30	-	<30	-	<30	-	<30	-
	10^{-6}	<30	-	<30	-	<30	-	<30	-
2	10^{-1}	186	$1.86x10^{4}$	TNTC	-	151	$1.51x10^{4}$	200	$2.00x10^{4}$
	10^{-2}	<30	-	107	$1.07x10^{5}$	<30	-	<30	-
	10^{-3}	74	$7.4x10^{5}$	<30	-	<30	-	<30	-
	10^{-4}	<30	-	<30	-	<30	-	<30	-
	10^{-5}	33	$3.3x10^{7}$	<30	-	<30	-	<30	-
	10^{-6}	<30	-	<30	-	<30	-	<30	-
3	10^{-1}	191	$1.91x10^{3}$	TNTC	-	108	$1.08x10^{4}$	214	$2.14x10^{4}$
	10^{-2}	<30	-	96	$9.6x10^{4}$	<30	-	<30	-
	10^{-3}	<30	-	<30	-	<30	-	<30	-
	10^{-4}	<30	-	<30	-	<30	-	<30	-
	10^{-5}	<30	-	<30	-	<30	-	<30	-
	10^{-6}	<30	-	<30	-	<30	-	<30	-

Appendix B: Morphological and Physiological Characteristics of Bacterial Isolates

B1: Morphological and physiological characteristics of week 2 bacterial isolates

Deep Water Culture Treatment Bacterial Isolates

TSA	Shape and Arrangement	Gram Stain	Capsule Stain	Endospore Stain	Motility Test	Aerotolerance
T2D1	Bacillus, Single	+	+	+	-	**O.A.**
T2D2	Bacillus, Streptobacillus	+	+	+	+	**M**
T2D3	Bacillus, Diplobacillus	+	+	-	-	**F.A.**
T2D4	Bacillus, Coccobacillus	+	+	+	-	**F.A.**
T2D5	Bacillus, Diplobacillus	+	+	+	+	**M**
T2D6	Bacillus, Diplobacillus	+	+	-	-	**O.A.**
LN						
L2D1	Bacillus, Coccobacillus	+	-	+	+	**M**
L2D2	Bacillus, Diplobacillus	+	-	+	-	**F. A.**

Soil Treatment Bacterial Isolates

TSA	Shape and Arrangement	Gram Stain	Capsule Stain	Endospore Stain	Motility Test	Aerotolerance
T2S1	Coccus, Diplococcus	+	+	-	+	O. A.
T2S2	Bacillus, Single	+	-	+	-	M
LN						
L2S1	Bacillus, Coccobacillus	+	+	+	+	F.A.
L2S2	Bacillus, Streptobacillus	+	-	-	-	F.A.
L2S3	Bacillus, Single	+	-	-	+	F.A.

B2: Morphological and physiological characteristics of week 3 bacterial isolates

Deep Water Culture Treatment Bacterial Isolates

TSA	Shape and Arrangement	Gram Stain	Capsule Stain	Endospore Stain	Motility Test	Aerotolerance
T3D1	Coccus, Tetrad	+	+	+	+	O.A.
T3D2	Bacillus, Coccobacillus	+	+	-	+	F.A.
T3D3	Bacillus, Single	+	-	+	+	F.A.
T3D4	Bacillus, Streptobacillus	+	+	-	+	M
T3D5	Single, Bacillus	+	+	+	+	F.A.
LN						
L3D1	Bacillus, Single	+	+	+	+	M
L3D2	Coccus, Single	-	-	+	+	M
L3D3	Bacillus, Diplobacillus	+	+	+	+	M
L3D4	Bacillus, Single	-	-	+	+	M
L3D5	Bacillus, Single	+	-	+	+	M
L3D6	Bacillus, Streptobacillus	+	-	-	+	O.A.
L3D7	Bacillus, Single	+	-	-	+	F.A.
L3D8	Coccus, Diplococcus	+	+	+	-	F.A.
L3D9	Bacillus, Single	+	+	+	+	F.A.
L3D10	Coccus, Single	+	-	-	+	F.A.

Soil Treatment Bacterial Isolates

TSA	Shape and Arrangement	Gram Stain	Capsule Stain	Endospore Stain	Motility Test	Aerotolerance
T3S1	Bacillus, Single	+	+	+	+	F.A.
T3S2	Coccus, Single	+	+	+	+	F.A.
T3S3	Bacillus, Streptobacillus	+	+	+	+	M
T3S4	Spirillum, Spirochete	+	+	+	+	M
T3S5	Bacillus, Single	+	+	+	+	M
T3S6	Bacillus, Streptobacillus	+	+	+	-	F.A.
T3S7	Bacillus, Coccobacillus	**Variable**	+	-	+	M
T3S8	Bacillus, Single	+	+	-	+	M
T3S9	Bacillus, Coccobacillus	+	+	+	+	M
T3S10	Coccus, Diplococcus	+	+	-	-	M
T3S11	Bacillus, Single	+	-	+	+	F.A.
LN						
L3S1	Bacillus, Single	+	+	+	+	O.A.
L3S2	Coccus, Single	+	+	+	-	F.A.
L3S3	Bacillus, Single	+	-	+	+	F.A.
L3S4	Bacillus, Single	+	+	-	+	F.A.
L3S5	Bacillus, Streptobacillus	+	+	-	-	M
L3S6	Coccus, Single	+	-	+	+	M
L3S7	Bacillus, Coccobacillus	+	-	-	+	M

B3: Morphological characteristics of week 5 bacterial isolates

Deep Water Culture Treatment Bacterial Isolates

TSA	Shape and Arrangement	Gram Stain	Capsule Stain	Endospore Stain	Motility Test	Aerotolerance
T5D1	Coccus, Single	+	+	+	+	F.A.
T5D2	Bacillus, Single	+	+	+	+	F.A.
T5D3	Bacillus, Streptobacilus	+	-	+	+	M
T5D4	Bacillus, Streptobacillus	+	+	-	+	M
T5D5	Bacillus, Single	+	+	+	+	F.A.
LN						
L5D1	Coccus, Single	+	+	-	+	M
L5D2	Bacillus, Single	+	+	-	+	M
L5D3	Bacillus, Streptobacillus	+	+	-	+	M
L5D4	Bacillus, Single	+	+	-	-	M
L5D5	Bacillus, Coccobacillus	+	+	+	+	F.A.
L5D6	Bacillus, Single	+	+	+	+	O.A.

Soil Treatment Bacterial Isolates

TSA	Shape and Arrangement	Gram Stain	Capsule Stain	Endospore Stain	Motility Test	Aerotolerance
T5S1	Bacillus, Single	+	+	+	+	F.A.
T5S2	Coccus, Single	+	+	-	-	F.A.
T5S3	Coccus, Streptococcus	+	+	+	+	M
T5S4	Bacillus, Streptobacillus	+	+	-	+	M
T5S5	Bacillus, Single	+	+	+	+	F.A.
T5S6	Bacillus, Single	+	+	+	+	F.A.
T5S7	Bacillus, Single	+	+	+	+	M
T5S8	Bacillus, Single	+	-	-	-	M
LN						
L5S1	Bacillus, Single	+	+	+	+	O.A.
L5S2	Bacillus, Single	+	-	+	-	F.A.
L5S3	Bacillus, Single	+	+	-	+	F.A.
L5S4	Bacillus, Streptobacillus	+	-	+	+	F.A.
L5S5	Bacillus, Single	-	+	-	+	M
L5S6	Bacillus, Single	+	+	+	+	M

Appendix C: Biochemical Test Results

C1: Week 2 deep water culture biochemical test results

			TSA				LN	
Test	**T2D1**	**T2D2**	**T2D3**	**T2D4**	**T2D5**	**T2D6**	**L2D1**	**L2D2**
Indole	-	-	-	-	-	-	-	-
Methyl Red	+	+	+	+	+	+	+	+
Vogues-Proskauer	-	-	+	+	+	+	+	-
Citrate	-	+	-	-	-	-	+	-
Nitrate Reduction	+	-	+	+	+	-	+	+
Denitrification		+				+		
Catalase	+	+	+	+	-	+	+	+
DNA	+	+	+	-	-	-	+	+
Starch	+	-	-	+	-	+	+	+
Casein	+	-	+	-	-	+	+	+
Urea	+	-	-	+	-	+	-	-
Gelatin	+	-	-	+	-	+	-	-
Bile Esculin	-	-	-	-	-	-	-	-
Phenylalanine Deaminase	-	-	-	-	-	-	-	-
Oxidation Fermentation	-	**O**	-	**O**	-	-	**O**	**O**
Sugar Fermentation								
PR Glucose	**A**	**A**	**A**	**A**	**A**	**A**	**A**	**A**
PR Lactose	**P**	**P**	**A**	**P**	**P**	**P**	**A**	**A**
PR Sucrose	**A**	**P**	**A**	**P**	**P**	**P**	**A**	**A**
PR Mannitol	**P**	**P**	**A**	**P**	**P**	**P**	**A/G**	**A**

C2: Week 2 soil treatment biochemical test results

	TSA		LN		
Test	**T2S1**	**T2S2**	**L2S1**	**L2S2**	**L2S3**
Indole	-	-	-	-	-
Methyl Red	-	-	-	-	-
Vogues-Proskauer	+	+	-	-	-
Citrate	+	-	+	+	+
Nitrate Reduction	+	+	-	+	-
Denitrification	-	-	+	-	+
Catalase	+	-	-	+	+
DNA	-	-	+	-	+
Starch	-	-	-	-	-
Casein	+	+	-	-	+
Urea	+	-	-	+	-
Gelatin	-	-	-	-	-
Bile Esculin	+	-	+	-	-
Phenylalanine Deaminase	-	-	-	-	-
Oxidation Fermentation	-	-	**O**	-	**O**
Sugar Fermentation					
PR Glucose	**A**	**A**	**P**	**P**	**P**
PR Lactose	**P**	**P**	**P**	**P**	**P**
PR Sucrose	**P**	**P**	**A**	**A**	**A**
PR Mannitol	**P**	**P**	**A**	**A**	**P**

C3: Week 3 deep water culture biochemical test results

	TSA					LN									
Test	**T3D1**	**T3D2**	**T3D3**	**T3D4**	**T3D5**	**L3D1**	**L3D2**	**L3D3**	**L3D4**	**L3D5**	**L3D6**	**L3D7**	**L3D8**	**L3D9**	**L3D10**
Indole	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Methyl Red	+	-	+	-	-	-	-	-	+	+	+	+	-	+	-
Vogues-Proskauer	+	-	+	-	-	+	-	+	+	+	+	+	-	-	-
Citrate	-	-	-	-	-	-	+	-	+	-	+	-	-	-	+
Nitrate Reduction	-	-	-	+	+	+	-	-	-	-	+	-	-	-	-
Denitrification	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-
Catalase	-	+	-	-	+	+	+	+	-	+	+	-	+	+	+
DNA	-	-	+	+	-	-	-	-	-	+	-	+	-	-	-
Starch	-	-	-	-	-	-	+	+	-	-	-	-	-	+	-
Casein	+	-	-	-	-	+	-	-	+	-	-	+	-	-	-
Urea	-	+	-	-	-	-	-	-	+	-	-	-	+	+	+
Gelatin	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-
Bile Esculin	-	-	-	-	-	+	+	+	-	+	-	-	-	-	-
Phenylalanine Deaminase	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Oxidation Fermentation	-	-	-	**F**	-	**O**	-	-	**O/F**	-			-	-	-
Sugar Fermentation															
PR Glucose	**A**	**A**	**A**	**A**	**A**	**A**	**A**	-/-	-/-	**A**	**A**	**A**	-/-	**A**	**A**
PR Lactose	**P**	**P**	**P**	**P**	**P**	**P**	**P**	**P**	**P**	**P**	**P**	**P**	**P**	**P**	**P**
PR Sucrose	**A/G**	**P**	**A**	**A**	**P**	**A**	-/-	-/-	**A**	**A**	**A**	**A**	-/-	**A**	**A**
PR Mannitol	**P**	**P**	**A**	**P**	**P**	**A**	**P**	**A**	**P**	**P**	**P**	**A**	**P**	**P**	**P**

C4: Week 3 soil treatment biochemical test results

	TSA											LN						
Test	**T3S1**	**T3S2**	**T3S3**	**T3S4**	**T3S5**	**T3S6**	**T3S7**	**T3S8**	**T3S9**	**T3S10**	**T3S11**	**L3S1**	**L3S2**	**L3S3**	**L3S4**	**L3S5**	**L3S6**	**L3S7**
Indole	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Methyl Red	+	-	+	+	-	-	-	-	-	+	-	+	+	-	-	-	-	-
Vogues-Proskauer	+	-	+	+	-	-	-	+	-	+	-	-	+	-	+	-	-	-
Citrate	-	+	-	-	+	+	-	+	-	-	-	+	+	+	+	+	-	+
Nitrate Reduction	+	-	+	-	-	-	+	-	-	-	-	-	-	-	-	+	+	-
Denitrification	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Catalase	+	+	+	-	-	+	+	-	+	+	+	+	-	+	-	+	-	+
DNA	+	-	-	+	-	-	-	-	+	-	-	-	+	+	+	+	+	-
Starch	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-
Casein	+	+	+	-	-	-	-	+	+	+	+	+	+	+	-	-	-	-
Urea	-	+	-	-	-	+	-	-	+	+	+	+	+	+	-	+	+	+
Gelatin	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-
Bile Esculin	+	+	+	-	-	+	-	-	-	+	-	-	-	+	+	-	-	-
Phenylalanine Deaminase	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Oxidation Fermentation	**-**	**-**	**O**	**O**	**-**	**F**	**O**	**O/F**	**-**	**-**	**-**	**O**	**O/F**	**-**	**-**			
Sugar Fermentation																		
PR Glucose	**A**	**P**	**A**	**A**	**A/G**	**A/G**	**A/G**	**A**	**A**	**A**	**A**	**A**	**A**	**A**	**A**	**A**	**A**	**A**
PR Lactose	**P**	**P**	**P**	**P**	**P**	**P**	**P**	**P**	**P**	**-/-**	**A**	**P**	**A**	**A**	**A**	**P**	**P**	**P**
PR Sucrose	**A**	**P**	**-/-**	**P**	**A**	**A/G**	**A**	**A**	**A**	**P**	**A/G**	**-/-**	**A**	**P**	**P**	**P**	**A**	**A**
PR Mannitol	**A**	**P**	**A**	**P**	**A**	**A**	**A**	**P**	**A**	**P**	**A**	**A**	**A**	**P**	**A**	**A**	**P**	**P**

C5: Week 5 deep water culture biochemical tests

	TSA					LN					
Test	**T5D1**	**T5D2**	**T5D3**	**T5D4**	**T5D5**	**L5D1**	**L5D2**	**L5D3**	**L5D4**	**L5D5**	**L5D6**
Indole	-	-	-	-	-	-	-	-	-	-	-
Methyl Red	-	-	+	+	-	-	+	-	-	-	+
Vogues-Proskauer	-	-	+	+	-	-	+	+	+	-	+
Citrate	-	-	-	-	+	+	+	+	-	-	-
Nitrate Reduction	-	-	-	-	-	-	+	+	+	+	-
Denitrification	-	-	-	+	-	-	-	-	-	-	
Catalase	+	+	+	+	-	+	+	+	-	-	+
DNA	+	+	-	-	+	+	-	+	-	+	+
Starch	-	-	-	+	-	-	-	+	-	-	-
Casein	+	+	+	-	+	+	-	-	-	+	-
Urea	+	-	+	-	-	-	+	+	+	+	+
Gelatin	-	-	-	-	-	-	-	-	-	-	+
Bile Esculin	-	-	+	-	+	-	-	-	+	+	+
Phenylalanine Deaminase	-	-	-	-	-	-	-	-	-	-	-
Oxidation	-	**O**	-	-	**O**	**O**	-	-	**O**	**O/F**	-
Fermentation											
Sugar Fermentation											
PR Glucose	**-/-**	**-/-**	**A**	**-/-**	**A**	**P**	**P**	**A**	**A**	**A**	**A**
PR Lactose	**P**	**P**	**P**	**P**	**P**	**P**	**P**	**P**	**P**	**P**	**P**
PR Sucrose	**P**	**P**	**A**	**P**	**P**	**A**	**A**	**P**	**P**	**A**	**A**
PR Mannitol	**A**	**P**	**A**	**P**	**P**	**P**	**A**	**P**	**P**	**P**	**P**

C6: Week 5 soil culture biochemical tests

	TSA								LN					
Test	**T5S1**	**T5S2**	**T5S3**	**T5S4**	**T5S5**	**T5S6**	**T5S7**	**T5S8**	**L5S1**	**L5S2**	**L5S3**	**L5S4**	**L5S5**	**L5S6**
Indole	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Methyl Red	+	-	-	+	+	+	-	+	+	-	+	+	-	+
Vogues-Proskauer	+	-	-	+	+	+	+	+	+	-	-	-	-	-
Citrate	-	-	+	+	-	-	+	+	-	-	+	+	+	-
Nitrate Reduction	+	+	-	+	-	-	-	-	-	-	-	-	-	-
Denitrification	-	-	-	-	-	-	-	+	-	-	-	+	-	-
Catalase	+	+	+	+	+	+	+	+	+	+	+	+	+	+
DNA	-	-	+	-	-	-	-	+	+	-	+	+	-	+
Starch	+	-	-	-	-	-	-	-	-	-	+	+	-	+
Casein	+	+	-	+	+	-	+	-	+	+	-	+	-	-
Urea	+	-	+	+	-	-	-	+	+	-	-	+	+	-
Gelatin	-	+	-	-	-	-	-	-	+	-	+	-	+	-
Bile Esculin	-	-	-	-	-	+	+	+	+	+	-	+	+	+
Phenylalanine Deaminase	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Oxidation	-	-	**O/F**	-	-	-	-	**O/F**	-	-	**O**	-	-	-
Fermentation														
Sugar Fermentation														
PR Glucose	**A**	**A**	**A**	**A**	**A**	**P**	**A**	**P**	**A**	**A**	**A**	**A**	**P**	**A**
PR Lactose	**P**	**P**	**P**	**P**	**P**	**P**	**P**	**P**	**P**	**P**	**P**	**P**	**A**	**P**
PR Sucrose	**A**	**A**	**A**	**P**	**P**	**P**	**P**	**P**	**A**	**A**	**P**	**P**	**A**	**A**
PR Mannitol	**P**	**P**	**A**	**A**	**P**	**A**	**A**	**A**	**P**	**A**	**A**	**P**	**P**	**P**

Appendix D: Presumptive Identification of Unknown Organisms

D1: Presumptive identification of week 2 deep water culture bacteria

TSA	
T2D1	*Corynebacterium xerosis*
T2D2	*Bacillus thuringiensis*
T2D3	*Renibacterium salmoninarum*
T2D4	*Corynebacterium pseudodiphtheriticum*
T2D5	*Sporolactobacillus inulinus*
T2D6	*Brochothrix thermosphacta*
LN	
L2D1	*Bacillus subtilis*
L2D2	*Corynebacterium xerosis*

D2: Presumptive identification of week 2 soil treatment bacteria

TSA	
T2S1	*Planococcus citreus*
T2S2	*Corynebacterium xerosis*
LN	
L2S1	*Sporolactobacillus inulinus*
L2S2	*Corynebacterium pseudodiphtheriticum*
L2S3	*Listeria denitrificans*

D3: Presumptive identification of week 3 deep water culture bacteria

TSA	
T3D1	*Sporosarcina halophila*
T3D2	*Listeria denitrificans*
T3D3	*Sporolactobacillus inulinus*
T3D4	*Lactobacillus delbrueckii*
T3D5	*Bacillus coagulans*
LN	
L3D1	*Bacillus brevis*
L3D2	*Enterobacter agglomerans*
L3D3	*Bacillus subtilis*
L3D4	*Enterobacter gergoviae*
L3D5	*Bacillus coagulans*
L3D6	*Cellulomonas biazotea*
L3D7	*Lactobacillus alimentarius*
L3D8	*Bacillus thuringiensis*
L3D9	*Bacillus coagulans*
L3D10	*Planococcus citreus*

D4: Presumptive identification of week 3 soil treatment bacteria

TSA	
T3S1	*Bacillus subtilis*
T3S2	*Sporosarcina ureae*
T3S3	*Bacillus subtilis*
T3S4	*Spirochaeta halophila*
T3S5	*Lactobacillus delbruekii*
T3S6	*Bacillus megaterium*
T3S7	*Cellulomonas biazotea*
T3S8	*Lactobacillus homohiochii*
T3S9	*Bacillus brevis*
T3S10	*Bacillus thuringiensis*
T3S11	*Bacillus brevis*
LN	
L3S1	*Bacillus megaterium*
L3S2	*Sporosarcina ureae*
L3S3	*Bacillus thuringiensis*
L3S4	*Lactobacillus plantarum*
L3S5	*Cellulomonas flavigena*
L3S6	*Sporosarcina ureae*
L3S7	*Cellulomonas biazotea*

D5: Presumptive identification of week 5 deep water culture bacteria

TSA	
T5D1	*Sporosarcina ureae*
T5D2	*Bacillus thuringiensis*
T5D3	*Bacillus brevis*
T5D4	*Listeria grayi*
T5D5	*Sporolactobacillus inulinus*
LN	
L5D1	*Listeria denitrificans*
L5D2	*Listeria denitrificans*
L5D3	*Listeria denitrificans*
L5D4	*Listeria denitrificans*
L5D5	*Sporolactobacillus inulinus*
L5D6	*Bacillus coagulans*

D6: Presumptive identification of week 5 soil treatment bacteria

TSA	
T5S1	*Bacillus coagulans*
T5S2	*Arthrobacter agilis*
T5S3	*Streptococcus agalactiae*
T5S4	*Bacillus brevis*
T5S5	*Bacillus coagulans*
T5S6	*Bacillus brevis*
T5S7	*Bacillus brevis*
T5S8	*Cellulomonas biazotea*
LN	
L5S1	*Bacillus coagulans*
L5S2	*Corynebacterium xerosis*
L5S3	*Cellulomonas biazotea*
L5S4	*Bacillus thuringiensis*
L5S5	*Enterobacter agglomerans*
L5S6	*Bacillus thuringiensis*