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COMPARISON OF BACTERIAL DIVERSITY IN WATER SAMPLES OF AQUACULTURE, AQUAPONIC AND HYDROPONIC SYSTEM

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ABSTRACT

Aquaponics is the production of aquatic organisms using less water while hydroponic is the production of vegetables and plants without soil. Microbial diversity in these culture systems includes bacteria, protists, fungi, and archaea. Among microbes, bacteria are a pivotal and crucial aspect of aquaponic, aquaculture, and hydroponic system, acts as the bridge connecting the waste of fish to the fertilizer for plants. Current research aims to compare the bacterial diversity in water samples of aquaculture, aquaponics, and hydroponic systems. For this purpose, water samples were taken from aquaculture, aquaponic, and hydroponic system. Aliquots of the samples were used for the isolation of bacterial species based on standard procedures. Colonies were purified after isolation by membrane filtration by twice subculturing using the method of streaking plate. Potential bacterial isolates were characterized and identified up to the specie level by following standard microbiological techniques. Different bacterial species were isolated from water samples during the study include *Aeromonas spp., Bacillus spp., Nitrosomnas spp., Nitrobacter spp., Psuedomonas spp., Acinetobacter spp., Enterobacter spp., Streptomyces spp., Escherichia coli* and *Staphylococcus spp. Bacillus spp*. present in all these systems. *Nitrosomnas spp*. and *Psuedomonas spp.*, show similarity in an aquaponic and hydroponic system. *Enterobacter spp., Streptomyces spp*., shows similarity in aquaculture and hydroponic system. It is observed that the water was full of beneficial and pathogenic bacteria which exhibited potential impact on the cultured fish and plant species.

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INTRODUCTION

The increasing population has triggered research on new agricultural practices in order to satisfy the need for healthy food (Fedoroff *et al*., 2010). "Aquaponic system is one of the technologies that has ecofriendly influence and is often taken as a mode for healthy food production system. This is the type of farming in which plants and fishes are grown together" (Endut *et al*., 2011). The ability to convert fish waste (nutrients, extra feed, excreta) from the aquaculture system to fertilizer is a benefit of the aquaponic system (Tyson *et al*., 2008). The system enables the production of high-quality organic vegetables, fruits, and meat by reducing the amount of water used. In addition, the demand for processed fertilizers has also decreased (Wongkiew *et al*., 2018). Media bed is more effective in the use of nitrogen as it provides surface area for the growth of bacteria than other types of system (Lennard and Leonard, 2006). Bacteria also prefer darkness, so darkening the biofilter will also help it function better

(Gregory *et al*., 2010). An aquaponic system requires bacterial diversity for fish and plants. Plants feed fish waste and decomposed food for nutrients. The system would perform poorly without bacteria; the fish may also die, as well as the crops would not flourish. In hydroponic, aquaponic, or aquaculture systems, bacteria are just as crucial as plants and fish. Bacteria convert fish waste into nitrates before plants can use these nutrients for growth. Bacteria, which act as the bridge connecting fish waste to plant fertilizers, are a critical and pivotal component of the aquaponic and hydroponic system. Some bacteria are pathogens of fish, plants, and humans. Optimal habitat for bacterial growth is created by the accumulation of fecal matter, organic and nitrogen compounds, and unconsumed food in biofilters (Rurangwa and Vedegem, 2015). In the absence of microbes, the system will continue to accumulate unconsumed food and ammonia, leading to fish fatalities. Bacteria grow well on the substrate or rocks that are present on the bottom surface of fish tanks. Rocks are used as a medium in which plants are grown. Fish waste and food settle at the bottom of the fish tank, and the bacteria work on this. While beginning an RAS, along with an aquaponic system, system cycling refers to the initial phase of establishing a bacterial colony. It requires 3 to 5 weeks throughout normal conditions; system cycling is a slow process that needs flexibility (Goddek *et al*., 2016). The ammonia, when incorporated into the system, serves as a basic source of food for the bacteria, which is a natural process that occurs in the system on its own. The bacteria form colonies and start to oxidize the ammonia into nitrite around 5–7 days of the very first incorporation of ammonia. The nitrite water levels might have begun to increase again after 5–7 days, attracting the bacteria (Daims *et al*., 2015). The cycling process is complete when the nitrite concentration is 0 mg/liter, the nitrate concentration is steadily increasing, and the ammonia level is even less than 1 mg/liter. This requires approximately 25–40 days in optimal circumstances, however, if water temperatures are low, it could require up to 2 months to complete. Enough colony of bacteria formed during that stage, and it is effectively transforming ammonia into nitrate. In aquaponics systems, biofilters and fish tanks are constructed similarly to RAS, with the goal of increasing nitrite (NO2) and total ammonia nitrogen (TAN) oxidation (Brown *et al*., 2013). As a result, all processes are likely to have the same nitrifying and denitrifying microbes. In aquaponics systems, bacteria can be found in any compartment: 1. Periphyton, 2. Roots of plants, 3. Bio filtrate 4. Feces of fish and media beds (Schmautz *et al*., 2017; Kasozi *et al*., 2020). The process of converting the waste of fish to ammonia (NH3), then nitrites, and finally nitrates is called the nitrogen cycle. The ability of bacterial diversity to colonize probably depends on water flow, dissolved oxygen (DO), material (metal or plastic), and aquaponic system design. Water quality can affect the behavior and composition of bacterial communities with in an aquaponic, hydroponic, and aquaculture system including DO, pH, nutrients, CO_2, lightening, feed, flow rate of water, electrical conductivity, and redox potentials (table 1) (Junge *et al*., 2017). Surface area refers to the square feet within a system that can house bacteria. Increasing surface area can increase the abundance and diversity of types of bacteria. Current research aims to compare the bacterial diversity in water samples of an aquaculture, aquaponic, and hydroponic system. Microbes are beneficial and harmful to fish and plants, so it is important to investigate the bacterial diversity in water. It is intended to distinguish bacterial diversity in aquaponic because of bacterial role in ammonification. Moreover, there is a dire need to have information about any pathogenic bacteria in the aquaponic system. So, some control measures may be adopted for the proper functioning of aquaponic and hydroponic systems.

Table 1. Ranges for Water quality parameters for bacterial growth.

Ranges for parameters			
pH of water	6-7	Ammonia NH_3	0ppm
Temperature of water	64-86°F	Dissolved oxygen	5-8ppm

MATERIALS AND METHODS

Sampling

Water samples were taken aseptically and labelled appropriately for the comparison of bacterial diversity from aquaponic, hydroponic and aquaculture. Analysis of these samples was carried out in the microbiological laboratory of Government College Women University.

Physicochemical and Bacteriological Analysis

The temperature, pH, DO, EC, and NH_3 of water samples were determined. Triplicates of the water sample were

serially diluted, and appropriate 1 ml sample size were inoculated into a Petri dish and incubated at 37°C for 24-48 hours (APHA, 1995). Total bacteria counts were determined from these sources, and Gram and spore staining was performed (Calheiros *et al.,* 2009a). Throughout the study, Winogradskys (WP I and WP II), Mac Conkey agar (MAC), eosin methylene blue (E. M. B.), and nutrient agar (NA) were commonly used. Subculture was then performed until pure isolates were transferred onto agar slant in McCartney or super bottles and kept in the refrigerator at 4°C as a stock culture for subsequent tests during identification. To avoid contamination, this procedure was carried out aseptically.

Characterization and Identification

There were bacterial colonies counted using a magnifying glass and the naked eye once the bacteria had grown over the plates. The surface, size, shape, colors, margins, and elevation of bacterial colony were all noted. The surface-plate counting method was used to determine colony forming units for bacterial count (Calheiros *et al.,* 2009a). All the pure isolates were also determined for their Gram staining, endospore, and acid-fast staining. The biochemical test was performed to identify the unknown cultures which include Catalase, Citrate test, Starch hydrolysis, urease, indole test, Mannitol fermentation, Glucose fermentation, Mannitol, Maltose fermentation, Ammonia utilization Oxidase, VP and Motility test (Collins *et al.,* 1989).

RESULTS

This study helps to determine the bacterial properties and some ecological parameters of water sources from the systems. Table 2 shows some physico-chemical properties including the dissolved oxygen (DO) ranges from of 6.9ppm (sample 1) to a 7.8ppm (sample 2), temperature ranges from low unit of 27°C (sample 1) to a high temperature of 28°C (sample 2), NH_3 ranges from low unit of 1.34 (sample 3) to a high 1.46ppm (sample 1) and the values of pH which range from pH 6.7 (sample 1) to a high range of pH 7.1 (sample 3). Table 3 shows the total bacterial counts of the samples. The bacterial load ranged from low of 17×10^4 cfu/ml in sample 3 to a high of 24×10^4 cfu/ml in sample 2. Morphological characteristics and some biochemical characteristics including Gram staining reaction were used to give probable identity of the isolate (Table 4). Different bacterial species obtained during the study include *Aeromonas spp., Bacillus spp., Nitrosomnas spp., Nitrobacter spp., Psuedomonas spp., Acinetobacter spp., Enterobacter spp., Streptomyces spp., Escherichia coli* and *Staphylococcus spp.* (Figure 1).

Table 2. Physicochemical analysis of water collected in various aquaponic, hydroponic, and aquaculture locations.

Water Sample point	Temp °C	DO ppm	EC ms	NH_3 ppm	pH
Recirculating fish tank 1 (sample 1)	27	6.9	3	1.46	6.7
Recirculating fish tank 2 (sample 2)	28	7.1	8.3	1.34	6.9
Media grow beds (sample 3)	28	7.8	8.2	1.34	7.1

Table 3. Total Bacterial counts (CFU/ml) of water samples.

Water Samples	Total bacterial count ($\times10^4$ cfu/mL)
1	19
2	24
3	17

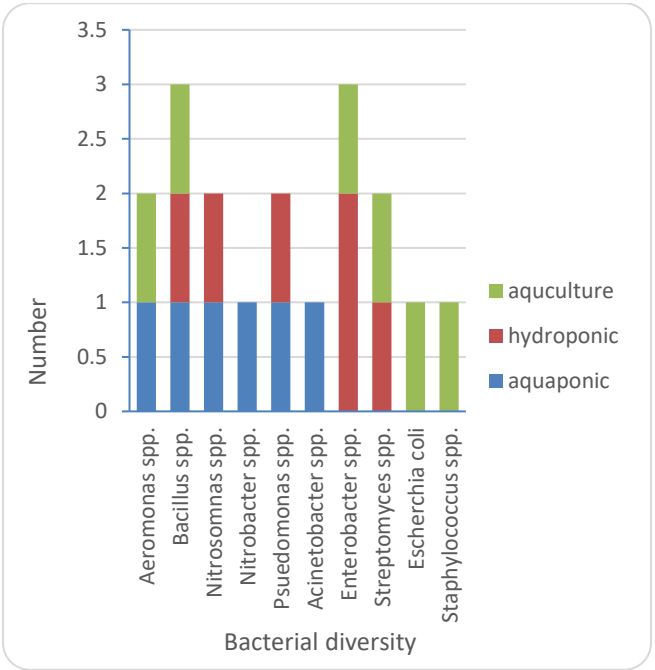

Figure 1. Comparison of isolated bacteria in aquaculture, aquaponic and hydroponic system.

DISCUSSION

Captive Bacteriological examination of selected sites of waters in the system of GC Women University FSD for the detection of various bacteria and their population could be found in different sites of systems was carried out in this study.

Table 4. Biochemical properties of water utilizing bacterial isolates.

Different sources	Isolates	Gram stain	Spore formation	Cell shape	Colony color	Motility	Methyl red	Catalase test	Oxidase test	Indole production	Vogus-proskour	Starch hydrolysis	Utilization of Glucose	Mannitol fermentation	Maltose	Citrate test	urease	Ammonia utilization	Identified bacteria
Aquaponic system	NA1	-	-	Short rods	Dark green	+	+	+	+	-	+		+	+	+				*Aeromonas spp.*
	NA2	+	+	Long Rods	White		-	+	-	-	-	+	+	-		-	+		*Bacillus spp.*
	NA3	-	-	Rods	Yellow	+	+	+	-	-	-					-	-	-	*Nitrosomnas spp.*
	NA4	-	-	Rod	Yellowish	-	+	+	-	-	-					-	-	+	*Nitrobacter spp.*
	NA5	-		short rod in cluster	Creamy white		-	+	+	-	+	+	-	+	+				*Psuedomonas spp.*
	NA6	-		Coccobacilli	White	-	-	+	-	-	-	-	+	-		+	variable		*Acinetobacter spp.*
Hydroponic system	NA7	-	-	short rod in cluster	grey like pale pink	+	-	+	-	-	+	-	+	+	-	+			*Enterobacter spp*
	NA8	-	-	short rod in chain	Whitish opaque	+	-	+	-	-	+	-	_	+	-	+			*Enterobacter spp*
	NA9	-	-	Rods	Yellow	+	+	+	-	-	-					-	-	-	*Nitrosomnas spp.*
	NA01	+	+	Rods	White		+	-	-	-	+	+	-	-		-	-		*Streptomyces spp.*
	NA02	+	+	Rods	White		+	-	-	-	+	+	-	-		-	-		*Bacillus spp.*
Aquaculture	NA03	-		short rod in cluster	Creamy white		-	+	+	-	+	+	-	+	+				*Psuedomonas spp.*
	NA04	-	-	Short rods	Dark green	+	+	+	+	-	+		+	+	+				*Aeromonas spp.*
	NA05	+	+	Rods	White		+	-	-	-	+	+	-	-		-	-		*Streptomyces spp.*
	NA06	-	-	short rod in cluster	grey like pale pink	+	-	+	-	-	+	-	+	+	-	+			*Enterobacter spp*
	NA07	-	-	Short rod	Whitish grey	-	+	+	-	+	-	-	+	-	+	-			*Escherchia coli*
	NA08	+	-	Cocci	Golden yellow	-	-	-	-	-	-	+	-	-	+	-			*Staphylococcus spp.*
	NA09	+	+	Rods	White		+	-	-	-	+	+	-	-		-	-		*Bacillus spp.*

Aquaculture organisms take food and secrete waste, which is then converted to nutrient for plant growth by nitrifying bacteria. In this study, water recirculation begins with aquaculture water aimed directly to a system for culturing nitrifying bacteria, accompanied by a hydroponic system as a water retention system for plant growth. Aquaculture microbial communities had inherent plant growth promoting characteristics that can be used as a natural insight to agriculture. Following Gram staining, all purified isolates were examined for cell shape, flagellation, motility, encapsulation, and spores. This study identified bacteria that look like *Aeromonas spp., Bacillus spp., Nitrosomnas spp., Nitrobacter spp., Psuedomonas spp., Acinetobacter spp., Enterobacter spp., Streptomyces spp., Escherichia coli* and *Staphylococcus spp.* species based on biochemical characterization. Bergey's manual of determinative bacteriology was used to identify the bacteria species isolated during this course of study (Buchanan and Cibbons, 1974). *Bacillus spp.* present in all this system. *Nitrosomnas spp.* and *Psuedomonas spp.,* show similarity in aquaponic and hydroponic system. *Enterobacter spp., Streptomyces spp.,* shows similarity in aquaculture and hydroponic system. Significant variations in its physiochemical properties, such as pH, water temperature, dissolved oxygen, hardness, chloride, and alkalinity, were observed in the current study. It appears that these variations in water quality parameters may favor disease outbreaks in fish culture farms (Walker, 2004). Infectious disease has been identified as one of the most significant constraints to efficient sustainable aquaculture, aquaponic, and hydroponic production, which focuses on food security, trade, socioeconomic development, market, and profitability. Pathogen persistence in the aquatic environment is also regarded as one of the most important factors in the transmission of infections, which eventually results in an acute outbreak of disease in fish tanks. Water samples were collected from various points of the aquaculture, aquaponic, and hydroponics systems that were adjacent to each other, so that the surrounding physio - chemical factors of the fish tanks regulated the amount and quality of microorganisms, which reflected the microbial load assessment. pH of any body of water should be neutral, ranging from 7.5 to 8.5. Fish have their own tolerable limits, and any changes in pH cause mass mortality. During the summer, the pH is slightly higher, but it is sufficient for the survival of fish, plants, and bacterial communities (Alikunhi, 1957). The concentration of dissolved oxygen is an important parameter used in assessing the suitability of a water body for supporting fish, plants, and bacterial communities (Banerjee and Srinivasan, 1983). Temperature is another important factor that has a greater impact on an aquatic ecosystem, and the ideal temperature for increased fish productivity was observed to be 20-30°C. However, the temperature obtained in this study ranged from 25 to 27°C. The comparison of the bacterial compositions of aquaculture, hydroponic, and aquaponic systems is gaining popularity (Schmautz *et al.,* 2017; Eck *et al.,* 2019). This discovery was motivated in part by the discovery of bacteria more closely associated with plant roots, such as *Lysobacter sp, Rhodobacterales* and *Rhizobiales*, which are known to benefit plant health and growth (Folman *et al.,* 2003). The identification of these bacteria is consistent with previous research on the bacterial composition of aquaponic systems (Schmautz *et al.,* 2017; Wongkiew *et al.,* 2018). Streptomyces, a relatively common bacterium, was found in aquaculture but not in the aquaponic system. Schmautz *et al*. (2017) reported the absence of Streptomyces in an aquaponic system and suggested that this was due to low phosphorus levels in the water caused by the plants' continuous uptake. Bacillus sp. was found in these systems, which are well-known probiotics that likely originated from their additions at the start of this study to grow the bio filter. Furthermore, *B. thuringiensis* was only found in the aquaponic system, most likely because of spraying the leaves with this product once a week to discourage caterpillars and other insects. Several Lactobacillus species, as well as fish pathogens such as *Aeromonas hydrophila*, *Vibrio sp.,* and *Pseudomonas aeruginosa*, were only found in the aquaponic system. This discovery suggested that the enriched nitrifying microbial community could be used as a microbial inoculum in organic hydroponics. As a result, the nitrification process using enriched nitrifying microorganisms is a practical model for managing microorganisms in organic hydroponics. Plant pathogens were discovered in the aquaculture system, but not in the aquaponic system. It may thus be worthwhile to investigate the effects of fish and plant probiotics on productivity. As a result, proper water quality parameters and good hygiene practices must be an exceptional source for preventing microbial diseases in aquaculture, aquaponics, and hydroponic systems. It should be noted that the outbreak of bacterial diseases occurs only when

the bacterial load in fish culture systems increases. It is also suggested that the culture species become more vulnerable as the bacterial load in the water increases. To avoid bacterial outbreaks, good cultural management practices and continuous monitoring of benthic microbes are essential. Ajayi and Okoh (2014) *Shigella spp., Pseudomonas* spp*., Klebsiella* spp*., Proteus* spp. and *Staphylococcus aureus, Citrobacter* spp., *Bacillus* spp*., Streptococcus* spp*.,* and *Enterobacter* spp., studied bacteriological status in aquaculture. According to Abedin *et al.,* (2020) *Vibrio spp., Pseudomonas spp., Staphylococcus spp., Edwardsiella spp., Aeromonas spp., Flavobacterium spp., Citrobacter spp.,* and *Enterobacter spp.* were the eight species of isolated bacteria with the highest frequency of occurrence in aquaculture. The average water temperature, pH, and ammonia were 27.3°C, 7.6 and 0.87mg/L, respectively, based on the physiochemical properties pond water samples. Within these five types of ponds, there was a significant difference in physiochemical parameters such as water temperature, pH, and ammonia. As a result, detecting fish diseases, beneficial or harmful bacteria, and other agents is critical for the conservation of water resources. According to the findings of Huang *et al.,* (2016) *Nitrosomonas* and *Nitrospira* were found in higher numbers. *Nitrosomonas* and *Nitrospira* appeared to play a significant part in the nitrification process. According to research of Sarada *et al.,* (2016) *Enterococci* were found in the mostly in aquaculture. Other bacteria found in the fishponds included *Bacillus subtilis, Staphylococcus aureus, Klebsiella, Micrococcus, Enterobacter, and E. coli*, *Enterobacter, Bacillus subtilis, Klebsiella* and *Micrococcus* look-alike bacteria were found in this study based on biochemical and phonetic characterization. The ponds were found to be heavily contaminated with harmful microorganisms, particularly bacteria, which pose a significant risk to the cultured fish. Chitmanat *et al.,* (2015) studied *Flavobacterium column* and *Aeromonas hydrophila* in an aquaponic system.

CONCLUSION

The purpose of the present work was the comparison of bacterial diversity in water samples of an aquaculture, aquaponic and hydroponic system. The experiment was done at the aquaponic system research area of GC Women University. Bacterial species identified from aquaponics, aquaculture and hydroponic system includes *Aeromonas spp., Bacillus spp., Nitrosomnas spp., Nitrobacter spp., Psuedomonas spp., Acinetobacter spp., Enterobacter spp., Streptomyces spp., Escherichia coli* and *Staphylococcus spp.* Water is most important in aquaculture, aquaponics, and hydroponic systems as it is a home for fish. It also maintains nutrient flow in aquaponics systems. The most important bacterial strains that are useful or hazardous in aquaculture, aquaponics, and hydroponic systems also utilize water as medium. Aquaculture microbial communities possessed inherent plant growth-promoting qualities that might be employed as a natural agricultural input. This study demonstrates the presence of some potentially harmful contaminants in water sources that pose an ecological risk and are harmful to human health. This knowledge could aid in the improvement of fish and plant production. As a result, much work should be done to reduce the microbial load of ponds and to ensure that the water used to supply is free of harmful bacteria to prevent harmful bacteria from entering the systems. Furthermore, prevention of diseases in fish and plants should be accomplished by improving culture practices and fish health management to ensure the best yields and product quality.

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