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Pathogenic Bacteria in *Oreochromis Niloticus* Var. Stirling Tilapia Culture

Huicab-Pech ZG, Castaneda-Chavez MR* and Lango-Reynoso F

National Technological Institute of Mexico/Technological Institute of Boca del Rio Veracruz, Mexico

***Corresponding author:** Castaneda-Chavez MR, National Technological Institute of Mexico/Technological Institute of Boca del Rio Veracruz, Mexico, Tel: +52 229 986 0189; E-mail: castanedaitboca@yahoo.com.mx

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Abstract

Tilapia *Oreochromis niloticus* is an aquaculture resource that represents one of the most popular crops in the world. However, species cultivation presents health problems, which are associated with the presence of pathogenic bacteria and causes high economic losses. The aim of this study was to determine the diversity of these bacteria at the genus level in the species *O. niloticus var.* Styrling during growing stage in the fattening and pre-fattening phases. Tilapia samples were collected and analyzed; each sample was subjected to a macroscopic external and internal observation of organs and tissues. Subsequently, samples were evaluated by microbiological tests using Trypticase Soy Agar (TSA), Thiosulfate Citrate Bile Salts Sucrose Agar (TCBS) and selective media (Pseudomonas sp. Group), and conventional biochemical tests aimed at the production of glucose, sucrose, lactose, oxidase, catalase, indole, ornithine and Gram staining. External analysis revealed clinical signs of disease such as skin bleeding, body ulceration, corneal opacity, and intestine and vesicle inflammation. Microbiological and biochemical analysis showed the presence of eleven bacterial genera known as *Arthrobacter sp., Enterococcus sp., Staphylococcus sp., Micrococcus sp., Streptococcus sp., Aeromonas sp., Pseudomonas sp., Edwardsiella sp., Flexibacter sp. and Flavobacterium sp.,* with a predominance of 55% Gram-negative bacilli in tilapia crops. According to the results, it is necessary to take preventive and corrective measures in order to avoid possible risks during production cycles, mainly when handling organisms. It is also important to promote good crop management practices and quality systems in production units to benefit the aquaculture sector.

Keywords: Diversity; Bacteria; Disease; Microbiological; Biochemical

Introduction

Aquaculture is considered an activity that contributes to the production of foods of high nutritional value, generating employment and economic income for the world population. In addition, it strengthens the source of inputs for the food industry and foreign exchange for the country [1]. During 2014, aquaculture reached 73.8 million tons with an estimated value of USD 160.2 billion [1]. According to the statistics, from 1970 to 2008 aquaculture production presented an annual growth of 8.3%, with a per capita consumption of 0.7 to 7.8 kg per person, which meant a 6.6% increase of the annual average. However the apparent fish consumption per capita will be 21.8 kg in 2025, this consumption is associated with the improvement of distribution channels, increase in fish production and urbanization [1]. At present, the aquaculture productive sector is made up of species that are grown in fresh water reaching a world production of 59.9%. Tilapia cultivation, as an introduced species, has favoured aquaculture development in recent years, because its cultivation shows high resistance in the chain production [1]. Among other characteristics, 99% of this species is grown outside its natural habitat and is positioned as the second species cultivated worldwide, mainly in countries such as Philippines, Indonesia, Thailand, Malaysia, China, Chile, Mexico, Ecuador, Brazil, and Colombia, with productions that exceed the cultivation of salmonids and carps [2]. Among commercial species are *O. niloticus, O. mossambicus, O. aureus, O. hornorum, Tilapia rendalli and Tilapia zilli* [1]. Tilapia cultivation in Mexico has shown an increase in production, favouring the country's economy with yields of 12,529 t [3]. Mexico reaches development levels ranging from the experimental scale as it happens with white fish, native mojarra, abalone, scallops, mussel, lobster and snail, to industrial-commercial production of species such as catfish, carp, tilapia, trout, oysters, shrimp and prawns. The state of Veracruz reports a production of tilapia *O. aureus and O. niloticus* of 74,659 kg and 121,459 kg, respectively [4].

The success of tilapia production is due to the fact that the species exhibits rapid growth, ease of propagation, tolerance to environmental conditions, easy acceptance of natural foods and dietary supplements, and resistance to diseases. *Oreochromis sp.* is resistant to bacterial diseases caused by stress as a result of high planting densities and minimal control in crop management. The state of disease in fish is completed from the appearance of clinical signs by bodily alterations and the behaviour of the organism. According to Rodriguez et al. [5], diseases are classified by physical, chemical and biological risks, and the disturbing result is due to the association of one or two factors, which causes a physiological alteration of the organism [6]. There are two categories of disease called infectious and non-infectious diseases. The first is related to the pathogenic organisms present in aquatic environment and in fish, which causes contagious diseases, therefore treatments to control bacterial outbreak are required. While non-infectious diseases are related to environment, for example those problems associated with biotic and abiotic factors, minimum management of good practices, deficits in the nutrition of the organisms, and genetic abnormalities in relation to the quality of progeny. MSD Animal Health says that the mortalities correspond to the increase of seed densities (number of fish/m^3) and species introduction are the result of the appearance of emerging diseases [7,8]. Snieszko [9] mentions that disease is related to the interaction between fish, the pathogen and the aquatic environment as habitat,

that is, when an organism is exposed to pathogenic bacteria in unfavourable environments, where poor quality of water or excess of organic matter prevails. The incidence of disease is higher because balance between host, guest and aquatic environment is broken [6]. However, fish exhibit a high bacterial diversity, a symbiotic effect among bacteria, which protects them to adapt to nutritional changes and assimilation of food in the digestive tract [10]. Among bacteria that cause mortality in tilapia pathogens of Flavobacterium columnare stand out, *Edwardsiella tarda, Aeromonas sp., Vibrio sp., Francisella sp., Streptococcus iniae, Streptococcus agalactiae, Vibrio anguillarum, V. harveyi, Photobacterium damsele subsp.* [11]. Some of these pathogens have a geographical distribution in tropical and temperate regions where warm water species, such as Nile tilapia, are commonly grown. Among the most important bacteria, the species Aeromonas hydrophila is the causal agent of hemorrhagic septicemia syndrome or red pest on skin [12], it is also considered as an opportunistic and contaminating pathogen in aquaculture environments. Aeromonas hydrophila has a prevalence of 10% and 85% at any stage of culture and is considered the most important bacterial agent in freshwater fish, especially rainbow trout, tilapia, including ornamental and marine fish [13-15]. Another pathogen is *Streptococcus agalactiae*, which is characterized by septicemia and meningoencephalitis in fish. It has a prevalence of 40% and 70% in stages of fry, juvenile and fattening. It is also reported in several species of fish around the world, due to its geographic distribution includes regions with temperate and tropical climate, as presented by Brazil, China, Malaysia and the United States [16-18]. Some diseases are caused by not applying sanitary protocols on growing farms, as well as the use of antibiotics as an almost mandatory preventive or corrective practice. In this context, the main objective was to identify bacterial diversity with pathogen potential in *O. niloticus var. Styrling* during pre-fattening and fattening phases, to suggest management alternatives related to the implementation of quality systems in the production processes of aquatic organisms.

Materials and Methods

Study area

Aquaculture production units are located between 18°53' 35.64" north latitude and 95°56' 40.89" west longitude, in the municipality of Alvarado, Veracruz, Mexico. The production units consist of 15 rustic ponds of 0.5 ha in average and 12 circular concrete ponds of 12 m in diameter with a capacity of 200 m^3. Aquaculture farms also have their own water system and a sedimentation lagoon with groundwater supply.

Collection of organisms

Organisms were collected in aquaculture production units in order to perform analyzes corresponding to the identification of pathogenic bacteria. The weight of each organism was taken into consideration to choose the ones with a final weight between 350 gr and 450 gr. The whole process was carried out under aseptic conditions. Each sample of organism was protected following the chain of custody, placed in polyethylene bags with water at 40% capacity. They were transported in containers at 4°C (NOM-109-SSA1-1994; [19]) to the Research and Aquatic Resources Laboratory of the Technological Institute of Boca del Río, Veracruz.

Taking physicochemical parameters of water in the in the culture ponds

Water parameters were measured monthly at the sampling points. Oxygen (mg/L), temperature (°C), salinity (ppm) and pH were recorded with a multi-parameter probe YSI 556 MPS. Nitrites (mg/L) and nitrates (mg/L) were determined with the CHEMets® Colourimetric Test Kit.

Bacterial identification and processing

Each organism was observed in the laboratory by macroscopic external for the identification of lesions in skin and fins. A ventral section was then made to observe internal organs such as gills, intestine, spleen, liver, gallbladder and kidney.

These organs were selected to determine bacterial genus and isolate pathogens. A minimum portion of the sample was taken with a platinum handle and seeded in duplicate by cross-streaking in boxes of Trypticase Soy Agar (TSA), Thiosulfate Citrate Bile Salts Sucrose Agar (TCBS) and selective media for the Pseudomonas group (Pseudomonas F Agar) The incubation time was 24-48 hrs at 34°C. After bacterial growth, some colonies were selected to perform Gram staining and biochemical and presumptive tests, mainly motility, indole, catalase, ornithine, triple sugar iron agar, methyl red, and Voges-Proskauer tests [20]. These tests are explained below:

For the Gram staining test a small sample was taken with a platinum handle and spread over the slide. The extension was fixed by heat, passing it gently over the flame of the burner until dry. Staining was performed with crystal violet or gentian violet, covering the sample homogeneously, this solution is allowed to work for one minute at 25°C. Briefly rinsed with water, air dried and Lugol was added for one minute, and then continue washing it with water. The discolouration was performed with alcohol for about 15 to 20 seconds and subsequently washed with water. Safranite was used, as contrasting staining, for 15 seconds, followed immediately with a water wash and allowed to dry. Direct observation, under a microscope, continued where Gram (+) bacteria showed a purple colour and Gram (-) bacteria a pink or red colour.

The catalase test was carried out with a sterile straight bacteriological loop, taken from the center of a pure colony of 18 to 48 hours and placed on a clean glass slide. Then, a drop of Pasteur 30% hydrogen peroxide (H_2O_2) was added. A positive result is evidenced by bubbling and no bubbling as negative.

Motility test, indole and ornithine (MIO) was performed in 3 ml tubes with MIO agar, in each tube the microorganism strains were seeded in the culture medium and incubated for 24 hours at 37°C. After 24 hours of incubation, the mobility test took as positive those that generated turbidity or growth beyond the line of sowing, while the negative ones were those that showed growth only in the line of seed. The indole test was determined by the Erlinch or Kovacs Reagent, the medium was allowed to warm to room temperature prior to inoculation. The tubes were inoculated with a platinum handle, where a portion of pure culture was transferred and incubated at 37°C for 40 to 48 hours. Then 0.5 mL of the Kovac Reagent was added and gently shaken to determine the production of Indol. It was taken as positive when adding the reagent presented the formation of a red colour band at the top of the medium. A yellow colour denotes a negative indole after addition of Kovacs Reagent.

A KIA agar was used for the Kligler test. By tilting, a loop of pure culture was taken from a well-isolated colony of the bacteria and in the culture medium agar it was stabbed to the bottom for later to perform a seeding striation on the bezel. They were incubated for 24 hrs. The results were observed to determine the different characteristics that this test indicated. It was also observed if it had bubble formation, which would indicate the production of gas and the blackening indicated the production of hydrogen sulphide.

For the methyl red tests 3 ml tubes and MR-VP broth were used as the culture medium. A bacterial colony was taken and seeded by shaking, with 24 hours incubation. After incubation, 5 drops of the red methyl indicator were added, the bright red colour indicates a positive reaction and the yellow colour indicates a negative one.

For the Voges-Proskauer (VP) test tubes were used with MR/VP broth where an aliquot of the colony was placed and seeded by shaking in the broth, with an incubation of 24 hrs. After the incubation, 0.2 ml of potassium hydroxide (KOH) was added; the positive result is indicated by a pink-red colour and the negative result by a yellow or copper colour.

As a presumptive test the presence of the cytochrome oxidase complex was determined with Bactident oxidase indicator strips (Merck, Merck KGaA Germany®) for each bacterial colony. The results scored positive showing a violet colour and negative when there were no changes in colouration [20].

Statistical analysis

Statistical analysis was performed using Excel and presence/absence of pathogens. The physicochemical parameters are presented as mean ± SE.

Results

Physicochemical parameters of crop water

The physicochemical parameters of water in pre-fattening and fattening ponds were found within the limits for the cultivation of Nile Tilapia *O. niloticus var. Styrling* (Tables 1 and 2).

Physicochemical parameters of the culture water						
	Temperature (°C)	**Oxygen (mg/L)**	**pH**	**Salinity (ppm)**	**Nitrites (mg/L)**	**Nitrates (mg/L)**
March	22.5 ± 1.12	7.5 ± 1.9	9.1	0.28	0.25	0.25
April	22.9 ± 0.91	7.7 ± 1.83	8.9	0.27	0.5	0.5
May	23.1 ± 0.77	7.4 ± 1.93	9.2	0.29	0.25	0.25

Table 1: Physicochemical parameters of water in pre-fattening ponds.

Physicochemical parameters of culture water						
	Temperature (°C)	**Oxygen (mg/L)**	**pH**	**Salinity (ppm)**	**Nitrites (mg/L)**	**Nitrates (mg/L)**
March	23 ± 2.34	7 ± 2.26	8.07	0.31	0.25	0.25
April	24 ± 1.14	6.9 ± 2.21	8.5	0.3	0.5	0.5
May	24.8 ± 0.97	7 ± 2.20	8.3	0.32	0.25	0.25

Table 2: Physicochemical parameters of water in fattening ponds.

External analysis of Tilapia *Oreochromis niloticus*

External analysis showed cynical signs of disease caused by handling in production units. The signs commonly found in the two phases of culture are related to bleeding skin, body depigmentation, frayed fins, distended gallbladder, liver discolouration, body ulceration, corneal opacity and intestine inflammation (Figure 1).

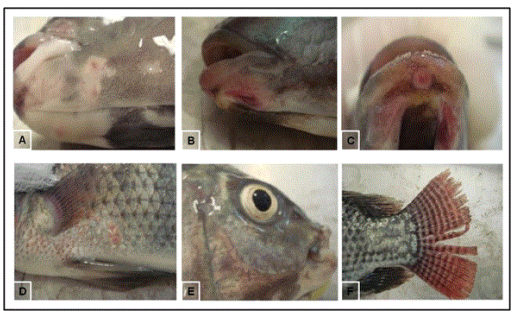

Figure 1: External signs of damage and anomalies caused by mismanagement and handling of organisms. A, B and C: Skin discolouration, D: Skin bleeding, E: Corneal Opacity, F: Frayed fins.

Taxonomic composition of bacteria found in species of Tilapia *Oreochromis niloticus*

Eleven bacterial genera were identified in pre-fattening and fattening tilapia, foremost among them *Arthrobacter sp., Enterococcus sp., Staphylococcus sp., Vibrios sp., Micrococcus sp., Streptococcus sp., Aeromonas sp., Pseudomonas sp., Edwardsiella sp., Flexibacter sp. and Flavobacterium sp.* According to the biochemical tests, Gram staining verified the presence of 55% Gram-negative organisms predominating *Aeromonas sp., Pseudomonas sp., Edwardsiella sp., Flexibacter sp., and Flavobacterium sp.;* and 45% Gram-positive organisms *Arthrobacter sp., Enterococcus sp., Staphylococcus sp., Micrococcus sp. and Streptococcus sp.* (Figures 2 and 3, Tables 3 and 4).

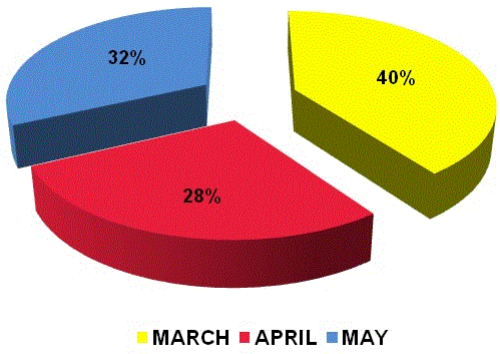

Figure 2: Distribution of bacterial pathogens in *O. niloticus* Var. Stirling.

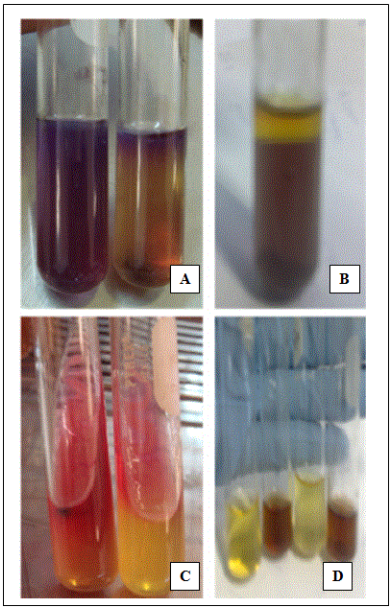

Figure 3: Reaction of biochemical tests. A: Ornithine test; B: Indole test; C: Triple sugar test; D: MR-VP test.

Conforming to presence/absence of pathogens, it is observed that in the sampling months of March, April and May, where *Aeromonas sp.*, *Pseudomonas sp.*, *Vibrios sp.*, and *Enterococcus sp.* were detected; March turned out to be the month with the highest incidence of bacterial genera compared to April and May (Tables 3 and 4).

The presence of five bacterial genera was registered, during the fattening stage, *Aeromonas sp., Pseudomonas sp., Vibrios sp., Enterococcus sp.,* and *Micrococcus sp.*, with a higher incidence in March, April and May. The highest presence of pathogens, during this stage, was determined mainly in March and May (Tables 3 and 4).

Months	Pre-fattening	Fattening
	Bacteria	
March	*Aeromonas sp.*	*Aeromonas sp.*
	Edwardsiella sp.	*Streptococcus sp.*
	Streptococcus sp.	*Staphylococcus sp.*
	Staphylococcus sp.	*Pseudomonas sp.*
	Pseudomonas sp.	*Vibrios sp.*
	Vibrios sp.	*Flexibacter sp.*
	Flexibacter sp.	*Enterococcus sp.*
	Arthrobacter sp.	*Micrococcus sp.*
	Enterococcus sp.	
April	*Aeromonas sp.*	*Aeromonas sp.*
	Pseudomonas sp.	*Edwardsiella sp.*
	Vibrios sp.	*Pseudomonas sp.*
	Arthrobacter sp.	*Vibrios sp.*
	Enterococcus sp.	*Enterococcus sp.*
		Micrococcus sp.
May	*Aeromonas sp.*	*Aeromonas sp.*

Table 3: Presence-Absence of pathogens in *O. niloticus* var. Styrling, Pre-fattening and fattening phases.

Bacteria	Gram		O	C	Mot	Ind	Orn	Lys	MR	VP
Arthrobacter sp.	+	C	+	-	-	-	-	-	-	-
Enterococcus sp.	+	C	+	-	-	-	-	+	-	-
Staphylococcus sp.	+	C	+	+	-	-	-	-	-	-
Micrococcus sp.	+	C	+	+	-	-	-	+	-	-
Streptococcus sp.	+	C	+	-	-	-	+	-	-	-
Aeromonas sp.	-	B	+	+	-	-	-	+	-	-
Vibrio sp.	-	B	-	+	+	+	+	+	+	-
Pseudomonas flourescens	-	B	+	+	-	-	-	+	-	-
Edwardsiella sp.	-	B	-	+	-	-	-	-	-	-
Flexibacter sp.	-	B	+	-	-	-	-	-	-	-

APRIL										
Enterococcus sp.	+	C	+	-	-	-	-	+	-	-
Staphylococcus sp.	+	C	+	+	-	-	-	-	-	-
Micrococcus sp.	+	C	+	+	-	-	-	+	-	-
Aeromonas sp.	-	B	+	+	-	-	-	+	-	-
Vibrio sp.	-	B	-	+	+	-	-	+	-	-
Pseudomonas sp.	-	B	+	+	-	-	-	-	-	-
Edwardsiella sp.	-	B	-	+	-	-	-	-	-	-
MAY										
Streptococcus sp.	+	C	+	-	-	-	+	-	-	-
Enterococcus sp.	+	C	+	-	-	-	-	+	-	-
Micrococcus sp.	+	C	+	+	-	-	-	+	-	-
Aeromonas sobria	-	B	+	+	+	-	+	+	-	-
Flavobacterium columnare	-	B	+	+	-	-	-	-	-	-
Pseudomonas sp.	-	B	+	+	-	-	-	+	-	-
Vibrios sp.	-	B	-	+	+	+	+	+	+	-
Edwardsiella sp.	-	B	-	+	+	+	+	+	-	-

Table 4: B: Bacillus: Coconuts, O: Oxidase, Ca: Catalase, Mot: Motility, Ind: Indol, Orn: Ornithine, Lys: Lysine, MR: Methyl Red, VP: Vogues-Proskeaur.

Discussion

During the study, it was shown that the presence of pathogenic bacteria is not significantly related to the physicochemical parameters (PQ) of water, i.e., the PQ parameters are not considered as indicative of disease in the production farm. In addition, they are within the optimal values for tilapia cultivation [21,22]. In contrast, *Aeromonas hydrophila* is related to sudden changes in temperature, dissolved oxygen and inadequate nutrition, as pointed out by Conroy [23-25] who indicate that the constant variation of physicochemical parameters is a stress factor that benefits the outbreak of disease caused by opportunistic bacteria. However, in the study there was no direct relationship with physicochemical parameters and the presence of bacterial genera.

External analysis showed clinical signs of disease, foremost among them skin hemorrhages, corneal opacity, body ulceration, liver discolouration, frayed fins and intestine and vesicle inflammation, these abnormalities are considered as the main symptoms of infection reported by Giordano, et al. [17,26,27], who observed signs such as skin alterations, anorexia, exophthalmia, corneal opacity, extension of the visceral cavity, bleeding and abdominal inflammation, hepatomegaly and splenomegaly. According to Soto [28] it is proven that bacteria are the cause of epithelial hyperplasia in gills, splenomegaly, renomegaly and necrosis in internal organs, mainly in spleen, heart, liver, kidney, brain and musculature. For example Yardimci, et al. [29-31] indicate that species *Aeromonas hydrophila* is associated with hemorrhages in gills and skin, weakness and anorexia, as well as vision loss by breaking' orbital eyes, the above agrees with those reported by Clavijo et al. [32], who reported that the presence of genus *Edwardsiella sp.,* causes septicemia in internal and external organs, including kidney, liver and spleen, skin, rectum, fins, abdominal inflammation, and opaque eyes. Some pathogens are transmitted horizontally as indicated by Mauel et al. [33], who pointed out that bacteria use water as a precursor vehicle, causing fish-to-fish outbreaks by direct contact. Newman [34] also mentions that the degree of pathogenicity depends on species resistance and environmental conditions. Because some pathogens are present in environments with high temperatures, poor water quality and accumulation of organic matter, these conditions allow bacterial adhesion and replication in host cells.

In this sense, the management and handling of organisms in this study is considered inadequate, since the activities carried out in the production phases do not comply with the Manual of Good Practices established by the National Service of Health, Safety and Agro-Food Quality [35], which demand the references established by the United Nations Organization (UNO), Food and Agriculture Organization (FAO), and the World Health Organization (WHO), through the Codex Alimentarius Commission; concentrated on water management, food handling, handling of chemicals and drugs, and product safety during harvesting. These safety measures reduce risks of biological, physical and chemical contamination and avoid possible losses caused by diseases, in this case losses by opportunistic bacteria.

In the pre-fattening and fattening phases, Gram-positive and Gram-negative bacteria predominated, which agrees with [36], which determine a prevalence of 77% of Gram negative bacilli in the intestinal flora of tilapia and a prevalence >10% of bacteria such as *Aeromonas hydrophila, Swewanella putrefaciens, Corynebacterium urealyticum, Escherichia coli and Vibrio Cholerae.* Some of the bacteria found in the present study are considered native to aquatic environment, such as *Aeromonas hydrophila* and *Vibrios sp* [36]. The presence of various bacterial genera varies according to the growth of organisms. However, the reason for its diversification is due to food consumption and water quality. Gram negative bacteria is the main cause of bacterial disease, for example Austin et al. [37] mention that genus *Aeromonas sp* causes *furunculosis* and *hemorrhagic septicemia* in skin, this coincides with Calvo et al. [38], who consider that Gram negative bacteria is listed at the margins of public health as well as its high impact by antibiotic resistance.

Pseudomonas sp., Staphylococcus sp. and *Aeromonas sp.* were found in pre-fattening and fattening phases. The incidence is related to tilapias grown in floating cages [28] also points out that these genera can provoke an epidemic outbreak due to its potential bacterial pathogen. Some reports by Al-Harbi et al. [39] indicate that the presence of bacteria in fish's digestive flora is normal. However, the outbreak of disease is related to the existence of a stress factor based on the interaction between fish, pathogens and aquatic environment as a natural habitat of the organism, as well as poor water quality or excess of organic matter factors which allows the incidence of disease to be greater, as mentioned by Huicab-Pech et al. [6]. Although fish exhibit high bacterial diversity, [9] points out that there is a symbiotic effect among bacteria, that is, the host adapts to nutritional changes and food assimilation in the digestive tract through bacterial balance.

Bacterial genera, found in the present work, belong to the lineage of species such as *Aeromonas hydrophila, Vibrio parahaemolyticus, Vibrio vulnificus, Vibrio cholerae, Streptococcus iniae, Pseudomonas spp., Edwardsiella sp., Flexibacter sp. and Flavobacterium sp.,* which present risk for human health; bacteria are autochthonous organisms

of the aquatic environment, including water and sediment. The presence, as an opportunistic pathogen, is due to the conditions of the aquatic environment and stress, as reported by Burr et al. [14,15,40-42] who consider that organisms under stress conditions are susceptible to the presence of opportunistic pathogens. These pathogens cause *hemorrhagic septicemia* and clinical signs of erratic or circling swimming, uncoordinated movements, anorexia or decreased appetite, exophthalmia, corneal opacity, visceral cavity extension, bleeding and abdominal inflammation, softening of the brain and liver, hepatomegaly and pallor in the organ, as well as splenomegaly and visceral adhesion commonly found in crops and studies at experimental level.

The genus *Sthapylococcus sp.* and *Micrococcus sp.* were presented in fattening organisms, however, according to Mhango, et al. [43,44] who relate lack of hygiene in the management of fish, since bacteria is common in human skin and in normal bacterial flora of freshwater fish. The culture of tilapia presented those bacteria that are in the NOM-115-SSA1-1994, reason why its infective activity is related to alimentary intoxications in human beings. In addition to that, some are classified like pathogens indicative of fecal contamination; such is the case of *Enterococcus sp., Vibrio sp.* and *Sthapylococcus* sp., as indicated by Soto [28], so that temperature variation, human settlements, amount of food supplied, nutritional quality and harvesting methods are related to the incidence of opportunistic bacteria in crops.

Conclusion

The occurrence of diseases is due mainly to several factors that act individually or jointly in an aquaculture crop, therefore, it is considered necessary the implementation of strategies for its optimal management, with the objective of achieving a sustainable production under safety and good management practices programs, as well as an alternative of effective vaccination as a preventive and corrective treatment. Having also the support of friendly treatments for crops and human being, in order to avoid direct and indirect losses, and to assure the production and success of *O. niloticus* crops.

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