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MICROBIOLOGICAL ANALYSIS OF TILAPIA AND WATER IN AQUACULTURE FARMS FROM SINALOA

ANÁLISIS MICROBIOLÓGICO DE TILAPIA Y AGUA EN GRANJAS ACUÍCOLAS DE SINALOA

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ABSTRACT

Tilapia is the most cultivated fish around the world. The fish's environment can harbor bacteria, especially coliforms, which are not normal biota of fish. These microorganisms are contamination indicator groups (IGs) reflecting the possible presence of pathogens, which can compromise the safety of fishery products. The IGs prevalence was estimated in tilapia and water from 29 aquaculture farms. Total and fecal coliforms (TC, FC), mesophilic aerobic bacteria (MAB), *S. aureus*, *E. coli, Salmonella* sp., and *Streptococcus* sp., were investigated. In tilapia, TC and FC were detected in 64% and 48% of the samples, ranging from 4.0×10^1 to 9.8×10^5, and from 1.0×10^1 to 6.4×10^3 CFU/g, respectively; MAB were detected in 90% of the samples. In water, TC and FC were detected in 57% and 35% of the samples, oscillating from 1.0×10^1 to 2.28×10^4, and from 1.0×10^1 to 1.2×10^3 CFU/mL, respectively. MAB were detected in 89% of the water samples. Mostly *E. coli* and *Enterobacter agglomerans* were detected. Generally, IGs concentrations meet the Mexican regulations; however, the tilapia's microbiological quality must be continuously monitored.

Keywords: Tilapia, Total coliforms, Fecal coliforms, Mesophilic aerobic bacteria, *E. coli***.**

RESUMEN

La tilapia es el pez más cultivado en el mundo. El ambiente de los peces puede albergar bacterias, especialmente coliformes, que no son biota normal piscícola. Estos microorganismos son grupos indicadores (GIs) de contaminación, reflejando la posible presencia de patógenos que pueden comprometer la inocuidad de los productos pesqueros. Se estimó la prevalencia de GIs en tilapia y agua de 29 granjas acuícolas. Se investigaron coliformes totales y fecales (CT, CF), bacterias mesófilas aeróbicas (BMA), *S. aureus*, *E. coli, Salmonella* sp. y *Streptococcus* sp. En tilapia, se detectaron CT y CF en 64% y 48% de las muestras, variando de 4.0 x 101 a 9.8 x 105, y de 1.0 x 101 a 6.4 x 103 UFC/g, respectivamente; las BMA se detectaron en 90% de las muestras. En agua, se detectaron CT y CF en 57% y 35% de las muestras oscilando de 1.0×10^1 a 2.28×10^4, y de 1.0×10^1 a 1.2×10^3 UFC/mL, respectivamente. Las BMA se detectaron en 89% de las muestras. Principalmente se detectaron *E. coli* y *Enterobacter agglomerans*. Generalmente, las concentraciones de los GIs cumplen las regulaciones mexicanas; sin embargo, la calidad microbiológica de la tilapia debe monitorearse continuamente.

Palabras clave: Tilapia, Coliformes totales, Coliformes fecales, Bacterias mesófilas aerobias, *E. coli*.

INTRODUCTION

Cultivation of aquatic organisms for human consumption is one of the food productive activities with higher development around the world, reaching growth percentages above other sectors such as agriculture and livestock. The demand for fish products has increased in recent years while in marine and inland waters capture has reached its extraction limit. Aquaculture is covering such deficit. In México, the aquaculture production levels for some species exceed that of capture production (Rábago-Castro, 2010).

The Food and Agricultural Organization in its 2016 world report on the state of fisheries and aquaculture (FAO, 2016) informed a total production of 167.2 million tons (93.4 from capture and 73.8 from aquaculture). In 2014, the aquaculture sector's contribution to the supply of fish for human consumption overtook that of wild-caught fish for the first time. Global fish production is supported by freshwater organisms such as tilapia, for which world demand is significantly increasing (FAO, 2016). In Mexico, tilapia culture ranks second only surpassed by shrimp farming (Mojica *et al*., 2010; CONAPESCA, 2015).

Tilapia poses the ability to grow in fresh, brackish or salt-water environments. However, it is very susceptible to microorganism attack, which can cause considerable economic losses. Moreover, fishing products are an important part of human diet which makes it important to ensure their microbiological quality and safety to prevent the onset of disease in consumers, while both the health of fish and the profitability of this activity, are protected.

In order to assess food microbiological quality, since detection of all microorganisms is not possible, contamination indicator organisms are sought (Campos, 1999). The indicator groups (IGs) are those who live in conditions sim-

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ilar to pathogenic agents (pH, temperature, nutrients, etc.) but are easier and economical to detect. In such a way, the presence of IGs is evidence that pathogens may be present (Vázques *et al.,* 2006).

The total coliform group (TC) indicates the possible presence of the Enterobacteriaceae family. These bacteria colonize the gastrointestinal tract of man and mammals, and are eliminated through the stool. Such contamination mainly comes through water and can cause disease in humans. The TC group includes aerobic and facultative anaerobic bacteria, bacillary, non-spore forming that ferment lactose with gas production in 48 h at 37 °C.

The fecal coliform (FC) group, include coliforms that can ferment lactose at 44.5 °C. These microorganisms, and particularly *Escherichia coli*, were selected as indicators of fecal contamination due to their relationship with the typhoid-paratyphoid group (*Salmonella typhi*) and their high concentration in different types of samples (water and foods). By being present almost exclusively in the feces of warm-blooded animals, these coliforms reflect better the presence of fecal contamination (Madigan *et al.,* 2009). Another IG is the mesophilic aerobic bacteria (MAB) or Heterotrophic plate count (HPC). These bacteria grow under aerobic conditions, at a temperature range between 35 and 40 °C and may be pathogenic or saprophytic; they are used as general indicators of the bacterial population that may be present in a product (Allen *et al.,* 2004) and reflect the hygiene with it has been handled.

Some salmonellosis outbreaks have been associated with seafood products contaminated with *Salmonella* sp. (Amagliani *et al.,* 2012). This bacterium became a challenge for the global food system from production to consumption (Bujjamma *et al.,* 2015). Other bacterium that can colonize fish and is related to gastrointestinal diseases in humans is *Shigella* sp. (Onyango *et al.,* 2009). Shigellosis has become a major health problem due to the minimal infectious dose of these microorganisms (10 cells of *S. dysenteriae*), among others (Baveja *et al.,* 2014).

Other contaminating bacterium related to the mishandling of products, is *Staphylococcus aureus*. In tilapia cultivated worldwide, *Staphylococcus* sp. causes diseases with high mortality rate (Austin y Austin, 2016). Moreover, in several countries in Central America, South America and the Caribbean the emergence of diseases associated with *Streptococcus* sp. in tilapia cultured in freshwater is confirmed, with mortalities up to 50%.

Because there are pathogenic bacteria in fish that can affect production and be transmitted to humans causing disease, it is necessary to ensure that the product meets the microbiological quality standards. The objective of this work was to investigate the presence of fecal contamination IGs or pathogenic bacteria for tilapia or humans (*e.g. Staphylococcus* sp., *Streptococcus* sp., *Salmonella* sp., and *Shigella* sp.) in Tilapia (adult and fry) and water (pond and supply) from aquaculture farms in Sinaloa.

MATERIALS AND METHODS

Study location and sampling

Samples were obtained from 29 aquaculture tilapia-producing farms along the State of Sinaloa, which is located at the Northwest of the Mexican Republic. The Sinaloa geographic coordinates are North 27°02', South 22°29' latitude north, East 105°23', West 109°28' longitude west. For sampling purposes, the Sinaloa state was divided into three zones (Fig. 1). Sixty-one tilapia samples were studied: 23 of fry, and 38 of adult fishes (each sample of fry was composed for 50 g of fish, and each representative sample of adult tilapia consisted of ten fishes, not for individual fishes). Regardless of the fish age, 17, 23 and 21 samples were collected from zones I, II, and III, respectively. Also, 65 water samples were analyzed: 36 of pond water and 29 of water supply (if available). Separately, 12, 24, and 29 water samples were collected in zones I, II and III, respectively. In the same order for the zones, 7, 14, and 15 samples of pond water were ob-

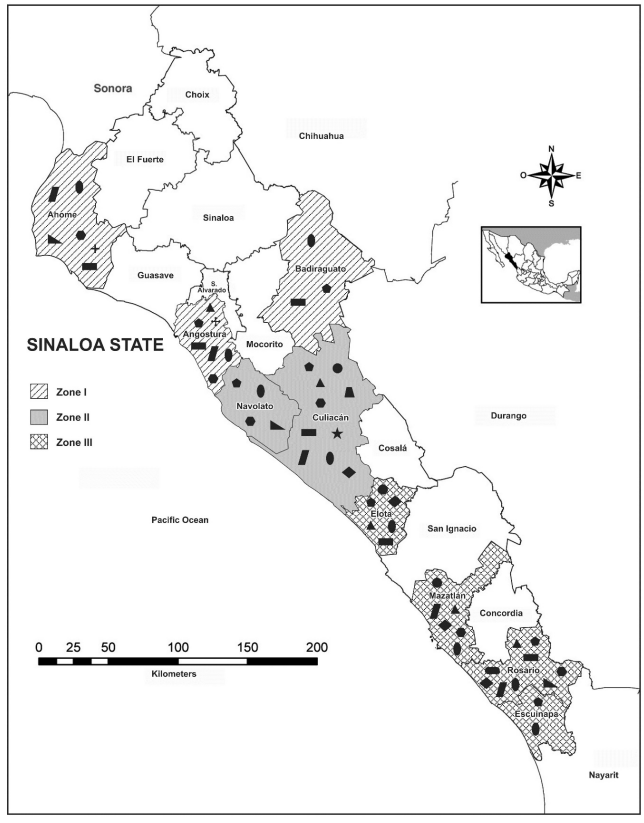

Figure 1. Microorganisms found in aquaculture farms (zones I, II and III) in the Sinaloa state. *E. coli* (⬮), *Enterobacter agglomerans* (▬), *Escherichia fergusonii* (◣), *Enterobacter gergoviae* (⬣), *Serratia odorifera* (⬟), *Citrobacter freundii* (▰), *Serratia liquefaciens* (●), *Enterobacter aerogenes* (◆), *Klebsiella ozaenae* (▲), *Plesiomonas shigelloides* (✚), *Streptococcus* sp.(⬬), *Staphylococcus* sp. (★), *S. aureus* (✣), *Bacillus* sp. (▲).

Figura 1. Microorganismos encontrados en las granjas acuícolas (zonas I, II, y III) en el estado de Sinaloa. *E. coli* (⬮), *Enterobacter agglomerans* (▬), *Escherichia fergusonii* (◣), *Enterobacter gergoviae* (⬣), *Serratia odorifera* (⬟), *Citrobacter freundii* (▰), *Serratia liquefaciens* (●), *Enterobacter aerogenes* (◆), *Klebsiella ozaenae* (▲), *Plesiomonas shigelloides* (✚), *Streptococcus* sp.(⬬), *Staphylococcus* sp. (★), *S. aureus* (✣), *Bacillus* sp. (▲).

tained. On the other hand 5, 10, and 14 supply water samples were collected for zones I, II, and III, respectively.

Sampling was carried out from November 2011 to May 2014. Sterile bags were used to capture live fish (with pond water for transportation to the laboratory) at room temperature. Pond and supply water samples were collected using 500 mL sterile bags (Whirl-Pak®) and placed in an ice cooler (4 °C). All samples were transported to the Unit of Research in Public Health from the School of Chemical and Biological Sciences of the Autonomous University of Sinaloa. At the laboratory, fish were sacrificed by cervical dislocation, and the collection data were recorded (place, date, time of collection, and the name of the production company), as well as some morphometric date (body width, standard length, and total length). Subsequently, tilapia samples (including the skin), were taken and processed within 2 – 10 h of sampling following aseptic techniques.

Detection of TC, FC, MAB and *S. aureus* by the Petrifilm™ plate method on tilapia and pond water

Tilapia samples were prepared as follows: 10 g of tilapia mass (fry or adults) including the skin were homogenized in peptone water; appropriate dilutions were prepared to inoculate one mL of them on 3M™ Petrifilm™ (3M Madrid, Spain). For pond water, appropriate dilutions were made, and one mL was inoculated on 3M™ Petrifilm plates. Incubation was carried out at 37 °C (for TC, MAB, and *S. aureus*) and 45 °C (for FC). After incubation, colonies were counted using a Québec type colony counter (CRAFT™, México) and the results were expressed as CFU/g (tilapia samples) or CFU/mL (water samples).

Detection of *E. coli, Salmonella* sp., *Staphylococcus* sp. and *Streptococcus* sp. in tilapia by the standard plating procedures

Twenty-five g of tilapia mass (fry or adults) including the skin were homogenized in GN (Gram Negative) Hajna enrichment broth and incubated for six h at 37 °C. Then, selective and differential media plates were inoculated for the presumptive isolation of *Salmonella* sp. and *Shigella* sp. [Salmonella-Shigella agar (SS), Bismuth Sulphite (BS), Brilliant Green (BG), and Xylose-Lysin-Deoxycholate agar (XLD)]. For the isolation of *E. coli* and other Enterobacteria, MacConkey agar was used. Additionally, Blood agar was used for the presumptive isolation of *Staphylococcus* sp. and *Streptococcus* sp. All culture medium were from Beckton-Dickinson™. Samples were inoculated by the plate extension method and incubated for 24 h at 37 °C.

Identification of the isolates by conventional biochemical test

For the biochemical identification of the isolates, the following media were used: Simon's citrate, KIA (Kligler agar), LIA (Lysine and Iron agar), MIO (mobility, indole, and ornithine), SIM (Sulfide, indole, and mobility), and urea, saccharose, and Malonate broths (Beckton-Dickinson™). Inoculation of bacterial isolates was according to each culture media specifications and incubated at 37 °C for 24 to 48 h. With the biochemical test results, genus and species of the isolates were determined. *S. aureus* and *Streptococcus* sp. were identified by Gram staining, catalase, coagulase, DNase, as well as by mannitol fermentation tests.

Statistical analyses

The measures of central tendency (average and proportions) were calculated. The accomplishment of the hypothesis and objectives was contrasted with the Pearson Chi-square test, and an analysis of variance was performed. A *p* value minor than 5% was considered statistically significant. All statistical analyses were performed using the Stata Intercooled version 14 software.

RESULTS

TC, FC, MAB, and *S. aureus* in adult tilapia and tilapia fry (Petrifilm™ plate method)

Independently of sampling zones, TC were detected in 64% of adult tilapia and fry samples (39/61) ranging from 4.0×10^1 to 9.8×10^5 CFU/g. FC were detected in 48% of the samples (29/61), ranging from 1.0×10^1 to 6.4×10^3 CFU/g. Separately, the TC average concentrations were 3.46×10^4 and 1.14×10^4 CFU/g for adult tilapia and fry, respectively (Fig. 2). In the same order, the FC average concentrations were 356 and 436 CFU/g (Fig. 2). On the other hand, MAB were detected in 90% of the samples (55/61) ranging from 1 $\times 10^3$ to 1.52×10^7 CFU/g. The MAB average levels were 1.08×10^6 and 1.09×10^6 CFU/g for adult tilapia and fry, respectively (Fig 2). Taking sampling zones into account, no significant difference in the concentrations of the IGs on tilapia between the zones (p= 0.62) was found. The highest concentration values for TC, FC, and MAB were recorded in zones II, III, and I, respectively (data not shown). The average level for each indicator group in tilapia and fry samples from zones I, II, and, III are shown in Table 1.

Eleven percent of the samples (7/61) were positive to *E. coli* with average concentrations of 18 and 14 CFU/g for adult tilapia and fry, respectively (Fig. 2). Additionally, *S. aureus* was detected in a sample of tilapia at a concentration of 30 CFU/g. From 61 samples of tilapia analyzed, 18% (11/61) showed FC levels exceeding the limits established by the Mexican standard for fresh and frozen fish (NOM-027-SSA1-1993; 400 NMP/g).

TC, FC, MAB, and *S. aureus* in pond and supply water detection by the Petrifilm™ plate method

Independently of sampling zones, TC were present in 57% of the water samples (37/65) ranging from 1.0×10^1 to 2.28×10^4 CFU/mL. FC were detected in 35% of the water samples (23/65) ranging from 1.0×10^1 to 1.2×10^3 CFU/mL. Separately, the TC average concentrations were 1.6×10^3 and 4.91×10^2 CFU/mL for pond and supply water, respectively (Fig. 2*). In the same order, the FC average concentrations were 1.31×10^2 and 42 CFU/mL (Fig. 2*). The MAB group was

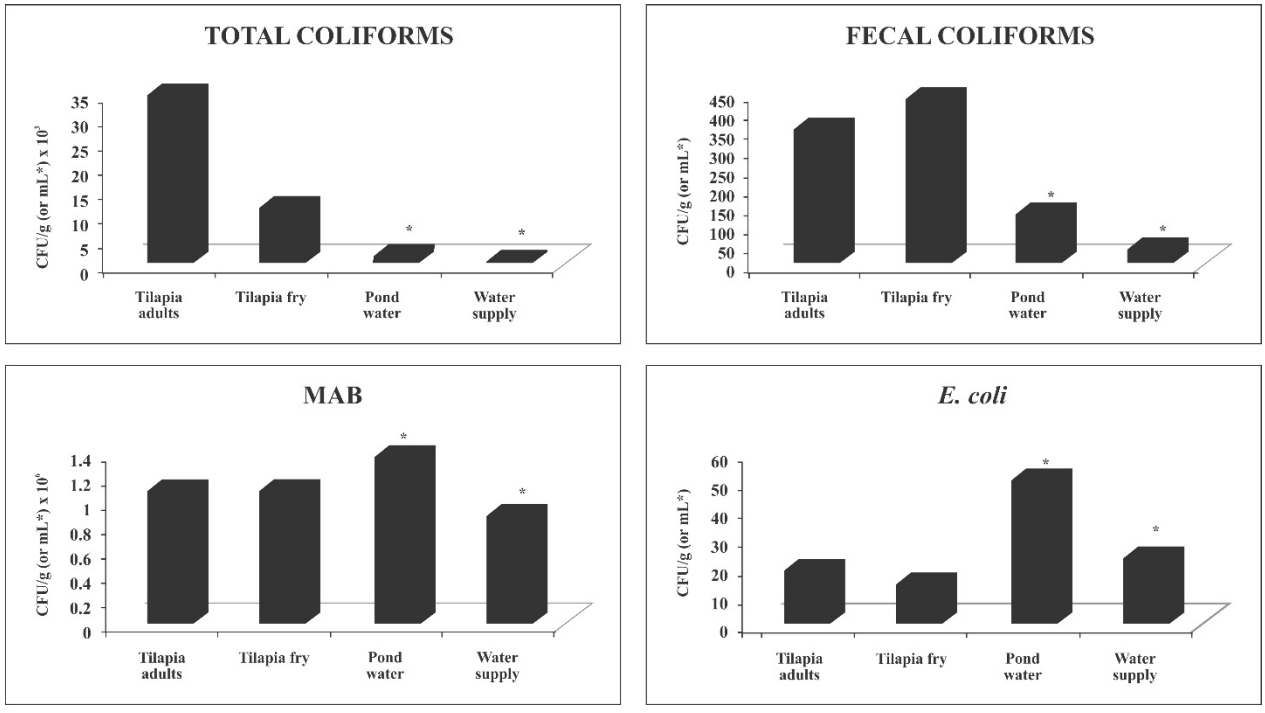

Figure 2. Global average concentrations (independent of the collection zone) of IGs (TC, FC, MAB, and *E. coli*) in adult tilapia, tilapia fry, pond water, and water supply. The average calculation considered all the sampling results of all the aquaculture farms. Results represented as CFU/g for tilapia and CFU/mL for water.

Figura 2. Concentraciones promedio globales (sin tomar en cuenta las zonas) de GIs (CT, CF y *E. coli*) en tilapia adulta, alevines, agua de estanque y agua de suministro. El promedio se calculó considerando los resultados de todos los muestreos en todas las granjas acuícolas. Los resultados se muestran como UFC/g para tilapia y como UFC/mL para el agua.

Table 1. Average concentrations of contamination IGs in samples of tilapia and water. No significant statistical difference was found by the variance analysis and Pearson Chi-square test in the indicator microorganism's concentrations between the zones, or between the sample types ($p > 0.05$).

Tabla I. Concentraciones promedio de GIs de contaminación en muestras de tilapia y agua. No se encontró diferencia estadística significativa mediante el análisis de varianza y la prueba Chi-cuadrada de Pearson en las concentraciones de microorganismos indicadores entre las zonas, o entre el tipo de muestra ($p > 0.05$).

SAMPLING ZONE	SAMPLE TYPE	INDICATOR GROUP		
		TC	FC	MAB
ZONE I	Adult tilapia (CFU/g)	1.768×10^3	1.46×10^2	5.058×10^5
	Fry (CFU/g)	6.95×10^2	1.22×10^2	3.922×10^6
	Pond water (CFU/mL)	2.14×10^2	1.17×10^2	3.775×10^5
	Supply water (CFU/mL)	1.2×10^1	4	4.200×10^4
ZONE II	Adult tilapia (CFU/g)	8.389×10^4	6.63×10^2	1.430×10^6
	Fry (CFU/g)	9.925×10^3	8.5×10^1	6.662×10^5
	Pond water (CFU/mL)	1.811×10^3	1.71×10^2	1.901×10^6
	Supply water (CFU/mL)	3.55×10^2	5.3×10^1	1.100×10^6
ZONE III	Adult tilapia (CFU/g)	1.828×10^4	2.57×10^2	1.326×10^6
	Fry (CFU/g)	1.706×10^4	8.43×10^2	3.037×10^5
	Pond water (CFU/mL)	2.177×10^3	1.01×10^2	1.225×10^6
	Supply water(CFU/mL)	7.60×10^2	4.9×10^1	1.014×10^6

detected in 89% of water samples (58/65) ranging from 1.0 x 10^4 to 9.05 x 10^6 CFU/mL. Separately, the MAB average concentrations were 1.35 x 10^6 and 8.76 x 10^5 CFU/mL for pond and supply water, respectively (Fig. 2*). Zone III recorded the highest value for TC and MAB while the highest concentration value for FC in water was registered in zone II (data not shown). The average concentration for each indicator group in pond and supply water samples taking into account zones I, II, and III are shown in Table 1.

Escherichia coli was detected in 14% of the samples (9/65) with average concentrations of 51 and 23 CFU/mL for pond and supply water, respectivly (Fig. 2*). Additionally, the bacterial genus *Micrococcus* sp. and *Kuthia* sp. were present in pond water.

Enterobacteria, *Staphylococcus* sp. and *Streptococcus* sp. detection in tilapia muscle by standard plate procedure

Presumptive colonies of *E. coli, S. aureus*, *Streptococcus* sp., and other bacteria were isolated in selective and differential media. The microorganisms were identified, and the more frequently isolated bacteria in adult fishes were *E. coli* (31), *Enterobacter agglomerans* (14), *Escherichia fergusonii* (11), *Enterobacter gergoviae* (7), *Serratia odorifera* (9) and *Citrobacter freundii* (6) (Fig. 3). In tilapia fry the more frequently isolated bacteria were *E. coli* (17) and *E. agglomerans* (7). *Staphylococcus aureus* was isolated in one sample of tilapia as well as *Streptococcus* sp. On the other hand, neither *Salmonella* sp. nor *Shigella* sp. were detected in any sample of adult or tilapia fry. Moreover, *Plesiomonas shigelloides* was identified in one sample of adult fish and *Bacillus* sp., a Gram-positive bacterium, was isolated from adult fishes (12 isolates).

Detection of *E. coli* in adult tilapia and fry by standard plating procedure

Escherichia coli was present in 81.6% of the adult fish's samples (31/38) and 73.9% of fry samples (17/23). In both cases, the concentration is under the limits for fecal coliform (represented by *E. coli*) in the Mexican standard (NOM-027-SSA1-1993; 400 NMP/g). No statistical difference was found between the concentrations of *E. coli* in adult fish and fry (p=0.479) at a significance level of 5%.

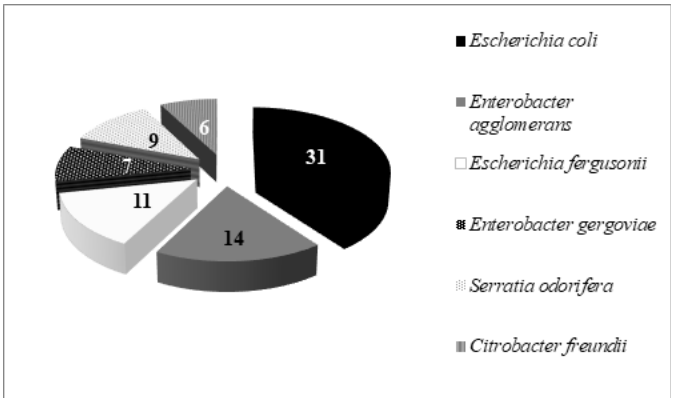

Figure 3. Number of isolates of each bacterium detected by standard plate method in adult fishes.
Figura 3. Número de aislados de cada bacteria detectada mediante el método de placa estándar en peces adultos.

DISCUSSION

To evaluate the results of the microbiological analysis in fish, the Mexican standard for the quality and safety of these products was taken as reference (NOM-027-SSA1-1993. Goods and services. Fishery Products. Fresh-chilled and frozen fish. Sanitary specifications). On the other hand, the water analysis results were contrasted with the Mexican rule NOM-001-SEMARNAT-1996 which establishes the maximum permissible limits of contaminants in wastewater discharges into federal waters and property. It is important to mention that there is no particular standard for water used in aquaculture ponds in Mexico. Therefore, the rule mentioned above governs the water used in aquaculture ponds.

Due to the nature of the microorganisms integrating the TC, FC and MAB groups, their concentrations are useful to predict the hygiene level during the production or handling of food (Allen *et al*., 2004). Moreover, the presence of these microorganisms, could affect the microbiological quality and the storage life of the fishery products.

The bacteria isolated in this work are mostly part of the intestinal biota of warm blood animals including humans. The aquaculture farms studied are open environments where the microorganisms can arrive to the ponds by rainfall runoff and directly from animal faeces, as suggested by Al-Harbi (2003). This fact explains the presence and concentrations of TC, FC and MAB detected in fish and water samples.

In general, we found more than one IG in a sample at concentrations under the maximum permissible limits in the Mexican standard NOM-027-SSA1-1993. Similar findings were reported in a study on *Oreochromis niloticus* performed in El Salvador (Romero y Romero, 2012). In that work, counts of indicator organisms (TC and FC) in pond fish were within the World Health Organization limits established for recreational water suitable for fish farming (2,000 and 1,000 NMP/mL). However, the authors found counts of *E. coli* exceeding the RTCA (Reglamento Técnico Centroamericano) 67.04.50:08 standards (10^2 CFU/g; RTCA 2009) in 89% of the samples (Romero y Romero, 2012). In comparison, 18% of the tilapia samples in our work showed FC concentrations exceeding the Mexican normative limit. In a work carried out in Cuba, the presence of these contamination IGs in fresh product was found within the framework of the Cuban standard for fresh fish NC 585:2008 (10^6 microorganisms/g at 30 °C) (Fuentes *et al.,* 2011).

In this work, *Salmonella* sp. was not found in any sample. In contrast, this bacterium was reported in 2% of the samples of tilapia in a study performed in Costa Rica (Morales *et al.,* 2004). Otherwise, *S. aureus* was detected in tilapia. Another study in tilapia farm found *S. aureus* in the hands of personal and muscle of the fish, and the authors suggested that the personal transmitted the bacteria to the fish (Romero y Romero, 2012). The presence of *S. aureus* implies a risk for the fish health since this bacterium can cause infections in

eye, brain, and kidney (Atyah *et al.,* 2010) severely affecting the farm profitability. Furthermore, in this work, we detected the presence of *Streptococcus* sp. in one tilapia sample. This microorganism is considered a potential pathogen for both tilapia and humans. In previous studies performed in Colombia (Pulido *et al.,* 2004), Saudi-Arabia (Al-Harbi y Uddin, 2005), Cuba (Fuentes *et al.,* 2011; Rubio-Limonta *et al.,* 2010) and México (Lara-Flores *et al.,* 2013), *Streptococcus* sp. was reported in tilapia. Pulido *et al.* (2004) showed the presence of *Streptococcus* sp. by transmission electron microscopy (TEM) in brain samples of tilapia during the study of an endemic mortality of tilapia in a freshwater aquaculture facility. When there is infection by this bacterium, fish show disoriented and erratic natatorium movements. This is because the *Streptococcus* sp. infection causes a meningoencephalitis, unilateral or bilateral exophthalmos with or without corneal opacity, and periocular hemorrhage. The diseased tilapias show clinical signs similar to hemorrhagic septicemia resulting from Gram-negative bacteria (Conroy, 2009). None of these symptoms was observed in any of the aquaculture farms studied.

On the other hand, Al-Harbi y Uddin (2005) reported a prevalence above 10% of S*treptococcus* sp. in brackish pond water, gills and intestine of healthy tilapia cultured in Saudi Arabia. Additionally, Fuentes *et al.* (2011) reported a 6 to 7% prevalence of *Streptococcus* sp. in tilapia (samples of skin, gills, intestine and muscle) cultured in floating cages in freshwater environments. A similar study in tilapia (samples of eye, brain, heart, spleen, liver, and kidney) from farms with intensive farming, during rainy and dry seasons, reported the detection of *Streptococcus* sp. in tilapia from the western, central and southern regions of Cuba in both seasons. Lara-Flores *et al.* (2013) reported a 13% prevalence of *Streptococcus* sp. in tilapia cultured in cages in Champotón, Campeche, México.

Plesiomonas sp. and *Staphylococcus* sp. are critical bacteria in public health due to the severity of some infections they cause. Even when they were detected at a very low frequency in this study (one isolate each), their presence makes necessary to maintain microbiological quality surveillance in tilapia culture and in general, in aquaculture (Allen *et al.,* 2004).

It is important to stand out that MAB isolated in this work are ubiquitous (present in all types of water, food, soil, vegetation, and air) and some of them are opportunistic pathogens (only in people with an extremely comprised immunological system). In agreement with this, the opportunistic nature of these bacteria is only associated with hospital-acquired infections, where the route of infection is not food or water ingestion but medical devices. In the MAB group (or HPC) all bacteria that use organic nutrients for growth (primary and secondary pathogens, as well as coliforms) are included. However, it is necessary to remark that all the MAB genera are common in foods, and humans ingest significant amounts of these microorganisms daily. Notably, this fact is inconsequential because the naturally occurring MAB group lacks virulence factors making irrelevant their presence for healthy people (Allen *et al.,* 2004).

According to the International Commission on Microbiological Specifications for Foods (ICMSF, 2005), very few pathogens are of concern in fresh fish. However, an adequate temperature control is critical for preventing or controlling the growth of aquatic and terrigenous pathogens that potentially may be present on fish products after processing including *Vibrio* sp., *Salmonella* sp., *S. aureus*, *Bacillus cereus*, *E. coli*, *Clostridium perfringens*, *Listeria monocytogenes* and S*higella* sp. None of these microorganisms represent a threat on chill stored and cooked fish. However, in areas where raw fish is consumed, strict hygiene conditions must be maintained, and contamination with bacteria with low infection dose must be avoided (ICMSF, 2005). The ICMSF for fresh and frozen fish set maximum limits as follows: a MAB limit of 10^7 CFU/g; 5 x 10^2 CFU/g for *E. coli* and 10^3 CFU/g for *S. aureus* (ICMSF, 1986). Most of the evaluated samples in this work are under these limits. However, since pathogens associated with chilled raw fish are primarily the same as those related to the initial microflora (ICMSF, 2005), and considering that mexican population traditionally consume dishes made with raw tilapia (*e.g*. ceviche), it is important to maintain surveillance protocols in fish farms to minimize the presence of microorganisms in fresh tilapia. It is important to highlight that some of these bacteria are opportunistic and could cause disease in immunocompromised people consuming these products without a proper cooking process (ICMSF, 2005).

Hernández *et al*. (2009) remarked the economic, social and alimentary value of the fishery in Mexico, where the aquaculture activities are a matter of national security. According to these authors, all the Mexican government levels are responsible for the establishment of inspection and surveillance programs in aquaculture systems. This fact should promote microbiological research in quaculture, which in turn, would increase the productivity of this activity.

CONCLUSIONS

The levels of IGs found in most of the samples are usually within the maximum permissible limit established in the Mexican standard for fresh and frozen fish. This is indicative that the tilapia harvested in Sinaloa is safe for human consumption regarding its microbiological quality. However, the presence of FC and MAB above the Mexican standard permissible limits in 18 and 3% of the samples, respectively, as well as presence of *S. aureus* and *Streptococcus* sp., highlights the need to pay particular attention to the management of fish in farms to ensure the product safety and protect the aquaculture production in Sinaloa.

For this and considering our results, the establishment of standardized monitoring protocols for ensuring the microbiological quality of culture water in all aquaculture farms is necessary, as well as protocols for fry and adult fishes management to avoid the presence or the increase of these microorganisms. The adhesion of the Mexican aquaculture

systems to the international quality and safety standards is important to maintain the profitability of this activity.

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