Int Aquat Res (2023) 15:135–144
https://doi.org/10.22034/IAR.2023.1973419.1362

ORIGINAL RESEARCH

Selection of native lactic acid bacteria with probiotic potential from Nile tilapia (*Oreochromis niloticus*) gastrointestinal tract

Ana Antonia Zapata . Ruth López-Alcántara . Myrna Leticia Bravo-Olivas . Rosa Chávez-Dagostino . Julia Ramos-Miranda . David Ulises Miranda-Hernández . Maurilio Lara-Flores

Received: 23 November 2022 / Accepted: 17 April 2023 / Published online: 25 April 2023

Abstract The main objective was to select and identify native lactic acid bacteria from the gastrointestinal tract of Nile tilapia *(Oreochromis niloticus)* to use as a possible probiotic. Seventeen Lactic Acid Bacteria (LAB) were isolated from the gastrointestinal tract of juvenile Nile tilapia. Six strains were selected and named in the first phenotypic and morphological selection process: C3, C4, C7, C15, C16, and C25. The strains C3, C7, and C25 presented high antagonism activity towards pathogens strain, high survival index against bile salts and acidity tolerances, optimum acid lactic production, and enzymatic activity for carbohydrates and protein substrate. Therefore, strains C3, C7, and C16 presented potential probiotics for Nile tilapia aquaculture. The biological effects of the strains in Nile tilapia will study in a future experiment where the strains will include in fish feed.

Keywords Probiotics . Lactic acid bacteria . Nile tilapia . *Oreochromis niloticus*

Introduction

Aquaculture is a commercial activity with exponential growth in the last decade. For example, in 2016, 80 million tons of fish were produced only for human intake. As a result, Tilapia is one of the most consumed fish in the world (Meidong et al. 2017; FAO 2018).

One of the principal systems of culture is intensive, where the organisms expose to different stress factors, which causes a loss in the productivity caused by disturbances in the immune system and physiological deficiencies (Lara-Flores 2011; Fečkaninová et al. 2017). Antibiotics will use to reduce the stress effects; however, these treatments cause adverse effects on the final products as toxicity when they consume and develop drug resistance of bacteria (Smith 2008). An alternative option is using probiotics because they can improve digestive and immunological processes and prevent diseases.

Ana Antonia Zapata
BEMAREMA´s Doctorate, Coast University Centre from Guadalajara University. Puerto Vallarta, Jalisco, Mexico

Maurilio Lara-Flores (✉)
Molecular Microbiology Laboratory, EPOMEX Institute, Autonomous Campeche University. San Francisco de Campeche, Campeche, Mexico
e-mail: maurlara@uacam.mx

Julia Ramos-Miranda
Ecology and Fisheries Laboratory, EPOMEX Institute, Autonomous Campeche University. San Francisco de Campeche, Campeche, Mexico

Ruth López-Alcántara
Biomedical Research Centre, Autonomous Campeche University. San Francisco de Campeche, Campeche, Mexico

Myrna Leticia Bravo-Olivas . Rosa Chávez-Dagostino
Biological Science Department, Coast University Centre from Guadalajara University. Puerto Vallarta, Jalisco, Mexico

David Ulises Miranda-Hernández
Agricultural Science Faculty, Autonomous Campeche University, Escarcega, Campeche, Mexico

Probiotics are "live microorganisms that confer a beneficial effect on the host" (Fuller 1989). These effects are performed by a graduality establishment of a mature intestinal microbiota, which could influence by digestive enzymes, diet, and environment (Lara Mantilla et al. 2016). Most probiotics used in aquaculture are lactic acid bacteria (LAB). LAB are considered safe growth promoters and prevent diseases in fish (Beck et al. 2015; Dawood and Koshio 2026; Lim et al. 2016; Pirarat et al. 2015). Therefore, this study's objective was to isolate, characterize, identify, and select lactic acid bacteria from the digestive tract of Nile tilapia (*O. niloticus*) to use as a probiotic.

Materials and methods

Sampling

Nile tilapia fingerlings (*O. niloticus*) (6±1 g weight) were selected. The fingerlings were culture in rustic geomembrane tanks in Chapingo's Postgraduate College (Champoton, Campeche, Mexico). The organisms will euthanize with benzocaine solution (400 ppm). The organisms were disinfected with benzalkonium chloride solution (1%) and washed twice with sterile saline solution (0.85%) (Grisez et al. 1997). All biological procedures will approve on March 15th, 2017, for the Ethics Committee of EPOMEX Institute with normative references on the Mexican Federal Norms NOM-01-1992-STPS-1993 and NOM-062-ZOO-1999. The fish were dissected in aseptic conditions to obtain the gastrointestinal tract. The intestines were homogenized and stored at 4° C until processing (Sugita et al. 1988).

Isolation of lactic acid bacteria as a possible probiotic

10 µl of homogenized intestines were inoculated in nutritive broth to isolate lactic acid bacteria (Difco, Becton Dickinson France S.A., Le Pont de Claix France) and incubated at 35° C for 24 hours; after the incubation, an aliquot was strained in Petri plates with TSA, KF (Difco, Becton Dickinson France S.A., Le Pont de Claix France), and MRS Agar (DIBICO®) and incubated 24 h at 35° C (Huys et al. 2001).

After the incubation, a colony was isolated from the culture to purify the bacteria. Next, the colonies will inoculate in MRS broth, and a Gram stain will make (Ringo et al. 2000); finally, to conserve the bacteria, an aliquot of 100 µl was inoculated in MRS and nutritive broth with 15% of glycerol.

Biochemical identification

The isolates were identified using the Mini-API (BIOMERIEUX) (Prasad et al. 1998). API-50 CH probe was used according to the manufacturer's instructions. Finally, the API-WBE V 5.1 software was used to interpret the results. The identification was confirmed when the match percentage was >90% (Fečkaninová et al. 2019).

Antagonism assay

Dopazzo's double-layer method, modified by Zapata and Lara-Flores (2012), is used. First, an aliquot of 50 µl of bacteria culture is inoculated in a Petri plate with TSA agar and incubated at 35° C for 24 h to obtain a macrocolony. After this, an aliquot of 10 µl of a pathogen culture was mixed in 10 ml of TSA 25% soft agar, poured into the Petri plate with the macrocolony, and incubated for 48 h at 35° C. Then, the inhibition halo was computed in square centimetres with the SketchAndCalc® software, and the inhibition percentage was obtained according to the following formula:

$$\text{Area}(\%) = \left(\frac{\text{area final } (\text{cm}^2)}{\text{area total } (\text{cm}^2)}\right)$$

The pathogen bacteria utilized in the antagonism assay were isolated from the water of culture of the tilapia fingerlings.

Cross antagonism assay

The intra-strains compatibility is verified, and the modified technique of Zapata and Lara-Flores (2012) was used. An aliquot of 10 µl of all the acid lactic bacteria cultures is dropped at a middle distance in the TSA agar Petri plate, except one will be the strain to challenge. The plates were incubated for 48 h at 35° C. After the incubation, in a tube with 10 ml of soft agar (TSA 25%), the challenge strain was inoculated, and the soft agar was mixed on the plates with macrocolonies and incubated at 35° C for 48 h. An inhibition halo confirms growth inhibition (Ramírez-López and Velez-Ruiz 2016).

Bile salts and pH tolerance

The pH and bile salts tolerance were performed by Guo et al. (2010) whit some modifications. An initial count of the colonies forming units (CFU) was made to know the initial density of the bacteria before the tests. In an Erlenmeyer flask, 100 ml of nutritive broth adjusted to 4 pH whit 6 N chloride acid was prepared for the pH tolerance test; 1 ml of culture bacteria was inoculated. The flasks were incubated at 35° C, and sampling was taken at 6, 12, and 24 h. For the bile salts tolerance assay, 100 ml Bright Green broth with 2% bile salts was prepared, and an aliquot of 1 ml of bacteria culture was inoculated and incubated at 35° C at 6, 12, and 24 h; a sampling was taken. The counts were made manually over TSA agar after 48 h of incubation at 35° C. Survival is calculated with the following equation (Bao et al. 2010):

$$(\%) = \left(\frac{\log \text{CFU } N_1}{\log \text{CFUN}_2}\right) \text{x}100$$

N_1=Final total cell number
N_0= Initial total cell number

Digestive enzyme analysis

The proteolytic, amylolytic, and lipolytic activity was evaluated using three specific culture media: Milk agar (TSA agar and 2% skim milk), Starch agar (TSA agar and 1% of starch), and cream agar (TSA agar and 1% natural cream extracted). In each plate with a specific medium, 50 ml of bacteria culture was dropped to make a macrocolony and incubated for 72 h at 35° C; The enzymatic activity was considered when a clear zone surrounding the macrocolony appeared (Afrilasari et al. 2016; Ramírez-López and Vélez-Ruiz 2016).

Catalase activity

Catalase activity of lactic acid bacteria was determined as described by Cohen et al. (1970) using bovine liver catalase as standard. H_2O_2 production was determined using the Amplez red Hydrogen Peroxide Assay Kit (Molecular Probes). Fluorescence was measured using excitation in the range 530-560 nm and emission at 590 nm.

Hemolytic activity

All the lactic acid bacteria were inoculated on MRS agar with 5% sterile defibrinated sheep blood and incubated at 35° C for 48 h. After the incubation, there was observed if had hemolysis signs (Argyri et al. 2013).

Acid lactic production

The evaluation was performed according to the NMX-F-511-1988 Mexican Normative with modifications. First, from a culture of 24 h of the lactic acid bacteria, 100 ml is taken and inoculated in a tube with nutritive

broth. Then, the tubes were incubated at 35° C for three h, and after incubation, an aliquot of 5 ml of culture was taken and placed in a flask with 50 ml of distilled water and three drops of phenolphthalein (1%). Subsequently, the solution was titled with sodium hydroxide solution (0.1 N) until its colour changed. Finally, the acidity was calculated as follows:

$$\text{Acidity g. L}^{-1}\text{(lactic acid)} = \left(\frac{\text{VxNx0.090}}{\text{M}}\right)\text{x1000}$$

V=cm^3 consumption of NaOH (0.1 N) in titration
M= Sample volume
0.090= Lactic acid meq

Statistical analysis

The antagonism assay and the lactic acid production data are analyzed using One-Way Variance Analysis (ANOVA). The statistical significance was performed through Tukey's test ($P<0.05$). The bile salts and pH tolerances were analyzed through a Gehan-Brelow-Wilcoxon test ($P<0.0001$). All tests were performed with Graph Pad Prism Software V.8.0.2 (Graph Pad Inc., USA). The complete analysis for the selection of probiotics was performed through a Correspondence Canonical Analysis (CCA) with all data using the Excel package XLSTAT (V.2015.1.03.16409).

Results

Acid-lactic bacteria identification

Seventeen strains with probiotic potential were obtained from 25 fingerlings. The strains present morphological characteristics of lactic acid bacteria like Gram-positive stain and negative reaction to catalase test. A representative's strains from each isolated group were selected as described in Table 1.Three isolation strains were selected (C3, C4, C7, C15, C16, and C25) for the biochemical identification with the Mini API 50 CHL Test using Api-Web™ software to interpret the results. The three strains showed 99.9% of the Lactobacillus genus.

Antagonisms assay

The strains C3, C7, and C16 presented significantly higher antagonisms (32%, 34%, and 27%, respectively) than strains C4, C15, and C25, with no presented antagonisms capacity ($P<0.05$) (Fig. 1, Table 2). The selected strains did not show antagonism activity themselves (Fig. 2).

Bile salts and pH tolerance

The strains C7 and C16 demonstrated a high resistance and survival rate to bile salts (100%) ($P<0.05$). On the other hand, strain C15 presented only a 75% of survival rate, and the C4 and C25 had below 50% of survival (Fig. 3, Table 2).The pH tolerance shows that C7 and C15 strains presented 100% of survival. However, C3, C4, and C25 strains presented low survival rates (Fig. 3, Table 2).

Table 1 Microscopic and morphologic characteristics from selected strains for assays Antagonisms assay

Strain	Gram stain	Catalase	Oxidase
C3	Bacillus +	-	-
C4	Coccobacilli +	-	-
C7	Bifidobacterium +	-	-
C15	Coccobacilli +	-	-
C16	Bacillus +	-	-
C25	Coco +	-	-

Digestive enzyme analysis

The proteolytic and amylolytic activity results were positive in all the strains tested. However, all the strains not presented lipolytic activity (Table 2).

Hemolytic activity

The strains did not present hemolytic activity (Table 2).

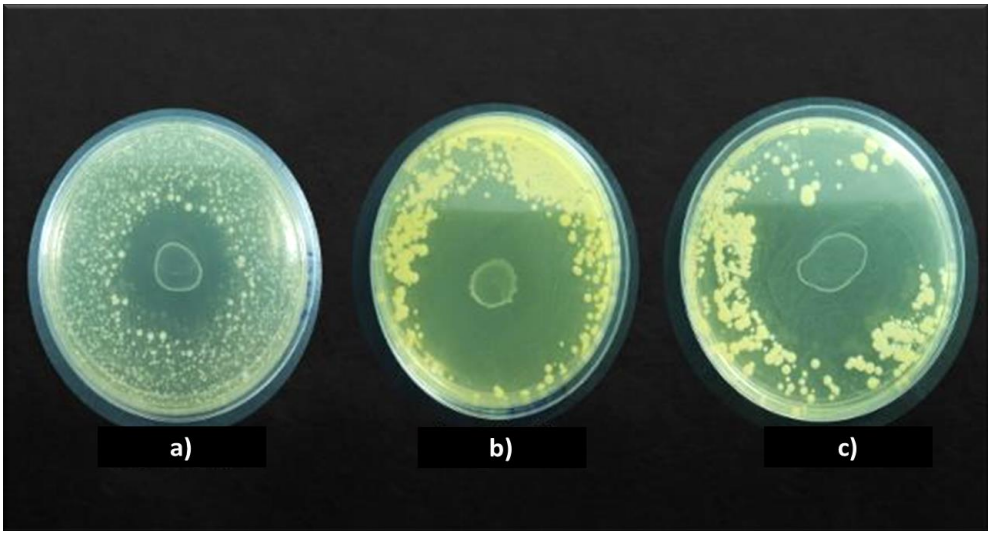

Fig. 1. Antagonistic activity of lactic acid strains against Mac B2 pathogen. A) C3, b) C7, c) C16

Table 2 Selection criteria for a probiotic strain

Strain	Antagonism (%)				Lactic acid production (g/L)			Enzymatic activity			Hemolytic activity	Survival (%)	
	Mac B2	MRS 2.1	SyS C1	TCBS 7.1	3	6	24	Protein	Lipid	Carbohydrate		Salt bile tolerance 2%	pH 4 tolerance
C3	40.3	3.3	37.1	21.3	1.08	0.90	1.08	+	-	+	Ɣ-Hemolisis	21.0	13.0
C4	0.0	0.0	0.0	0.0	1.17	0.72	0.90	+	-	+	Ɣ-Hemolisis	63.0	45.0
C7	32.9	18.9	48.6	24.5	1.8	0.81	0.72	+	-	+	Ɣ-Hemolisis	100.0	100.0
C15	0.0	0.0	16.8	0.0	1.26	0.72	0.9	+	-	+	Ɣ-Hemolisis	75.0	100.0
C16	39.8	3.8	44.6	4.1	1.08	0.72	1.08	+	-	+	Ɣ-Hemolisis	100.0	42.0
C25	0.0	0.0	0.0	0.0	1.17	0.72	0.72	+	-	+	Ɣ-Hemolisis	38.0	31.0

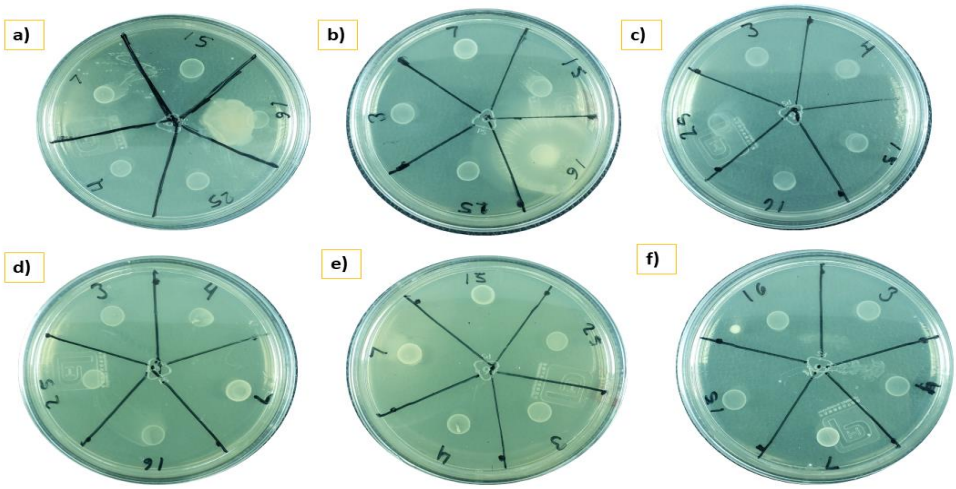

Fig. 2. Selected strain cross antagonism. a) C3 challenge strain, b) C4 challenge strain, c) C7 challenge strain, d) C15 challenge strain, e) C16 challenge strain, f) C25 challenge strain

Acid lactic production

All the strains presented an optimums production of acid lactic; however, the C7 strain presented a higher production than others at three hours of incubation. On the other hand, at 6 and 24 h of incubation, the C7 strain presented a similar production to the other strains (Fig. 4, Table 2).

Correspondence canonical analysis (CCA)

The Correspondence Canonical Analysis (CCA) (Fig. 5) showed that the C3 strain has high antagonistic activity, better acid lactic production at 24 h of incubation, and low resistance to pH test. On the other hand, the C7 strain has a medium antagonistic activity, high production of acid lactic at three hours of incubation, and high resistance to bile salts and pH tests.

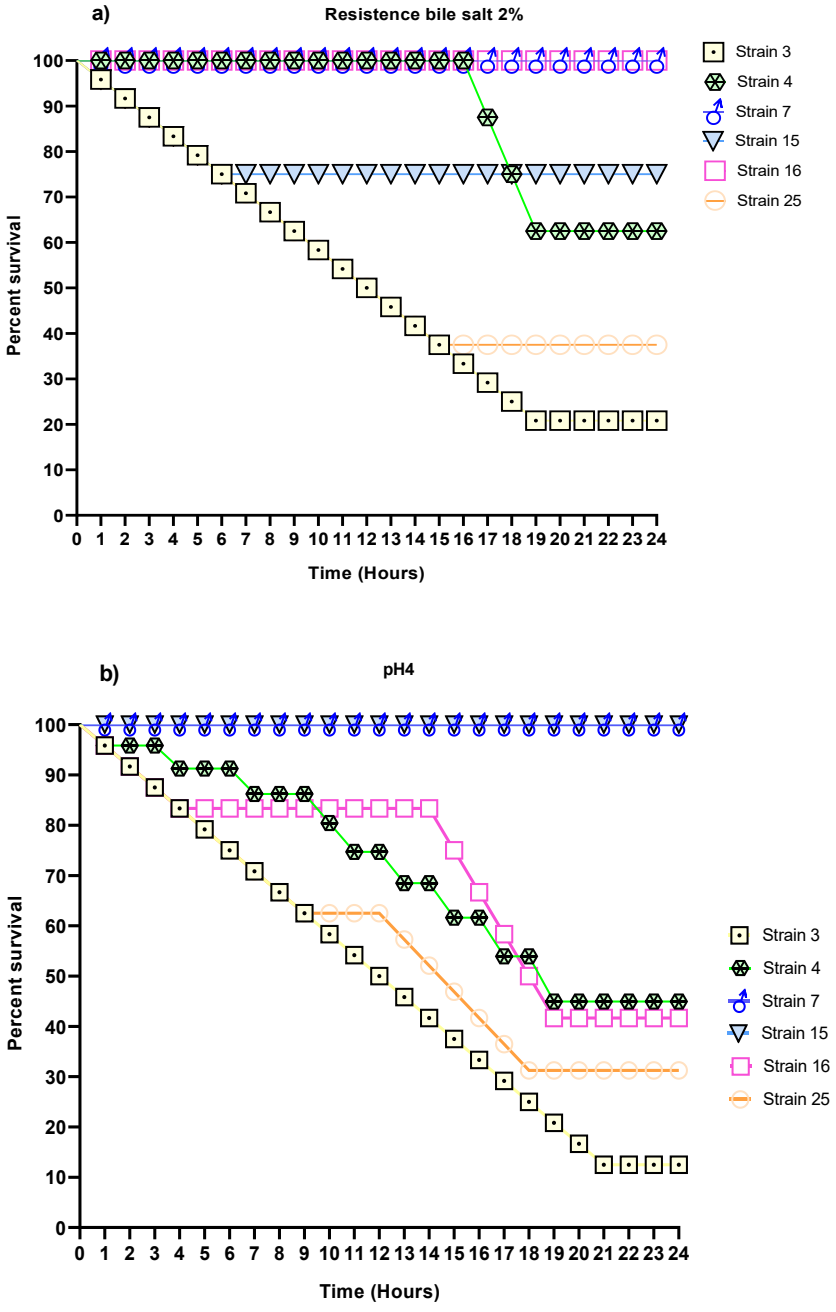

Fig. 3. a) Survival percentage resistance to 2% bile salts. b) Survival percentage resistance to acid ambient (pH 4)

Discussion

The health state of culture fish depends mainly on their developing environment. In aquatic organisms, gastrointestinal tract colonization in the early stage of life usually happens after hatching, and a healthy microbiota is s primordial phenomenon to maintain the health of the organisms.

The establishment of intestinal microbiota in aquatic organisms depends on the genetic and nutritional stage of the host and environmental factors. This community comprises facultative, strictly anaerobic, and acid-lactic bacteria (Ringo and Gatesoupe 1998; Gómez and Balcázar 2008). In aquaculture, probiotics demand detailed assessments of the potential benefits for the culture organisms. These leads to asses new

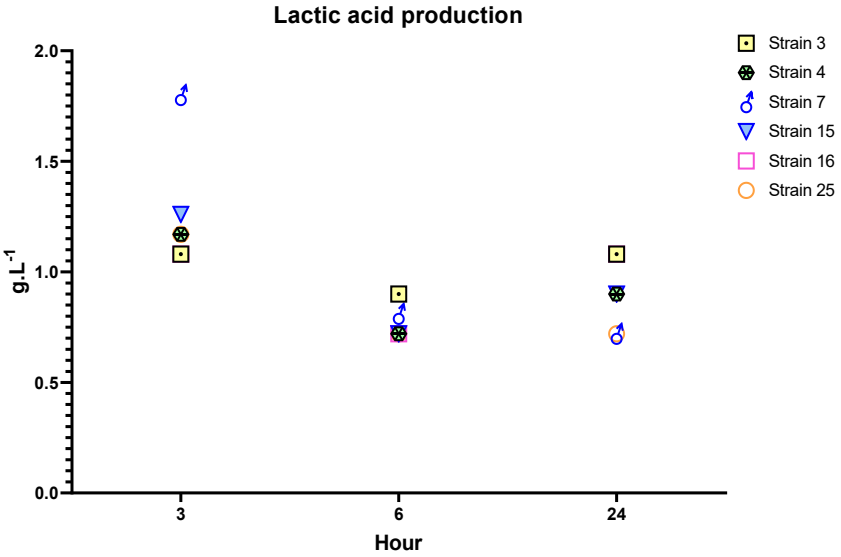

Fig. 4. Kinetics from acid lactic production through the time from selected strain

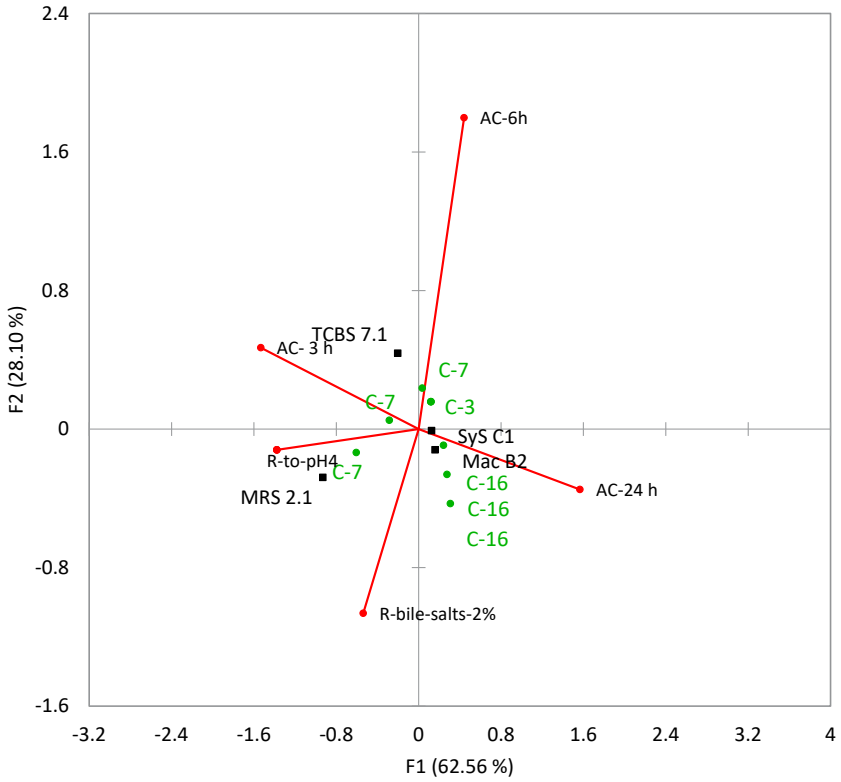

Fig. 5. Diagram from correspondence canonical analysis of selection criteria vs strains

bacterial strains with probiotic possibilities *in vitro* and *in vivo* assays (Huang et al. 2020). Furthermore, this leads to the study of the traits of native lactic acid bacteria that can be included in the design of probiotics that could be used in aquatic organisms based on the host specificity and to can apply under similar culture environments or hosts (Nayak 2010; Argyri et al. 2013; Huang et al. 2020). A wide diversity of probiotics without characterizing have been successfully used to improve the performance and illness resistance in terrestrial animals and a few in aquatic organisms, and though their beneficial effects in fishes have been demonstrated, they do not be approved completely yet (Gatesoupe 2000; Verschuere et al. 2000; Merrifield et al. 2010; do Vale Pereira et al. 2017).

Probiotics benefit organisms by improving nutrient assimilation efficiency and organic substances, delaying the colonization of pathogens, and keeping a mature, healthy, and beneficial microbiota (Gómez and Balcázar 2008).In this study, strains C3, C7, and C16 have high antagonisms. The bacteriostatic activity is a functional requirement in probiotic strains. It is a prerequisite to assess the probiotic efficiency, whereby any potential probiotic should have high bacteriostatic efficiency (Chauhan and Singh 2019). This antibacterial effect could be through substances such as bacteriocins and siderophores, lysozymes, proteases, and organic acids (Kabir, 2009; Fgaier and Eberl 2011; Zapata and Lara-Flores 2012; Ringo 2020). The lactic acid bacteria must overcome chemical and physical barriers during their transit through the gastrointestinal tract as gastric acidity and bile toxicity (Chauhan and Singh 2019; Huang et al. 2020). The low pH tolerance appears to be a specific attribute of the lactic acid bacteria (Argyri et al. 2013).

The resistance bile salts are considered an important property to colonize the host gastrointestinal tract because this process could damage the bacterial cell due to the structural modification in the cell membrane (Chauhan and Singh 2019). The survival of probiotic strains and the capacity to establish in the small intestine once overcome the acid fluid from the stomach is the key to their colonization and exerting effect (Kavitha et al. 2018). The strains C7, C15, and C16 were none affected by the stress process to which they are *in vitro* submitted with high resistance. Some studies describe bile salt tolerances for the cell membrane structure because the fatty acids' contents confer hydrolysis resistance (Murga et al. 1999; Mamianetti et al. 1999; Begley et al. 2006).

The intestinal tract in aquatic organisms is a dynamic ecosystem of diverse microorganisms that is vital to the host's growth, development, and immunity.The aquatic environment and the nutrients in the gastrointestinal tract of the aquatic organisms are appropriate for bacterial development, mainly of the enzymatic diversity that takes part in several digestive processes. For example, the microbiota can produce exogenous substances such as proteolytic, amylolytic, and lipolytic enzymes that can take part in the digestion of macromolecules such as proteins, carbohydrates, and lipids, promoting nutritional benefits to the host (Nayak 2010; Kavitha et al. 2018). In addition, these bacteria can help digest substances like cellulose that the host cannot digest and could be an alternative energy source. Ray et al. (2012) describe several studies on probiotic enzymatic activity and its effect on fish nutrition.

Biosecurity is an important aspect that is necessary to consider since aquaculture production is part of the human alimentary industry. At the global level, existing regulations about the use of probiotics for probiotic products (Verschuere et al. 2000; FAO/OMS 2002; do Vale Pereira et al. 2007). In this study, all the strains did not be hemolytic, and due to their other positive attributes, strains C3, C7, and C16 may be considered good candidates to use as probiotics. In addition, more than one strain established an antagonistic behaviour against pathogens, resistance to the transit through the gastrointestinal tract, and the potential ability to catabolize the main biologic macromolecules present in the diet nutrients.

After determining the probiotic properties of lactic acid bacteria isolated from the gastrointestinal tract of Tilapia by metabolic criteria, future *in vivo* assays are necessary to validate their real biologic potential as probiotics. In addition, the comprehension of the action mechanisms of these bacteria, identifying their potential probiotic and the benefits on aquaculture production.

The results of this work support the importance of a selection of native microorganisms with high specificity levels and probiotics abilities that can be re-introduced subsequently to the host and try to demonstrate through future assays and with a better bacteria characterization to get evidence to improve the production process in the aquaculture industry.

The main objective of probiotic use in aquaculture is to reduce or eradicate indiscriminate antibiotic use and other substance that could affect the aquatic organisms in the culture systems.

Acknowledgements This publication forms part of an investigation from Guadalajara University in collaborative work with Campeche University through of Postgraduate Doctorate in Science in Biosystematics, Ecology, and Management of Natural and Agrarian Resources (BEMARENA). The first author thanks the postgraduate grant number 483468 granted by the CONACYT. To all the Institutions for the support in carrying out this investigation

Conflict of interest The authors declare no conflict of interest.

References

Afrilasari W, Widanarni W, Meryandini A (2016) Effect of probiotic *Bacillus megaterium* PTB 1.4 on the population of intestinal microflora, digestive enzyme activity, and the growth of catfish (*Clarias sp.*). HAYATI J Biosci 23(4):168-172. https://doi.org/10.1016/j.hjb.2016.12.005

Argyri AA, Zoumpopoulou G, Karatzas KAG, Tsakalidou E, Nychas GJE, Panagou EZ, Tassou C (2013) Selection of potential probiotic lactic acid bacteria from fermented olives by in vitro tests. Food Microbiol 33(2):282-291. https://doi.org/10.1016/j.fm.2012.10.005

Askarian F, Zhou Z, Olsen RE, Sperstad S, Ringo E (2012) Culturable autochthonous gut bacteria in atlantic salmon (*Salmo salar* L.) fed diets with or without chitin. characterization by 16S rRNA gene sequencing, ability to produce enzymes and *in vitro* growth inhibition of four fish pathogens. Aquaculture 326:1-18. https://doi.org/10.1016/j.aquaculture.2011.10.016

Bao Y, Zhang Y, Zhang Y, Liu Y, Wang S, Dong X, Wang Y, Zhang H (2010) Screening of potential probiotic properties of *Lactobacillus fermentum* isolated from traditional dairy products. Food Control 21(5):695-701. https://doi.org/10.1016/j.foodcont.2009.10.010

Beck BR, Kim D, Jeon J, Lee SM, Kim H, Kim O-J, Lee JI, Suh BS, Do HK, Lee KH, Holzapfel WH, Hwang JY, Kwon MG, Song SK (2015) The effects of combined dietary probiotics *Lactococcus lactis* BFE920 and *Lactobacillus plantarum* FGL0001 on innate immunity and disease resistance in olive flounder (*Paralichthys olivaceus*). Fish Shellfish Immunol 42(1):177-183. https://doi.org/10.1016/j.fsi.2014.10.035

Begley M, Hill C, Gahan CGM (2006) Bile salt hydrolase activity in probiotics. Appl Environ Microbiol 72(3):1729-1738. https://doi.org/10.1128/AEM.72.3.1729-1738.2006

Chauhan A, Singh R (2019) Isolation and evaluation of putative probiotic strains from different teleost to prevent *Pseudomonas aeruginosa* infection in infection in *Cyprinus carpio*. Aquac Res 50:3616-3627.

Cohen G, Dembiec d, Judtih M (1970) Measurement of catalase activity in tissue extracts. Anal Bioch 34:30-38. 10.1016/0003-2697(70)90083-7

Dawood MA, Koshio S, Ishikawa M, Yokoyama S, El Basuini MF, Hossain MS, Nhu TH, Dossou S, Moss AS (2016) Effects of dietary supplementation of *Lactobacillus rhamnosus* or/and *Lactococcus lactis* on the growth, gut microbiota and immune responses of red sea bream, *Pagrus major*. Fish Shellfish Immunol 49:275-285. https://doi.org/ 10.1016/j.fsi.2015.12.047

Dawood MAO, Koshio S (2016) Recent advances in the role of probiotics and prebiotics in carp aquaculture: A review. Aquaculture 454:243–251. https://doi.org/10.1016/j.aquaculture.2015.12.033

FAO/OMS (2002) Informe del grupo de trabajo conjunto FAO/OMS sobre borrador de directrices para la evaluación de los probióticos en los alimentos. Available at: file:///C:/Users/Acer/Documents/paty/homework1/PROBIOTICOS_OPS_2006.PDF (Accessed: 4 September 2020).

FAO (2018) El estado mundial de la pesca y la acuicultura 2018, contribución a la seguridad alimentaria y la nutrición para todos. Roma. Available at: ftp://ftp.fao.org/docrep/fao//007/y5600s/y5600s00.pdf

Feckaninová A, Koscova J, Mudronova D, Popelka P, Toropilova J (2017) The use of probiotic bacteria against *Aeromonas infections* in salmonid aquaculture. Aquaculture 469:1-8. http://dx.doi.org/10.1016/j.aquaculture.2016.11.042

Feckaninová A, Koscová J, Mudronová d, Schisterová P, Maruscaková IC, Popelka P (2019) Characterization of two novel lactic acid bacteria isolated from the intestine of rainbow trout (*Oncorhynchus mykiss*, Walbaum) in Slovakia. Aquaculture 506:294-301. https://doi.org/10.1016/j.aquaculture.2019.03.026

Murga MLF, Bernik D, de Valdez GF, Disalvo AE (1999) Permeability and stability properties of membranes formed by lipids extracted from *Lactobacillus acidophilus* frown at different temperatures. Arch Biochem Biophys 364(1):115-121. https://doi.org/10.1006/abbi.1998.1093

Fgaier H, Eberl HJ (2011) Antagonistic control of microbial pathogens under iron limitations by siderophore producing bacteria in a chemostat setup. J Theor Biol 273(1):103-114. https://doi.org/10.1016/j.jtbi.2010.12.034

Fuller R (1989) Probiotics in man and animals. J Appl Bacteriol 66(5):365-378

Gatesoupe FJ (2000) Uso de probióticos en acuacultura. in avances en nutrición acuicola (Eds) Civera-Cerecedo R, Perez-Estrada CJ, Ricque-Marie D, Cruz-Suárez LE. La Paz B.C.S., México: Memorias del Iv Simposium Internacional de Nutrición Acuícola. Available at: https://www.uanl.mx/utilerias/nutricion_acuicola/IV/archivos/27gates.pdf (Accessed: 12 October 2019)

Giri SS, Sukumaran V, Oviya M (2013) Potential probiotic *Lactobacillus plantarum* VSG3 improves the growth, immunity and disease resistance of tropical freshwater fish, *Labeo rohita*. Fish Shellfish Immunol 34(2):660-666. https://doi.org/10.1016/j.fsi.2012.12.008

Gomez GD, Balcazar JL (2008) A review on the interactions between gut microbiota and innate immunity of fish. FEMS Immunol Med Microbiol 52(2):145-154. https://doi.org/10.1111/j.1574-695x.2007.00343.x

Grisez L, Reyniers J, Verdonck L, Swings J, Olivier F (1997) Dominant intestinal microflora of sea bream and sea bass larvae, from two hatcheries, during larval development. Aquaculture 155:387-399. https://doi.org/10.1016/S0044-8486(97)00113-0

Guo XH, Kim JM, Nam HM, Park SY, Kim JM (2010) Screening lactic acid bacteria from swine origins for multi-strain probiotics based on *in vitro* functional properties. Anaerobe 16(4):321-326. https://doi.org/10.1016/j.anaerobe.2010.03.006

Huang J, Zhang W, Hu Z, Liu Z, Du T, Dai, Xion T (2020) Isolation, characterization and selection of potential probiotic lactic acid bacteria from faeces of wild boar, native pig and commercial pig. Livest Sci 237:104036. https://doi.org/10.1016/j.livsci.2020.104036

Huys L, Dhert P, Robles R, Ollevier F, Sorgeloos P, Swings J (2001) Search for beneficial bacterial strains for turbot (*Scophthalmus maximus* L.) larviculture. Aquaculture 193:25-37. https://doi.org/10.1016/S0044-8486(00)00474-9

Kabir SML (2009) The role of probiotics in poultry industry. Int J Mol Sci 10(8):3531-3546. https://doi.org/10.3390%2Fijms10083531

Kavitha M, Raja M, Perumal O (2018) Evaluation of probiotic potential of *Bacillus spp.* Isolated from the digestive tract of freshwater fish *Labeo calbasu* (Hamilton, 1822). Aquac Rep 11:59-69. https://doi.org/10.1016/j.aqrep.2018.07.001

Lara-Flores M (2011) The use of probiotics in aquaculture: an overview. Int Res J Microbiol 2(12):471-478

Lara Mantilla CH, Vellojin Furnieles J, Garcia Perez D, Pertuz Bulevas V (2016) Desempeño del crecimiento y sobrevivencia de larvas de *Oreochromis ssp.* Utilizando un probiótico en el alimento. Rev Colomb Biotecnol 18(1):90-94. https://doi.org/10.15446/rev.colomb.biote.v18n1.57717

Lim SM, Jeong JJ, Jang SE, Han MJ, Kim DH (2016) A mixture of the probiotic strains *Bifidobacterium longum* CH57 and *Lactobacillus brevis* CH23 ameliorates colitis in mice by inhibiting macrophage activation and restoring the Th17/Treg balance. J Funct Foods 27:295-309. https://doi.org/10.1016/j.jff.2016.09.011

Mamianetti A, Garrido D, Carducci CN, Cristina Vescina M (1999) Fecal bile acid excretion profile in gallstone patients. Medicina 59(3):269-273

Meindong R, Doolgindachbaporn S, Sakai K, Tongpim S (2017) Isolation and selection of lactic acid bacteria from Thai indigenous fermented foods for use as probiotics in tilapia fish *Oreochormis niloticus*. Aquac Aquarium Conserv Legis 10(2):455-463

Merrifield DL, Dimitroglou A, Foey A, Davies SJ, Baker RTM, Bogwald J, Castex M, Ringo E (2010) The current status and future focus of probiotic and prebiotic applications for salmonids. Aquaculture 302:1–18. https://doi.org/10.1016/j.aquaculture.2010.02.007

Nayak SK (2010) Probiotic and immunity: A fish perspective. Fish Shellfish Immunol 29(1):2–14. https://doi.org/10.1016/j.fsi.2010.02.017

NMX-F-511-1998 (no date) Alimentos determinación de acidez en leche reconstituida. Normas mexicacanas. dirección general de normas. Available at: https//colpos.mx/bancodenormas/mexicanas/NMX-F-511.1988.pdf (Accessed: 22 August 2020)

Pérez-Sánchez T, Balcázar JL, García Y, Halaihel N, Vendrell D, de Blass I, Merrifield DL, Ruiz-Zarzuela I (2011) Identification and characterization of lactic acid bacteria isolated from rainbow trout, *Oncorhynchus mykiss* (Walbaum), with inhibitory activity against *Lactococcus garvieae*. J Fish Dis 34(7):499-507. https://doi.org/10.1111/j.1365-2761.2011.01260.x

Pirarat N, Pinpimai K, Rodkhum C, Chansue N, Ooi EL, Katagiri T, Maita M (2015) Viability and morphological evaluation of alginate-encapsulated *Lactobacillus rhamnosus* GG under simulated tilapia gastrointestinal conditions and its effect on growth performance, intestinal morphology and protection against *Streptococcus agalactiae*. Anim Feed Sci Tecnol 207:93-103. https://doi.org/10.1016/j.anifeedsci.2015.03.002

Prasad J, Gill H, Smart J, Gopal PK (1998) Selection and characterization of *Lactobacillus* and *Bifidobacterium* strains for use probiotics. Int Dairy J 8(12):993-1002. https://doi.org/10.1016/S0958-6946(99)00024-2

Ramírez-López C, Vélez-Ruiz JF (2016) Aislamiento, caracterización y selección de bacterias lácticas autóctonas de leche y queso fresco artesanal de cabra. Información Tecnológica 27(6):115-128. http://dx.doi.org/10.4067/S0718-07642016000600012

Ray AK, Ghosh K, Ringo E (2012) Enzyme-producing bacteria isolated from fish gut: A review. Aqua Nutr 18(5):465-492. https://doi.org/10.1111/j.1365-2095.2012.00943.x

Ringo E, Bendiksen HR, Wesmajervi MS, Olsen RE, Jansen PAS, Mikkelsen H (2000) Lactic acid bacteria associated with the digestive tract of Atlantic salmon (*Salmo salar* L). J Appl Microbiol 89:317–322. https://doi.org/10.1046/j.1365-2672.2000.01116.x

Ringo E (2020) Probiotics in shellfish aquaculture. Aquac Fish 5:1–27. https://doi.org/10.1016/j.aaf.2019.12.001

Ringo E, Gatesoupe FJ (1998) Lactic acid bacteria in fish: A review. Aquaculture 160:177–203. https://doi.org/10.1016/S0044-8486(97)00299-8

Smith P (2008) Antimicrobial resistance in aquaculture. Rev Sci Tech 27(1):243–264

Sugita H, Tsunohara M, Ohkoschi T, Deguchi Y (1988) The establishment of intestinal microflora in developing goldfish (*Carassius auratus*) of culture ponds. Microb Ecol 15:333-344

Do Vale Pereira G, Da Cunha DG, Pedreira Mourino JL, Rodiles A, Jaramillo-Torres A, Merrifield DL (2017) Characterization of microbiota in *Arapaima gigas* intestine and isolation of potential probiotic bacteria. J Appl Microbiol 123(5):1298-1311. https://doi.org/10.1111/jam.13572

Vershiuere L, Rombaut G, Sorgeloos P, Verstraete W (2000) Probiotic bacteria as biological control agents in aquaculture. Microl Mol Biol Rev 64(4):655-671. https://doi.org/10.1128%2Fmmbr.64.4.655-671.2000

Zapata AA, Lara-Flores M (2012) Antimicrobial activities of lactic acid bacteria strains isolated from Nile tilapia intestine (*Oreochromis niloticus*). J Biol Life Sci 4(1):164-171. https://doi.org/10.5296/jbls.v4i1.2408