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Effect of *Pediococcus parvulus* and *Candida parapsilosis* on growth and survival of tilapia, *Oreochromis niloticus* and *Oreochromis* sp.

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This study examined the effect of probiotic lactic acid bacteria (LAB) and yeast on growth and survival of tilapias, *Oreochromis niloticus* and *Oreochromis* sp. LAB and yeast were included in the diet. Microorganisms were identified at molecular level. The first bioassay (120 days, weight = 0.18 ± 0.03 g) was done with *Oreochromis* sp. with a control group, and animals fed with the probiotics (5×10^4, 1×10^6 and 1×10^7 CFU/g feed). The second bioassay lasted for 92 days with juveniles of *Oreochromis* sp. (weight 4.09 ± 0.99 g) and *O. niloticus* (weight 6.12 ± 0.86 g) and consisted of a control group and animals fed with probiotics (5×10^4 CFU/g) daily, every 10 days, and during the first 10 days only. LAB belonged to *Pediococcus parvulus* and yeast to *Candida parapsilosis*. In the first bioassay, no significant differences were found in survival and weight of *Oreochromis* sp. In the second bioassay, no significant differences were found in survival, but, in growth, fish fed with probiotics every 10 days (*O. niloticus*) and fish fed with probiotics daily and every 10 days (*Oreochromis* sp.) grew significantly better than the control group. The results indicated that LAB and yeast can be used as a feed additive every 10 days to reduce costs of commercial cultures.

Key words: *Oreochromis niloticus*, tilapia, probiotics, lactic acid bacteria, yeast.

INTRODUCTION

Aquaculture has become the fastest-growing sector of food production in the world; it has grown at an average rate of 8.9% per year since 1980s (FAO, 2012). Among cultured fish, tilapia has become very important in the last decades. Most produced tilapia is consumed in domestic markets in production areas, especially in rural Asia, Africa and South America. It plays a crucial role in food security and poverty alleviation in these regions. However, the demand of tilapia is growing in nontraditional and nonproducing countries (Vannuccini, 2001).

During the last years, efforts have been made in Mexico to develop the tilapia aquaculture industry, both rural and commercial. Therefore, in 2005, tilapia represented the second largest Mexican aquaculture product with 67 993 t/year, only exceeded by shrimp production, which reaches 90 041 t/year (Fitzsimmons, 2000; SAGARPA, 2005).

Although, tilapias are relatively more resistant to diseases than other cultured fish, many pathogenic organisms still plague them, affecting their production (Farmer and Hickman, 1992). Disease outbreaks are being increasingly recognized as a major constraint in aquaculture

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production and trade, affecting the economic development of the sector in many countries. Conventional approaches, such as the use of disinfectants and antimicrobial drugs, have had limited success in the prevention or cure of aquatic diseases (Subasinghe, 1997). However, there is widespread concern that the intense use and misuse of antibiotics in aquaculture have led to the emergence and selection of resistant bacteria (Inglis, 1996; Defoirdt et al., 2007). An alternative to prevent and control pathogenic bacteria is the use of probiotics. Probiotics are live microorganisms that have a beneficial effect on the host by increasing the immune response, or by the improvement of the use of feed and the environmental quality (Verschuere et al., 2000). Currently, the most common probiotics used in aquaculture belong to *Lactobacillus* sp., *Bifidobacterium* sp., *Vibrio* sp., *Saccharomyces* sp., *Enterococcus* sp. and *Bacillus* sp. (Kumar et al., 2006); which are administered, by enrichment of live foods, added to the diet or to the culture water (Panigrahia et al., 2005).

In recent years, some attempts have been made to obtain probiotics (bacteria and yeast) for the tilapia culture and successful effects were observed in growth promotion, immune stimulation and reducing the incidence of diseases (Lara-Flores et al., 2003; El-Haroun et al., 2006; Aly et al., 2008; Apún-Molina et al., 2009; Lara-Flores et al., 2010). In this sense, the aim of this study was to evaluate probiotic bacteria and yeast in terms of the growth performance and survival of *Oreochromis niloticus* and *Oreochromis* sp.

MATERIALS AND METHODS

Lactic acid bacteria and yeast strain

Lactic acid bacteria Lta2, Lta6, Lta8, Lta10 (LAB) (Apún-Molina et al., 2009) and yeast (Lt6) (Apún-Molina, unpublished data) used in this work as probiotics were originally isolated from the intestine of the tilapia, *O. niloticus*.

Molecular identification of yeast

DNA extraction was performed as follows: 50 µl of isolate was inoculated in 100 ml of MRS broth in an Erlenmeyer flask and incubated at 35°C for 18 to 24 h. The cells were collected by centrifugation at 3000 *g* for 15 min. The cell pellet was washed three times with buffer, 10 mM Tris-HCl (pH 8.0), and excess buffer was removed. The cell pellet was lyophilized and pulverized in a mortar. Cells were placed in a sterile Eppendorf tube and 500 µl of 50 mM HEPES (N-2-Hydroxyethylpiperazine-N-2-Ethane Sulfonic Acid) solution was added and stirred with a vortex for 30 s. Fifty microliters of lysozyme solution (10 mg/ml of distilled water) were added and incubated for 1 h at 37°C. Subsequently, 50 µl of 10% SDS solution was added, mixed by inversion and incubated at 65°C for 15 min; then, 500 µl of Tris-EDTA solution was added, and homogenized by inversion. The mixture was deproteinized by phenolization, and then the DNA was precipitated with 1/10 volumes of 3 M sodium acetate (pH 5.2) and 2.5 volumes of cold absolute ethanol. The DNA was recovered by centrifugation and washed with 70% ethanol. Subsequently, DNA was resuspended in 250 µl of TE (10 mM Tris, pH 8.0 and 0.1 mM EDTA) solution.

18S, 5.8S and 28S ribosomal genes (yeast)

The primers (ITS4 5'-TCCTCCGCTTATTGATATGC-3' and ITS5 5'-GGAAGTAAAAGTCGTAACAAGG-3' (White et al., 1990) used in the PCR amplified a genome fragment of 350 bp. Reaction mixture and amplification was performed according to White et al. (1990). Amplification was done in a thermocycler Tpersonal (Biometra, Goettingen, Germany) using the following program: initial denaturation (94°C for 5 min), 30 cycles of denaturation (94°C for 1 min), annealing (50°C for 1 min), extension (72°C for 45 s), and a final extension (72°C for 7 min). Aliquots of the PCR products were analyzed in 1% agarose gel electrophoresis stained with ethidium bromide, visualized under UV light, and photographed. PCR products were cleaned with QIAquick PCR Purification Kit (Invitrogen, Carlsbad, CA, USA).

Molecular identification of LAB

DNA extraction was performed with Bactozol kit (MRC, Cincinnati, OH, USA), and a 1500-bp fragment of the 16S rRNA gene was amplified by using primers 27f and 1492r (Jensen et al., 2002). PCR products were cleaned with spin columns and quantified with Quant-iT™ dsDNA HS kit (Invitrogen). PCR products were tested for DNA sequencing.

Sequencing

Ribosomal genes were sequenced using the primers reported above. Purified PCR products were sent for sequencing in an automated sequencer (Applied Biosystems 3730xl, Foster City, CA, USA). Sequences were subjected to BLAST searches (Altschul et al., 1990) by using the National Center for Biotechnology Information GenBank database.

Phylogenetic analysis

Sequence and phylogenetic analyses were performed with the Molecular Evolutionary Genetics Analysis software (MEGA5) (Tamura et al., 2011). The phylogenetic tree was constructed in the program MEGA5 using the neighbor-joining method (Saitou and Nei, 1987). The tree topology was evaluated on 1000 replicates (bootstraps) with MEGA5 software. Additionally, the sequences were compared with the MegAlign DNASTAR® software (Version 2.0, Madison, Wisconsin, USA).

LAB

Sequences of the 16S gene were aligned with *Pediococcus*, *Lactobacillus*, *Weisella* and *Lactococcus* sequences by using MEGA5 software. *Thermotoga maritima* sequence was used as outgroup to root the tree.

Yeast

Partial sequences of 18S, 5.8S and 28S genes were aligned with *Candida* and *Saccharomyces* sequences by using MEGA5 software. *Aspergillus niger* sequence was used as outgroup to root the tree.

Preparation of experimental diet with LAB and yeast

Microorganisms were quantified before been added to feed. The probiotic mixture of LAB and yeast, in the same proportion, were sprayed on commercial feed (Silver Cup®, Mexico, 45% protein).

Dry Oil® (Innovaciones Acuícolas, S.A. de C.V., Culiacán, Mexico) was used as adhesive and feed attractant. Feed was dried at room temperature for 5 h and stored at 4°C in a refrigerator for 8 days. Most of the microorganisms in feed remain viable under the storage conditions (data not shown) (Apun-Molina et al., 2009).

Experimental design

Hormone-treated fries were obtained from a private farmer (Aquatic Depot, S.A. de C.V., Guadalajara, Mexico) and acclimated for 5 days in two outdoor 1000-L plastic tanks with 800 L aerated freshwater. To evaluate the effect of probiotic microorganisms on growth performance and survival of tilapia, the outdoor culture system consisted of 2000-L plastic tanks with 1200 L aerated freshwater. The two bioassays were conducted as a completely randomized design with four treatments in triplicate. The fish were fed *ad libitum* with floating micro-pellets containing 45% protein. Water exchange was about 80% weekly. Photoperiod was 12:12 h light: dark cycle. Values of temperature (HI 98127 pHep, Hanna Instruments, Woonsocket, RI, USA), pH (HI 98127 pHep, Hanna Instruments) and dissolved oxygen (YSI model 55 Oxygen meter, Yellow Spring Instruments, Yellow Springs, OH, USA) were deter-mined weekly. Water samples for nitrite, nitrate and ammonia determinations were analyzed monthly following the Strickland and Parsons (1968) method. Growth in weight was monitored monthly by weighing all fish per tank in a balance (Scout Pro SP 601, Ohaus Corporation, Pine Brook, NJ, USA). Mortality was recorded daily.

Bioassay with *Oreochromis* sp.

Bioassay lasted for 120 days and the initial weight of fries was 0.18 ± 0.03 g. Treatments were as follows: (1) control group, fish fed with commercial feed plus Dry Oil®; (2) fish fed with commercial feed sprayed with probiotic mixture (5×10^4 CFU/g); (3) fish fed with commercial feed sprayed with probiotic mixture (1×10^6 CFU/g); (4) fish fed with commercial feed sprayed with probiotic mixture (1×10^7 CFU/g). At the beginning of the experiment, each treatment had three replicates of 120 tilapias, 40 organisms per tank. However, after 30 days of culture, the tilapia number was reduced to 60 organisms per treatment, 20 organisms per tank.

Bioassay with *O. niloticus* and *Oreochromis* sp.

Bioassay lasted for 92 days with juveniles of *Oreochromis* sp. (weight 4.09 ± 0.99 g) and *O. niloticus* (weight 6.12 ± 0.86 g). Treatments were as follows: (1) fish fed daily with commercial feed plus Dry Oil®; (2) fish fed daily with commercial feed sprayed with probiotic mixture (5×10^4 CFU/g); (3) fish fed with commercial feed sprayed with probiotic mixture (5×10^4 CFU/g) every 10 days; (4) fish fed with commercial feed sprayed with probiotic mixture (5×10^4 CFU/g) only during the first 10 days. In treatments 3 and 4, fish were fed with commercial feed plus Dry Oil® when they were not fed with probiotics. At the beginning of the experiment, each treatment had three replicates of 240 (120 *Oreochromis* sp. and 120 O. *niloticus*) tilapias, 80 organisms per tank. However, after 30 days of culture, the tilapia number was reduced to 120 (60 *Oreochromis* sp. and 60 *O. niloticus*) organisms, 40 organisms per tank.

Absolute growth, absolute growth rate and specific growth rate

The absolute growth (AG), absolute growth rate (AGR) and specific growth rate (SGR) were calculated based on the following formulas:

$AG\ (g) = W\text{-}W_0$

$AGR\ (g/d) = (W\text{-}W_0)/(t\text{-}t_0)$

$SGR\ (\%/d) = 100\ (\log W - \log W_0)/t$

Where, W_0 represents the initial body weight of tilapia fry, W represents the final body weight of tilapia and t represents time in days.

Statistical analysis

A one-way analysis of variance (ANOVA) was used to examine growth differences among treatments. Survival data were arcsine transformed according to Daniel (1997). When significant differences were found, Tukey's HSD test was used to identify the source of these differences ($P < 0.05$).

RESULTS

The sequences obtained for the 16S rRNA gene were used as a framework for the identification and classification of probiotic bacteria. Results showed that LAB isolates (Lta2, Lta6, Lta8 and Lta10) had identities of 93.7, 94.1, 94.0 and 93.8% with *Pediococcus parvulus*, respectively. Among the isolates there were identities above 99.5%. The phylogenetic tree groups Lta2, Lta6, Lta8 and Lta10 with *P. parvulus* (Figure 1).

The sequences obtained for the ribosomal genes (18S, 5.8S and 28S**)** were used as a framework for the identification and classification of yeast. The isolate Lt6 showed an identity of 100% with *Candida parapsilosis*. The phylogenetic tree clearly groups Lt6 with *C. parapsilosis* (Figure 2).

Bioassay with *Oreochromis* sp.

Results obtained during the first bioassay showed that fish growth did not increase significantly in the presence of bacteria and yeast. Also, treatments with microorganisms did not show significant differences on survival as compared to fish fed commercial feed (Table 1) ($P > 0.05$).

During the culture period, the water temperature was 26.37 ± 0.80°C, dissolved oxygen was 8.67 ± 0.43 mg/l, pH 8.45 ± 0.26, nitrites 0.21 ± 0.02 mg/l, nitrates 0.71 ± 0.04 mg/l and ammonia 0.44 ± 0.01 mg/l. The physicchemical water parameters recorded during the study were within the recommended tolerance range of Nile tilapia (Jiazhao, 1991; Popma and Lovshin, 1996).

Bioassay with *O. niloticus* and *Oreochromis* sp.

Table 2 summarizes the results obtained during the second bioassay for *O. niloticus*. Weight at harvesting (WH) showed that fish fed daily with commercial feed sprayed with probiotic mixture (5×10^4 CFU/g feed) in treatment 2 was significantly higher than in the control group ($P = 0.02$). No significant differences were found among treatments with probiotics. No significant differen-

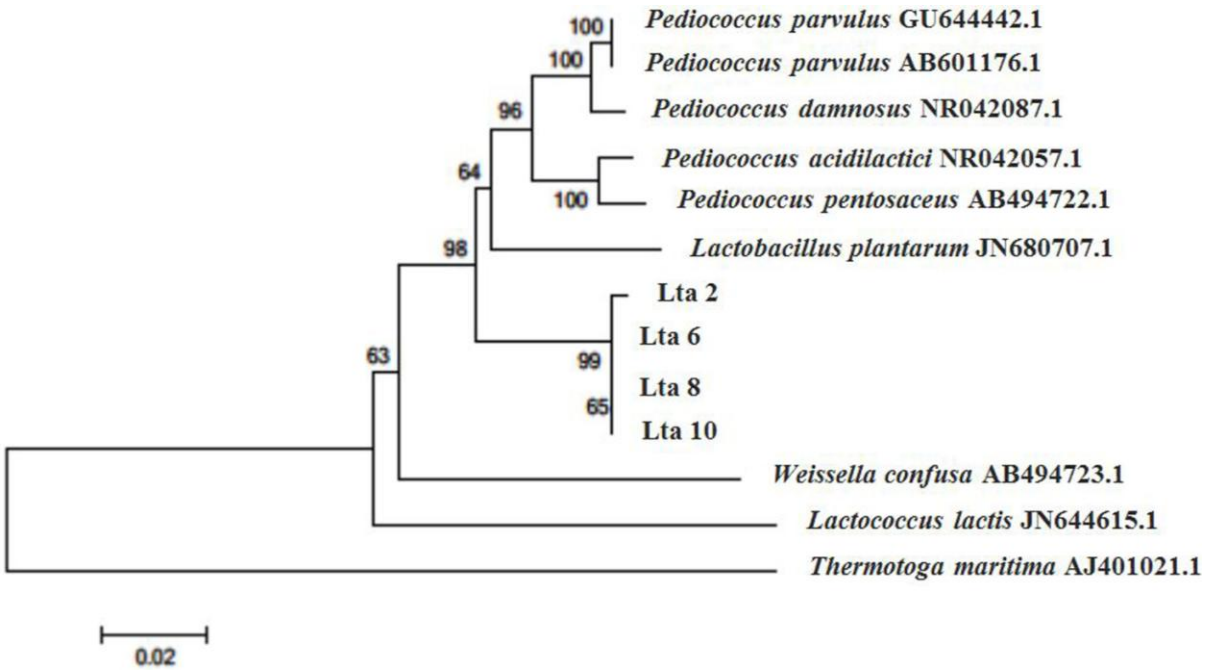

Figure 1. Phylogenetic tree (neighbor-joining) for LAB of *O. niloticus* and different sequences of LAB (accession numbers in GenBank are indicated) derived from 16S rRNA gene. *Thermotoga maritima* was used as outgroup. The numbers at the nodes indicate the levels of bootstrap support based on 1000 replicates. Bar = sequence divergence.

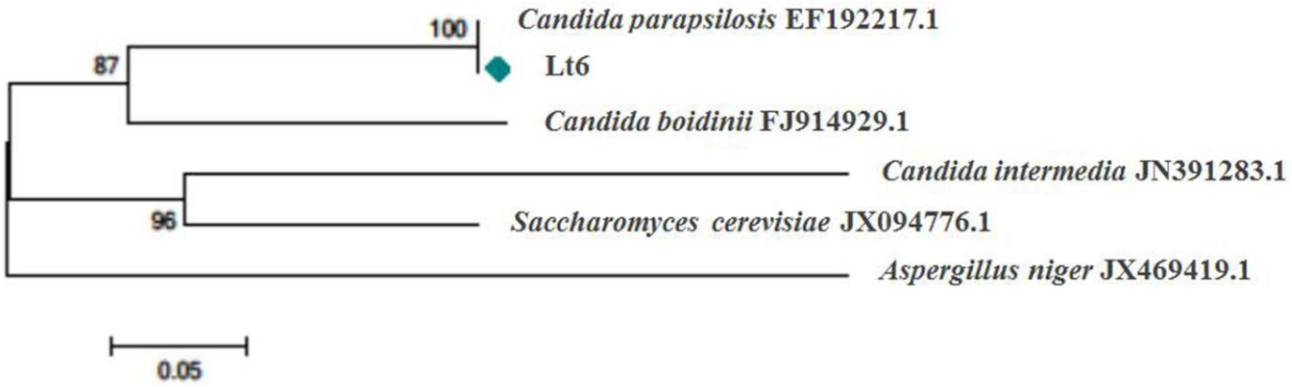

Figure 2. Phylogenetic tree (neighbor-joining) for yeast of *O. niloticus* and different sequences of yeast (accession numbers in GenBank are indicated) derived from 18S, 5.8S and 28S rRNA genes. *Aspergillus niger* was used as outgroup. The numbers at the nodes indicate the levels of bootstrap support based on 1000 replicates. Bar = sequence divergence.

Table 1. Growth performance and survival of *Oreochromis* sp. fed diets supplemented with LAB and yeast.

Mean value	Treatment			
	Control[1]	$(5 \times 10^4)^2$	$(1 \times 10^6)^3$	$(1 \times 10^7)^4$
Weight at harvesting (g ± SE)	99.5 ± 2.5	97.2 ± 3.1	96.6 ± 4.5	97.3 ± 4.0
Absolute Growth (g ± SE)	99.4 ± 2.5	97.1 ± 3.0	96.4 ± 4.4	87.2 ± 4.0
Absolute Growth Rate (g/d ± SD)	0.8 ± 0.0	0.81 ± 0.0	0.81 ± 0.0	0.73 ± 0.0
Specific Growth Rate (%/d ± SD)	6.0 ± 0.0	5.9 ± 0.0	5.9 ± 0.1	5.99 ± 0.0
Survival (% ± SD)	92.1 ± 8.6	76.5 ± 5.2	85.7 ± 4.3	82.6 ± 12.2

[1]Fish fed with commercial feed plus Dry Oil®; [2]fish fed with commercial feed sprayed with probiotic mixture (5×10^4 CFU/g feed); [3]fish fed with commercial feed sprayed with probiotic mixture (1×10^6 CFU/g feed); [4]fish fed with commercial feed sprayed with probiotic mixture (1×10^7 CFU/g feed). SD = Standard deviation, SE = standard error.

Table 2. Growth performance and survival of *O. niloticus* fed diets supplemented with LAB and yeast.

Mean value	Treatment			
	Control[1]	(5 x 10^4)[2]	(5 x 10^4)[3]	(5 x 10^4)[4]
Weight at harvesting (g ± SE)	80.6 ± 2.7[a]	89.7 ± 2.4[b]	86.2 ± 2.6[ab]	84.8 ± 2.4[ab]
Absolute Growth (g ± SE)	74.1 ± 2.5	83.3 ± 2.2	80.8 ± 2.3	78.5 ± 2.1
Absolute Growth Rate (g d^{-1} ± SD)	0.8 ± 0.0	0.91 ± 0.0	0.88 ± 0.0	0.86 ± 0.0
Specific Growth Rate (% d^{-1} ± SD)	2.3 ± 0.0[a]	2.4 ± 0.0[a]	2.6 ± 0.0[b]	2.44 ± 0.0[a]
Survival (%)	100	100	100	100

[1]Fish fed daily with commercial feed plus Dry Oil®; 2Fish fed daily with commercial feed sprayed with probiotic mixture (5 × 10^4 CFU/g feed); [3]fish fed with commercial feed sprayed with probiotic mixture (5 × 10^4 CFU/g feed) every 10 days; [4]fish fed with commercial feed sprayed with probiotic mixture (5 × 10^4 CFU/g feed) only during the first 10 days. SD = Standard deviation. SE = standard error. Values with different superscript in the same row are statistically different (P < 0.05).

Table 3. Growth performance and survival of *Oreochromis* sp. fed diets supplemented with LAB and yeast.

Mean value	Treatment			
	Control[1]	(5 x 10^4)[2]	(5 x 10^4)[3]	(5 x 10^4)[4]
Weight at harvesting (g ± SE)	40.9 ± 1.8[a]	46.8 ± 2.3[ab]	48.0 ± 1.7[b]	45.9 ±1.8[ab]
Absolute Growth (g ± SE)	36.8 ± 0.9	42.7 ± 2.0	44.1 ± 0.8	41.6 ± 3.4
Absolute Growth Rate (g d^{-1}± SD)	0.40 ± 0.0	0.46 ± 0.0	0.48 ± 0.0	0.45 ± 0.0
Specific Growth Rate (% d^{-1}± SD)	2.1 ± 0.0[a]	2.3 ± 0.0[b]	2.3 ± 0.0[b]	2.2 ± 0.1[ab]
Survival (% ± SD)	91.6 ± 2.3	96.6 ± 2.1	88.3 ± 4.5	95 ± 3.2

[1]Fish fed daily with commercial feed plus Dry Oil®; [2]fish fed daily with commercial feed sprayed with probiotic mixture (5 × 10^4 CFU/g feed); [3]fish fed with commercial feed sprayed with probiotic mixture (5 × 10^4 CFU/g feed) every 10 days; [4]fish fed with commercial feed sprayed with probiotic mixture (5 × 10^4 CFU/g feed) only during the first 10 days. SD = Standard deviation. SE = standard error. Values with different superscript in the same row are statistically different (P < 0.05).

ces were found among treatments in the absolute growth (AG) and absolute growth rate (AGR). Specific growth rate (SGR) of fish fed with probiotics every 10 days was significantly higher than in the control group (P = 0.0003), in fish fed with probiotics daily (P = 0.010), and in fish fed with probiotics only during the first 10 days (P = 0.002). No mortalities were registered in all treatments.

Results (Table 3) obtained for *Oreochromis* sp. showed that WH in fish fed every 10 days (treatment 3) with probiotic mixture (5 × 10^4 CFU/g feed) was significantly higher than in the control group (P = 0.04). No significant differences were found among treatments in AG and AGR. Specific growth rate of fish fed with probiotics daily and every 10 days was significantly higher than in the control group (P = 0.03, P = 0.01, respectively). Treatments with microorganisms did not show significant differences on survival as compared to fish fed commercial feed.

During the culture period, the water temperature was 25.08 ± 0.11°C, dissolved oxygen 7.53 ± 0.51 mg/l, pH 8.2 ± 0.08, nitrites 0.19 ± 0.16 mg/l, nitrates 0.56 ± 0.09 mg/l, and ammonia from 1.44 ± 0.72 mg/l. The physic-chemical water parameters recorded during the study were within the recommended tolerance range of Nile tilapia (Jiazhao, 1991; Popma and Lovshin, 1996).

DISCUSSION

In the last years, efforts have been made to find alternatives to antimicrobials as growth promoters in aquaculture. In this sense, the use of beneficial microorganisms (probiotics) is increasing (Verschuere et al., 2000; Balcázar et al., 2006; Zhou et al., 2010).

The molecular identification of the four LAB (Lta2, Lta6, Lta8, and Lta10) isolated from the intestine of the tilapia *O. niloticus* (Apún-Molina et al., 2009) showed that they belong to the species *P. parvulus* with identities of 93.7, 94.1, 94.0 and 93.8%, respectively. *P. varvulus* showed a high relatedness to *Pediococcus damnosus* and *Pediococcus inopinatus* (Dobson et al., 2002). Therefore, according to the identity percentages observed, it is recommended to use other methods different from the 16S rRNA gene, such as 16S-23S internally transcribed spacer regions and the heat shock protein (HSP)60 gene for delineating the species (Dobson et al., 2002). *Pediococcus* is a lactic acid bacteria, belonging to the family Lactobacillacea (Garvie, 1986). The only report on the probiotic effect of *P. parvulus* (Lta2, Lta6, Lta8 and Lta10) on *O. niloticus* was done by Apún-Molina et al. (2009).

The yeast isolate (Lt6) showed an identity of 100% with

the species *C. parapsilosis*. *C. parapsilosis* is a normal human commensal, and has been isolated from non human sources (Weems, 1992) such as insects, soil, domestic animals and marine environments (Fell et al., 1967). There are no reports on the use of this species as probiotic in aquaculture.

In aquaculture, growth rate is an important factor because it reflects the production yield (Oduleye, 1982). In the first bioassay with *Oreochromis* sp., treatments with the probiotic mixture did not enhance growth (SGR) as compared with the control group. Similarly, Günther and Jiménez-Montealegre (2004) found that *Bacillus subtilis* added to the feed does not improve growth of *O. niloticus*. Conversely, in the second bioassay, SGR of juveniles of *O. niloticus* and *Oreochromis* sp. fed with the probiotic mixture was significantly higher than in the control group. In *O. niloticus*, SGR of fish fed with probiotics every 10 days was significantly higher than in the control group, in fish fed with probiotics daily, and in fish fed with probiotics only during the first 10 days. In *Oreochromis* sp., SGR of fish fed with probiotics daily and every 10 days was significantly higher than in the control group. It is important to note that fish in the first bioassay weighed 0.18 ± 0.03 g at the beginning of the bioassay and were fed daily with increasing concentrations of probiotics per gram of feed. On the other hand, in the second bioassay, juveniles of *Oreochromis* sp. and *O. niloticus* weighed 4.09 ± 0.99 and 6.12 ± 0.86 g, respectively, and were fed with only one probiotic concentration. It is possible that LAB reduced pH of the fish digestive tract in the first bioassay, but not in the second one. To this regard, it is known that the pH of the digestive tract of juveniles in some species is alkaline at an early age and becomes acid with maturation (Walford and Lam, 1993; Yúfera et al., 2004; Darias et al., 2005). The improved growth in weight observed in the second bioassay is similar to those found in *Cyprinus carpio* fed diets supplemented with *Streptococcus faecium* (Bogut et al., 1998); *Oreochromis niloticus* fed diets supplemented with *Lactobacillus acidophilus*, *Streptococcus faecium* and *Saccharomyces cerevisiae* (Lara-Flores et al., 2003); *O. niloticus* fed diet with the probiotic Biogen® consisting of *Bacillus* sp. and *Lactobacillus* sp. (El-Haroun et al., 2006); and *O. niloticus* fed with 5×10^4 CFU/g feed of the LAB used in this study (Apún-Molina et al., 2009).

According to Cahill (1990), the microorganisms present in the intestinal tract generally seem to be those from the environment or the diet. In this work, probiotics administered daily and every 10 days had a beneficial effect on fish growth; however, when fish were fed with probiotic during the first 10 days only (second bioassay), growth did not improve. According to the above results, research is needed on the ability of microorganisms to adhere to gut fish. In this sense, Verschere et al. (2000) mentioned that the beneficial effects of probiotics are temporal. Probiotics need to attach to the intestinal tract to remain in the cultured animal to exert their beneficial effects. Also, attached probiotics need to multiply to influence the gastrointestinal microbiota of their host (Cahill, 1990; Andlid et al., 1998; Mack et al., 1999; Forestier et al., 2001; Ouwehand et al., 2001).

The results obtained in the present study indicate that LAB and yeast can be used as a feed additive every 10 days to reduce costs in commercial cultures.

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