International Journal of Aquaculture, 2016, Vol.6, No.2, 1−9
http://ija.biopublisher.ca

Research Article Open Access

In Vivo Evaluation of Indigenous Enterococci Strains on Biometrical Parameters of *Piaractus mesopotamicus* Embryos and Larvae

Guidoli M.G.[1,2,3], Hernández D.Q.[1], Boehringer S.I.[2], Sánchez S.[1], Nader-Macías M.E.F.[3]

1 Instituto de Ictiología del Nordeste, Argentina
2 Cátedra de Microbiología, Facultad de Ciencias Veterinarias, Universidad Nacional del Nordeste, Argentina
3 Centro de Referencia de Lactobacilos, Argentina
Corresponding author Email: fnader@cerela.org.ar

International Journal of Aquaculture, 2016, Vol.6, No.2 doi: 10.5376/ija.2016.06.0002
Received: 19 Mar., 2016
Accepted: 29 Apr., 2016
Published: 05 May, 2016

Preferred citation for this article:
Guidoli M.G., Hernández D.Q., Boehringer S.I., Sánchez S. and Nader-Macías M.E.F., 2016, In Vivo Evaluation of Indigenous Enterococci Strains on Biometrical Parameters of *Piaractus mesopotamicus* Embryos and Larvae, 6(2): 1-9 (doi: 10.5376/ija.2016.06.0002)

Abstract The use of autochthonous microorganisms as probiotics is a novel and promising alternative for the preventive application of antibiotics. Lactic acid bacteria (LAB) are one of the most successfully microorganisms used as probiotics and applied in different niches. The aquaculture of Piaractus mesopotamicus in Northern Argentina has increased exponentially, but the use of this native species in production systems led to a deficit in the availability of larvae for breeding and fattening. The objective of the present study was to evaluate the effect of the addition of two different formulae containing autochthonous beneficial LAB strains on the survival, mean weight and biomass of larvae under laboratory intensive breeding. Results indicate that the bacterial administration does not cause any significant effect on the variables evaluated. However, statistical analysis showed a tendency of the 6x107 CFU L-1 dose of one of the formula to stimulate an increase of mean weight and biomass. On the other hand, the average values of biometrical parameters obtained with the second formula, indicate that the optimum dose is 6x104 CFU L-1. These results and the histological confirmation of the harmfulness of the strains, allowed us to select these doses for their use in a combined probiotic mixture to be tested in future assays.

Keywords Aquaculture; Beneficial effects; *Enterococcus faecium*; Larvae, *Pediococcus acidilactici*; *Piaractus mesopotamicus*; Probiotics

1 Introduction

The use of native species in the aquaculture of Northeastern Argentina had shown an increased productivity, allowing their predominance in the National fish production chart. *Piaractus mesopotamicus* is the most cultivated specie in this region and the most farmed fish in the country since 2012 (Dirección de Acuicultura, 2013), with 2 017.45 tons produced in 2013, representing a 52.22% of the Argentinian aquaculture production (Dirección de Acuicultura 2014). The inclusion of indigenous fishes indicates an advantage supported by their resistance to the environmental conditions. However, they were not deeply studied, leaving gaps in some critical issues such as animal development, behavior, nutritional requirements and susceptible diseases. These facts and the high demand of larvae, juveniles and sexually mature adults, generated a deficit in the number of animals available. Thus, the requirement of techniques aimed to increase the production in aquaculture facilities becomes a gap in the area. Antibiotics are one of the applied strategies, used as growing factors, anti-infectious agents and also for tranquilization (FDA, 1998; Serrano, 2005). However, the European Union (EU), under the European Food Safety Authority (EFSA), have regulated the use of additives in animal feeding and prohibited antibiotics as additives in foods (EFSA, 2008). These regulations are now applied in most of the continents, supported mainly by various items: resistance transference (FAO/NACA/WHO, 1997), toxicity of residues (SOU, 1997), allergies and effects on human intestinal microbiota (Serrano, 2005) and environmental risks (EC, 1996). Although expected, these regulations have generated an urgent problem with many difficulties to solve: the search of more extensive production systems without the requirement of using additives, and/or the application of natural, novel and safe products to get similar results to those previously obtained with antibiotics.

The use of probiotics is widely applied in order to replace the use of chemotherapeutic agents in animal production, and to restore the indigenous microbiota. In aquaculture systems, probiotics were described as "a live microbial adjunct which has a beneficial effect on the host by modifying the host associated or ambient microbial community, by ensuring improved use of feed or enhancing its nutritional value, by enhancing the host response toward disease or by improving the quality of its ambient environment" (Verschuere et al., 2000), and later defined for a wide variety of hosts (ISAPP, 2011). Several beneficial effects were reported for different aquatic species: inhibitory activity against pathogens, nonspecific immune response stimulation, immunomodulatory effect, decrease of mortality and increase of growth rate and production (Gullian et al., 2004; Vieira et al., 2010; Nayak and Mukherjee, 2011; Rahman et al., 2011; Lakshmi et al., 2013).

Different bacterial genus, mainly those classified as lactic acid bacteria (LAB), including lactobacilli, enterococci and related (Bifidobacteria) have been potentially evaluated as probiotics (Mego et al., 2005; Marcinakova et al., 2006). Among enterococci, only *Enterococcus faecium* is included in most of the major probiotic products commercially available for aquatic animal nutrition in the EU (Bogut et al., 2000; Chang and Liu, 2002; Vahjen et al., 2007; Gatesoupe, 2008; Abumourad et al., 2014). *Pediococcus acidilactici* is a Food Grade Microorganism (FGM) frequently isolated from dairy products. As they are included in the Qualified Presumption of Safety (QPS) and found in a variety of ecosystems, are commonly used as probiotic for humans (Balgeri et al., 2013), terrestrial (Giancamillo et al., 2008; Mikulski et al., 2012), and aquatic animals (Castex et al., 2010; Neissi et al., 2013 and 2015; Giannenas et al., 2015; Ramos et al., 2015).

In previous studies *E. faecium* strains CRL 1937, CRL 1938, CRL 1940 and CRL 1941 and *P. acidilactici* strain CRL 1939 were isolated from healthy specimens of *P. mesopotamicus*, selected as potentially probiotic based on their "in vitro" beneficial properties and grouped into two formulae: LAB containing strains isolated in summer (*P. acidilactici* CRL 1939 and *E. faecium* CRL 1940 and CRL 1941) and ENT containing enterococci isolated in winter (*E. faecium* CRL 1937, CRL 1938) (Guidoli et al., 2015). As there are no previous descriptions of autochthonous strains as probiotics in this fish species, the aim of this work was to evaluate the effect of these two formulas on the survival, mean weight, biomass and histological parameters of *P. mesopotamicus* larvae. Another purpose was to select the optimum doses and stages of administration of each formula for the design of a multi-strain probiotic product.

2 Materials and Methods

2.1 Strains and formulae

Autochthonous *E. faecium* CRL1937, CRL1938, CRL1940 and CRL1941 and *P. acidilactici* CRL1939 strains were isolated from diverse body areas of healthy *P. mesopotamicus* specimens during different seasons and selected as potentially probiotic microorganisms based on their ability to express beneficial properties in *in vitro* tests (Table 1) (Guidoli et al., 2015). Strains were grouped into two formulae (LAB and ENT) according to their characteristics and the season in which were isolated (Table 1). All the microorganisms were included in the CERELA strain collection and in the Laboratorio de Sanidad Animal of the Estación Experimental Agropecuaria Rafaela belonging to the Instituto Nacional de Tecnología Agropecuaria (INTA) under Budapest treaty for patent aims (Conicet and Unne, 2013).

Table 1 Origins and beneficial properties of the potentially beneficial microorganisms assayed

Strain	Season	Anatomical part	Fish Weight (g)	Beneficial properties	Formulae (Selection criteria)
E. faecium CRL 1937	Winter	Medium Intestine	195.25	Inhibition of pathogens and food borne microorganisms	ENT (Selected enterococci isolated in winter)
E. faecium CRL 1938					
P. acidilactici CRL1939	Summer	Hole fish homogenate	0.379	H_2O_2 production and inhibition of pathogens and food borne microorganisms	LAB (Selected Lactic Acid Bacteria isolated in summer)
E. faecium CRL 1941			0.451		
E. faecium CRL 1940		Gills	1,399	High hydrophobicity and autoaggregation indexes	

2.2 Bacterial suspensions

The lactic acid bacteria (LAB) were grown daily in 400 mL LAPTg broth (Raibaud et al., 1963) and incubated at 37℃ in static conditions for 8 h. Bacterial cells were harvested by centrifugation at 3,000 g for 10 min at 4℃, washed twice with sterile distilled water and suspended to obtain the required viable cells concentration.

2.3 Live food preparation

One L freshwater containing a suspension of 1 g of brine shrimp cysts *Artemia* sp., 15 g of NaCl and 2 g of sodium bicarbonate was submitted to an incubation process under intense aeration and lightening for 24 h. Live hatched nauplii were harvested from the bottom of the hatching device by sedimentation, filtered to obtain those between 250 and 150 μm size and suspended in freshwater. Aliquots of the nauplii suspension previously obtained were counted in order to obtain their approximate number and concentration of the solution (Torrentera and Tacon, 1989).

2.4 Fish reproduction

P. mesopotamicus larvae were obtained by controlled reproduction from broodstock of the Instituto de Ictiologia del Nordeste (Corrientes, Argentina). Spawning was induced by injection of pituitary extract from the of *Prochilodus lineatus* (5 and 1.5 mg pituitary gland kg^{-1} of body weight in females and males, respectively) according to Da Silva et al.(1988). The sexual gametes were obtained by the stripping technique, mixed immediately in a bowl, suspended into fresh water for 5 minutes, washed twice and suspended at the desired concentration (Gómez et al., 2014). Aliquots of 1 mL were counted in order to obtain an approximate concentration of fecundated eggs.

2.5 Experimental design and sampling

Experimental units were settled as 5 L plastic fishbowls with a constant recirculation system and approximately 300 fecundated eggs. The formulae were administered in three different concentrations, named as: 4 ($6x10^4$ CFU L-1), 7 ($6x10^7$ CFU L-1) and 10 ($6x10^{10}$ CFU L-1) of each strain. Also at different stages of the biological cycle of larvae: E (from the time of fecundation of the eggs until the beginning of the exogenous feeding, 5 days), L (from the beginning of the exogenous feeding until the end of the assay in the laboratory, from day 5 up to day 15) and E&L (from the fecundation of the eggs until the end of the assay in laboratory, from day 0 up to day 15). Control units (CTRL) were performed with no addition of bacteria.

During stage E the bacterial suspension was added directly to the fish bowls four times a day with previous stop of water recirculation which was restarted one hour after. In stage L, bacteria were co-incubated with live food during two hours previous to administration; this procedure was performed *ad libitum* four times a day. Water recirculation was interrupted before alimentation and restored one hour after. Water quality determinations of pH, dissolved oxygen and temperature were performed daily in each experimental unit.

2.6 Sampling

At day 15, larvae were counted and weighed to obtain values of survival, mean weight and biomass. To evaluate the normal development, macroscopic evaluations were performed with binocular magnifier Optical Kyowa model SDZ. For histological studies, 10 larvae per treatment were collected and anaesthetized by chilling on ice, fixed in Bouin's solution (saturated picric acid 3000 ml, formaldehyde 1000 ml, glacial acetic acid 200 ml) for 12 h, washed twice with alcohol 70° and maintained in this solution until processing. Samples were then routinely processed for histology, stained with haematoxylin and eosin and analyzed by light microscopy using a Leica DM500 microscope, a Leica ICC50 digital camera and the Leica Application Suite 3.4.1 image analysis system (Culling, 1974).

The procedures and experimental protocols applied to the animals in this work were in accordance with the ethical principles of animal experimentation, and approved according to protocol n.0019/14-2011-02204 and

14-2012-03865 by the Ethics and Biosafety Committee of the School of Veterinary Sciences of the Northeast National University (UNNE) of Argentine.

2.7 Statistical evaluation

All the assays were performed by triplicate using a completely randomized design. Each replicate corresponds to different parents, excluding the genetic factor of the experiment. Statistical analyses were carried out using Statistica 6.0 for Microsoft Windows. Comparisons were performed, first, by a one way ANOVA including the ten experimental groups followed by a control vs. treatments comparison. Later, results were compared, excluding the control, by using a factorial two-way ANOVA with subsequent post hoc test in order to evaluate the main effects of doses and stages as well as the interactions between them. When interaction and significant effects were not detected, results were evaluated by orthogonal polynomials for trend analysis.

3 Results

The one way ANOVA of survival, mean weight and produced biomass of larvae administered with the microbial mixture of LAB does not indicate significant differences between treatments and control group ($p>0.05$) (Table 2). On the other hand, the two ways ANOVA analysis does not show neither interaction between doses and stages nor significant effect of dose or stage over any of the variables analyzed ($p>0.05$) (Figure 1). However, the low parameters in some of the treatments with dose 7 and 10 allowed to select the LAB suspension (composed by *E. faecium* strains CRL1940 and CRL1941 and *P. acidilactici* CRL 1939), containing $6x10^4$ CFU L-1 of each strain as the most suitable for its incorporation to a composite probiotic formula to be tested in future assays (Figure 2).

Table 2 One way ANOVA of mean weight, survival and biomass of *P. mesopotamicus* larvae administered with LAB mixture in different doses and stages

Variable	N	SS	df	MS	F	p-value
Mean weight (mg)	30	5.99	9	0.62	2.40	0.0494
Survival (%)	30	5336.48	9	592.94	1.47	0.2241
Biomass (mg)	30	275674.00	9	30630.44	1.88	0.1151

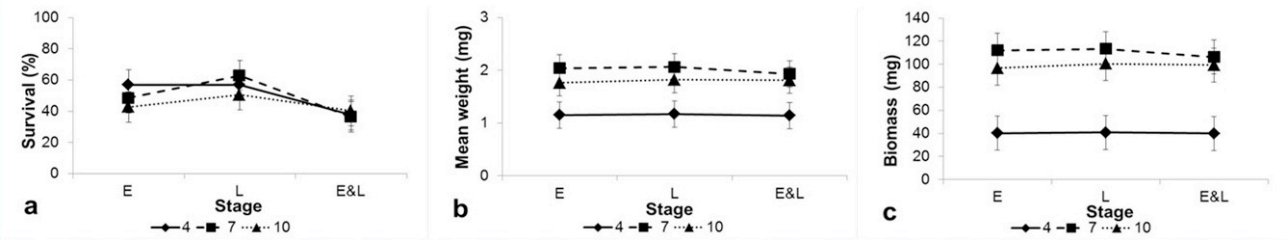

Figure 1 Two-ways assay results showing the stage-dose interaction on survival

Note: (a) mean weight (b) and biomass (c) of LAB suspension on *Piaractus mesopotamicus* parameters. Vertical bars indicate SE

The one-way ANOVA of the administration of ENT suspension (Table 3) shows no significant differences in the survival percentage of the specimens ($p>0.05$), with similar values after all the treatments (Figure 3). The two-ways ANOVA analysis shows neither dose-phase interaction nor significant effect of the stage of administration ($p>0.05$) (Figure 4). However, the orthogonal polynomials for trend analysis of the dose effect detected a significant effect of the linear and quadratic contrasts of the mean weight and biomass ($p>0.05$) (Table 4). The quadratic contrast showed to be the best model to describe the polynomial function for both variables due to the fact that the highest estimated values belonged to dose $6x10^7$ CFU L-1.

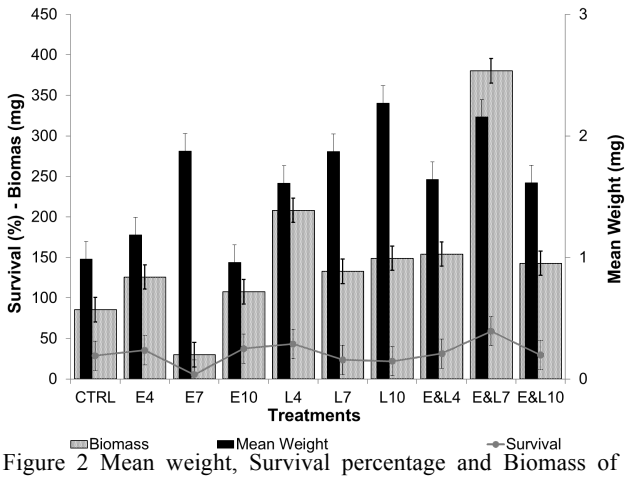

Figure 2 Mean weight, Survival percentage and Biomass of *Piaractus mesopotamicus* after bacterial treatments with LAB suspension on day 15

Note: Vertical bars indicate Standard Error of means (SE)

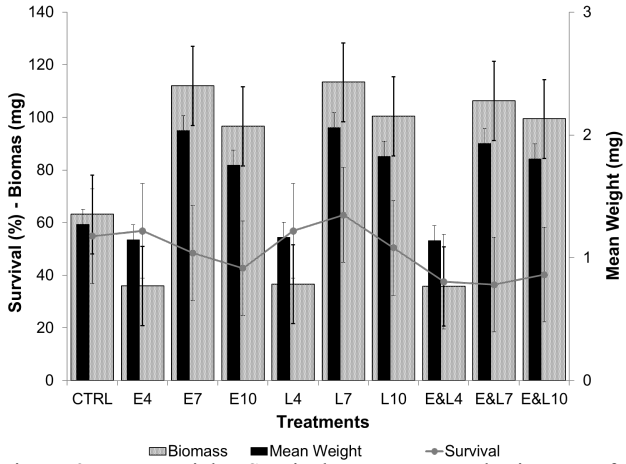

Figure 3 Mean weight, Survival percentage and Biomass of Piaractus mesopotamicus after bacterial treatments with ENT suspension on day 15

Note: Vertical bars indicate Standard Error of means (SE)

Table 3 One way ANOVA of mean weight, survival and biomass of *P. mesopotamicus* larvae administered with ENT mixture in different doses and stag

Variable	N	SS	df	MS	F	p-value
Mean weight (mg)	30	4.02	9	0.45	2.65	0.0332
Survival (%)	30	2278.53	9	253.17	1.01	0.4667
Biomass (mg)	30	26648.93	9	2960.99	5.01	0.0013

Table 4 Polynomial function coefficients for the mean weight and biomass of *Piaractus mesopotamicus* versus dose of microorganisms

	Mean Wight	Biomass
Polynomial function	- 0.7772 + 0.8216*dose - 0.0095*dose^2	- 111.8 +64.369*dose - 4.5525*dose^2
R^2	51.47%	68.25%

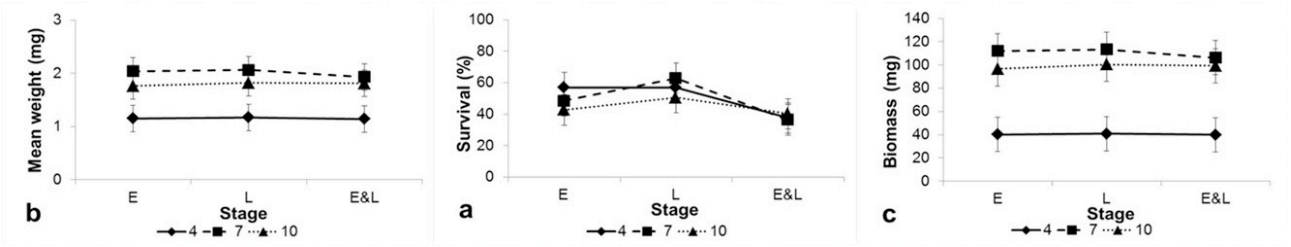

Figure 4 Two-ways assay results showing the stage-dose interaction on survival (a), mean weight (b) and biomass (c) of ENT suspension on Piaractus mesopotamicus parameters. Vertical bars indicate SE

Note: (a), mean weight (b) and biomass (c) of ENT suspension on Piaractus mesopotamicus parameters. Vertical bars indicate SE

The macroscopic evaluation of the animals showed no visible alterations of the normal development and/or behavior of the fishes in any of the doses of bacterial administered. The histological evaluation indicated no modifications of the normal structure of major organs, aggrupation of microorganisms or translocation to any of the organs under evaluation when comparing each treatment with control. Histology of stomach, liver and intestine showed no significant differences between control animals and those treated with the dose 4 of LAB and dose 7 of ENT during the three different stages assayed. They are resumed in Figure 5.

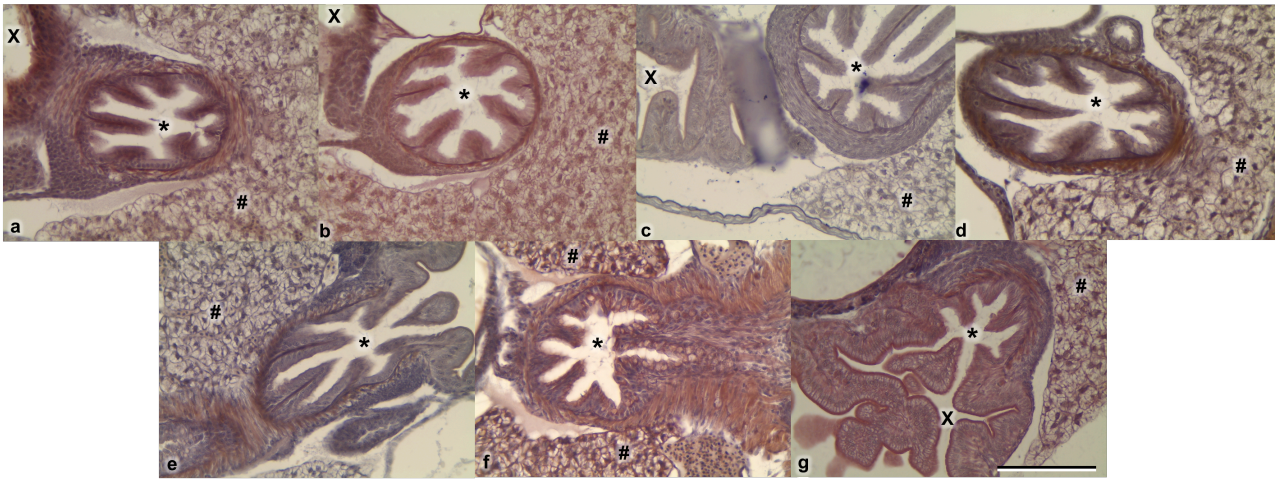

Figure 5 Histology of stomach (*), liver (+) and intestine (#) of control
Note: (a) compared with animals treated with 6x107 CFU L1 of each strain of ENT suspension at stages E; (b), L (c) and E&L (d) and treated with 6x104 CFU L-1 of each strain of LAB suspension at stages E; (e), L (f) and E&L (g). Bars= 50 µm

4 Discussion

The use of probiotics in aquaculture has increased exponentially in the last years (Verschuere et al., 2000; Reid et al., 2003; Romalde et al., 2005). However, commercial probiotics in fish culture are relatively ineffective. Most products include non-autochthonous strains unable to survive or remain viable in the intestinal environment (Ridha and Azad, 2015). Different authors have shown from long time ago (Kotarsky and Savage, 1979, Marteau, 2011) and demonstrated lately by the application of molecular biology techniques, the specie-specificity of the microbioma, or autochtonous microbiota, and also related to each specific tract of mucosa (Chi et al., 2014). It is essential the isolation and study of putative native probiotic microorganisms that are part of the microbiome of each specific host. Such strains have higher possibilities to survive and remain because they have already resisted these environmental conditions (Ghosh et al., 2007). Based on previous *in vitro* evaluations, our research group has selected five autochthonous lactic acid bacteria (three *E. faecium* and one *P. acidilactici* strains) as novel putative probiotics (Guidoli et al., 2015). However, the definitive application and clinical evidence of their beneficial effects should be evaluated through *in vivo* assays (ISAPP, 2011). Therefore, in this work we evaluated the effect of the administration of two different formulae composed by beneficial autochthonous microorganisms isolated on two seasons on the survival, mean weight, biomass and histological parameters of *P. mesopotamicus* larvae.

There are three critical items related to probiotic application: the stage of the biological cycle, the optimal dose and the way in which they should be administered to the host. Pasteris et al. (2012) suggested that the colonization of the skin (or scams) and the gastrointestinal tract of fishes occurs together with the ontogeny, as a way to be incorporated into the microbioma of each specie. The microbial adhesion to the eggs' surface is, then, the main factor to determine the development of the epibiota. Afterwards, the ingestion of microorganisms in larval stages results in the establishment of a dominant intestinal microbiota that seems to persist during the fish life. Unfortunately, there is no general consensus on the other two critical items cited before. Bibliographic references suggest doses of administration from $1x10^3$ to $1x10^9$ CFU and different ways of administration such as balanced food or environmental water (Brunt and Austin, 2005; Bagheri et al., 2008). The last critical item is the way of administration, being widely used the food (Brunt and Austin, 2005; Bagheri et al., 2008). In this work, the selected beneficial microorganisms were added at different doses, stages and systems of administration.

Although ANOVA analysis did not show significant differences or interactions of different treatments with ENT suspension and control, the trend analysis of the dose effect showed significant differences for the mean weight and biomass. This test showed the quadratic contrast as the best model to describe the polynomial function for both variables, indicating the dose 7 ($6x10^7$ CFU L-1 of each strain) as the most effective one. The better effect of

medium doses (quadratic effect) was described by several authors in aquaculture (Bagheri et al., 2008; Faramarzi et al., 2011; Kapareiko et al., 2011).

Statistical evaluations of the results of administering BAL suspension did not show significant differences. None of the treatments with dose 4, regardless stage of administration, showed values of survival, mean weight and biomass higher than the control group. Then, we selected dose 4 as the most adequate to be incorporated in future assays. This result, although not expected, support the concept that a higher number of probiotic cells included in diets and host intestine does not necessarily result in a highest or improved growth, survival and protein efficiency ratio of animals (Bagheri et al., 2008).

The macroscopic observations of the animals did not show modifications in their behavior and/or development of those treated with the probiotic strains when compared with control. The histological evaluation demonstrated no translocation or aggrupation of microorganisms, studies that needs to be performed to determine the safety of the strains used as probiotics (Pasteris et al., 2009; ISAPP, 2011). Histological studies of major organs and tissues were comparable to those described as normal for each stage (Mendoza et al., 2013). The results obtained indicate that the administration of the two formulae in the doses herein evaluated did not affect the normal development and function of animals.

Then, the dose 7 ($6x10^7$ CFU L-1 of each strain) of the ENT suspension (composed by strains *E. faecium* CRL 1937 and *E. faecium* CRL 1938) and dose 4 ($6x10^4$ CFU L-1 of each strain) of the LAB suspension (composed by strains *E. faecium* CRL 1940 and CRL 1941 and *P.acidilactici* CRL 1939) were selected to be included in a potentially probiotic mixture to be assayed *in vivo* in future assays. And a combination of these strains with three autochthonous *Bacillus subtilis* strains, were registered for patent aims (Conicet and Unne, 2013).

Acknowledgments

The Argentinian National Council of Scientific and Technical Researches (Consejo Nacional de Investigaciones Científicas y Técnicas–CONICET), projects numbers PIP 632 and PIP 744, together with the National Agency for Scientific and Technological Promotion (Agencia Nacional de Promoción Científica y Tecnológica–ANPCyT) through the Fund for Scientific and Technological Research (Fondo para la Investigación Científica y Tecnológica–FONCyT); projects numbers PICTO-UNNE-2007-161, PICTO-UNNE-2011-198, PICT-543, PICT-1088 and PICT-1187; financed this study. Some of the results of this paper were included in a patent presentation in Argentina (INPI Exp 20130103820)

References

Abumourad I.M.K., Kenwy A.M., Ibrahim T.B., Hanna M.I., and Soliman W.S., 2014, Enterococcus faecium probiotic as a growth promoter and its impact on the expression of the host innate immune in cultured Oreochromis niloticus, Research Journal of Pharmaceutical, Biological and Chemical Sciences, 5(2): 1747-1761

Bagheri T., Hedayati S.A., Yavari V., Alizade M., and Farzanfar A., 2008, Growth, survival and gut microbial load of Rainbow Trout (Onchorhynchus mykiss) fry given diet supplemented with probiotic during the two months of first feeding, Turkish Journal of Fisheries and Aquatic Sciences, 8: 43-48

Bogut I., Milakovic Z., Brkic S., Novoselic D., and Bukvic Z., 2000, Effects of Enterococcus faecium on the growth rate and content of intestinal microflora in sheat fish (Silurus glanis), Veterinarni Medicina International Journal for Veterinary and Biomedical Science, 45: 107-109

Brunt J., and Austin B., 2005, Use of a probiotic to control lactococcosis and streptococcosis in rainbow trout, Oncorhynchus mykiss (Walbaum), Journal of Fish Diseases, 28: 693-701

http://dx.doi.org/10.1111/j.1365-2761.2005.00672.x

PMid:16336470

Castex M., Lemaire P., Wabete N., and Chim L., 2010, Effect of probiotic Pediococcus acidilactici on antioxidant defenses and oxidative stress of Litopenaeus stylirostris under Vibrio nigripulchritudo challenge, Fish and Shellfish Immunology, 28(4): 622-631

http://dx.doi.org/10.1016/j.fsi.2009.12.024

PMid:20045735

Chang C.I., and Liu W.Y., 2002, An evaluation of two probiotic bacterial strains, Enterococcus faecium SF68 and Bacillus toyoi, for reducing edwardsiellosis in cultured European eel, Anguilla Anguilla, Journal of Fish Disease, 25: 311-315

http://dx.doi.org/10.1046/j.1365-2761.2002.00365.x

Chi C., Liu J.Y., Fei S.Z., Zhang C., Chang Y.Q., Liu X.L., and Wang G.X., 2014, Effect of intestinal autochthonous probiotics isolated from the gut of sea cucumber (Apostichopus japonicus) on immune response and growth of A. japonicas, Fish and Shellfish Immunology, 38: 367-373

http://dx.doi.org/10.1016/j.fsi.2014.04.001
PMid:24727198
CONICET (Consejo Nacional de Investigaciones Científicas y Técnicas), and UNNE (Universidad Nacional del Nordeste), 2013, Composición para mejorar la cría de peces de agua dulce y método de mejoramiento de la cría. Argentinian Patent 20130103820, 22 Oct 2013
Culling C.F.A., 1974, Handbook of histopathological and histochemical techniques. Butterworths & Co Publishers Ltd., London
PMCid:PMC1646058
Da Silva A.B., Vinatea J.E., and Alcantara F., 1988, Manual de reproducción de peces Colossoma (pacú y tambaqui). FAO's Trust Fund, Proyect GCP/RLA/075/ITA, Tarapoto, Perú
Di Giancamillo A., Vitari F., Savoini G., Bontempo V., Bersani C., Dell'Orto V., and Domeneghini C., 2008, Effects of orally administered probiotic Pediococcus acidilactici on the small and large intestine of weaning piglets. A qualitative and quantitative micro-anatomical study, Histology and Histopathology, 23(6): 651-64
PMid:18366003
Dirección de Acuicultura, 2013, Dirección Nacional de Planificación Pesquera. Subsecretaría de Pesca y Acuicultura de la Nación. Diciembre 2013. Cited 05 Oct 2015
Dirección de Acuicultura, 2014, Dirección Nacional de Planificación Pesquera. Subsecretaría de Pesca y Acuicultura de la Nación. Agosto 2014. Cited 05 Oct 2015
EC (European Commission), 1996, Council directive 96/23/EC on measures to monitor certain substances and residues thereof in live animals and animal products and repealing. Cited 05 Oct 2015
EFSA (European Food Safety Authority), 2008, Technical guidance: Update of the criteria used in the assessment of bacterial resistance to antibiotics of human or veterinary importance, The EFSA Journal, 732: 1-15
FAO/NACA/WHO, 1997, Joint Study Group. Food safety issues associated with products from aquaculture, WHO Technical Report Series 799
Faramarzi M., Kiaalvandi S., Lashkarbolooki M., and Iranshahi, F., 2011, The investigation of Lactobacillus acidophilus as probiotics on growth performance and disease resistance of rainbow trout (Oncorhychus mykiss), America-Eurasian Journal of Scientific Research, 6(1): 32-38
FDA (Food and Drug Administration), 1998, Aquaculture Drugs. In: Fish and Fishery Products Hazards and Control Guide, 2nd edn. FDA, Washington
Gatesoupe F.J., 2008, Updating the importance of lactic acid bacteria in fish farming: natural occurrence and probiotic treatments, Journal of Molecular Microbiology and Biotechnology, 14: 107-114
http://dx.doi.org/10.1159/000106089
PMid:17957117
Ghosh S., Sinha A., and Sahu C., 2007, Isolation of putative probionts from the intestines of Indian major carps, Israelian Journal of Aquaculture, 59(3): 127-132.
Giannenas I., Karamaligas I., Margaroni M., Pappas I., Mayer E., Encarnação P., and Karagouni E., 2015, Effect of dietary incorporation of a multi-strain probiotic on growth performance and health status in rainbow trout (Oncorhynchus mykiss), Fish Physiology and Biochemistry, 41(1): 119-128.
http://dx.doi.org/10.1007/s10695-014-0010-0
PMid:25411048
Gómez M.I., Sánchez S., and Fuentes C.M., 2014, Shrinkage of Prochilodus lineatus (Valenciennes, 1847) larvae preserved in either ethyl-alcohol or formalin in relation to their developmental stage and feeding condition, Journal of Applied Ichthyology, 30: 140-144
http://dx.doi.org/10.1111/jai.12308
Guidoli M.G., Santinón J.J., Pasteris S.E., Sánchez S., and Nader-Macías M.E.F., 2015, Isolation and selection of potentially beneficial autochthonous bacteria for Piaractus mesopotamicus aquaculture activities, Journal of Bioprocessings and Biotechniques, 5: 9, http://dx.doi.org/10.4172/2155-9821.1000254
http://dx.doi.org/10.4172/2155-9821.1000254
Gullian M., Thompson F., and Rodriguez J., 2004, Selection of probiotic bacteria and study of their immunostimulatory effect in Penaeus vannamei, Aquaculture, 233(4): 1-14
http://dx.doi.org/10.1016/j.aquaculture.2003.09.013
Hernández Serrano P., 2005, Responsible use of antibiotics in aquaculture, FAO Fisheries Technical Paper, 469
ISAPP (International Scientific Association for Probiotics and Prebiotics) www.isapp.net. Cited 05 Oct 2015
Kapareiko D, Lim HJ, Schott EJ, Hanif A., and Wikfors G.H., 2011, Isolation and evaluation of new probiotic bacteria for use in shellfish hatcheries, Effects of a Vibrio sp. probiotic candidate upon survival of oyster larvae (Crassostrea virginica) in pilot-scale trials, Journal of Shellfish Research, 30(3): 617-625.
http://dx.doi.org/10.2983/035.030.0304
Kotarski S.F., and Savage D.C., 1979, Models for study of the specificity by which indigenous lactobacilli adhere to murine gastric epithelia. Infection and Immunity, 26(3): 966-75
PMid:160893 PMCid:PMC414713
Lakshmi B., Viswanath B., and Gopal S., 2013, Probiotics as antiviral agents in shrimp aquaculture. Journal of Pathogens
http://dx.doi.org/10.1155/2013/424123
Marcinakova M., Simonova M., Strompfova V., and Lukova A., 2006, Oral application of Enterococcus faecium strain EE3 in healthy dogs, Folia Microbiológica, 51(3): 239-242
http://dx.doi.org/10.1007/BF02932129
Marteau P., 2011, Evidence of probiotic strain specificity makes extrapolation of results impossible from a strain to another, even from the same species, Annals of Gastroenterology and Hepatology, 2(1): 34-36
Mego M., Majek J., Konecova R., Ebringer L., Cierniková S., Rauko P., Kovác M., Trupl J., Slezák P., and Zajac V., 2005, Intramucosal bacteria in colon

cancer and their elimination by probiotic strain Enterococcus faecium M-74 with organic selenium, Folia Microbiológica, 50: 443-447
http://dx.doi.org/10.1007/BF02931427

Mendoza M.A., Corredor J.C., and Romero Hurtado C.S., 2013, Histological Study of the Digestive System in Different Stages of Development of Pacu (Piaractus brachypomus), Revista Médica Veterinaria, 25: 21-38

Mikulski D., Jankowski J., Naczmanski J., Mikulska M., and Demey V., 2012, Effects of dietary probiotic (Pediococcus acidilactici) supplementation on performance, nutrient digestibility, egg traits, egg yolk cholesterol, and fatty acid profile in laying hens, Poultry Science, 91(10): 2691-700
http://dx.doi.org/10.3382/ps.2012-02370
PMid:22991559

Nayak S.K., and Mukherjee S.C., 2011, Screening of gastrointestinal bacteria of Indian major carps for a candidate probiotic species for aquaculture practices. Aquaculture Research, 42(7): 1034-1041
http://dx.doi.org/10.1111/j.1365-2109.2010.02686.x

Neissi A., Rafiee G., Nematollahi M., Razavi S.H., and Maniei F., 2015, Influence of supplemented diet with Pediococcus acidilactici on non-specific immunity and stress indicators in green terror (Aequidens rivulatus) during hypoxia, Fish and Shellfish Immunology, 45(1): 13-8
http://dx.doi.org/10.1016/j.fsi.2015.04.008
PMid:25891275

Neissi A., Rafiee G., Nematollahi M., and Safari O., 2013, The effect of Pediococcus acidilactici bacteria used as probiotic supplement on the growth and non-specific immune responses of green terror, Aequidens rivulatus. Fish and Shellfish Immunology, 35(6): 1976-80
http://dx.doi.org/10.1016/j.fsi.2013.09.036
PMid:24161762

Pasteris S.E., Bühler M.I., and Nader-Macías M.E., 2012, Probióticos en acuicultura. In: Ramos A., Nader-Macías M.E.F., Monteoliva M. (eds) Probióticos y Salud, 1st edn, Diaz de Santos, Madrid

Pasteris S.E., Roig Babot G., Otero M.C., Bühler M.I., and Nader-Macías M.E., 2009, Beneficial properties of lactic acid bacteria isolated from a Rana catesbeiana hatchery, Aquaculture Research, 40: 1605-1615
http://dx.doi.org/10.1111/j.1365-2109.2009.02261.x

Rahman S., Khan S.N., Naser M.N., and Karim M.M., 2011, Safety issues of isolated probiotic natured bacteria from Bangladesh coastal waters for controlling shrimp diseases. Journal of Scientific Research, 3(3): 659-668
http://dx.doi.org/10.3329/jsr.v3i3.7750

Raibaud P., Galpin J.V., Ducluzeau R., Mocquot, G., and Oliver, G., 1963, Le genre Lactobacillus dans le tube digestif du Rat. II Caracte'res de souches heterofermentaires isolates de rats "Holo" et "Gnotoxeniques", Annals of Microbiology, 124: 2223-2235

Ramos M.A., Gonçalves J.F., Batista S., Costas B., Pires M.A., Rema P., and Ozório R.O., 2015, Growth, immune responses and intestinal morphology of rainbow trout (Oncorhynchus mykiss) supplemented with commercial probiotics. Fish and Shellfish Immunology, 45(1): 19-26
http://dx.doi.org/10.1016/j.fsi.2015.04.001
PMid:25865055

Reid G., Sanders M.E., Gaskins H.R., Gibson G.R., Mercenier A., Rastall R., Roberfroid M., Rowland I., Cherbut C., and Klaenhammer T.R., 2003, New scientific paradigms for probiotics and prebiotics. Journal of Clinical Gastroenterology, 37: 105-118
http://dx.doi.org/10.1097/00004836-200308000-00004
PMid:12869879

Ridha M.T., and Azad I.S., 2015, Effect of autochthonous and commercial probiotic bacteria on growth, persistence, immunity and disease resistance in juvenile and adult Nile tilapia Oreochromis niloticus, Aquaculture Research, 1–11
http://dx.doi.org/10.1111/are.12726

Romalde J.L., Ravelo C., López-Romalde S., Avenda-o-Herrera R., Magari-os B., and Toranzo A.E., 2005, Vaccination strategies to prevent emerging diseases for Spanish aquaculture, Developments Biologicals, 121: 85-95

SOU (Statens Offentliga Utrednigar-National Public Investigations from Sweden), 1997, Toxicological and related effects of antibacterial feed additives. Document N 1997:132

Torrentera L., and Tacon A., 1989, La producción de alimento vivo y su importancia en acuacultura. Una diagnosis. FAO-Italia

Vahjen W., Taras D., and Simon O., 2007, Effect of the Probiotic Enterococcus faecium NCIMB10415 on Cell numbers of total enterococcus spp., E. faecium and E. faecalis in the Intestine of Piglets, Current Issues in Intestinal Microbiology, 8: 1-8
PMid:17489433

Verschuere L., Rombaut G., Zorruelos P., and Verstraete W., 2000, Probiotic bacteria as biological control agents in aquaculture, Microbiology and Molecular Biology Reviews, 64(4): 655-671
http://dx.doi.org/10.1128/MMBR.64.4.655-671.2000
PMid:11104813 PMCid:PMC99008

Vieira, F.N., Buglione C.C., and Mourino J.P.L., 2010, Effect of probiotic supplemented diet on marine shrimp survival after challenge with Vibrio harveyi, Arquivo Brasileiro de Medicina Veterinária e Zootecnia, 62(3): 631-638
http://dx.doi.org/10.1590/S0102-09352010000300019