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ANIMAL SCIENCE

Autochthonous and allochthonous lactic acid bacteria: action on the hematological and intestinal microbiota for two species of Astyanax genus

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Abstract: The objective of this work was to evaluate the effect of autochthonous and allochthonous lactic acid bacteria (LAB) in two species of lambaris (*Astyanax bimaculatus* and *Astyanax fasciatus*), and to investigate the effects on intestinal microbiota and hematological changes. Two experiments were carried out, one for each lambari species, both assays were divided into three treatments: autochthonous LAB, allochthonous LAB and control. The 10% inoculum was included on diet in the LAB treatments and sterile medium for control. After 30 days for *A. bimaculatus* LAB indigenous changes all bacteria groups analyzed, while allochthonous LAB just decrease Staphylococcus spp. count. Though for *A. fasciatus* his autochthone LAB reduced the staphylococcal count. In hematology, for *A. bimaculatus* autochthonous LAB showed a higher number of thrombocytes, lymphocytes, monocytes and total leukocytes in the circulatory system than the control. Though for *A. fasciatus* his autochthone LAB showed a higher number of total lymphocytes and leukocytes than the control, while *Lactobacillus* sp. acting as an allochthone, it did not differ among treatments. In conclusion, both LAB (*Lactobacillus* sp. and *L. lactis*) promoted more beneficial changes in the microbiota and hematological profile when they act as an autochthone probiotic, demonstrating a probiotic-associated host.

Key words: probiotic, host-associated, microbiota, hematology, *Astyanax bimaculatus*, *Astyanax fasciatus*.

INTRODUCTION

Probiotics can be used to prevent disease and improve the health and immunomodulation of the fish's immune system. Its main purpose is to promote the biosafety of aquaculture farming systems (Hoseinifar et al. 2018). There is a great diversity of microorganisms with probiotic potential / characteristics, however just as important as the probiotic characteristics is its origin. Van Doan et al. (2020) cited that host-associated vs. terrestrial probiotics, host specificity is an important subject to mention and the adhesion of some treatments of bacteria, such as lactic acids, wouldn't be feasible in different hosts, a strain which is suitable as pig probiotic may not be active in chick, cow and other animals (Fuller 1989).

In aquaculture host-associated probiotics can be defined by bacteria originally isolated from the rearing water or host gastrointestinal tract. Although there is some evidence demonstrating beneficial effects of host-associated probiotics vs. probiotics isolated from other origins. In aquaculture, there is no sure, if host-associated probiotics are more effective than probiotics from other sources (Lazado et al. 2015, Van Doan

et al. 2020). Thus, the objective of this work was to evaluate the effect of autochthonous and allochthonous lactic acid bacteria (LAB) in two species of lambaris (*Astyanax bimaculatus* and *Astyanax fasciatus*), and to investigate the effects on intestinal microbiota and hematological changes.

METHODOLOGY

Two experimental design were completely randomized, using the *Lactobacillus* sp. strain CPQBA 1168-15 DRM-01 with proven probiotic effect *in vitro* and *in vivo* indigenous *A. bimaculatus* (Jatobá et al. 2017, Moraes et al. 2018), *L. lactis* probiotic effect *in vitro* (Unpublished data) indigenous *A. fasciatus*, 240 juveniles (120 of each species), with a mean weight of 6.92 ± 0.31 g (Protocol number 0005/2013 approved by animal ethics committee).

Fish were distributed into 24 experimental units (20 L aquariums) of 10 fish each and equipped with a recirculation aquaculture system with thermostat (27° C) and biological canister filter, 12 experimental units for each species. The experimental units were divided into two assays, 12 for *A. bimaculatus* and the others for *A. fasciatum*. The first assay had three treatments: diet supplemented with *Lactobacillus* sp., diet with *L. lactis* and without supplementation (control) for *A. bimaculatus*; and the second assay had three group diet supplemented with *Lactobacillus* sp., diet with *L. lactis* and without supplementation (control) for *A. fasciatus*. According to the protocols established by Jatobá et al. (2011), 10% of inoculum was included in the probiotic diets with *Lactobacillus* strain and MRS medium (Lactobacillus MRS Broth, HiMedia Laboratories Pvt., India), and only sterile MRS medium (Lactobacillus MRS Broth, HiMedia Laboratories Pvt., India) in the control diet. Probiotic diets were only used when concentrations ≥ 1.0 x 10^7 CFU.g^{-1} were observed.

The fish were fed three times a day with 2.5% of their biomass for food management according Moraes et al. (2018). Dissolved oxygen was maintained above 4.0 mg.L^{-1} throughout the experiment, the temperature and pH ranged between 26.7-27.2 °C and 6.9-7.1, while the toxic ammonia remains below 0.11 mg.L^{-1}.

After 30 days of rearing and a 24 h period of starvation, all fish were anesthetized with Eugenol (50 mg.L^{-1}) and euthanized by cerebral concussion. Five were used for the microbiological assays and the others were used for hematological assays.

For microbiological assays of the intestinal tract, the guts from five fish were removed and pooled to microbiological tests. The pooled fish guts were homogenized and serially diluted 1:10 in 0.65% of NaCl sterile saline. Samples from each dilution were cultured in PCA (Plate Count Agar, HiMedia Laboratories Pvt. Ltd., India), TCBS (Agar Thiosulfate Citrate Bile Sucrose, HiMedia Laboratories Pvt. Ltd., India), Cetrimide (Cetrimide Agar, HiMedia Laboratories Pvt. Ltd., India), BPA (Baird Parker Agar, HiMedia Laboratories Pvt. Ltd., India) and then incubated for 24 h at 30° C, as well as MRS (Lactobacillus MRS Agar, HiMedia Laboratories Pvt. Ltd., India), followed by incubation for 48 h at 35° C, for viable culturable heterotrophic bacterial counts, including *Vibrio* spp., *Pseudomonas* spp., *Staphylococcus* spp. and LAB, respectively.

Another five fish were used for hematological analysis, approximately 1.0 mL of blood was drawn from the caudal vein of each fish for the preparation of blood smears, in duplicate, and the following hematological analyses: determination of hematocrit by standard microhematocrit method and total hemocyte count by Neubauer hemocytometer. Blood smear slides were stained with Giemsa and May

Grünwald stain (Rosenfeld 1947) for total and differential leukocyte count.

Data were submitted to the Kolmogorov-Smirnov test to determine if data distribution was within the normality curve and Levene's test to verify homoscedasticity. For the data obtained that met the prerequisites of normality and homoscedasticity, ANOVA was applied to observe the occurrence of significant differences among treatments. Positive results were submitted to the Student–Newman–Keuls (SNK) test for means separation, and microbiological data were $\log_{(x+1)}$ transformed. For all evaluations, 5% of significance was used.

RESULTS AND DISCUSSION

The use of LAB has a positive effect registered in several species, such as sea bass, *Centropomus* spp. (Barbosa et al. 2011), tilapia, *Oreochromis* spp., (Yamashita et al. 2017, Jatobá et al. 2008, 2011), shrimps, *Litopenaeus vannamei*, (Vieira et al. 2007), bullfrog (*Lithobates catesbeianus*) (Pereira et al. 2017, 2018), yellow tail lambari *Astyanax bimaculatus* (Moraes et al. 2018, Jatobá et al. 2017), due to its ability to colonize the digestive tract, altering the natural dominance of the microbiota intestinal and promoting improvement in the animals immune system (Jatobá et al. 2008, Vieira et al. 2008). These results are related to the high specificity between the probiotic microorganism and the host, since all strains used in these studies were isolated from the animals under study.

On the other hand, studies demonstrate that the use of allochthonous bacteria can also have good results and a positive role in fish welfare (Ridha & Azad 2012), however there is a consensus that strains of allochthonous LAB should be evaluated regarding its ability to colonize the intestine of the target species, as well as providing benefits on host health. The addition of LAB as a dietary probiotic usually changes the host microbiota. Nowadays, most probiotic candidates are derived from the mucosal layers the autochthonous bacteria of aquatic animals (Lazado et al. 2015, Van Doan et al. 2020). Reason that highlights the understanding of the effect host-associated probiotic for aquatic animals.

Both LABs used in this work colonize the intestinal tract of both species, however their results showed a host-associated affinity. For *A. bimaculatus* LAB indigenous changes all bacteria groups analyzed, while allochthonous LAB just decrease *Staphylococcus* spp. count, and LAB count was higher for treatment with *Lactobacillus* sp. (autochthone LAB) than *L. lactis* (allochthonous LAB). Though for *A. fasciatus* his autochthone LAB reduced the staphylococcal count and allochthonous LAB only colonized the intestinal tract (Table I). There are many examples for species of fish that have been used autochthonous LAB as dietary probiotic, Jatobá et al. (2011) worked with *L. plantarum* to Nile tilapia, Mouriño et al. (2016) used *Weissella cibaria* to hybrid South American catfish (*Pseudoplatystoma reticulatum* × *P. corruscans*), as well as Moraes et al. (2018) studied the effects of the same LAB in this work for *A. bimaculatus*. In all these studies, autochthonous bacteria demonstrated a great capacity to colonize the intestinal tract of fish, corroborating with the data of this research.

Similar results were observed in hematology, which for *A. bimaculatus* autochthonous LAB showed a higher number of thrombocytes, lymphocytes, monocytes and total leukocytes in the circulatory system than the control, while *L. lactis* (allochthone) did not differ between treatments (control or *Lactobacillus* sp.), except for the reduction in the number of monocytes. Though for *A. fasciatus* his autochthone LAB showed a higher number of total lymphocytes

Table I. Bacterial count of the intestinal tract (Log UFC/g) of *Astyanax bimaculatus* and *A. fasciatus* fed diet supplemented with autochthone and allochthone probiotic.

	[1]*Lactobacillus sp.*	[2]*L. lactis*	Control
	A. bimaculatus		
Total Heterotrophic Bacteria	4,2 ± 0,5b	5,9 ± 1,0a	5,9 ± 0,6a
Staphylococcus spp.	4,2 ± 0,2b	3,6 ± 0,5c	5,5 ± 0,4a
Pseudomonas spp.	3,0 ± 0,3b	5,1 ± 1,1a	4,3 ± 0,6a
Vibrio ssp.	3,3 ± 0,2b	4,4 ± 0,7a	4,0 ± 0,2a
Lactic Acid Bacteria	4,8 ± 0,7a	3,8 ± 0,3b	2,4 ± 0,1c
	A. fasciatus		
Total Heterotrophic Bacteria	5,5 ± 1,1	6,1 ± 0,1	5,9 ± 0,8
Staphylococcus spp.	4,8 ± 1,9a	3,5 ± 0,2b	4,6 ± 0,2a
Pseudomonas spp.	3,9 ± 0,7	4,1 ± 0,1	4,2 ± 0,5
Vibrio ssp.	3,8 ± 0,2	4,1 ± 0,4	3,7 ± 0,9
Lactic Acid Bacteria	4,4 ± 1,7b	3,9 ± 0,3b	2,2 ± 0,1a

***Different letters indicate significant differences (P<0.05) between treatments in ANOVA and SNK test. [1]Autochthone for *A. bimaculatus* and allochthone for *A. fasciatus*. [2] Allochthone for *A. bimaculatus* and autochthone for *A. fasciatus*.**

and leukocytes than the control, while *Lactobacillus* sp. acting as an allochthone, it did not differ among treatments (Table II), despite having colonized the intestinal tract of this species.

Changes in the hematological profile are commonly observed in studies with dietary probiotics for fish (Lazado et al. 2015, Moraes et al. 2018, Van Doan et al. 2020), in addition to the origin of the strains and duration of treatment, the time of action and frequency of supply (Jatobá et al. 2018a, b, 2020) are crucial to assess the effect of these microorganisms on the hosts. However, the results suggest that the presence of LAB in the intestinal tract does not guarantee beneficial hematological changes to the hosts. This fact corroborates with Jatobá et al. (2018b) evaluated the frequency in the supply of probiotic (same strain) to *A. bimaculatus* showed that the frequency of 100% improved the growth performance due to changes in the microbiota and hematology, while the frequency of 25%, despite colonizing the intestinal microbiota, it was not able to alter the hematological profile or promote growth.

In conclusion, both LAB (*Lactobacillus* sp. and *L. lactis*) promoted more beneficial changes in the microbiota and hematological profile when they act as an autochthone probiotic, demonstrating a probiotic-associated host relationship. However, the use of autochthone LAB as a probiotic should not be ruled out, thus assays must be carried out to ascertain that colonization of the intestinal tract will promote other benefits for the hosts.

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Table II. Hematological parameters of *Astyanax bimaculatus* and *A. fasciatus* fed diet supplemented with autochthone and allochthone probiotic.

	Total and differential count	[1]*Lactobacillus* sp.	[2]*L. lactis*	Control
A. bimaculaturs	Thrombocytes (x 10^3.μL^{-1})	43,81 ± 8,23b	36,87 ± 5,06ab	27,94 ± 7,15a
	Leucocytes (x 10^3.μL^{-1})	31,24 ± 8,34b	23,63 ± 3,66ab	18,55 ± 5,01a
	Lymphocytes (x 10^3.μL^{-1})	25,64 ± 5,18b	19,63 ± 2,89ab	16,65 ± 4,44a
	Monocytes (x 10^3.μL^{-1})	4,17 ± 0,29b	2,40 ± 0,68a	1,01 ± 0,82a
	Neutrophiles (x 10^3.μL^{-1})	1,43 ± 0,24	1,52 ± 0,33	0,89 ± 0,38
	Erythrocytes ($10^6 \mu L^{-1}$)	1,27 ± 0,13	1,43 ± 0,15	1,36 ± 0,21
	Hematocrit (%)	21,70 ± 0,49	21,30 ± 0,41	19,8 ± 0,41
A. fasciatus	Thrombocytes (x 10^3.μL^{-1})	9,85 ± 2,29	10,44 ± 4,87	13,38 ± 6,29
	Leucocytes (x 10^3.μL^{-1})	25,51 ± 4,82ab	28,66 ± 3,51b	21,82± 3,61a
	Lymphocytes (x 10^3.μL^{-1})	24,61 ± 5,98ab	27,7 ± 1,50b	20,87 ± 3,68a
	Monocytes (x 10^3.μL^{-1})	0,79 ± 0,54	0,47 ± 0,16	0,42 ± 0,32
	Neutrophiles (x 10^3.μL^{-1})	0,11 ± 0,13	0,46 ± 0,19	0,52 ± 0,54
	Erythrocytes ($10^6 \mu L^{-1}$)	1,61 ± 0,40	1,75 ± 0,17	1,61 ± 0,30
	Hematocrit (%)	24,45 ± 0,58	22,52 ± 1,55	23,75 ± 1,02

*** Different letters indicate significant differences (P<0.05) between treatments in ANOVA and SNK test. [1]Autochthone for *A. bimaculatus* and allochthone for *A. fasciatus*. [2] Allochthone for *A. bimaculatus* and autochthone for *A. fasciatus*.**

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Author contributions

Both authors worked on writing, planning and statistical analysis. Adolfo Jatobá processed the microbiological analyzes, while Gabriel Jesus the hematological analyzes.