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Short Communication

Selection and isolation of bacterium with probiotic potential from the Amazon ornamental fish *Hypancistrus* sp. (Siluriformes, Loricariidae)

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ABSTRACT

The aim of this study was to select autochthonous bacteria with probiotic potential from the gastrointestinal tract of King Tiger Pleco (*Hypancistrus* sp.) for future applications in captive production. For the isolation of lactic acid bacteria, 12 specimens (8.31 ± 0.28 cm and 12.60 ± 1.13 cm) were used, whose intestines were removed, macerated, and homogenized in sterile saline. A total of 21 strains were isolated from the intestinal tract on MRS agar, of which five strains (St1, St2, St3, St4, and St5) were selected for in-vitro tests. Three strains (St2, St3, and St5) showed the highest values of maximum growth per hour, with a final concentration of 109 CFU.mL^{-1} in 24 hours. However, only the strains St3 and St5 had the highest growth ($p < 0.05$) in the presence of NaCl (0.5 to 1.5%) and pH in the range of 5 and 6. For bile salts, the greatest resistance observed was for strain St5. In conclusion, this is the first report of the isolation of autochthonous bacteria for a Loricariidae, recommending the bacterium *Enterococcus faecium* as probiotic in the aquaculture creation of King Tiger Pleco.

Keywords: Aquaculture; Production; Welfare; Teleostei; King Tiger Pleco; L333.

Seleção e isolamento de bactéria com potencial probiótico do peixe ornamental amazônico *Hypancistrus* sp. (Siluriformes, Loricariidae)

RESUMO

Este estudo teve como objetivo a seleção de bactérias autóctones com potencial probiótico do trato gastrointestinal do cascudo ornamental amazônico King Tiger Pleco (*Hypancistrus* sp.) para futuras aplicações em produção em cativeiro. Para o isolamento de bactérias lácticas, foram utilizados 12 espécimes (8,31 ± 0,28 cm e 12,60 ± 1,13 cm), cujos intestinos foram retirados, macerados e homogeneizados em soro fisiológico estéril. O total de 21 cepas foi isolado do trato intestinal em ágar MRS, das quais cinco cepas (St1, St2, St3, St4 e St5) foram selecionadas para os testes *in vitro*. Três cepas (St2, St3 e St5) apresentaram os maiores valores de crescimento máximo por hora, com concentração final de 10^9 UFC.mL^{-1} em 24 horas, contudo somente as cepas St3 e St5 tiveram o maior crescimento ($p < 0,05$) na presença de NaCl (0,5 a 1,5%) e pH na faixa de 5 e 6. Já para os sais biliares, a maior resistência observada foi para a cepa St5. Em conclusão, este é o primeiro relato do isolamento de bactérias autóctones para um Loricariidae, recomendando a bactéria *Enterococcus faecium* como probiótico na criação do acari-pão.

Palavras-chave: Aquicultura; Produção; Bem-estar; Teleostei; acari-pão; L333.

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INTRODUCTION

The commercialization of ornamental fish has become a profitable activity with the annual increase of aquarists, who constantly demand healthy species with color and shape patterns (Abe et al., 2019; Raut et al., 2020). Among the ornamental fish species, the King Tiger Pleco, known by the code L333 (*Hypancistrus* sp.), belongs to the Loricariidae family, order Siluriformes, and is an endemic species of the Xingu River in the Amazon region. Its body is surrounded by bone plates and has a coloration with black bands and a background that varies in yellow and white. It is of great interest to the international ornamental fish market (Ramos et al., 2015; Reis et al., 2021), reaching values of US$ 79.99 per fish (Aqua Imports, 2021).

The increase in ornamental fish farming with the aim of increasing productivity and providing more fish units to the market, consequently, provides a higher incidence of diseases, mainly of bacterial origin. This occurs due to the increase in stocking density, use of unbalanced feed, changes in the physical and chemical parameters of the water, among other factors, which lead to the proliferation of the disease, resulting in fish mortality and economic loss (Cardoso et al., 2020; Preena et al., 2022).

Therefore, probiotics are strategies applied in ornamental fish farming with promising results. Thus, *Lactobacillus plantarum*, *Enterococcus faecium*, *Lactococcus lactis* and *Lactobacillus acidophilus* are used in fish feeding (Sousa et al., 2019; Paixão et al., 2020b; Yamashita et al., 2020). They provide the modulation of the intestinal microbiota, increased growth and immune system, as well as resistance of host against diseases (Sousa et al., 2019; Couto et al., 2022; Preena et al., 2022).

However, to be considered a probiotic, the bacterium must have the ability to colonize the intestine and must resist the adverse conditions of the gastrointestinal tract (Balcázar et al., 2006; Sousa et al., 2020). Therefore, in-vitro tests such as the evaluation of pH, NaCl, bile salts and antagonism are the first steps to select strains resistant to the physiological actions of the animal, and, to be considered bacteria with probiotic potential (Paixão et al., 2020a; Yamashita et al., 2020), to promote beneficial effects to the fish (Sousa et al., 2019; Couto et al., 2022).

There is no information on the use of probiotics in the rearing of the King Tiger Pleco, productions protocols and health strategies for the species during the rearing cycle. Thus, the objective this study was to select in-vitro autochthonous bacteria with probiotic potential from the gastrointestinal tract of King Tiger Pleco (*Hypancistrus* sp.) for future applications in captive production.

MATERIAL AND METHODS

For the isolation of lactic acid bacteria, 12 healthy King Tiger Pleco (L333) specimens (8.31 ± 0.28 cm and 12.60 ± 1.13 cm) donated by the company Arapaima were used. The fish were anesthetized with benzocaine solution (20 $mg \cdot L^{-1}$) and euthanized by brain concussion to remove the intestine. All procedures performed on the animals were approved by the Universidade Federal do Pará ethics committee (under protocol no. 9202300420).

Individually, the intestines were removed, macerated, homogenized in sterile saline solution (0.65%), and serially diluted (factor 1:10), and 100 µL of dilutions from 10^{-1} to 10^{-3} were inoculated in a petri plate containing MRS agar (Rogosa and Sharpe Man) with aniline blue (1%). The plates were kept in an oven for 48 hours at 35°C. Strains were selected according to morphology (cocci and bacilli), gram positive, affinity for aniline blue, and catalase negative. Later, the cultivated colonies were purified and maintained in MRS broth.

The growth kinetics was determined for each strain isolated, and the strains were inoculated in MRS broth, incubated for 24 hours at 35°C. Every two hours the growth of the bacteria was evaluated by the spectrophotometry method (absorbance of 630 nm), and an aliquot (100 µL) was inoculated in a petri plate containing MRS agar, incubated for 48 hours at 35°C. Absorbance values were converted into colony forming units (CFU) to determine growth rate and final inoculum concentration (CFU.mL-1).

For the in-vitro resistance tests, the experiments were carried out in a completely randomized design with different concentrations of pH (4, 5, 6, 8 and 9), NaCl (0; 0.5; 1; 1.5; 2; 2.5 and 3%), and the presence of bile salts (5% w/v), all assays with four replications. After bacterial growth (24 hours at 35°C), the percentage reduction in absorbance (630 nm) was analyzed by spectrophotometry (Paixão et al., 2020a; Lopes et al., 2022).

For the antagonism test, *Aeromonas hydrophila* (CPQBA22808 DRM), *Pseudomonas aeruginosa* (ATCC27853), *Streptococcus agalactiae* (LAQUA), *Aeromonas jandaei* (LAQUA), and *Staphylococcus aureus* (ATCC 29213) were used, and a completely randomized design was carried out with four replications. The selected strains were inoculated on MRS agar and incubated for 48 hours at 35°C. After bacterial growth, four 0.8-cm disks were removed (each disk a repetition) and superimposed on petri plate containing TSA agar (Tryptone Soja) inoculated with the pathogens, incubated at 35°C for 48 hours. After bacterial growth, the zone inhibition (mm) against to the pathogens of each isolated strain was measured. The strain with the best results in the tests was identified by the MALDI-TOF (mass spectrometry) technique. The procedure for removal, identification, cultivation, growth kinetics and in-vitro testing of the strains were established according to the procedure adapted from Lopes et al. (2022) and Paixão et al. (2020a).

The data from the in-vitro test and antagonism were submitted to the test of normality (Shapiro-Wilk) and homoscedasticity (Levene's) assumptions, and then submitted to analysis of variance (ANOVA). Later, the Tukey's test was performed ($p < 0.05$) for comparison between means.

RESULTS

The total of 21 strains were isolated from the intestinal tract of the King Tiger Pleco (L333 *Hypancistrus* sp.), on MRS agar. From them, five strains were selected (St1, St2, St3, St4, and St5), and they presented characteristics (gram positive, catalase negative and affinity for aniline blue) for in-vitro assays. Three strains (St2, St3, and St5) had the highest values of maximum growth per hour, with a final concentration of 109 CFU.mL-1 in 24 hours (Table 1). In the in-vitro assay, strains St3 and St5 showed the highest growth values (p < 0.05) against NaCl, mainly at concentrations of 0.5 to 1.5%, as well to pH, in the ranges of 5 to 6 (Table 1). In the presence of bile salts, the greatest resistance was observed only for the St5 strain (Table 1).

Regarding the antagonism against pathogenic bacteria, all strains isolated showed inhibitory capacity, with better results for the St5 strain, that presented the highest inhibition halo, above 12 mm, for all tested pathogens, mainly against *A.hydrophila* (16, 89 ± 0.32 mm) (Table 2).

The bacterial strain (St5) with the best results in-vitro assays and greater inhibitory capacity to pathogens was identified as *E. faecium* 20218 CHB (MALDI-TOF Biotyper Microflex Bruker).

Table 1. Values (mean ± standard deviation) of in-vitro tests of maximum growth rate per hour (MGR/h), concentration (CFU/mL) and percentage of resistance (%) of the strains isolated of the King Tiger Pleco, L333 (*Hypancistrus* sp.), at different concentrations of NaCl, pH and bile salts*.

Strains substances	St_1	St_2	St_3	St_4	St_5	*P-value*
TCM/h	0.67 ± 0.02b	0.82 ± 0.03a	0.80 ± 0.03a	0.65 ± 0.04b	0.80 ± 0.02a	0.00344
Final Concentration	1.9 ± 0.14 x10^8b	2.1 ± 0.11 x10^9a	2.2 ± 0.18 x10^9a	2.1 ± 0.19 x10^8b	2.1 ± 0.11 x10^9a	0.01382
NaCl (0.5)	63.02 ± 4.62b	67.64 ± 3.56b	78.9 ± 1.83ab	70.75 ± 3.45b	80.85 ± 2.55a	0.00226
NaCl (1.0)	60.51 ± 2.20c	65.16 ± 1.92b	75.48 ± 1.42a	63.37 ± 1.33b	74.01 ± 1.55a	0.00487
NaCl (1.5)	44.32 ± 2.17c	52.39 ± 2.87b	68.38 ± 2.02a	43.60 ± 1.89c	66.10 ± 1.77a	0.00107
NaCl (2.0)	39.77 ± 1.75b	50.28 ± 2.02a	52.15 ± 1.79a	40.58 ± 1.61b	52.07 ± 1.95a	0.02409
NaCl (2.5)	37.28 ± 0.82c	46.12 ± 1.87b	50.98 ± 1.99a	48.37 ± 2.79ab	51.62 ± 1.72a	0.02191
NaCl (3.0)	28.72 ± 0.86c	33.25 ± 1.04b	42.24 ± 1.12a	34.81 ± 1.59b	41.94 ± 0.88a	0.00418
pH 4	12.21 ± 1.05b	12.77 ± 0.95b	23.29 ± 1.04a	11.98 ± 1.45b	23.30 ± 0.92a	0.00164
pH 5	68.49 ± 0.82b	66.38 ± 1.14c	77.15 ± 0.93a	65.92 ± 0.72bc	78.37 ± 0.74a	0.00101
pH 6	72.71 ± 1.42b	71.15 ± 0.82b	84.32 ± 1.02a	69.94 ± 1.59b	85.11 ± 1.472a	0.00012
pH 8	41.84 ± 0.57a	39.94 ± 0.37b	40.19 ± 1.12ab	33.18 ± 1.59c	41.26 ± 0.45a	0.00418
pH 9	31.55 ± 0.76b	32.74 ± 1.97b	37.52 ± 0.66a	22.29 ± 1.95c	37.93 ± 0.68a	0.00677
Bile Salts	49.85 ± 2.29 b	33.85 ± 3.14c	54.88 ± 3.47ab	34.86 ± 3.26c	57.83 ± 3.29a	0.00341

*Different letters in the same column differ statistically by Tukey's test (5%).

Table 2. Inhibition zone of strains with probiotic potential isolated from King Tiger Pleco, L333 (*Hypancistrus* sp.), against pathogenic bacteria*.

Strains	*Aeromonas hydrophila*	*Aeromonas jandaei*	*Pseudomonas aeroginosa*	*Streptococcus agalactiae*	*Staphylococcus aureus*
St_1	9.12 ± 0.64c	10.87 ± 0.82b	13.89 ± 0.65b	11.84 ± 0.62b	12.66 ± 0.71b
St_2	9.85 ± 0.82c	9.78 ± 0.758b	10.02 ± 0.68c	10.98 ± 0.79b	14.02 ± 0.37a
St_3	12.17 ± 0.77b	12.44 ± 0.76a	14.34 ± 0.89ab	13.45 ± 0.29a	12.46 ± 0.28b
St_4	13.05 ± 1.09b	9.31 ± 0.87b	9.58 ± 0.55c	11.22 ± 0.66b	11.21 ± 1.04b
St_5	16.89 ± 0.32a	12.58 ± 0.82a	15.21 ± 0.38a	13.16 ± 0.18a	14.62 ± 0.42a
p-value	0.00421	0.01011	0.00145	0.00102	0.00211

*Values (mean ± standard deviation) with different letters in the same column differ statistically by Tukey's test (5%).

DISCUSSION

The use of probiotics in diets for ornamental fish has shown promising results in intestinal modulation, improvement of the immune system, growth, and resistance to pathogenic diseases (Ahmadifard et al., 2019; Sousa et al., 2020; Wu et al., 2023). These beneficial effects are related to colonization in the host's intestine, one of the criteria for a bacterium to be considered probiotic; it can be influenced by the amount offered in the diet (CFU) and the physiological actions of the animal (Dias et al., 2018; Paixão et al., 2020b).

The importance of in-vitro tests is related to the select possibility strains with resistance to growth in the face of chemical barriers, allowing greater chances of colonizing the animal's intestine (Paixão et al., 2020a; Yamashita et al., 2020). For the selection of these strains with probiotic potential, the used culture medium provides guidance on the target group of bacteria to be educated and which are subsequently evaluated in in-vitro assays, such as the MRS medium, which is used for the selection of lactic acid bacteria (Dias et al., 2019; Lopes et al., 2022; Paixão et al., 2020a). In the present study, the bacterium *E. faecium* (St5) showed growth in the presence of NaCl, pH and bile salts, a promising characteristic for resistance to the physiological actions of the organism, allowing colonization of the intestinal tract and beneficial effects for the host.

The pH of the gastrointestinal tract of fish is one of the factors that modulates the intestinal microbiota. In its stomach, whose acidity is higher, gastric juice has the ability to break bacterial membranes (Sylvain et al., 2016; Solovyev et al., 2018), reducing the chances of colonization in the animal's intestine. However, the St5 strain (*E. faecium*) showed growth in all pH ranges, with values above 70% at pH 5 and 6, similar to the results for the bacterium *L. plantarum* (Paixão et al., 2020a). But these results differ from the findings by Dias et al. (2019), who observed a limitation for the growth of strains with probiotic potential at pH 4 and 5.

The stability of the ionic concentration is essential for the physiological homeostasis of animals, and ionic changes in fish can affect bacterial viability, which can cause the rupture of the membrane of these organisms in the digestive system (Griffith et al., 2018; Mortezaei et al., 2020; Tian, L. et al., 2020). This effect may reduce the number of probiotic bacteria available for colonization, as observed by Vieira et al. (2013), who, at the concentration of 1.5% of NaCl, did not have the growth of the probiotic bacterium *L. plantarum*. In the present study, strains with probiotic potential grew at all concentrations of NaCl (0.5 to 3%), with emphasis on St5 (*E. faecium*). The same occurred in growth resistance of the *E. genus* as a probiotic, which was observed for the species *Pterophyllum scalare* and *Nannostomus beckfordi* (Dias et al., 2019; Lopes et al., 2022).

In the food digestion process, bile salts act in the emulsification of lipids in the fish intestine, which consequently have antibacterial activity capable of modulating the intestinal microbiota (Tian, Y. et al., 2020). In the present study, St5 strain (*E. faecium*) showed growth above 50% in bile salts, and similar results were found for *E. faecium* of *P. scalare*. However, Paixão et al. (2020a) observed a 70% reduction in strains with probiotic potential isolated from *A. ocellaris* in the bile salts assay. Thus, the resistance of *E. faecium* bacteria to bile salts is related to the gene expression of the components BsrXRS and LiaFSR (Zhou et al., 2019).

Disease resistance is one of the most important beneficial effects in the rearing of fish fed diets containing probiotics (Tang et al., 2019). Therefore, the antagonism test is essential to select strains with inhibitory potential, through the release of substances (such as reuterin and bacteriocins) against pathogenic bacteria (Klimko et al., 2020; Paixão et al., 2020b). In this study, the St5 strain (*E. faecium*) inhibited the growth of all tested pathogens, with the highest inhibition halo for *A. hydrophila* (16.89 ± 0.32 mm). The antagonistic activity of this bacterium is related to the release of enterocins (P and TJUQ1), lactic acids and hydrogen peroxide (Braiek et al., 2017; Yoswaty et al., 2020), which act by inhibiting *A. hydrophila*, *E. durans*, *P. aeruginosa* and *S. agalactiae* (Dias et al., 2019; Lopes et al., 2022).

Therefore, based on the criteria of this study, it was possible to select a lactic acid strain (St5: *E. faecium*) with high probiotic potential for the aquaculture of King Tiger pleco (L333), as it is autochthonous and has the ability to withstand chemical conditions for bacterial growth and by antagonistic action to pathogens.

CONCLUSION

This is the first report of the isolation of autochthonous lactic acid bacteria for The King Tiger Pleco (L333), recommending the bacterium *E. faecium* as a potential probiotic for creation of King Tiger Pleco.

CONFLICT OF INTERESTS

The authors have no conflict of interest to declare.

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AUTHORS' CONTRIBUTIONS

Conceptualization: Reis GRA; **Data curation:** Reis GRA; Abe HA; Sousa NC; **Writing – original draft:** Reis GRA; Abe HA; **Writing – review & edition:** Abe HA; Sousa NC; Rocha RM.

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REFERENCES

Abe, H.A.; Dias, J.A.R.; Sousa, N.D.C.; Couto, M.V.S.D.; Reis, R.G.A.; Paixão, P.E.G., Fujimoto, R.Y. 2019. Growth of Amazon ornamental fish *Nannostomus beckfordi* larvae (Steindachner, 1876) submitted to different stocking densities and feeding management in captivity conditions. *Aquaculture Research*, 50(8): 2276-2280. https://doi.org/10.1111/are.14108

Ahmadifard, N.; Rezaei Aminlooi, V.; Tukmechi, A.; Agh, N. 2019. Evaluation of the impacts of long-term enriched Artemia with Bacillus subtilis on growth performance, reproduction, intestinal microflora, and resistance to Aeromonas hydrophila of ornamental fish Poecilia latipinna. *Probiotics and Antimicrobial Proteins*, 11: 957-965. https://doi.org/10.1007/s12602-018-9453-4

Aqua Imports (2021). *L333 yellow King Tiger Pleco (Hypancistrus sp. L333)*. Available at: https://www.aqua-imports.com/product/l333-yellow-king-tiger-pleco-hypancistrus-sp-l333/. Accessed on: June 1, 2021.

Balcázar, J.L.; De Blas, I., Ruiz-Zarzuela, I.; Cunningham, D.; Vendrell, D.; Muzquiz, J.L. 2006. The role of probiotics in aquaculture. *Veterinary Microbiology*, 114(3-4): 173-186. https://doi.org/10.1016/j.vetmic.2006.01.009

Braiek, O.B.; Ghomrassi, H.; Cremonesi, P.; Morandi, S.; Fleury, Y.; Le Chevalier, P.; Ghrairi, T. 2017. Isolation and characterisation of an enterocin P-producing *Enterococcus lactis* strain from a fresh shrimp (*Penaeus vannamei*). *Antonie van Leeuwenhoek*, 110(6): 771-786. https://doi.org/10.1007/s10482-017-0847-1

Cardoso, P.H.; Moreno, L.Z.; Oliveira, C.H.; Gomes, V.T.; Silva, A.P.S.; Barbosa, M.R.; Moreno, A.M. 2020. Main bacterial species causing clinical disease in ornamental freshwater fish in Brazil. *Folia Microbiologica*, 66: 231-239. https://doi.org/10.1007/s12223-020-00837-x

Couto, M.V.S.; Sousa, N.C.; Abe, H.A.; Cunha, F.S.; Meneses, J.O.; Paixão, P.E.G.; Mouriño, J.L.P.; Martins, M.L.; Cordeiro, C.A.M.; Fujimoto, R.Y. 2022. Dietary supplementation of Probiotic Enterococcus faecium improve resistance in Arapaima gigas against Aeromonas hydrophila. *Aquaculture Research*, 53(9): 3453-3463. https://doi.org/10.1111/are.15852

Dias, J.A.R.; Abe, H.A.; Sousa, N.C.; Couto, M.V.; Cordeiro, C.A.; Meneses, J.O.; Fujimoto, R.Y. 2018. Dietary supplementation with autochthonous *Bacillus cereus* improves growth performance and survival in tambaqui *Colossoma macropomum*. *Aquaculture Research*, 49(9): 3063-3070. https://doi.org/10.1111/are.13767

Dias, J.A.R.; Abe, H.A.; Sousa, N.C.; Silva, R.D.F.; Cordeiro, C.A.M.; Gomes, G.F.E.; Ready, J.S.; Mouriño, Martins, M.L.; Carneiro, P.C.F.; Maria, A.N.; Fujimoto, R.Y. 2019. *Enterococcus faecium* as potential probiotic for ornamental neotropical cichlid fish, *Pterophyllum scalare* (Schultze, 1823). *Aquaculture International*, 27(2): 463-474. https://doi.org/10.1007/s10499-019-00339-9

Griffith, M.B.; Zheng, L.; Cormier, S.M. 2018. Using extirpation to evaluate ionic tolerance of freshwater fish. *Environmental Toxicology and Chemistry*, 37(3): 871-883. https://doi.org/10.1002/etc.4022

Klimko, A.I.; Cherdyntseva, T.A.; Brioukhanov, A.L.; Netrusov, A.I. 2020. *In vitro* evaluation of probiotic potential of selected lactic acid bacteria strains. *Probiotics and Antimicrobial Proteins*, 12(3): 1139-1148. https://doi.org/10.1007/s12602-019-09599-6

Lopes, E.M.; Silva, A.V.; Barros, F.A.L.; Santos, A.F.L.; Cordeiro, C.A.M.; Couto, M.V.S.; Paixão, P.E.G.; Fujimoto, R.Y.; Sousa, N.C. 2022. In vitro bacterial probiotic selection from Nannostomus beckfordi, an Amazon ornamental fish. *Arquivo Brasileiro de Medicina Veterinária e Zootecnia*, 74(1): 111-116. https://doi.org/10.1590/1678-4162-12179

Mortezaei, F.; Royan, M.; Allaf Noveirian, H.; Babakhani, A.; Alaie Kordghashlaghi, H.; Balcázar, J.L. 2020. *In vitro* assessment of potential probiotic characteristics of indigenous Lactococcus lactis and Weissella oryzae isolates from rainbow trout (*Oncorhynchus mykiss* Walbaum). *Journal of Applied Microbiology*, 129(4): 1004-1019. https://doi.org/10.1111/jam.14652

Paixão, P.E.G.; Couto, M.V.S.; Costa Sousa, N.; Abe, H.A.; Dias, J.A.R.; Meneses, J.O.; Cunha, F.S.; Mouriño, J.L.P.; Martins, M.L.; Fujimoto, R.Y. 2020a. *In vitro* selection of autochthonous lactic acid bacterium from clownfish *Amphiprion ocellari*s. *Aquaculture Research*, 51(2): 848-851. https://doi.org/10.1111/are.14396

Paixão, P.E.G.; Couto, M.V.S.; Costa Sousa, N.; Abe, H.A.; Reis, R.G.A.; Dias, J.A.R.; Meneses, J.O.; Cunha, F.S.; Santos, T.B.R.; Silva, I.C.S.; Medeiros, E.S.; Fujimoto, R.Y. 2020b. Autochthonous bacterium *Lactobacillus plantarum* as probiotic supplementation for productive performance and sanitary improvements on clownfish *Amphiprion ocellaris*. *Aquaculture*, 526: 735395. https://doi.org/10.1016/j.aquaculture.2020.735395

Preena, P.G.; Dharmaratnam, A.; Swaminathan, T.R. 2022. A peek into mass mortality caused by antimicrobial resistant *Edwardsiella tarda* in goldfish, *Carassius auratus* in Kerala. *Biologia*, 77(4): 1161-1171. https://doi.org/10.1007/s11756-022-01007-9

Ramos, F.M.; Araújo, M.L.G.; Prang, G.; Fujimoto, R.Y. 2015. Ornamental fish of economic and biological importance to the Xingu River. *Brazilian Journal of Biology*, 75(3 Suppl. 1): 95-98. https://doi.org/10.1590/1519-6984.02614BM

Raut, S.M.; Kumar, M.; Bhatt, B.P.; Singh, J.; Kumar, T. 2020. Potential and Opportunity for Ornamental Fishes in North Bihar. *Biotica Research Today*, 2(7): 677-679.

Reis, R.G.A.; Alves, P.C.J.; Abe, H.A.; Sousa, C.N.; Paixão, P.E.G.; Palheta, G.D.A.; Melo, N.F.A.C.; Fujimoto, R.Y.; Luiz, R.K.; Takata, R. 2021. Feed management and stocking density for larviculture of the Amazon ornamental fish L333 king tiger pleco Hypancistrus sp. (Siluriformes: Loricariidae). *Aquaculture Research*, 52(5): 1995-2003. https://doi.org/10.1111/are.15047

Solovyev, M.M.; Izvekova, G.I.; Kashinskaya, E.N.; Gisbert, E. 2018. Dependence of pH values in the digestive tract of freshwater fishes on some abiotic and biotic factors. *Hydrobiologia*, 807(1): 67-85. https://doi.org/10.1007/s10750-017-3383-0

Sousa, N.C.; Couto, M.V.S.; Abe, H.A.; Paixão, P.E.G.; Cordeiro, C.A.M.; Monteiro Lopes, E.; Fujimoto, R.Y. 2019. Effects of an Enterococcus faecium-based probiotic on growth performance and health of Pirarucu, *Arapaima gigas*. *Aquaculture Research*, 50(12): 3720-3728. https://doi.org/10.1111/are.14332

Sousa, N.C.; Monteiro, J.A.D.S.E.; Felipe, L.A.; Lima, L.D.S.F.A.; Peterson, B.C.A.M.C.; Santos, E.G.P.E.; Couto, M.V.S. 2020. Enriched Artemia Nauplii with Commercial Probiotic in the larviculture of Angelfish *Pterophyllum scalare* Lichtenstein (1823). *Journal of Fisheries Science*, 2(1): 17-21. https://doi.org/10.30564/jfsr.v2i1.1569

Sylvain, F.É.; Cheaib, B.; Llewellyn, M.; Correia, T.G.; Fagundes, D.B.; Val, A.L.; Derome, N. 2016. pH drop impacts differentially skin and gut microbiota of the Amazonian fish tambaqui (*Colossoma macropomum*). *Scientific Reports*, 6(1): 32032. https://doi.org/10.1038/srep32032

Tang, Y.; Han, L.; Chen, X.; Xie, M.; Kong, W.; Wu, Z. 2019. Dietary supplementation of probiotic *Bacillus subtilis* affects antioxidant defenses and immune response in grass carp under *Aeromonas hydrophila* challenge. *Probiotics and Antimicrobial Proteins*, 11(2): 545-558. https://doi.org/10.1007/s12602-018-9409-8

Tian, L.; Tan, P.; Yang, L.; Zhu, W.; Xu, D. 2020. Effects of salinity on the growth, plasma ion concentrations, osmoregulation, non-specific immunity, and intestinal microbiota of the yellow drum (*Nibea albiflora*). *Aquaculture*, 528, 735470. https://doi.org/10.1016/j.aquaculture.2020.735470

Tian, Y.; Gui, W.; Koo, I.; Smith, P.B.; Allman, E.L.; Nichols, R.G.; Patterson, A.D. 2020. The microbiome modulating activity of bile acids. *Gut Microbes*, 11(4): 979-996. https://doi.org/10.1080/19490976.2020.1732268

Vieira, F.D.N.; Jatobá, A.; Mouriño, J.L.P.; Vieira, E.A.; Soares, M. 2013. *In vitro* selection of bacteria with potential for use as probiotics in marine shrimp culture. *Pesquisa Agropecuária Brasileira*, 48(8): 998-1004. https://doi.org/10.1590/S0100-204X2013000800027

Yamashita, M.M.; Ferrarezi, J.V.; Vale Pereira, G.; Júnior, G.B.; Silva, B.C.; Pereira, S.A.; Martins, M.L.; Mouriño, J.L.P. 2020. Autochthonous vs allochthonous probiotic strains to *Rhamdia quelen*. *Microbial Pathogenesis*, 139, 103897. https://doi.org/10.1016/j.micpath.2019.103897

Yoswaty, D.; Effendi, I.; Titani, M.R. 2020. Enterococcus gallinarum, a new antibioticproducing bacterium against fish pathogenic bacteria, isolated from mangrove mud snake (*Cerberus rynchops*). *Aquaculture, Aquarium, Conservation & Legislation*, 13(6): 3769-3777.

Wu, L.; Wang, L.; Cui, S.; Peng, Z.; Liu, Z.; Li, M.; Han, Y.; Ren, T. 2023. Effects of dietary compound probiotics and heat-killed compound probiotics on antioxidative capacity, plasma biochemical parameters, intestinal morphology, and microbiota of Cyprinus carpio haematopterus. *Aquaculture International*, 31: 2199-2219. https://doi.org/10.1007/s10499-023-01080-0

Zhou, L.; Wang, L.; Tian, P.; Bao, T.; Li, L.; Zhao, X. 2019. The LiaFSR and BsrXRS systems contribute to bile salt resistance in *Enterococcus faecium* isolates. *Frontiers in Microbiology*, 10: 1048. https://doi.org/10.3389%2Ffmicb.2019.01048