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Enterococcus faecium as potential probiotic for ornamental neotropical cichlid fish, *Pterophyllum scalare* (Schultze, 1823)

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Abstract

The production of ornamental fishes has been intensified to attend the global market. With this expansion, an increase in infections and infirmities has been observed, most of which are attributed to pathogenic bacteria. Dietary supplements to improve growth and immunity of these animals have been introduced as a safe way to control and prevent disease outbreaks. This study therefore aims to isolate, identify, select and evaluate strains of lactic acid bacteria that show potential as probiotics for *Pterophyllum scalare*. Of 16 initial isolates, five strains were molecularly identified as *Enterococcus faecium*. Profiles of probiotic candidate strains were based on: catalase test and hemolytic activity; in vitro tolerance responses to NaCl (0.5; 1.0; 1.5; 2.0; 2.5 and 3.0%), pH (4, 5, 6, 8, and 9), and bile salts (5%); pathogen inhibition halo size maximum growth rate; and final counts of viable cells. In vivo effectiveness of the best performing strain in vitro was determined by growth and survival parameters for post-larvae over a period of 40 days. All strains fulfilled the criteria as probiotics in animals, with Strain 4 showing the best results in vitro, and improving growth and viability of fish in vivo.

Keywords Aquaculture · Ornamental fish · Production · Sanitation · Specific species

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Introduction

The intensification of animal production has led to an increase in the occurrence of infections and mass mortalities, resulting in economic losses and reduced efficiency of the production chain in aquaculture (Kotob et al. 2016; Madani et al. 2018). Pathogenic bacteria are attributed to be the cause of this problem, opportunistically infecting fish weakened by poor sanitation, high stocking density, poor food management, and the consequent stress of the animals in confinement (Assis et al. 2017; Doan et al. 2018).

In view of this, antibiotics are frequently administered to prevent and combat bacterial pathogens in aquaculture. Unfortunately, in many cases, they are used negligently, often resulting in the development of undesirable resistant pathogen strains (Mello et al. 2013; Maradonna et al. 2015; Doan et al. 2018), and acting a pollutant in the effluent from these systems (Cyrino et al. 2010).

In aquaculture, the use of probiotics has become a safe and efficient alternative method to combat and/or prevent outbreaks of infection, often resulting in improvements both in the immune response and growth of the host (Verschuere et al. 2000; Jatobá and Mouriño 2015). However, the selection of a probiotic requires the isolation and testing of various microorganisms, with strains originating from animals themselves to produce the best results (Balcázar et al. 2008). Benefits to fish health or growth were observed in *Poeciliopsis gracilis* and *Rhamdia quelen* fed with rations supplemented with probiotics derived from each of these host species (Hernandez et al. 2010; Souza et al. 2012). This work supports the theory that obtaining probiotic strains that are specific to the host species is fundamental for the development of a product with accurate information described for dosage and duration of supplementation considering factors including: method of application; bacterial strain; size and age of fish; water quality parameters; stocking density; and nutrition and type of rearing system (Doan et al. 2017; Doan et al. 2018).

For the species *Pterophyllum scalare* there is only one record involving probiotic supplementation of feed, with *Bacillus* spp. shown to increase resistance to the bacterial pathogen *Aeromonas hydrophila* (Monroy-Dosta et al. 2010). However, other positive effects were not encountered, and other strains of potential probiotics were not tested.

Therefore, the aim of this study was to isolate and select in vitro strains of Lactic Acid Bacteria (LAB) with probiotic potential derived directly from the ornamental Amazonian angelfish *Pterophyllum scalare* (Schultze, 1823), a species of great importance in both the national and international markets (Prang 2007), and determine the best candidate for effective improvement of growth performance and survival of post-larvae in vivo.

Materials and methods

Isolation of LAB strains with probiotic potential

Strains of LAB were isolated from the digestive tract of ten healthy juvenile *P. scalare* (length: 21.95 mm ± 0.17 S.D.; weight: 775.71 mg ± 0.15 S.D.), obtained from natural reproduction in captivity. The animals were subjected to 24 h of fasting to clear the intestine. They were then euthanized by spinal column section and externally disinfected using 70% ethanol solution before dissection to remove 0.1 g of intestinal fragments under sterile conditions.

The collected material was macerated in saline solution (NaCl 2%) weight volume^{-1} and transferred to Falcon tubes in Man Rogosa Sharpe (MRS) culture medium in broth (1:10), homogenized in a vortex tube-type agitator, incubated anaerobically for 24 h at 35 °C. After bacterial growth, cultures were plated in MRS Agar medium following the methods of Ramirez et al. (2006) and Jatobá et al. (2008).

Potential pathogenicity of strains was assayed based on catalase production and hemolytic activity. Catalase positive strains were identified using hydrogen peroxide in freshly grown bacteria according to Jatobá et al. (2008) and hemolytic activity assayed using MRS Agar medium enriched with 5% fish blood incubated at 35 °C for 48 h, with hemolytic strains characterized by a zone of hemolysis around newly cultivated colonies (Silva et al. 2011). Potentially pathogenic strains were discarded form further analyses.

Species identification

Genetic material used for identifying strains was isolated from pure isolates maintained in semi-solid state at the Laboratory for Probiotics at the Federal University of Pará. Prokaryote DNA extraction was performed using the method of Sambrook et al. (1989), adapted by Jin (2006). Quantification was performed using the fluorescence method after electrophoresis of 4 μl of the product run on a 1% agarose gel (Sambrook and Russell 2001).

The gene *Phenylalanyl-tRNA synthase* (*PheS*) was amplified by Polymerase Chain Reaction (PCR) using the primers: pheS-21-F (5′ CAYCCNGCHCGYGAYATGC 3′) and pheS-23-R (5′ GGRTGRACCATVCCNGCHCC 3′), shown to be efficient for taxonomic analysis of LAB (Naser et al. 2005). The PCR conditions involved an initial denaturing phase of 5 min at 95 °C followed by 30 cycles comprising: denaturation at 95 °C for 60 s; primer annealing at 58 °C for 90 s; and extension at 72 °C for 90 s. This was then followed by a final extension step at 75 °C for 5 min.

PCR products were sequenced using the dideoxiterminal method (Sanger et al. 1977), on an ABI 3500 XL automated sequencer using the BigDye kit (ABI Prism TM Dye Terminator Cycle Sequencing—PE Applied Biosystems, Carlsbad, CA, USA).

Sequences were aligned and edited using the software BioEdit (Hall 1999). The FASTA format of the sequence was then submitted to the *Basic Local Alignment Search Tool* (BLAST), comparing the new sequences generated to existing data in GenBank (http:/www.ncbi.nlm.nih.gov/). Similar sequences were downloaded and aligned to allow production of a phylogram. The phylogenetic relationships were estimated by constructing a neighbor-joining tree, using 1000 bootstrap pseudoreplicates, in MEGA v. 6.05 (Tamura et al. 2013).

In vitro selection

Four in vitro challenge tests were performed, the isolated strains were activated in sterile Falcon tubes with MRS broth (1:10) that was adapted to provide different saline concentrations (0; 0.5; 1.0; 1.5; 2.0; 2.5 and 3%), pH values (4, 5, 6, 8, and 9), or the presence of bile salts (5% weight volume^{-1}), before incubating at 35 °C for 24 h (Vieira et al. 2013).

Inhibition of pathogens

The antibacterial capacity of LAB strains was evaluated using the halo inhibition method, determining the diameter in mm of the inhibitory zone of the probiotic strains (Hjelm et al.

2004), using the model of Ramirez et al. (2006) and Vieira et al. (2013). For this, four disks with diameters of 0.8 cm were removed from Agar plates containing LAB strains and placed onto Agar Typtone Soya (TSA) media plates that had just been seeded with one of the following pathogens: *Aeromonas hydrophyla* (ATCC 7966), *Pseudomonas aeroginosa* (ATCC 27853), *Enterococcus durans* (ATCC 19432), *Escherichia coli* (D363), *Staphylococcus aureus* (ATCC 29213), and *Micrococcus luteus* (A270). These plates were then incubated at 30 °C for 48 h before measuring the inhibitory zones.

Growth kinetics

Cultures were seeded in triplicate for each LAB strain. Analyses were made by removing a sample of 3 ml of culture medium every 2 h after seeding until 24 h had passed. Growth was determined by spectrophotometer absorbance readings at 630 nm (Jatobá et al. 2008).

Concentrations of the solutions were converted to Colony Forming Units (CFU ml^{-1}) by taking duplicate 100 μl aliquots of all samples. These were then seeded in MRS Agar culture medium using serial dilution and incubated at 35 °C for 48 h to determine the CFU ml^{-1}. From these data, we calculated the maximum growth rate and doubling time of the strains following the method of Jatobá et al. (2008).

In vivo effectiveness

The strain with the best in vitro results was used as test in vivo supplementation effects. For this, 120 healthy juvenile *Pterophyllum scalare* were selected (total length 12.25 ± 1.03 mm, standard length 10.00 ± 0.72 mm, height 4.75 ± 0.61 mm, and weight 24.5 ± 0.80 mg) from breeding stock. Fish were distributed in 12 10-l tanks, each containing ten fish and supplied with aeration. Experimental diets were prepared using probiotic strains of *E. faecium* grown in liquid culture medium (MRS) at 35 °C up to the concentration of 2×10^9 CFU ml^{-1}. To achieve accurate final concentrations in the diet (2×10^8 CFU ml g^{-1} and 2×10^6 CFU ml g^{-1}), the bacterial suspension was diluted in the same culture medium. After dilution, the bacterial suspension was slowly added to commercial feed gradually mixing in a laminar airflow chamber under sterilized conditions, as described by Jatobá et al. (2008). The feed was oven-dried at 35 °C and stored at 6–10 °C until use. To ensure high probiotic levels in the supplemented feed, fresh feed was prepared every 7 days. A control feed formulated under the same conditions in MRS medium but without using the LAB strain was also produced, resulting in four replicate tanks for each of the three treatments.

Feed was provided ad libitum four times per day (08:00; 11:00; 14:00; 17:00 h) during an experimental period of 40 days. The commercial feed used presented the following chemical composition: Moisture 15%; crude protein 39.5%; lipids 6.5%; crude fiber 2.5%; Ash 8.5%; calcium 2.8%; and phosphorus 5.3%.

Partial water changes (40% of total water volume) were performed approximately 40 min after the last meal of each day to remove excess organic material and maintain water quality. To monitor water quality, measures were made on alternating days for temperature and conductivity (using a YSI 550 probe), dissolved oxygen (using a YSI 30 probe), and pH (using an AKROM digital probe). Total ammonia was measured every 10 days using the HANNA HI 96700 kit.

At 20 days and 40 days, experimental animals were counted, weighed, and measured to determine the growth parameters: Total Length (TL), Total Length Gain (TLG) = current TL − initial TL, Standard Length (SL), Standard Length Gain (SLG) = current SL − initial SL, Height (H), Height Gain (HG) = current H − initial H, Weight (W), Weight Gain (WG) = current W − initial W, Specific Growth Rate (SGR) = 100 × [(ln current W − ln initial W) / number of days], Feed Conversion Rate (FCR) = Feed given / weight gain, and uniformity of the lot (Furuya et al. 1998) as well as survival.

Data analyses

The data from the in vitro challenges and microbiological counts were square root–transformed before performing statistical tests. Analysis of variance (ANOVA) was used to assess differences in the results obtained between strains and treatments. When ANOVA results indicated $p < 0.05$, a Tukey test (at 5%) was used to compare means. Analyses were performed in the program ESTAT.

Results

Identification of species

A total of 16 morphotypes of LAB with potential for probiotic use were isolated from the gastrointestinal tract of *P. scalare*. Of these, five Gram-positive cocci did not produce catalase or show hemolytic activity and proceeded to in vitro trials. The isolated strains were molecularly identified by BLAST analysis as *Enterococcus faecium*, with no divergence from sequences registered in GenBank for this species and high bootstrap support in a phylogram including possible related species (Fig. 1).

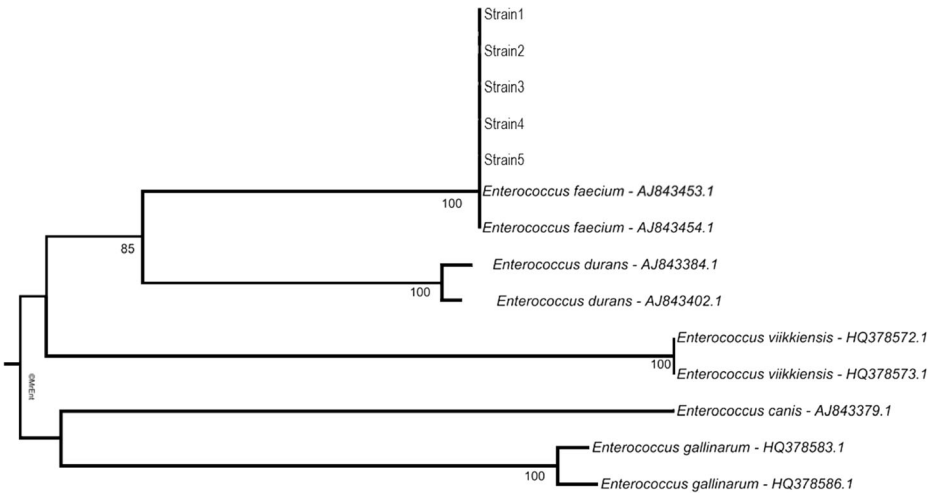

Fig. 1 Neighbor-joining phylogram of the probiotic strains (strains 1–5) isolated from *P. scalare* and related species as determined by BLAST similarity. Analysis based on 334 base pairs of the *PheS* gene with % bootstrap support based on 1000 pseudoreplicates indicated below nodes

Chemical tolerance

All isolated strains presented tolerance to the diverse range of NaCl concentrations and bile salts ($p < 0.05$), although some strains were more tolerant than others. Strains 2 and 4 presented lower tolerance under NaCl challenges but strain 4 presented the greatest tolerance under the pH (especially at extreme values) and bile salt challenges where many other strains showed much lower tolerance or no tolerance at all (Table 1).

Inhibition of pathogens

All candidate strains presented halo inhibition against the pathogens analyzed, however, significant differences ($p < 0,05$) were observed in the halo size produced by each strain. Candidate strains 2, 4, and 5 produced the largest inhibition halos against pathogens *S. aureus*, *P. aeroginosa*, *E. coli*, and *A. hydrophila* (Table 2). The smallest inhibition halos were observed in strains 1 and 3, with pathogens *A. hydrophila* and *S. aureus*, respectively.

Growth kinetics

Strains 2, 3, and 4 showed the highest final counts though had slightly lower growth rates and longer doubling times than strains 1 and 5. However, the coefficient of variation was greater in these measures, with standard deviations for strain 4 indicating considerable overlap with strain 5 in terms of the range of these metrics (Table 2).

In vivo effectiveness

Application of candidate strain 4 to *Pterophyllum scalare* in vivo resulted in faster growth in fish fed feeds supplemented with *E. faecium* compared to fish given control feed. During the first 20 days, the effects were evident in the parameters Total Length, Standard Length, Standard Length Gain, Height, Weight Gain, Specific Growth Rate, and Food Conversion Rate ($p < 0.05$). At the end of the experiment (40 days), the Food Conversion Rate was still significantly lower (better) in fish given feed containing the probiotic supplement. Similarly, growth parameters Total Length, Standard Length, Total Length Gain, Height, Weight, and Weight Gain continued to be significantly higher than the values obtained for the control group (Table 3).

Probiotic supplemented feeds did not alter water quality variables ($p > 0.05$) with mean values maintained for temperature (27.8 ± 0.2 °C), pH (7.3 ± 0.4), dissolved oxygen (6.65 ± 0.32 mg L^{-1}), conductivity (215.39 ± 3.21 μS cm^{-1}), and total ammonia (0.3 ± 0.12 mg L^{-1}).

Discussion

Previous studies have also indicated the value of *E. faecium*, the species isolated and molecularly identified here, as a probiotic supplement. It promotes host growth and immune responses as well as being a predominant member of the gastrointestinal flora of many animals (Tarasova 2010; Sun et al. 2010). Specifically, a number of target species for aquaculture including Nile tilapia (*Oreochromis niloticus* Linnaeus, 1758), trout (*Oncorhynchus mykiis* Walbaum, 1792), and flatfish (*Paralichthys olivaceus* Temminck and Schlegel, 1846) that

Table 1 Relative growth of Lactic Acid Bacteria strains isolated from the digestive tract of the ornamental fish *Pterophyllum scalare* under chemical challenges with different concentrations of NaCl, pH values, and with bile salts (BS, 5% weight/volume). Values are mean (± standard deviation) percentage of the growth compared to the same strain grown under control conditions (NaCl 0%, pH 7, no bile salts)

STRAIN	NaCl 0.5%	NaCl 1.0%	NaCl 1.5%	NaCl 2.0%	NaCl 2.5%	NaCl 3.0%	pH 4
1	57.9 ± 3.58^{B}	58.1 ± 7.83^{B}	57.3 ± 6.99^{B}	41.2 ± 6.05^{C}	56.6 ± 7.00^{B}	68.5 ± 5.96^{A}	0.0 ± 0.0^{B}
2	34.1 ± 6.91^{C}	28.9 ± 2.69^{C}	24.7 ± 3.73^{C}	21.9 ± 3.00^{D}	15.9 ± 3.65^{D}	14.5 ± 3.62^{C}	0.0 ± 0.0^{B}
3	80.3 ± 3.04^{A}	72.8 ± 1.64^{A}	74.3 ± 6.28^{A}	68.7 ± 4.85^{A}	69.9 ± 1.57^{A}	59.6 ± 4.67^{A}	0.0 ± 0.0^{B}
4	34.4 ± 4.56^{C}	32.4 ± 1.54^{C}	33.6 ± 3.11^{C}	28.3 ± 2.45^{CD}	26.7 ± 1.99^{C}	23.6 ± 1.53^{C}	25.9 ± 0.32^{A}
5	66.7 ± 4.94^{B}	70.6 ± 4.78^{A}	67.1 ± 8.61^{AB}	57.3 ± 8.05^{B}	51.0 ± 6.21^{B}	44.8 ± 8.42^{B}	0.0 ± 0.0^{B}
F	**	**	**	**	**	**	**
CV (%)	8.78	8.37	11.88	11.63	8.99	12.17	35.21

STRAIN	pH 5	pH 6	pH 8	pH 9	BS
1	0.0 ± 0.0^{B}	17.5 ± 1.89^{BC}	16.5 ± 1.66^{C}	0.0 ± 0.0^{C}	10.8 ± 0.18^{C}
2	0.0 ± 0.0^{B}	19.1 ± 2.89^{B}	23.8 ± 2.73^{B}	8.8 ± 1.02^{B}	11.2 ± 0.78^{C}
3	23.3 ± 2.40^{A}	13.2 ± 0.59^{CD}	1.1 ± 0.69^{D}	7.1 ± 2.92^{B}	17.0 ± 0.42^{BC}
4	15.8 ± 2.24^{A}	46.1 ± 1.00^{A}	36.5 ± 3.75^{A}	34.4 ± 1.39^{A}	62.9 ± 6.88^{A}
5	22.8 ± 9.50^{A}	10.3 ± 2.58^{D}	26.2 ± 2.20^{B}	7.1 ± 1.37^{B}	23.2 ± 1.31^{B}
F	**	**	**	**	**
CV (%)	28.05	9.4	11.7	14.27	17.36

Different letters indicate significant differences in responses by each strain based on Tukey tests ($p < 0.05$). * Significant F statistic. CV: Coefficient of Variation

Table 2 Pathogen in vitro halo inhibition and growth kinetics for Lactic Acid Bacteria strains developed from the digestive tract of the ornamental fish *Pterophyllum scalare*. Mean inhibition halo (mm ± standard deviation) produced with cultures of *Staphylococcus aureus* (SA), *Enterococcus durans* (ED), *Micrococcus luteus* (ML), *Pseudomonas aeroginosa* (PA), *Escherichia coli* (EC), and *Aeromonas hydrophila* (AH), and values middle with standard deviation (±) for Final bacterial counts (FBC—registered as number of colony forming units, CFU, per ml), maximum growth rate per hour (MV h^{-1}) and duplication time (DT)

Strain	SA	ED	ML	PA	EC	AH
1	15.4 ± 1.07^{A}	12.1 ± 1.18^{A}	15.7 ± 1.69^{BC}	14.1 ± 0.80^{AB}	12.8 ± 0.70^{A}	11.9 ± 1.48^{B}
2	14.0 ± 0.70^{A}	13.2 ± 1.69^{A}	12.2 ± 0.74^{C}	15.7 ± 0.79^{A}	13.1 ± 0.41^{A}	13.5 ± 0.37^{A}
3	11.0 ± 0.55^{B}	14.6 ± 1.13^{A}	16.5 ± 1.58^{B}	13.9 ± 0.82^{B}	12.5 ± 1.05^{A}	12.9 ± 0.22^{AB}
4	14.7 ± 1.41^{A}	11.9 ± 0.62^{A}	15.9 ± 0.99^{BC}	14.3 ± 0.89^{AB}	12.7 ± 1.10^{A}	14.0 ± 0.31^{A}
5	16.8 ± 2.09^{A}	14.0 ± 1.43^{A}	25.4 ± 2.85^{A}	15.4 ± 0.79^{AB}	13.2 ± 1.16^{A}	13.6 ± 0.35^{A}
F	**	Ns	**	*	Ns	**
CV (%)	8.96	9.61	10.73	5.61	7.24	5.48
STRAIN	FBC (CFU/mL × 10^9)		MV h^{-1}		DT (h)	
1	0.98 ± 0.31^{B}		0.11 ± 0.12^{A}		6.06 ± 0.38^{B}	
2	1.92 ± 0.64^{A}		0.07 ± 0.18^{B}		8.68 ± 0.67^{A}	
3	1.92 ± 0.75^{A}		0.08 ± 0.22^{B}		7.90 ± 0.87^{AB}	
4	1.88 ± 0.82^{A}		0.08 ± 0.01^{B}		8.11 ± 1.71^{AB}	
5	1.14 ± 0.11^{B}		0.09 ± 0.02^{AB}		7.17 ± 0.26^{AB}	
F	**		**		*	
CV (%)	7.06		11.59		12.35	

Different letters indicate significant differences in responses by each strain based on Tukey tests ($p < 0.05$).
* Significant F statistic. CV: Coefficient of Variatio; Ns: not significant

were tested with supplements of *E. faecium* have shown improved growth (weight gain and food conversion efficiency) and immune responses compared to control treatments (Merrifield et al. 2010; Kim et al. 2012).

However, probiotic colonization and survival depends on the environmental conditions during their application and ingestion by the target host species. Various osmotic and chemical barriers can rupture their cell membranes or influence their growth rate (Erkkilä and Petäjä 2000; Pennacchia et al. 2004). The resistance of strains to a range of conditions during the preparation, distribution, and digestion of feed is therefore fundamental knowledge when developing a strain for commercial use (Martins et al. 2005; Nithya and Halami 2013). Of the five strains isolated here, strain 4 was found to be the most resistant considering its tolerance across all challenges.

The antagonistic effect of probiotic strains is probably related to their production of antibacterial compounds including lactic acid and bacteriocins. The latter are considered to be the most important inhibitors of Gram-positive species (*S. aureus*, *E. durans* and *M. luteus*) (Gillor et al. 2008). However, these substances may not inhibit Gram-negative species (Vásquez et al. 2005), including *P. aeroginosa*, *E. coli*, and *A. hydrophila*. Growth inhibition of these Gram-negative species has been related to the production of hydrogen peroxide, as well as organic and acetic acids by LAB (Vásquez et al. 2005; Sugita et al. 2007). The inhibitory function of *E. faecium* may also be related to the microbiological sensor quorum, or bacterial community. The formation of high concentrations of chemical signals are crucial for population level responses of microorganisms as the detection of these signals can activate or deactivate gene expression, altering the growth of pathogenic colonies (Boyer and Wisniewski-Dye 2009).

A fundamental characteristic for useful probiotics is that they should be ingested at a reasonable concentration inside feed, with targets of between 10^8 and 10^9 CFU g^{-1}, (ANVISA 2017). In in vitro tests of growth kinetics using strains of *E. faecium* and *L. helveticus*, greater

Table 3 Mean and standard deviation for growth parameters of juvenile *Pterophyllum scalare*, provided with feed (one of two feeds supplemented with probiotic *Enterococcus faecium* at different concentrations or control feed) for 40 days. TL, Total Length; UTL, Uniformity of Total Length; TLG, Total Length gain; SL, Standard Length; USL, Uniformity of Standard Length; SLG, Standard Length gain; H, Height; UH, Uniformity of Height; HG, Height gain; W, Weight; UW, Uniformity of Weight; WG, Weight gain; SGR, Specific Growth rate; FCR, Food conversion rate; S, Survival

Parameter	Treatment					
	Control 20 days	10^6	10^8	Control 40 days	10^6	10^8
TL (mm)	15.8 ± 0.34 B	16.75 ± 0.24 A	16.25 ± 0.23 AB	19.7 ± 0.69 B	22.6 ± 1.13 A	21.78 ± 0.34 A
UTL (%)	100 ± 10.00 A	100 ± 7.14 A	100 ± 0.00 A	100 ± 10.00 A	100 ± 0.00 A	100 ± 5.56 A
TLG (mm)	3.55 ± 1.10 A	4.65 ± 1.12 A	4.20 ± 0.76 A	3.8 ± 0.92 B	5.9 ± 1.25 A	5.4 ± 0.37 AB
SL (mm)	11.85 ± 0.36 B	12.92 ± 0.30 A	12.55 ± 0.20 A	15.8 ± 0.93 B	17.5 ± 0.38 A	17.1 ± 0.71 AB
USL (%)	94.4 ± 6.86 A	100 ± 0.00 A	100 ± 0.00 A	94.44 ± 9.69 A	100 ± 0.00 A	100 ± 0.00 A
SLG (mm)	1.85 ± 0.76 B	3.25 ± 0.49 A	2.60 ± 0.71 AB	4.2 ± 1.01 A	4.6 ± 0.55 A	4.5 ± 0.87 A
H (mm)	6.4 ± 0.21 B	6.9 ± 0.18 A	6.9 ± 0.12 A	8.65 ± 0.63 B	10.16 ± 0.83 A	9.8 ± 0.23 AB
UH (%)	94.4 ± 9.69 A	100 ± 0.00 A	100 ± 5.56 A	86.60 ± 8.43 A	100 ± 10.00 A	100 ± 0.00 A
HG (mm)	1.83 ± 0.74 A	2.19 ± 0.30 A	2.20 ± 0.36 A	2.2 ± 0.77 A	3.4 ± 0.87 A	3.0 ± 0.28 A
W (mg)	0.593 ± 0.10 A	0.641 ± 0.80 A	0.577 ± 0.10 A	1.093 ± 0.10 C	1.761 ± 0.10 A	1.450 ± 0.01 B
UW (%)	84.40 ± 10.96 A	85.71 ± 3.67 A	100 ± 6.25 A	94.44 ± 17.69 A	63.57 ± 19.56 A	70.83 ± 13.46 A
WG (mg)	0.308 ± 0.00 B	0.433 ± 0.00 A	0.369 ± 0.00 AB	0.549 ± 0.20 B	1.094 ± 0.20 A	0.837 ± 0.20 AB
SGR (%)	3.52 ± 0.35 B	5.79 ± 1.23 A	4.97 ± 1.41 AB	1.67 ± 0.52 A	2.42 ± 0.28 A	2.22 ± 0.39 A
FCR	1.84 ± 0.40 A	1.15 ± 0.21 B	1.44 ± 0.21 AB	2.78 ± 0.60 B	1.46 ± 0.12 A	1.50 ± 0.27 A
S (%)	72.5 ± 17.07 A	82.5 ± 15.00 A	82.5 ± 9.57 A	72.5 ± 17.07 A	82.5 ± 15.00 A	82.5 ± 9.57 A

Different letters in each row for each time period are significantly different from each other as determined by Tukey test ($p < 0.05$)

CFU g^{-1} values were obtained with *E. faecium* (Kinouchi et al. 2012). These values were similar to the values obtained in the present study using isolates from *P. scalare*, where growth to above 5×10^8 CFU ml^{-1} was obtained for all strains, attending the demand for use in functional probiotic feed production (ANVISA 2017).

Fast bacterial growth and low duplication times are important factors for probiotics that will compete with undesired microorganisms for space and nutrients (Lorentz et al. 2006), as well as being important for efficient commercial production of the probiotic strains (Vine et al. 2004; Vieira et al. 2013). Although strains 1 and 5 generally grew slightly faster, the overlap in measures between strains 4 and 5 indicates that this difference should not result in practical difficulties for production and uptake by the host.

Finally, the value of the probiotic *E. faecium* for improving growth of fish was confirmed by the in vivo study using feed containing strain 4, regardless of your concentration 2×10^6 or 2×10^8 CFU g^{-1} in the diet. We hypothesize that the probiotic used here improves nutrient uptake from the feed, as described for other probiotics that have been found to contribute to increased growth and better food conversion rates (Ringo et al. 2010; Jatobá and Mouriño 2015). In tilapia given feed supplemented with *Bacillus subtilis*, greater bacterial density in the gastrointestinal tract was shown to alter intestinal pH, reduce adhesion of pathogenic bacteria, reduce the presence of pathogenic bacteria in the intestinal mucosa, and act directly on the nutritional uptake of the host by promoting production of vitamins and exogenous digestive enzymes (Essa et al. 2010).

Conclusion

LAB strains isolated from *Pterophyllum scalare* were identified as belonging to the species *Enterococcus faecium* with Strain 4 was found to be the most resistant to chemical challenges, presents moderate to good inhibition of all pathogens tested, grew to produce high final counts and resulted in improved growth of juvenile *P. scalare* when supplemented to feed, thus representing the most promising strain for probiotic production.

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Compliance with ethical standards

Experiment approved by ethical committee, number 03.13.09.015.00.00 (attached).

Conflict of interest The authors declare that they have no conflict of interest.

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