Sustainable recovery of protein-rich liquor from shrimp farming waste by lactic acid fermentation for application in tilapia feed

J. C. M. Ximenes^{1,2} · D. C. Hissa¹ · L. H. Ribeiro¹ · M. V. P. Rocha³ · E. G. Oliveira⁴ · V. M. M. Melo^{1,2}

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Abstract

The biotransformation of shrimp head waste into ingredients for Nile tilapia (*Oreochromis niloticus*) post-larvae (PLs) diet formulations was evaluated herein. A novel consortium of lactic acid bacteria, comprising the strains *Lactobacillus futsaii* LAB06 and *L. plantarum* LAB14, selected based on kinetic growth parameters, was applied in the fermentation of shrimp head waste. After 48 h, the highest lactic acid production was 100 g L⁻¹, allowing for maximum recovery of chitin and protein-rich liquor. The liquor was added to commercial powdered fish feed at 15, 30, and 45% (w/w) and offered to Nile tilapia PL during 28 days. The inclusion of 30% provided the best results for Nile tilapia PL survival, weight and length gains, specific growth rate, and biomass, proving that the fermented liquor can be incorporated, leading to economic benefits and contributing to the reduction of environmental pollution caused by the improper disposal of shrimp waste.

Keywords Shrimp waste · Fish feed · Nile tilapia · Lactic acid fermentation · Recycle

Introduction

Nile tilapia (*Oreochromis niloticus*) is the most cultivated fish species in Brazil, with an approximate production of 207,400 t in 2014 [1]. Fish diet can make up to 40–70% of production costs, representing the main cost in intensive tilapia farming [2]. In the current context, in which the demand for fish meat is increasing, the aquaculture industry is searching for alternative and sustainable materials to aggregate to fish diets, in order to lower cultivation costs and maintain nutritional profiles, improving animal growth performance and health [3]. Many studies have focused on the formulations of less expensive, locally available, plant- and animal-derived proteins,

such as palm kernel meal [4], cottonseed meal [5], rendered animal [6], and industrial shrimp waste [7].

Shrimp is a very important product in Brazilian aquaculture, with a production of 90,000 t in 2014 [1]. This activity produces huge amounts of wastes, since approximately 40–50% of shrimp weight is discarded (head, tail, vein/viscera, and shell). These wastes contain valuable commercial products, such as the polysaccharide chitin, proteins, minerals, lipids, and pigments, which constitute the shrimp exoskeleton [8]. Thus, recycling shrimp waste represents an innovative and cost-effective way to reduce environmental pollution caused by the improper disposal of these highly perishable wastes in the coastal area, in addition to obtaining added value products.

Traditionally, shrimp farming wastes are chemically processed with strong acids and bases, acting, respectively, in the demineralization and deproteinization of shrimp exoskeletons, in order to recover chitin and other constituents [9]. The fermentative metabolism of lactic acid bacteria (LAB) has been exploited as an alternative to transform shrimp farming waste into added value products [10–12]. Calcium compounds in the shrimp waste are dissolved by the lactic acid and thereby the proteins and other constituents can be extracted by microbial proteases from the waste. Therefore, the lactic acid produced in situ by carbohydrate fermentation is a suitable substitute for the strong chemicals traditionally used [9] and cheaper than using commercial lactic acid.

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✉ V. M. M. Melo
vmmelo@ufc.br

¹ Laboratório de Ecologia Microbiana e Biotecnologia, Departamento de Biologia, Universidade Federal do Ceará, Av. Humberto Monte 2977, Campus do Pici, Bloco 909, Fortaleza, CE 60455-000, Brazil

² Rede Nordeste de Biotecnologia (RENORBIO), Fortaleza, Brazil

³ Departamento de Engenharia Química, Universidade Federal do Ceará, Fortaleza, Brazil

⁴ Departamento de Engenharia de Pesca, Universidade Federal do Ceará, Fortaleza, Brazil

To enhance the economic viability of the biological recycling of shrimp waste, the optimization of the lactic fermentation process is crucial, leading to high acid yields in a shorter period of time. Therefore, this study aimed to identify and select high-yielding acid strains of lactic acid bacteria for the lactic acid fermentation of shrimp head intending the demineralization and deproteinization of the waste for obtaining protein-rich liquor for inclusion in Nile tilapia post-larvae feed.

Material and Methods

Lactic acid bacteria

Six LAB strains isolated from handmade cheese (LAB06, LAB07, LAB08, and LAB09) and pepper sauce (LAB11 and LAB14) from a local market in Fortaleza, Ceará, Brazil, were evaluated. The strains were stored at -80°C in Man, Rogosa and Sharpe (MRS) broth with 20% glycerol (v/v).

Shrimp head waste

Pacific white shrimp head (*Litopenaeus vannamei*) waste was donated by the Cajucoco Aquaculture and Agribusiness, located in the state of Ceará, Brazil, and maintained frozen until use. The fresh waste proximate composition analysis [13] showed $76.92 \pm 0.9\%$ of moisture, $3.75 \pm 0.11\%$ of ash, $13.02 \pm 0.46\%$ of protein, and $1.3 \pm 0.35\%$ of lipids.

Molecular identification of the bacterial strains

Genomic DNA was extracted using the cetyl trimethylammonium bromide protocol (CTAB) [14], and the 16S rRNA gene was amplified by polymerase chain reaction (PCR) using universal primers 27F (5'-AGA GTT TGA TCC TGG CTC AG -3') and 1525r (5'-AAG GAG GTG ATC CAG CC-3') [15]. The PCR was completed with a final volume of 25 μL containing 100 ng of DNA (template), 20 mM Tris-HCl (pH 8.4), 50 mM KCl, 3.0 mM MgCl_2 , 0.2 mM of each dNTP, 0.5 mM of each primer, and 0.04 units of Taq DNA polymerase (MBI Fermentas Inc., USA). The reactions were performed in a thermocycler programmed for an initial denaturation step of 4 min at 94°C , followed by 35 cycles for 1 min each at 94°C , 1 min at 55°C , and 2 min at 72°C . The last cycle was followed by a final extension step of 10 min at 72°C .

Additionally, the housekeeping genes of the RNA polymerase α -subunit (*rpoA*) and phenylalanine-tRNA synthetase (*pheS*) α -subunit were amplified using primers 21F (5'-ATG GAR ATY AAA GAA TTT CC -3') and 23R (5'-ACH GTR TTR ATD CCD GCR CG-3') for *rpoA*, and 21F (5'-HCG CGY CAY GAY ACA ATG C-3') and 23R (5'-GGC ACA TGR HCG ACC ATC CC-3') for *pheS* [16]. The PCR was completed with a final volume of 30 μL containing 100 ng of genomic

DNA (template), 20 mM Tris-HCl (pH 8.4), 50 mM KCl, 3 mM MgCl_2 , 0.2 mM of each dNTP, 0.5 mM of each primer, and 0.04 units of Taq DNA polymerase (MBI Fermentas Inc., USA). PCRs were performed in a thermocycler programmed for an initial denaturation step of 5 min at 95°C followed by 3 cycles for 1 min each at 95°C , 2 min at 46°C , and 1 min at 72°C , supplemented by a further 30 cycles of 35 s each at 95°C , 1 min at 46°C , and 1 min at 72°C . The last cycle was followed by a final extension step of 10 min at 72°C . These conditions were used for both genes, except for the annealing temperature, which was 56°C for *rpoA*.

All PCR products were purified using the Wizard® SV Gel and PCR Clean-Up System (Promega Corporation, Madison, WI, USA) and subjected to sequencing at Macrogen (Seoul, Korea) using primers 27F, 1525r, 518F (5'-GCA CCA GGC GCC GTA ATA CG-3') and 800R (5'-TAA TAC CAG GGT ATC TCC-3') for the 16S rRNA gene and *rpoA*-21F (5'-ATG GAR ATY TTC GAA AAA CC-3'), *rpoA*-23R (5'-ACH GTR TTR ATD CCD GCR CG-3'), *pheS*-21F (5'-HCG CGY CAY GAY ACA ATG C-3'), and *pheS*-23R (5'-GGC ACA TGR HCG ACC ATC CC-3').

The partial high quality (Phred > 20) sequences were applied for the generation consensus sequences using the Codon Code Aligner 5.1 package [17, 18].

Biochemical identification of the bacterial strains

The biochemical identification of the LAB strains was performed with the API 50 CH gallery system (BioMérieux, Marcy-l'Étoile, France) using the *Lactobacillus* API 50 CHL (BioMérieux, Marcy-l'Étoile, France) medium, according to the manufacturer's instructions.

LAB cultivation in MRS broth containing sucrose

The LAB strains were cultured in a MRS broth [19], in which glucose was replaced by sucrose [12], aiming to acclimate the strains to the same carbon source that would be used in the shrimp waste fermentation process. The modified MRS was composed of sucrose (20 g L^{-1}), peptone (10 g L^{-1}), meat extract (10 g L^{-1}), yeast extract (5 g L^{-1}), polysorbate 80 (1 g L^{-1}), ammonium citrate (2 g L^{-1}), sodium acetate (5 g L^{-1}), magnesium sulfate (0.1 g L^{-1}), manganese sulfate (0.05 g L^{-1}), dipotassium phosphate (2 g L^{-1}), and distilled water (1 L), pH 7.0.

The bacterial strains were removed from the stock at -80°C and inoculated into 10 mL of modified MRS broth for 16 h, at room temperature. Subsequently, the cultures were streaked on modified MRS agar for 48 h at 37°C to obtain isolated colonies. Following this step, three colonies were transferred to 100 mL of modified MRS broth and cultivated at 37°C under agitation at 150 rpm for 16 h, since it was noticed that agitation increased biomass yield. Next, the

optical density of this culture was adjusted to 0.1 at 600 nm (UV-Vis Spectrophotometer Thermo Scientific, Genesys 6), corresponding to 1×10^8 CFU (colony-forming units) mL^{-1} , and 20 mL were inoculated in 180 mL of modified MRS broth and incubated under stirring at 150 rpm at 37 °C for 48 h. Every 2 h, 2 mL aliquots were removed to evaluate absorbance, pH, sucrose, and lactic acid concentrations. For biomass determinations, a 48-h culture prepared under the same conditions was centrifuged at 10,000g for 10 min, and the pellet was washed with sterile distilled under centrifugation at 10,000g for 10 min (three times). The biomass was then resuspended in 10 mL of sterile distilled water and three 2 mL aliquots were dried in an oven at 100 °C for 24 h to obtain dry weight values. Data were then used to construct a biomass versus absorbance at 600 nm plot.

The sucrose and lactic acid concentrations were measured by high performance liquid chromatography (HPLC) (Waters, MA, USA), on an HPLC system equipped with a refractive index detector (Waters 2414) using an Aminex HPX-87H column (Bio-Rad, CA, USA). Sulfuric acid 5 mM was used as the mobile phase at a 0.5 mL min^{-1} flow at 65 °C. The sample injection volume was 20 μL . The lactic acid and sucrose peaks were determined by comparison to commercial standards (Sigma-Aldrich, MO, USA).

Lactic fermentation kinetic parameters

The experimental data (biomass, substrate, and product concentrations) were used to determine the kinetic parameters [20] of the fermentation. The volumetric lactic acid productivity (Q_p , $\text{g L}^{-1} \text{h}^{-1}$) was calculated as the ratio of the maximum concentration of lactic acid obtained ($P_{\max}$ g L^{-1}) and the fermentation time at which $P_{\max}$ is reached (t , h).

$$Q_p = P_{\max}/t$$

Sugar conversion to lactic acid ($Y_{P/S}$, g g^{-1}) was defined as:

$$Y_{P/S} = P_f/(S_0 - S_f)$$

where P_f (g L^{-1}) is the acid lactic concentration and S_0 (g L^{-1}) and S_f (g L^{-1}) are sugar concentrations at the beginning and end of the fermentation process, respectively.

Sugar conversion (SC) was calculated as:

$$\text{SC (\%)} = [(S_0 - S_f)/S_0] \times 100$$

Substrate conversion into biomass ($Y_{X/S}$, g g^{-1}) was defined as:

$$Y_{X/S} = (X_f - X_0)/(S_0 - S_f)$$

where X_0 (g L^{-1}) and X_f (g L^{-1}) are the biomass at the beginning and end of fermentation, respectively.

Acid lactic conversion into biomass ($Y_{X/P}$, g g^{-1}) was defined as:

$$Y_{X/P} = (X_f - X_0)/(P_f - P_0)$$

where P_0 (g L^{-1}) and P_f (g L^{-1}) are the acid lactic concentrations at the beginning and end of the fermentation process, respectively.

Solid-state fermentation of shrimp head waste

For solid-state fermentation, 500 g of non-sterilized shrimp heads were crushed and mixed with 10% sucrose (w/w) and 5% of bacterial inoculum adjusted to 1×10^8 CFU mL^{-1} (v/w) according to Cira et al. [12]. Fermentation was performed in Erlenmeyer flasks at 30 °C for 48 h, and aliquots were removed for pH, sucrose, lactic acid, and viable cell evaluation at predetermined times.

The bacterial strains were tested alone and in consortium. For the consortium preparation, equal volumes of each culture, with an optical density of 0.1 at 600 nm, were mixed before use. After the analysis of the preliminary data, the best consortium was selected based on the lactic acid production at 48 h and used in the fermentation of 2 Kg of crushed shrimp head in a horizontal reactor (5-Kg capacity), aiming at obtaining sufficient products to prepare feed for Nile tilapia post-larvae.

Experimental feed formulation

The experimental diets offered to post-larvae Nile tilapia were formulated with commercial powdered feed (Nutreco, Amersfoort, Netherlands) with the addition of different proportions (0%, 15%, 30%, and 45% (w/v)) of the fermented shrimp liquor, adjusted to 55% of crude protein and 3500 Kcal Kg^{-1} in order to attend the nutritional requirements of Nile tilapia post-larvae [7, 21]. After complete homogenization of the ingredients, the feeds were dried at 60 °C for 24 h, until constant weight. The powdered diets received 60 mg Kg^{-1} of the androgenic hormone 17 α -methyltestosterone (Gen-shi, Osaka, Japan) and were stored protected from light in a refrigerator until use.

Chemical analyses

Aliquots of crude chitin, fermented liquor, and commercial and experimental diets were analyzed in the commercial laboratory LABTEC (Sao Paulo, Brazil) for the determination of the proximate composition based on Association of Official Analytical Chemists (AOAC) standard methods [13]. The mineralization (MD) and deproteinization (DP) percentages of

shrimp waste were calculated by the method described by Rao and Stevens [22].

Nile tilapia post-larvae cultivation

The cultivation experiment was conducted at the Aquaculture Laboratory, located in the Department of Fisheries Engineering, University Federal of Ceará, Fortaleza, Brazil, according to the Brazilian Federal Law on Animal Experimentation [23]. For the assay, 5000 post-larvae (PLs) were acquired through a donation from the Aquaculture Research Center (CPAQ/DNOCS, Pentecoste, Ceara, Brazil), immediately after they absorption of the yolk sac. In the laboratory, the PLs were acclimated for 20 min. After acclimatization, PLs measuring 10.01 ± 0.06 mm were selected, counted, and randomly transferred to 16 tanks containing 100 L of dechlorinated freshwater, comprising a total of 200 PLs per tank (2 PLs L^{-1}). The tanks were maintained with continuous aeration, and the water was changed daily in order to remove excreta and residual feed. In the first 14 days, 10% of the water in each tank was replaced with dechlorinated water once a day, while in the last 14 days, the same volume of water was replaced twice a day.

The experiment was conducted in a completely randomized design with four diet treatments (level of liquor inclusion to the feed) and four repetitions (tanks). The treatments were identified as T0 (commercial feed without liquor), T15 (15% of liquor added), T30 (30% of liquor added), and T45 (45% of liquor added).

During the first 7 days, feeding rates corresponded to 25% of total weight per day, and from the eighth day, feeding rates corresponded to 20% of total weight per day. The amount of feed was fractionated into six daily meals, and the fish were hand-fed regularly at every 2 h for 28 days. Dead fish were removed daily, and mortality rates were registered.

Twenty PLs were taken from each tank every 7 days for biometric analyses. For measurements, the fishes were anesthetized by immersion in a 50 mg L^{-1} eugenol solution (Biodinamica, Brazil). After analysis, the PLs were placed in clean water to recover from anesthesia, transferred to a prophylactic bath (0.9% NaCl solution) for 1 min, and finally placed in the tanks (Vidal et al., 2008). This data allowed for the determination of survival (%), specific growth rate ($\% \text{ day}^{-1}$), weight gain (mg day^{-1}), length gain (mm day^{-1}), and feed conversion factor [7].

Temperature ($^{\circ}\text{C}$), dissolved oxygen (DO , mg L^{-1}), pH, and salinity were monitored twice a day, using a digital probe (Hanna HI 9828, Sao Paulo, Brazil) immediately before water replacement. In addition, before beginning the experiment, 13 days and 27 days later, water samples were collected from each tank for the determination of total ammonia (mg L^{-1}), nitrite (mg L^{-1}), nitrate (mg L^{-1}), and orthophosphate ($\mu\text{g L}^{-1}$) concentrations [24].

Statistical analyses

All data were analyzed by a one-way analysis of variance (ANOVA) with Tukey's post-test with a confidence interval of 95% ($p < 0.05$), using the ORIGIN 8.0 software package.

Results and discussion

Molecular and biochemical identification of lactic acid bacteria

Based on the 16S rRNA gene sequence analysis, the six evaluated lactic acid bacterial (LAB) strains were assigned within the *Lactobacillus* (LAB06, LAB07, LAB08, LAB09, and LAB14) and *Pediococcus* (LAB11) genera. The partial 16S rRNA gene sequences were deposited in the Genbank with the accession numbers FJ05310, FJ05311, FJ05312, FJ05313, FJ05315, and FJ05318, respectively. Because the strains could not be clearly identified at the species level, we added the analysis of the housekeeping genes *rpoA* (accession numbers KT245157, KT245158, KT245159, KT245160, and KT245161, respectively) and *pheS* (accession numbers KT245162, KT245163, KT245164, KT245165, and KT245166, respectively) and biochemical tests for species identification [16, 25, 26]. These combined approaches allowed for the accurate identification of strains LAB07, LAB08, LAB09, and LAB14 as *L. plantarum*; LAB06 was assigned as *L. futsaii* and LAB11 as *P. acidilactici*.

It is noteworthy that *L. futsaii* was recently described as a novel *Lactobacillus* species. This species was isolated from fermented mustard products traditionally prepared by the Hakka tribe in Taiwan [25].

LAB growth in synthetic MRS broth

Initially, the production of lactic acid was evaluated in a MRS broth containing sucrose, since the aim of this study was to select the best LAB strains to produce lactic acid in a semi-solid fermentation of crushed shrimp head using sucrose as carbon source. Sucrose was chosen due to its low price and fermenting potential aiming the posterior scale-up, as previously showed by Cira et al. [12].

The kinetic parameters of bacterial growth in the synthetic medium are displayed in Table 1. Overall, only LAB06 differed from the other strains, mostly due to its lowest biomass and acid lactic yield, although displayed the best cell product ($Y_{P/X}$) and cell substrate ($Y_{X/S}$) conversion rates. The other strains showed similar growth performance in modified MRS containing 10% sucrose. Given these preliminary results, we decided to test all six species in shrimp waste fermentation conditions.

Table 1 Kinetic and yield parameters calculated during LAB growth in synthetic MRS broth containing sucrose as carbon source

Parameter	Strain					
	LAB06	LAB07	LAB08	LAB09	LAB11	LAB14
$\mu_{\max}$ (h ⁻¹)	0.07	0.07	0.08	0.07	0.08	0.07
$X_{\max}$ (g L ⁻¹)	2.91 ± 0.04 ^a	6.38 ± 0.60 ^b	6.98 ± 0.24 ^c	7.28 ± 0.53 ^d	7.65 ± 0.09 ^e	6.75 ± 0.44 ^b
$S_{\max}$ (g L ⁻¹)	10.34 ± 0.12 ^a	4.48 ± 0.08 ^b	5.02 ± 0.13 ^c	2.76 ± 0.15 ^d	5.14 ± 0.19 ^c	4.78 ± 0.09 ^b
$P_{\max}$ (g L ⁻¹)	10.93 ± 0.45 ^a	19.14 ± 0.03 ^b	18.94 ± 0.68 ^c	16.99 ± 0.44 ^d	20.24 ± 0.86 ^c	19.95 ± 1.06 ^b
pH	4.11 ± 0.01 ^a	3.80 ± 0.01 ^b	3.80 ± 0.01 ^b	3.80 ± 0.01 ^b	3.80 ± 0.01 ^b	3.80 ± 0.01 ^b
$Y_{P/X}$ (g g ⁻¹)	3.32	2.25	2.12	2.60	1.99	2.37
$Y_{X/S}$ (g g ⁻¹)	0.36	0.51	0.59	0.43	0.66	0.47
$Y_{P/S}$ (g g ⁻¹)	1.20	1.15	1.26	1.12	1.31	1.13
SC (%)	51.85	79.8	77.6	87.6	78.9	78.5
Q_p (g L ⁻¹ h ⁻¹)	0.45	0.87	0.78	0.71	0.84	0.83

$\mu_{\max}$, specific growth rate; $X_{\max}$, maximum production cell concentration; $S_{\max}$, maximum sucrose consumption; $P_{\max}$, maximum product concentration; $Y_{P/X}$, yield of product based on cell growth; $Y_{X/S}$, conversion of substrate to cells; $Y_{P/S}$, conversion of substrate to product; SC, sugar conversion; Q_p , productivity

$p < 0.05$

LAB performance in shrimp head fermentation

Shrimp head fermentation was initially tested in Erlenmeyer containing 500 g of shrimp waste and 10% sucrose as the carbon source. The main lactic acid producers were LAB06, LAB07, and LAB14, especially LAB06, that produced 100 g L⁻¹ within 48 h (Fig. 1a). All strains presented similar sucrose consumption (Fig. 1b) decreased the pH to 4.5–4.3 (Fig. 1c) at the end of fermentation, due to lactic acid production. The HPLC analysis indicated that all strains are homofermentative, since only lactic acid was detected as the final product.

Shirai et al. [27] reported that initial sucrose concentrations, inoculum size, and fermentation time have a significant effect on lactic acid production during shrimp waste fermentation. In addition, when the sugar concentration is greater than 10% (w/w), a prolonged lag phase is observed, due to a decrease in the water activity of the system. In this study, we used 10% sugar and at 8 h of fermentation; all the cultures entered the log phase. This feature is interesting, since the LAB strains promote a shift in the fermentation pattern by the acidification of the medium that inhibits the indigenous bacteria from the waste, preventing putrefaction.

Overall, *L. plantarum* and *P. acidilactici* performances were superior to other performances reported elsewhere, mostly since, comparatively, they produced higher yields of lactic acid in less time [11, 28, 29]. It is noteworthy that no reports are available for comparison on the use of *L. futsaii* in the fermentation of shrimp waste. This is the first occurrence of this species in the west and its first application in shrimp waste recycling.

It has been well established that lactic bacteria strains with commercial potential for industrial application should demonstrate yields above 100 g L⁻¹ lactic acid [10, 30]. All the other evaluated strains (LAB08, LAB09, and LAB11) presented lactic acid production around 80 g L⁻¹ at 48 h of fermentation, which are outstanding results, but not good enough for industrial production. Therefore, these results combined to the kinetic growth analysis allowed us to select LAB06, LAB07, and LAB14 as the most promising for further evaluation regarding if these strains could produce more lactic acid when brought together in a consortium. The lactic acid forms complexes with Ca²⁺ leading to the dissolution of calcium compounds in the shells, facilitating the recovery of proteins and other constituents from shrimp exoskeletons by the microbial proteases [27]. It is important to emphasize that the production of lactic acid in situ by carbohydrate fermentation is cheaper than using commercial lactic acid.

LAB consortia evaluation in shrimp waste fermentation

The tested consortia were LAB67 (LAB06 + LAB07), LAB614 (LAB06 + LAB14), and LAB6714 (LAB06 + LAB07 + LAB14). Considering that LAB06 presented the highest lactic acid production at 48 h, it was included alone as a reference. The fermentation occurred in flakes containing 500 g of crushed shrimp heads supplemented with 10% sucrose (w/w) and 5% inoculum (v/w), during 48 h, at 30 °C. The growth curves are displayed in Fig. 2. The comparative analysis indicated that the consortium LAB614 differed from the other consortia, reaching 100 g L⁻¹ lactic acid production in

Fig. 1 Lactic acid bacterial strain profiles throughout the semi-solid fermentation of crushed shrimp head waste with the addition of 10% sucrose as carbon source. **a** Lactic acid production (g L⁻¹). **b** Sucrose consumption (g L⁻¹). **c** pH

48 h. It is noteworthy that LAB614 at 24 h displayed the lowest production of acid. Nevertheless, between 24 and 32 h, it showed a 240% increase, and the production of lactic acid increased 430% between 24 and 48 h. This behavior demonstrates how metabolic cooperation among microorganisms determines their metabolic streamlining. It is well known that different microorganisms achieve one or more improvements over each other, such as a broader substrate range, improved efficiency, productivity, and reduction in nutritional requirements. Therefore, the use of mixed strains in fermentations can provide useful combinations of metabolic pathways for the use of complex substrates, such as shrimp waste, with high acid production [10, 31].

John et al. [32] reported that mixed *L. casei* and *L. delbrueckii* cultures could effectively replace pure cultures to increase lactic acid production during cassava waste fermentation. Duan et al. [33] also reported the best performance of the consortium formed by *L. acidophilus*, *L. bulgaricus*, and *Streptococcus thermophilus* for shrimp waste fermentation. However, the lactic acid production peak detected by those

authors occurred at 120 h of experiment. Comparatively, the consortium formed by *L. futsaii* (LAB06) and *L. plantarum* (LAB14) in the current study showed better performance, since they produced higher lactic acid in less time.

The fermentation of 2 Kg of shrimp head with the LAB614 consortium yielded 15% (w/w) of chitin and 85% (w/w) of protein-rich liquor. The chitin was recovered partially free of protein (84.8% deproteinization) and minerals (88.6% of demineralization) within 48 h. Cira et al. [12] obtained similar results, but at 6 days of fermentation. Pacheco et al. [28] reported better deproteinization (94%) and demineralization (92%) results, but at 96 h of fermentation.

The proximate composition of the liquor is displayed in Table 2. The liquor was a reddish liquid paste, with a shrimp smell and flavor. It was largely comprised of lipids, minerals, and hydrolyzed proteins from the combined action of the acid lactic and endogenous proteases from the microorganisms present in the shrimp skeleton [22, 27, 28].

As reported previously, shrimp waste protein is rich in essential amino acids [34] and peptides, and the amino

Fig. 2 Comparative acid lactic production (g L^{-1}) and sucrose consumption (g L^{-1}) during semi-solid fermentation of crushed shrimp head waste by selected bacterial consortia L67 (●), L614 (▲), L6714 (◆), and by the L6 strain alone (■)

acids and carotenoids present in liquor are expected to be responsible for its antioxidant activity [35]. In addition, the oil extracted from shrimp head contains valuable polyunsaturated fatty acids (PUFA) [36]. Therefore, liquor can be used as an ingredient in aquaculture feed formulations aiming at beneficial effects.

Growth performance of Nile tilapia post-larvae fed fermented shrimp liquor

The comparison between the proximate composition of the liquor and the experimental diets expressed on a dry basis is presented in Table 2. Moisture content varied among the different experimental feeds, with values of 7.5%, 12.7%, 19.7%, and 23.3%, respectively, for T0, T15, T30, and T45. This variation, however, did not alter diet composition (on a dry matter basis), formulated to provide the same number of calories ($3500 \text{ kcal kg}^{-1}$) and nutrients.

The biometric parameters of the PLs at the 28th day of cultivation indicated no statistical difference between control and all experimental treatments ($p > 0.05$). The average in weight gain was $26.08 \text{ mg day}^{-1}$, in biomass was 49.57 mg , in length gain was 0.86 mm day^{-1} , in specific growth rate was

12.47%, and in feed conversion rate was 1.11. Even with similar specific growth rate, T15 and T30 were indicated as the best treatments, since the accumulated gain weight at the second and third weeks were greater. Besides, T45 did not show good buoyancy in the water and therefore is not technically suitable for feeding the fish larvae.

It is noteworthy that the specific growth rate values observed in the current study are higher than ones reported by Leal et al. [21] and Fasakin et al. [6].

The survival percentage (71.63%) was similar to those observed by Toyama et al. [37], which reported survival rate of 67.88% for tilapia PLs fed diets supplemented with vitamin C.

Regarding feed conversion, the results of this study are better than the ones presented by Fasakin et al. [6] that evaluated the inclusion of animal ingredients in the formulation of tilapia diets. Cavalheiro et al. [7] suggested that shrimp head silage can replace fish meal in tilapia feed, as an economical alternative, without affecting its nutritional quality. Our results support this statement and provide additional data on the priceless value of shrimp head-fermented liquor as ingredients for Nile tilapia post-larvae feed.

PLs showed initial weight average of $12.13 \pm 0.68 \text{ mg}$ and size average of $10.01 \pm 0.06 \text{ mm}$. At the end of the cultivation

Table 2 Chemical composition of the liquor and experimental diets, expressed on a dry basis

Nutrient	Value in the experimental diets (%)				
	Liquor	T0	T15	T30	T45
Protein	59.2 ± 0.52	55.0 ± 0.56	55.3 ± 0.53	55.7 ± 0.49	55.2 ± 0.48
Lipids	9.0 ± 0.04	4.4 ± 0.07	4.9 ± 0.06	5.1 ± 0.07	4.7 ± 0.06
Minerals	19.6 ± 0.01	14.6 ± 0.01	14.8 ± 0.01	14.3 ± 0.01	14.8 ± 0.01

T0: commercial feed (control); T15, T30 and T45: experimental diets with the inclusion of 15%, 30%, and 45% of the fermented liquor, respectively

(28th day), they reached weight and size average, respectively, of 304.6 mg and 25.99 mm for T0 (commercial control diet) and 334.85 mg and 27.02 mm for T30 (added 30% of liquor). As T15 and T30 were statistically similar, the treatment with the higher inclusion of liquor was chosen. PLs weight, size, and biomass throughout the experiment differed significantly ($p < 0.05$) in the second and third weeks among the groups fed with diets containing 15% and 30% of liquor compared to control. Nevertheless, T30 has the advantage of reducing the amount of commercial feed, being therefore advantageous regarding cost and sustainability. Kubitza [38] discussed that adequate nutrition during the sexual reversion phase for commercial purposes should generate PLs with an average final weight of at least 240 mg, a value exceeded herein. Furthermore, the average growth rates observed herein are about 30% higher than the ones reported by Lunsdedt et al. [39].

The water monitoring indicated that temperature (27.43 ± 0.47 °C), dissolved oxygen (6.42 ± 0.46 mg L⁻¹), pH (8.14 ± 0.18), and salinity (0.25 ± 0.03) in the tanks were constant throughout the experiment and within the recommended standards for fish farming [24]. The analyses also indicate no significant difference between the treatments concerning total ammonia (0.82 ± 0.59 mg L⁻¹), nitrite (2.03 ± 1.01 mg L⁻¹), nitrate (12.17 ± 3.15 mg L⁻¹), and orthophosphate (118.17 ± 25.12 µg L⁻¹), demonstrating that liquor supplementation did not influence these parameters.

Conclusions

The bacterial consortium comprising *Lactobacillus futsaii* LAB06 and *L. plantarum* LAB14 stands out as novel competitive product for recovering chitin and others shrimp constituents by lactic acid fermentation without previous or subsequent chemical steps. High yields of lactic acid (100 g L⁻¹) in a short time (48 h), coupled with the acid tolerance of the strains, summarize its most striking features. The obtained fermented protein-rich liquor may comprise up to 30% of the commercial diet of Nile tilapia post-larvae without compromising the biometric parameters, which means the replacement of 30% of the commercial feed. Overall, the results highlight the feasibility to biotransform shrimp waste into value added product, creating economic benefits and aiding in the proper disposal of waste from shrimp farming.

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