



## FERMENTATION OF LEMNA SP. FLOUR BY PRESUMPTIVE BACILLI AND *Pediococcus pentosaceus* SUITABLE AS PROTEIN SOURCE FOR WHITE SHRIMP (*Litopenaeus vannamei*) DIETS

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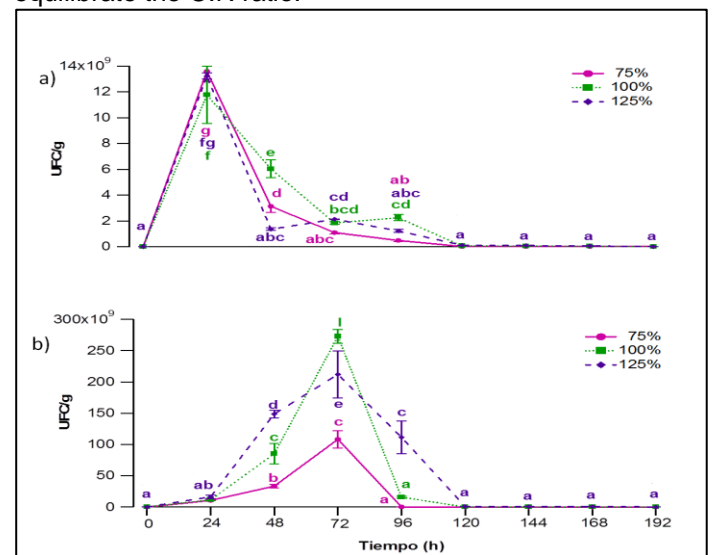
**Introduction.** Duckweed (*Lemna* sp.) has high protein content and the amino acid profile is similar to animal protein (1). Additionally, there are fermentation studies on protein sources with low digestibility, in which microorganisms are used to hydrolyze unusual substrates as feathers to improve the digestibility (2). For example, fermentation of duckweed flour by *Bacillus* sp. decreases fiber and antinutritional factors.

In this study, duckweed was evaluated as a potential source of protein for the preparation of whiteleg shrimp diets by improving their nutritional quality through a fermentation process.

**Methods.** Lactic acid bacteria (LAB) were isolated from *Litopenaeus vannamei* and *L. stylirostris* juveniles and characterized with biochemical (Gram stain, hemolytic activity, extracellular enzymatic activity, hydrophobicity, bacterial growth kinetics, salt tolerance and CFU/mL) and molecular tests (16S ribosomal gene). *Lemna* sp. was fermented by *Pediococcus pentosaceus* and presumptive bacilli. Also, pH, reducing sugars, tannins, and total microbial count were determined.

**Results.** Two LAB isolates were obtained from the hepatopancreas of *L. vannamei* and four from the gut of *L. stylirostris*. All isolates were Gram positive without enzyme activity. The LAB grew well on MRS broth with concentrations of 0.5 to 2.5% NaCl. Regarding growth, the exponential phase of isolates began between 6 at 12 h. The values of CFU/mL for each isolate were from  $2.3 \times 10^9$  and  $6.0 \times 10^9$  CFU/mL. Microbial adhesion to p-Xylene was between  $35.52 \pm 12.71$  and  $83.46 \pm 3.74\%$ . LAB isolates have hydrophobic structures in the cell wall which are capable of establishing hydrophobic interactions (3). The obtained bacterial sequences showed homologies of 93.5% to 99.9% with sequences of BAL. The isolate BALLvHp2 (*P. pentosaceus*) and presumptive bacillus, previously isolated and characterized, were used in the fermentation of duckweed flour, with different percentages of molasses and humidity. The best bacterial growth was obtained at 10% molasses, while the growth at different percentages of moisture was similar, with differences at specific time points of fermentation (Fig.1). Proximate analysis of fermented flour showed percentages of moisture between  $6.9 \pm 0.2$  and  $8.5 \pm 0.2$ , protein was between  $21.0 \pm 0.1$  and  $27.9 \pm 0.1$ , crude fiber was between  $3.4 \pm 0.2$  and  $6.6 \pm 0.2$ , and ash was between  $28.6 \pm 1.8$  and  $36.3 \pm 1.8$ .

The energy was between  $3144.7 \pm 21.6$  and  $3361.3 \pm 23.9$  cal/g. The tannins ranged from  $1.04 \pm 0.1$  and  $1.18 \pm 0.1$  %. Fermented flour showed lower protein content than unfermented flour because bacteria utilize flour protein to equilibrate the C:N ratio.



**Fig. 1.** Growth kinetics of isolates BA4 (a) and BALLvHp2 (b) in *Lemna* flour added with molasses (10%) and with different humidity percentages (75, 100, and 125). Means $\pm$ SD. Different literals denote significant differences.

**Conclusions.** *P. pentosaceus* (BALLvHp2) and presumptive bacilli showed high growth in *Lemna* flour. The high protein content of duckweed flour showed that this plant can be used as protein source for shrimp diets. Additionally, the fermentation of *Lemna* flour decreases the percentage of crude fiber and ash that deteriorate the palatability and digestibility of diets for shrimp.

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