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## Microbial biofloc as source of probiotic bacteria for the culture of *Litopenaeus vannamei*

Gabriela Soltes Ferreira<sup>a,\*</sup>, Norha Constanza Bolívar, Scheila Anelise Pereira, Cristhiane Guertler, Felipe do Nascimento Vieira, José Luiz Pedreira Mouriño, Walter Quadros Seiffert.

<sup>a</sup>Laboratório de Camarões Marinhos, Departamento de Aquicultura, Centro de Ciências Agrárias, Universidade Federal de Santa Catarina, Brasil.

\*Corresponding author. Tel:+554837214103. E-mail address: gabisoltes@yahoo.com.br (Ferreira, G.S.). Servidão dos Coroas s/n (fundos), Barra da Lagoa, 88061-600, Florianópolis, SC, Brazil.

### ABSTRACT

The objective of this study was to isolate and identify bacteria of the genus *Bacillus* sp. from a super-intensive culture system with *Litopenaeus vannamei* microbial flakes, and evaluate their potential of affecting the water quality, performance and immunological parameters when added to the water and diet. *Bacillus* spp. isolates were evaluated *in vitro* for the ability to inhibit pathogens, antagonistic activity. After selection, *Bacillus* spp. was added to the water to assess the influence in the formation and maintenance of microbial flakes in post-larvae culture. In order to verify the performance and hemato-immunological parameters of *L. vannamei* for 42 days of culture, they were incorporated in formulated diets. Four bacteria were isolated from the microbial flakes super-intensive system: *Bacillus thuringiensis* CPQBA 571-12 DRM 06 (>99%), *Bacillus licheniformis* CPQBA 571-12 DRM 07 (>99%), *Bacillus licheniformis* CPQBA 571-12 DRM 08 (>99%) and *Bacillus cereus* CPQBA 571-12 DRM 09 (>99%). *Bacillus licheniformis* CPQBA 571-12 DRM 07 was the only isolate that showed *in vitro* inhibitory characteristics against the pathogen *V. alginolyticus* (BCCM 2068) with a 20 mm diameter inhibition zone and the isolates did not show any antagonistic activity. For the test of formation and maintenance of microbial aggregates generated in the post-larvae culture super-intensive system, a significant difference was observed ( $p<0.05$ ) in the microbiological count of the water on the seventh day of testing, when the total count of *Vibrio* spp. in water was lower in the groups treated with *Bacillus* spp. ( $1\times 10^4$  CFU.mL<sup>-1</sup>) than in the control group. In the fattening test, supplementation with *Bacillus* spp. did not affect the performance of shrimp, however, there were no significant differences ( $p<0.05$ ) in the immunological parameters, *Bacillus* spp. strains were able to increase the total count of hemocytes, the serum binder content and the total protein in serum, and decrease the hemolymph phenoloxidase (PO) activity. There was also an increase ( $p<0.05$ ) in the intracellular production of superoxide anion stimulated by laminarin ( $\beta$ -1,3 glucans) and PMA (Phorbol myristate acetate) compared to the baseline. The conclusion is that the Gram-positive bacteria *Bacillus* spp., isolated from microbial bioflakes, are important for the cultivation and maintenance of the health and growth of *L. vannamei*, and can be used as probiotics or as biocontrol for water in super-intensive culture systems.

**Keywords:** Biofloc; *Bacillus* sp.; shrimp; health; probiotic.

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