

Title: IDENTIFICATION AND PROBIOTIC SELECTION OF *Bacillus* spp. ISOLATED FROM SHRIMP GUT (*Litopenaeus vannamei*)

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Abstract:

Shrimp farming is an important economic activity in Brazilian coastal areas. However, shrimps developed in rearing systems are more likely to develop bacterial infections, and vibriosis has been identified as one of the most serious disease problem. The use of antibiotics, in this case, shows to be limited due to their relative efficacy and possible development of resistant strains. For this reason, the use of probiotic strains isolated from shrimp gut may represent an advance in controlling diseases in shrimp farming. *Bacillus* spp. is ubiquitous in the marine environment and it is successfully used as probiotic in aquaculture. The present study aimed to enumerate, isolate and identify *Bacillus* spp. of intestinal microbiota of the shrimp *Litopenaeus vannamei*, and to evaluate the *in vitro* the production of antagonistic substances. Dilutions of intestinal contents from 12 adult animals were plated onto BHI agar, and colonies were presumptive characterized as *Bacillus* spp. by Gram-staining and catalase activity and then counted. The identification was done by partial sequencing of rRNA16S and by fatty acid methyl ester (FAME) analysis. To select candidate for probiotic use, the isolates were submitted to *in vitro* antagonism assay by the agar double layer diffusion method, using pathogenic vibrio to shrimp as indicator. The population levels of catalase-negative and Gram positive rods ranged from 4 to 6 log₁₀ CFU/g of intestinal contents. Sequencing of rRNA 16S did not allow separation by species for *Bacillus subtilis* group. However, FAME analysis allowed the identification of *B. subtilis* (8), *Bacillus atropheus* (1) and *Bacillus megaterium* (2). These strains inhibited the growth of *Vibrio alginolyticus*, *Vibrio parahaemolyticus* and *Vibrio harveyi*, but did not antagonize *Vibrio anguillarum*. Concluding, *Bacillus* spp. with antagonistic characteristics against pathogenic indicators were isolated from the intestinal microbiota of healthy shrimp which could be used for disease control.

Keywords: *Litopenaeus vannamei*, *Bacillus* spp., probiotic

Support: CNPq, FAPEMIG, PRP-q/UFMG and Potiporã Aquacultura S.A.