Sanghamitra Nayak 2013: Application of *Bacillus* spp. to Control the Pathogenic Bacteria of Aquaculture. Doctor of Philosophy (Fisheries Science), Major Field: Fisheries Science, Department of Fishery Biology. Thesis Advisor: Associate Professor Chalor Limsuwan, Ph.D. 155 pages.

The present study was aimed to select potential probionts from five different strains of *Bacillus* spp. The test pathogens included *Vibrio* spp. *of V. vulnificus, V. fluvialis, V. parahaemolyticus, V. alginolyticus, V. mimicus* and *V. cholerae* (non 01), which were isolated from the white feces disease infected pacific white shrimp (*Litopenaeus vannamei*), and *V. harveyi* which was isolated from the diseased post larvae of pacific white shrimp .*V. vulnificus* and *V. fluvialis* included two strains of yellow and green respectively. *Aeromonas hydrophila* was isolated from diseased Nile Tilapia (*Oreochromis niloticus*). The probiont strains were *Bacillus subtilis* B1, *B. pumilus* B2, *B. subtilis* B3, *B.subtilis* B4, and *B. subtilis* B5. The test of antagonism was carried out by cross streak and agar well diffusion plate assay (AWDA). Antagonistic activities were observed in B1, B2 and B5 against *A. hydrophila* by B1 and against all the *Vibrio* species by the other two strains. B1 and B5 were found to antagonize by inhibition to the pathogens while B2 colonized on the pathogens in cross streak method. In AWDA, these three probionts produced an antimicrobial substance evidenced by the presence of a clear zone after respective hours of incubation with the pathogens. B2 and B5 were found to retain antimicrobial activity up to seven days while B1 up to five days. There was no antagonism found in B3 and B4 against the selected pathogens. Based on the results of cross streak and AWDA, the probiontsB1, B2 and B5 were subjected to co-culture experiments where all the *Bacillus* species were cultured individually with the target pathogen for 120 hours and compared with the monoculture of each strain to determine the potentiality of competitive exclusion of the test probionts for the target pathogens. It was observed that B1 was able to reduce the growth of *A. hydrophila*by about 61.81%, while B2 and B5 inhibited the growth of *Vibrio* spp. by more than 90 and 85%, respectively, at the end of 120 hours of co-culture. For the characterization and partial purification of the antimicrobial substance the B2 was selected on the basis of the residual antimicrobial activity by the critical dilution method against the selected target pathogenic strain. The Cell free neutralized supernatant (CFNS) of B2 showed the moderate thermostability and could be stable up to 70°C for 60minutes and activity was greatly reduced while exposed to 80°Cfor 20minutes .The antimicrobials of B2 showed a stable activity within the pH range of 6 – 10 under room temperature (approximately 30°C) and 4°C . The residual antimicrobial activity of the crude CFNS showed a salinity tolerance up to 7 % of sodium chloride under 4 °C. The activity was absolutely loss while exposed to proteolytic enzyme like proteinase K and pepsin and there was no loss of activity while exposed to lipase. The partial purification of the CFNS was done by the amberlite XAD - 16 absorption after which it was subjected to SDS – PAGE for the determination of the molecular weight. The SDS –PAGE revealed a single protein band approximately 17 kDa. Initial characterization could categorize the antimicrobial substance in CFNS of B2 as bactericin like inhibitory substance. This study indicated that owing to the biochemical properties of B2 and antagonism produced by B2 and B5 against a wide range of pathogenic *Vibrio* spp. and *A . hydrophila* by B1 made these strains potential as a probiotic agent in aquaculture.

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