

Vichai Domrongpokkaphan 2009: Strain Selection and Production of *Bacillus* spp. against *Vibrio* spp. for Black Tiger Shrimp Cultivation. Doctor of Philosophy (Biotechnology), Major Field: Biotechnology, Department of Biotechnology. Thesis Advisor: Associate Professor Penkhae Wanchaitanawong, Ph.D. 200 pages.

Twenty-five isolates of *Bacillus* spp. from hepatopancreas of black tiger shrimp were screened for their antimicrobial activity against ten strains of shrimp pathogenic *Vibrio* spp. by agar well diffusion assay. Four isolates of B17, B19, B21 and B25 which were identified as *B. amyloliquefaciens*, *B. pumilus*, *B. amyloliquefaciens* and *B. megaterium* were found to against all pathogenic strains with the average diameter of inhibition zones ranging from 1.03 to 2.20 cm and the minimum inhibitory concentration (MIC) was  $5.0 \times 10^7$ ,  $5.0 \times 10^7$ ,  $5.0 \times 10^7$  and  $5.0 \times 10^6$  cfu/ml, respectively. Moreover, the MIC was determined by co-culture method in nutrient broth. B17 and B21 exhibited the growth inhibition of *V. harveyi* VH05 with MIC of  $5.0 \times 10^6$  and  $5.0 \times 10^7$  cfu/ml, respectively. Furthermore, the optimal condition for the growth of B17, B19, B21 and B25 were investigated in shake flask. It was found that modified molass medium with Sandril salt and 5% monosodium glutamate with addition of 0 to 15 g/l NaCl was suitable for their growth at 30 °C to 37 °C. The effect of pH on growth of B17, B21 and B25 was also investigated in 2 litre fermentor. The pH 6.0 was found to be optimum for their growth with the maximum specific growth rate of 0.4985, 0.4387 and 0.4938 h<sup>-1</sup>, respectively. Spray drying of B17 with different growth media, growth phase and carriers were studied. After spray drying the highest survival rate of 9.28% was obtained from the cultivation of culture for 36 h in nutrient broth. Combination of 15% maltodextrin and 5% monosodium glutamate as carrier media showed the highest survival rate (63.48%) after spray drying. Under storage at 4 °C and 30 °C for 6 months, the results showed that the strain could survive better at lower storage temperature. Moreover, the spray drying and storage had no effect on the ability of the strain to produce antimicrobial substances. *In vivo* studies, the effect of B17, B21 and B25 on survival of black tiger shrimp was examined. Fresh cells of each strain were added to shrimp diets with 10<sup>8</sup> and 10<sup>6</sup> cfu/g. After a 28-day feeding trial with *Bacillus* supplemented and non-supplemented (control) feeds and challenging shrimps with *V. harveyi* VH05, the treatment group with 10<sup>8</sup> cfu/g exhibited higher survival than that with 10<sup>6</sup> cfu/g. However, B17 has the highest potential application for controlling the pathogen. The treatment with B17 decreased final mortality to 23.08-42.86% depending on cell concentration, compared with 69.23% in the control group. Similar results were observed when spray-dried B17 was used. Shrimp exhibited no difference in survival (70.83-54.55%).

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Thesis Advisor's signature

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