

Characterization of *Bacillus subtilis* and *B. licheniformis* potentials as probiotic bacteria in Vanamei shrimp feed (*Litopenaeus vannamei* Boone, 1931)

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Abstract. Andriani Y, Safitri R, Rochima E, Fakhrudin SD. 2017. Characterization of *Bacillus subtilis* and *B. licheniformis* potentials as probiotic bacteria in Vanamei shrimp feed (*Litopenaeus Vannamei* Boone, 1931). *Nusantara Bioscience* 9: 188-193. This study aimed to characterize the *Bacillus subtilis* and *Bacillus licheniformis* potentials as probiotic bacteria in Vanamei shrimp feed (*Litopenaeus vannamei* Boone, 1931). A two-phase experimental descriptive method in a laboratory scale was applied in this study. The first phase was the bacterial growth curve observation of *B. subtilis* and *B. licheniformis*. The second phase was the characterization of the two bacteria in terms of their tolerance to an acidic condition, temperature, and bile salt concentration. The measured parameter was the number of colonies in predetermined acidic conditions, temperatures, and bile salt concentrations. The number of growing bacterial colonies was calculated using the total plate count method. Results show that *B. subtilis* had an optimal growth point at the eighth hours, achieving a total population of 6.138×10^{13} CFU/mL, while the optimal growth point for *B. licheniformis* was reached at the tenth hour with 4.299×10^{13} CFU/mL. *Bacillus subtilis* and *B. licheniformis* were also tolerant at 40, 50, and 60°C. The acid tolerance testing was conducted at pH of 2, 4, and 6. It was revealed that *Bacillus subtilis* were intolerant to acidic condition at pH = 2, in which the number of colonies decreased drastically. On the contrary, *B. licheniformis* was tolerant to pH = 2, which was evident from the absence of a drastic decrease in the number of colonies. Both *B. subtilis* and *B. licheniformis* were tolerant to pH 4 and 6. High tolerance to 0.3% and 0.5% bile salt concentration was observed in both *Bacillus subtilis* and *B. Licheniformis* with a high number of colonies of 10^{10} CFU/mL. Therefore, it is concluded that both *B. subtilis* and *B. licheniformis* can be developed into probiotics.

Keywords: *Bacillus licheniformis*, *Bacillus subtilis*, feed, probiotic, Vanamei shrimp

INTRODUCTION

Vanamei Shrimp (*Litopenaeus vannamei* Boone, 1931) is a type of shrimps commonly cultivated in Indonesia. One of the challenges faced when cultivating Vanamei shrimp is the high mortality rate due to disease attacks, such as *white spot disease* (WSD), which leads to reduced shrimp production and creates a loss for the farmer. One of the approaches that can be applied to cope with this condition is through the use of probiotics (Gatesoupe 1999). Probiotics are commonly applied through feed used in shrimp cultivation. The addition of probiotic bacteria in the feed will be useful to regulate the existing microbe environment in the intestine, prevent the growth of an intestinal pathogenic microorganism, and improve feed efficiency by releasing enzymes that are able to improve feed digestion process (Anwar et al. 2016). A good digestion process will optimize the use of feed consumed, leading to increased feed efficiency and reduced the amount of feed organic waste in the form of feces and other metabolism waste, e.g. urine and ammonia. This will maintain the quality of water.

The types of bacteria that are potentially used as probiotic bacteria include, among others, *Bacillus subtilis*

and *B. licheniformis*. *Bacillus* is ubiquitous and widely distributed in most places, including in shrimp digestive tract. These bacteria are very suitable to use because they do not produce toxin, easy to grow, do not need expensive substrates, able to thrive in high temperature, and do not produce any metabolic side product. The development of *Bacillus*-based probiotics in Vanamei shrimp cultivation has a good prospect and potential (Linggarjati et al. 2013). The stages that have to be passed before using microbes as probiotics are, among others, viability testing, temperature tolerance testing, acid tolerance testing, bile salt tolerance testing, antimicrobial testing, autoaggregation testing, and co-aggregation testing. This study, however, only focused on testing the microbial tolerance to acid, temperature, and bile salt concentration. These tests are expected to be able to convince us that the microbes can survive and thrive in shrimp digestive tract by the ability to tolerate the temperature, acidity, and bile salt concentration in the tract. The aim was to characterize the potential use of *Bacillus subtilis* and *Bacillus licheniformis* as probiotic bacteria in Vanamei (*Litopenaeus vannamei* Boone, 1931) shrimp feed through observation of the probiotic bacterial growth curve, followed by acid, temperature, and bile salt tolerance testing.