ABSTRACT

Esti Handayani Hardi. Potential Vaccine Candidate of *Streptococcus agalactiae* for Prevent Streptococcosis on Nila Tilapia (*Oreochromis niloticus*). Under direction of Enang Harris, Sukenda, Angela M. Lusiastuti.

The main purpose of this research was to find a vaccine to protect *S. agalactiae* infection. The research was divided into five steps. First experiment, characteristic test displayed that this bacteria was Gram positive, oxidative fermentative positive, negative catalase and motility, grow well on media containing NaCl 6.5%, possed two type of haemolytic: β-haemolytic and non-haemolytic. The capability of both bacteria to hydrolyze sugar was different: β-haemolytic could hydrolyze more sugars than non-haemolytic. Based on phenotypic test, *S. agalactiae* could be divided into two groups, capsulated [isolate 2, 4 and 5] and non-capsulated bacteria [isolate 1 and 3]. Second experiment, to evaluate virulence of each *S. agalactiae* isolate to nile tilapia. After intraperitoneally injected (0.1 mL/fish) into 30 fish, the non-haemolytic demonstrated more virulent than β-haemolytic. It caused faster mortality, clinical symptoms, severe behavior changes and pathological changes macroscopically and microscopically. The third experiment, toxicity of extracellular products (ECP) of *S. agalactiae* was tested in cultured nile. Toxicity test of ECP to know the virulence factor of *S. agalactiae* was still limited. It was found that after tested on 15 fish, through intraperitoneal injection of 0.1 mL/fish, ECP from both bacteria caused changes in swimming pattern, response to food, external changes and histopathology. Extracellular products of *S. agalactiae* non-haemolytic type (BHIA and BHI 24 h) and β-haemolytic type (BHI 72 h) caused mortality 12 hours after injection and the mortality continued till day 7th of culture. Silver staining of sodium dodecyl sulphate-polyacrylamide gels to *S. agalactiae* revealed that predominant 51.8–69.6 kDa bands were present in BHIA ECP fraction. The 69.6 kDa was absent from the BHI ECP. Total protein on non-haemolytic *S. agalactiae* ECP are 28.18 ppm on BHIA medium and 13.64 ppm on BHI medium. Whereas β-haemolytic *S. agalactiae* ECP are 2.73 ppm on BHIA medium and 8.18 ppm on BHI medium. The conclusion from the research that ECP was virulent factor on β-haemolytic and non-haemolytic *S. agalactiae* in fish. Fourth and five experiment, the effectiveness of a *S. agalactiae* vaccine in tilapia (*O. niloticus*) was evaluated for the prevention of streptococcal disease. The vaccine was prepared from formalin-killed whole cell and concentrated ECP of β-haemolytic and non-haemolytic of *S. agalactiae*. Vaccination trial was conducted through intraperitoneal (IP) injection into fish. Fish vaccinated with whole cell, ECP and mix of them were challenged by IP injection with $10^3$ colony-forming units (CFU)/fish of β-haemolytic and $10^5$ CFU/fish of non-haemolytic *S. agalactiae*. The highest RPS is formed on vaccination with mix whole-cell and extracellular product vaccine of *S. agalactiae* β-haemolytic tipe. The conclusion is vaccination with *S. agalactiae* β-haemolytic tipe more effective to protect tilapia against Streptococcosis than non-haemolytic of *S. agalactiae* vaccine.

Keywords: characteristic, pathogenicity, ECP, vaccination, *S. agalactiae*, *O. niloticus*