

ABSTRACT

Febriana Rahmawati, 24020121130095. Co-infection Detection Assay of *Aeromonas hydrophila* and *Streptococcus agalactiae* in Nile Tilapia (*Oreochromis niloticus*) using Single and Duplex PCR. Under the guidance of Siti Nur Jannah and Hesty Novita.

Aeromonas hydrophila and *Streptococcus agalactiae* are pathogens that can cause co-infection in Nile tilapia with high mortality rates, making accurate pathogen detection critically important. The objective of this study was to characterize *A. hydrophila* and *S. agalactiae* isolates from Nile tilapia, develop a single and duplex PCR method for detecting co-infection of *A. hydrophila* and *S. agalactiae*, and evaluate Koch's *postulate* test results in tilapia. The research methods employed included bacterial isolate culture, biochemical tests, DNA extraction, *single* PCR assays, duplex PCR optimization, limit of detection testing, and Koch's *postulate* evaluation. Two primer pairs used in the PCR assays targeted specific sequences of the aerolysin gene and 16S rRNA gene to detect *A. hydrophila* and *S. agalactiae*, respectively. The results demonstrated that *A. hydrophila* was characterized as a Gram-negative bacterium with glossy yellowish colonies, catalase-oxidase positive, and β -hemolytic, while *S. agalactiae* was identified as a Gram-positive bacterium with transparent white colonies, catalase-oxidase negative, and β -hemolytic. The single and duplex PCR method successfully detected simultaneous co-infection of *A. hydrophila* and *S. agalactiae*, evidenced by the presence of DNA bands at 417 bp for *A. hydrophila* and 220 bp for *S. agalactiae*. Limit of detection testing revealed that *A. hydrophila* DNA was detectable at 4.3 ng/mL concentration, while *S. agalactiae* was detectable at 7.1 ng/mL. Koch's *postulate* confirmed that co-infection of *A. hydrophila* and *S. agalactiae* was the causative agent of disease in Nile tilapia.

Keywords: *Aeromonas hydrophila*, *Streptococcus agalactiae*, *Co-infection*, *Nile Tilapia*, *Duplex PCR*