ABSTRACT

Nabilah Evelina Dewi. 24020121190052. Rapid and Specific Detection Development of *Aeromonas hydrophila* and *Aeromonas veronii* in Nile Tilapia Fish Using Single and Duplex PCR. Under the supervision by Dr. Siti Nur Jannah, S. Si, M. Si. and Dr. Hessy Novita, S.Pi., M.Si.

Aeromonas hydrophila and *Aeromonas veronii* are pathogens capable of co-infecting Nile tilapia simultaneously. This study aims to develop Single PCR and Duplex PCR methods for the rapid and specific detection of *Aeromonas hydrophila* carrying the *aerolysin* gene and *Aeromonas veronii* with the *rpoB* gene in Nile tilapia (*Oreochromis niloticus*). These study was conducted to evaluate the efficiency of Duplex PCR compared to Single PCR in simultaneously detecting both bacteria, making it an ideal tool for aquaculture pathogen surveillance, with target bands at 417 bp for *Aerolysin* gene and 224 bp for *rpoB* gene. The results showed that *A. hydrophila* and *A. veronii* isolates exhibited distinct colony morphologies on TSA, GSP, RS, and MacConkey media and were confirmed as Gram-negative, β-hemolytic bacteria, catalase and oxidase positive. Single PCR successfully detected the *aer* gene (417 bp) for *A. hydrophila* and the *rpoB* gene (224 bp) for *A. veronii*, with clear bands at optimal annealing temperature of 58°C in 30 cycle. The *Limit of Detection* (LOD) for duplex PCR was 10^{-5} dilution, while single PCR reached up to 10^{-10}. Koch's postulates confirmed the pathogenicity of both bacteria, with co-infected fish showing clinical signs such as hemorrhage, exophthalmia, and internal organ damage, highlighting the importance of early detection for disease management in aquaculture.

***Keyword*:** Nile Tilapia (*Oreochromis niloticus*), *Aeromonas hydrophila*, *Aeromonas veronii*, *duplex* PCR, *Aerolysin* gene, *rpoB* gene.