Development of Gold Nanoparticles based Genosensor for the Detection of *Aphanomyces Invadans* in Fish

ABSTRACT

Aphanomyces invadans is a pathogen that causes Epizootic Ulcerative Syndrome (EUS) in fish. It is a devastating disease affecting the commercially important wild and cultured freshwater and estuarine finfish species worldwide. Conventional method for the detection of *Aphanomyces invadans* was found to be more time consuming and labor intensive. Hence, an electrochemical genosensor based on sandwich hybridization assay which offer rapid and sensitive detection has been developed to detect the DNA oligonucleotides and PCR products from fungus *Aphanomyces invadans.* Two methods, step-by-step and premix sandwich hybridization methods were tested with linear DNA targets to determine the best signal response. Eventually, premix sandwich hybridization method was chosen for the PCR products detection as it was shown to be more sensitive and easier to perform. This assay depended on the hybridization of the single stranded target DNA with complementary oligonucleotide probe using modified gold nanoparticles in advance and later hybridized with the complementary oligonucleotide probe that has been immobilized on the surface of screen-printed electrode (SPE). Anodic stripping and differential pulse voltammetry (DPASV) were used to detect the gold nanoparticles labeling at which its concentration is directly proportional to the concentration of target DNA. Different concentration of linear DNA target was tested for both the methods. The limit of detection obtained for the premix hybridization assay was 0.5 fM (3.014×10^3 DNA molecules) while the step-by-step sandwich assay was 5 fM (6.0815×10^4 DNA molecules). For the PCR products detection, various probes which

targeted at different regions of the 208 bp PCR products had been designed and tested in order to realize the best position for the DNA hybridization reactions. The combination of bottom capture probe with bottom reporter probe gave the highest signal due to minimal steric hindrance for hybridization. The limit of detection achieved for the PCR products detection was 1 pM (6.1676 x 10^6 DNA molecules). The developed genosensor had shown to be a promising tool for providing a rapid, inexpensive and sensitive detection method for the fish pathogen. Hence, it can be used as a surveillance tool to monitor the spread of EUS in the aquaculture industry.