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Molecular Detection of the Harmful Raphidophyte *Chattonella subsalsa* Biecheler by Whole-Cell Fluorescence *in-situ* Hybridisation Assay

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Abstract: Species of the genus *Chattonella (Raphidophyceae)* are a group of marine protists that are commonly found in coastal marine waters. Some are known as harmful microalgae that form noxious blooms and cause massive fish mortality in finfish aquaculture. In Malaysia, blooms of *Chattonella* have been recorded since the 1980s in the Johor Strait. In this study, two strains of *Chattonella* were established from the strait, and morphological examination revealed characteristics resembling *Chattonella subsalsa*. The molecular characterization further confirmed the species' identity as *C. subsalsa*. To precisely detect the cells of *C. subsalsa* in the environment, a whole-cell fluorescence *in-situ* hybridisation (FISH) assay was developed. The species-specific oligonucleotide probes were designed *in silico* based on the nucleotide sequences of the large subunit (LSU) and internal transcribed spacer 2 (ITS2) of the ribosomal DNA gene (rDNA). The best candidate signature regions in the LSU-rRNA and ITS2-rDNA were selected based on hybridisation efficiency and probe parameters. The probes were synthesised as biotinylated probes and tested by tyramide signal amplification with FISH (FISH-TSA). The results showed the specificity of the probes toward the target cells. FISH-TSA has been proven to be a potential tool in the detection of harmful algae in the environment and could be applied to the harmful algal monitoring program.

Keywords: *Chattonella*, harmful algal bloom, oligonucleotide probe, ribosomal DNA, fluorescence *in situ* hybridisation (FISH)

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