TROPICAL LIFE SCIENCES RESEARCH

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

Authors:

Winnie Lik Sing Lau, Sing Tung Teng, Hong Chang Lim, Kieng Soon Hii, Sandric Chee Yew Leong, Chui Pin Leaw* and Po Teen Lim*

***Correspondence:** cpleaw@um.edu.my; ptlim@um.edu.my

Submitted: 15 February 2022; **Accepted:** 11 August 2022; **Published:** 31 March 2023

To cite this article: Winnie Lik Sing Lau, Sing Tung Teng, Hong Chang Lim, Kieng Soon Hii, Sandric Chee Yew Leong, Chui Pin Leaw and Po Teen Lim (2023). Molecular detection of harmful raphidophyte *Chattonella subsalsa* Biecheler by whole-cell fluorescence *in situ* hybridisation assay. *Tropical Life Sciences Research* 34(1): 99–120. https://doi.org/10.21315/tlsr2023.34.1.7

To link to this article: https://doi.org/10.21315/tlsr2023.34.1.7

Highlights

- Two strains of the harmful raphidophyte *Chattonella subsalsa* were established from in the Johor Strait.
- A whole-cell fluorescence *in situ* hybridisation (FISH) assay targeting *C. subsalsa* cells was developed based on the nucleotide sequences of the LSU rDNA and ITS2.
- The species-specific probes developed showed specificity toward the target cells, thus having the potential to detect this harmful microalga in the environment.

Tropical Life Sciences Research, 34(1), 99–120, 2023

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

[1]Winnie Lik Sing Lau, [2]Sing Tung Teng, [3]Hong Chang Lim, [1]Kieng Soon Hii, [4]Sandric Chee Yew Leong, [1]Chui Pin Leaw* and [1]Po Teen Lim*

[1]Bachok Marine Research Station, Institute of Ocean and Earth Sciences, University of Malaya, 16310 Bachok, Kelantan, Malaysia
[2]Faculty of Resource Science and Technology, Universiti Malaysia Sarawak, 94300 Kota Samarahan, Sarawak, Malaysia
[3]Institute of Biodiversity and Environmental Conservation, Universiti Malaysia Sarawak, 94300 Kota Samarahan, Sarawak, Malaysia
[4]St. John's Island National Marine Laboratory, Tropical Marine Science Institute, National University of Singapore, Singapore 119227

Submitted: 15 February 2022; **Accepted:** 11 August 2022; **Published:** 31 March 2023
To cite this article: Winnie Lik Sing Lau, Sing Tung Teng, Hong Chang Lim, Kieng Soon Hii, Sandric Chee Yew Leong, Chui Pin Leaw and Po Teen Lim (2023). Molecular detection of harmful raphidophyte *Chattonella subsalsa* Biecheler by whole-cell fluorescence *in situ* hybridisation assay. *Tropical Life Sciences Research* 34(1): 99–120. https://doi.org/10.21315/tlsr2023.34.1.7
To link to this article: https://doi.org/10.21315/tlsr2023.34.1.7

Abstract: Species of the genus *Chattonella (Raphidophyceae)* are a group of marine protists that are commonly found in coastal 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 (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)

*Corresponding author: cpleaw@um.edu.my, ptlim@um.edu.my

Abstrak: Spesies genus Chattonella (Raphidophyceae) ialah sekumpulan protista marin yang biasa ditemui di perairan laut pantai. Sesetengahnya dikenali sebagai mikroalga berbahaya yang membentuk ledakan alga berbahaya dan menyebabkan kematian ikan secara besar-besaran dalam akuakultur ikan sirip. Di Malaysia, ledakan alga Chattonella telah direkodkan sejak tahun 1980-an di Selat Johor. Dalam kajian ini, dua strain Chattonella telah didirikan dari selat, dan pemeriksaan morfologi mendedahkan ciri-ciri yang menyerupai *Chattonella subsalsa*. Pencirian molekul seterusnya mengesahkan identiti spesies sebagai *C. subsalsa*. Untuk mengesan dengan tepat sel-sel *C. subsalsa* di dalam persekitaran, ujian penghibridan in-situ berpendarfluor (FISH) ke atas sel keseluruhan telah dibangunkan. Prob oligonukleotida spesies telah direka secara spesifik secara siliko berdasarkan jujukan nukleotida subunit besar (LSU) dan spacer transkripsi dalaman 2 (ITS2) gen DNA ribosom (rDNA). Calon terbaik kawasan tanda dalam LSU-rRNA dan ITS2-rDNA telah dipilih berdasarkan kecekapan penghibridan dan parameter prob. Prob telah disintesis sebagai prob biotinilasi dan diuji dengan penguatan isyarat tyramide dengan FISH (FISH-TSA). Keputusan menunjukkan kekhususan prob ke atas sel sasaran. FISH-TSA telah terbukti sebagai alat yang berpotensi dalam pengesanan alga berbahaya di alam sekitar dan boleh digunakan untuk program pemantauan alga berbahaya.

Kata kunci: *Chattonella*, kembangan alga yang berbahaya, oligonukleotid prob, DNA ribosom, penghibridan *in situ* berpendarfluor

INTRODUCTION

Harmful algal bloom (HAB), also known as “red tide”, occurs when harmful microalgae grow in high biomass in the water column, causing severe consequences such as food poisoning syndromes in humans who consume algal toxins-contaminated seafood and massive mortality of marine organisms (Hoagland *et al.* 2002). Paralytic shellfish poisoning has been the focus of attention in Malaysia, as it has been linked to the majority of human intoxication cases (Lim *et al.* 2012; Usup *et al.* 2012; Yñiguez *et al.* 2021). Several causative dinoflagellates, including Pryodinium bahamense Plate, Alexandrium tamiyavanichii Balech, A. minutum Halim, and Gymnodinium catenatum Graham, have been documented throughout the Malaysian waters (Leaw *et al.* 2005; Lim *et al.* 2007). Nonetheless, other algal-related incidents have been documented in Malaysia, such as massive fish kills in aquaculture farms (Lim *et al.* 2012; 2014; Teng *et al.* 2016; Yñiguez *et al.* 2021; Lum *et al.* 2021). The majority of these events have been linked to marine harmful dinoflagellates, such as *Margalefidinium polykrikoides* (Margalef) *Gómez,* Richlen, and Anderson*, Noctiluca scintillans* (Macartney), Kofoid & Swezy, and *Karlodinium australe* Salas, Bolch*,* and Hallegraeff (Lim *et al.* 2014; Teng *et al.* 2016).

Among the harmful microalgae, several groups of raphidophytes have been recognized as harmful to marine organisms (Lum *et al.* 2021). Members of the genus *Chattonella Biecheler* are among those that have caused severe damage to the aquaculture industries in many coastal countries, for instance Japan (Okaichi 2003; Imai & Yamaguchi 2012). The first record of *Chattonella* bloom has been reported on the Malabar Coast, India, while the most severe fish kill event