定量实时PCR检测有害刺胞藻的检测——Karlodinium australi

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SUMMARY

We investigated a harmful algal bloom (HAB) associated with the massive fish kills in Johor Strait, Malaysia, which recurred a year after the first incident in 2014. This incident has urged the need for a rapid and precise method in HAB monitoring. In this study, we develop a SYBR green-based real-time PCR (qPCR) to detect the culpable dinoflagellate species, Karlodinium australi. Species-specific qPCR primers were designed in the gene region of the second internal transcribed spacer of the ribosomal RNA gene (rDNA). The species specificity of the primers designed was evaluated by screening on the non-target species (Karlodinium veneficum, Takayama spp., and Karenia spp.) and no cross-detection was observed. The extractable gene copies per cell of K. australi determined in this study were 19 998 ± 505 (P < 0.0001). Estimation of cell densities by qPCR in the experimental spiked samples showed high correlation with data determined microscopically (R² = 0.93). Using the qPCR assay developed in this study, we successfully detected the 2015 bloom species as K. australi. Single-cell PCR and rDNA sequencing from the field samples further confirmed the finding. With the sensitivity as low as five cells, the qPCR assay developed in this study could effectively and rapidly detect cells of K. australi in the environmental samples for monitoring purpose.

Key words: fish kill, Malaysia, ribosomal RNA gene, second internal transcribed spacer.

INTRODUCTION

Rapidly expanding aquaculture activities and industrialization in coastal regions may have contributed to an increase in nutrient loadings, and resulted in localized eutrophication. Together with favorable physical conditions, this will promote rapid growth of phytoplankton, and algal bloom is likely to occur (Hall et al. 2008). Some of these algal blooms are known to cause harm by producing biotoxins that can intoxicate humans or kill marine organisms. Thus, rapid harmful algal detection tools are needed for regular monitoring and early prediction of these harmful algal blooms (HABs).