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ORIGINAL ARTICLE

Biology

Pseudo-nitzschia species (Bacillariophyceae) identification and delineation using mitochondrial *cox*1 gene sequences as compared to LSU rDNA

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Abstract An attempt was made to infer the phylogeny of *Pseudo-nitzschia* species by using the mitochondrial-encoded gene, cytochrome c oxidase subunit I (*cox*1), and comparing it with the nuclear-encoded large subunit ribosomal DNA (LSU rDNA). A pair of primers targeting *Pseudo-nitzschia cox*1 was designed in silico and used to infer the molecular phylogeny of *Pseudo-nitzschia*. The primer pair was tested using genomic DNAs isolated from six species of *Pseudo-nitzschia* from Malaysia. The phylogenetic inference of *cox*1 was then compared to the LSU rDNA phylogeny. Phylogenetic reconstructions of both data sets revealed monophyly of *Pseudo-nitzschia* species complexes. The range of genetic divergences among *Pseudo-nitzschia* species were higher in the *cox*1 data set (3.5–20.4 %) compared to the LSU rDNA data set (0.1–8.8 %). The present study suggests that high genetic divergence in *cox*1 of *Pseudo-nitzschia* species could be a useful genetic marker for DNA bar coding.

Keywords *cox*1 · Diatom · LSU rDNA · Primer · *Pseudo-nitzschia*

Introduction

Diatoms are an important component of the marine phytoplankton, in terms of both abundance and species richness [1]. While most diatom species are important ecologically, several species are known to cause amnesic shellfish poisoning (ASP). The first incident of ASP was reported in Canada, in 1987; three deaths and 105 cases of acute human intoxication were documented after consuming the contaminated blue mussels (*Mytilus edulis* Linnaeus) [2]. The neurotoxin, domoic acid (DA), $C_{15}H_{21}NO_6$, was later found to be produced by the diatom, *Pseudo-nitzschia multiseries* (Hasle) Hasle (formerly known as *Nitzschia pungens* f. *multiseries*) [3]. People who consumed the contaminated shellfish in relation to ASP showed similar symptoms to Alzheimer's disease, i.e. loss of short-term memory [2]; other symptoms included diarrhea, nausea, vomiting, abdominal cramps and headache.

In Malaysia, there have been no reported cases of ASP. Screening of ASP-toxin production among the cultured strains of *P. pungens* (Grunow *ex* Cleve) Hasle showed undetectable levels of DA [4], even though the species is commonly found in Malaysian waters [5, 6]. High species diversity of *Pseudo-nitzschia* species in Malaysian coastal waters was reported by Teng et al. [5], with more than 23 species documented. Among the 23 species, six species have been identified from the waters as new morphotypes, and described as *P. batesiana* H.C. Lim, Teng, Leaw *et* P.T. Lim, *P. circumpora* H.C. Lim, Leaw *et* P.T. Lim, *P. fukuyoi* H.C. Lim, Teng, Leaw *et* P.T. Lim, *P. kodamae* Teng, H.C. Lim, Leaw *et* P.T. Lim, *P. lundholmiae* H.C. Lim, Teng,

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