



Rich molecular diversity of ribosomal RNA genes in *Pseudo-nitzschia* species (Bacillariophyceae) revealed through single-strain high throughput sequencing

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ABSTRACT

Ribosomal RNA genes (rDNAs) are widely used as molecular markers in phytoplankton identification and diversity analysis, showing significant efficacy in metabarcoding studies. Recent analysis of amplicon sequencing results of the 18S rDNA V4 domain in multiple strains of the toxigenic *Pseudo-nitzschia multistriata* revealed that, in addition to the presence of a dominant copy of 18S rDNA V4 in each strain, there also exists a large number of low-abundance 18S rDNA V4 sequence variants, indicating the existence of high levels of intragenomic variations (IGVs). Many of these low-abundance variants identified in *P. multistriata* also show high similarity to that of other *Pseudo-nitzschia* species. In this study, we analyzed the molecular diversity of 18S rDNA V4 sequences of additional 56 clonal strains of nine *Pseudo-nitzschia* species collected along the coast of China and the South China Sea using high-throughput amplicon sequencing. The results showed that each strain harbors high levels of IGVs as expected. Although a common dominant sequence variant is often shared by all strains of the same species, these variants are usually also found at low frequencies in other species, suggesting that IGVs in these diatoms, like most protists, are linked to the biological processes of genome evolution. Understanding the nature of IGVs is not only critical for ascertaining the species evolution, but also for precise interpretation of the molecular diversity revealed in metabarcoding studies.

1. Introduction

Species of the pennate diatom genus *Pseudo-nitzschia* are mostly cosmopolitan and play a crucial role as primary producers in the marine biogeochemical cycles [1–6]. *Pseudo-nitzschia* species have attracted worldwide attention after a deadly incident resulting in three deaths and more than a hundred hospitalizations in Prince Edward Island in Canada in 1987 [7]. Of the 61 species described thus far, 29 have been demonstrated to be toxigenic by producing the neurotoxin domoic acid (DA), which can accumulate in shellfish and cause amnesic shellfish poisoning (ASP), and affects marine lives and human health [6–11]. In China, sampling effort has resulted in the identification of over 40

Pseudo-nitzschia species, ten of which are known to be toxigenic (*P. bipertita*, *P. cacciantha*, *P. cuspidata*, *P. fraudulenta*, *P. fukuyoi*, *P. lundholmiae*, *P. multiseries*, *P. pseudodelicatissima*, *P. punctonis*, and *P. simulans*) [9,12–17]. The cosmopolitan presence of toxigenic *Pseudo-nitzschia* species prompted the establishment of a series of monitoring plans for toxigenic *Pseudo-nitzschia* species and DA in aquatic products to mitigate their adverse impacts [8]. Because the toxigenic potential of *Pseudo-nitzschia* species varies (reviewed in [7]), it is important to identify and monitor the specific toxic species.

Ribosomal gene markers including the SSU (18S), LSU (28S), ITS1, ITS2, and 5.8S rDNAs have been widely used for distinguishing species of *Pseudo-nitzschia*, each having its own advantages and disadvantages

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