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Toxicity comparison among four strains of *Margalefidinium polykrikoides* from China, Malaysia, and USA (belonging to two ribotypes) and possible implications

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ABSTRACT

The unarmored dinoflagellate *Margalefidinium polykrikoides* is a well-known cosmopolitan harmful alga that forms intensive blooms causing massive fish kills and mortality of other marine animals. Globally distributed populations of *M. polykrikoides* have been categorized into four ribotypes: East Asian, American/Malaysian, Philippines, and Mediterranean, according to their characteristic sequences in LSU rRNA gene and primary geographic distributions, while no discernible morphological feature has been observed to distinguish. Here we examined the toxicity of four strains of *M. polykrikoides* including MPJZB-C3 and MPJZB-D6 (East Asian ribotype, from China), CP1 (American/Malaysian ribotype, from USA), and MPCoKK23 (American/Malaysian ribotype, from Malaysia), to three aquatic animals including the marine medaka (*Oryzias melastigma*), rotifer (*Brachionus plicatilis*, L-type), and brine shrimp (*Artemia salina*). With equivalent initial cell densities ranged from 1000 to 7000 cells mL^{-1}, CP1 and MPCoKK23 displayed acute toxicity in a dose-response manner to the three target animals (100% mortality at higher cell densities) within 24 h, MPJZB-C3 also displayed acute toxicity to the fish and rotifer in 24 h (up to 50% mortality) when the cell densities were above 3000 cells mL^{-1}. The strain MPJZB-D6 did not exhibit significant toxicity to all three animals in 24 h, but, in 120 h, caused 50–100% mortalities in fish when the cell density was >3000 cells mL^{-1} and 17% mortality in brine shrimp when the cell density was >5000 cells mL^{-1}. Statistical analysis of the data indicated that the strains CP1 and MPCoKK23 exhibited significantly higher toxicity (50–100% mortalities) to all three animals than the strains MPJZB-C3 and MPJZB-D6 (0–100% mortality) did within 24 h exposure, while, however, the difference in toxicity between the American and Malaysian strains and that between the two Chinese strains were both not significant in terms of the mortality of test animals. While the cell-free culture media (by filtration) and sonicated cultures were less toxic to test animals than the live cultures for all *M. polykrikoides* strains, sonicated cultures were even less toxic than the cell-free culture media. Among the three target animals, the marine medaka exhibited most sensitive to the toxicity, followed by rotifer and then brine shrimp. Collectively, while our results demonstrated that all strains, or both ribotypes, of *M. polykrikoides* are toxic, the results may also suggest the differences in toxicity to three test animals among the four cultures of *M. polykrikoides* reflect the inherent difference in toxicity between East Asian and American/Malaysian ribotypes, which stimulated us to propose a further investigation on whether or not the four different ribotypes also differ inherently in their toxicity in the future, via comparing more strains of all four ribotypes and relevant genetic characterizations.

1. Introduction

Harmful algal blooms (HABs) have increased in frequency, duration, and distribution in recent decades around the world (Heisler et al., 2008; Anderson et al., 2012; Glibert et al., 2018; Cortes-Altamirano et al., 2019). The causes of HABs include external causes such as eutrophication in coastal waters and global warming (Heisler et al., 2008; Glibert et al., 2018), and the characteristic traits of individual bloom-forming species, such as high nutrient absorption capacity and rapid proliferation ability, mixotrophy (Glibert and

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