Harmful Algae 63 (2017) 109–118

Contents lists available at ScienceDirect

Harmful Algae

journal homepage: www.elsevier.com/locate/hal

# Control of ichthyotoxic *Cochlodinium polykrikoides* using the mixotrophic dinoflagellate *Alexandrium pohangense*: A potential effective sustainable method

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ARTICLE INFO

*Article history:*
Received 14 September 2016
Received in revised form 3 February 2017
Accepted 3 February 2017
Available online 17 February 2017

*Keywords:*
Biological control
Harmful algal bloom
Olive flounder
Red tide

ABSTRACT

Red tides dominated by *Cochlodinium polykrikoides* often lead to great economic losses and some methods of controlling these red tides have been developed. However, due to possible adverse effects and the short persistence of their control actions, safer and more effective sustainable methods should be developed. The non-toxic dinoflagellate *Alexandrium pohangense* is known to grow well mixotrophically feeding on *C. polykrikoides*, and populations are also maintained by photosynthesis. Thus, compared with other methods, the use of mass-cultured *A. pohangense* is safer and the effects can be maintained in the long term. To develop an effective method, the concentrations of *A. pohangense* cells and culture filtrate resulting in the death of *C. polykrikoides* cells were determined by adding the cells or filtrates to cultured and natural populations of *C. polykrikoides*. Cultures containing 800 *A. pohangense* cells $ml^{-1}$ eliminated almost all cultured *C. polykrikoides* cells at a concentration of 1000 cells $ml^{-1}$ within 24 h. Furthermore, the addition of *A. pohangense* cultures at a concentration of 800 cells $ml^{-1}$ to *C. polykrikoides* populations from a red-tide patch resulted in the death of most *C. polykrikoides* cells (99.8%) within 24 h. This addition of *A. pohangense* cells also lowered the abundances of total phototrophic dinoflagellates excluding *C. polykrikoides*, but did not lower the abundance of total diatoms. Filtrate from 800 cells $ml^{-1}$ *A. pohangense* cultures reduced the population of cultured *C. polykrikoides* by 80% within 48 h. This suggests that *A. pohangense* cells eliminate *C. polykrikoides* by feeding and releasing extracellular compounds. Over time, *A. pohangense* concentrations gradually increased when incubated with *C. polykrikoides*. Thus, an increase in the concentration of *A. pohangense* by feeding may lead to *A. pohangense* cells eliminating more *C. polykrikoides* cells in larger volumes. Based on the results of this study, a 1 $m^3$ stock culture of *A. pohangense* at 4000 cells $ml^{-1}$ is calculated to remove all *C. polykrikoides* cells in ca. 200 $m^3$ within 6 days. Furthermore, maintenance of *A. pohangense* populations through photosynthesis prepared *A. pohangense* to eliminate *C. polykrikoides* cells in future red-tide patches. Moreover, incubation of *A. pohangense* at 2000 cells $ml^{-1}$ with juvenile olive flounder *Paralichthys olivaceus* for 3 days did not result in the death of fish. Therefore, the method developed in this study is a safe and effective way of controlling *C. polykrikoides* populations and can be easily applied to aqua-tanks on land.



## 1. Introduction

The mixotrophic dinoflagellate *Cochlodinium polykrikoides* often forms red-tide patches in the waters of many countries and has caused great losses in aquaculture industries in Korea, Japan, Canada, and other countries (Onoue et al., 1985; Qi et al., 1993; Kim et al., 1999, 2000; Zingone and Enevoldsen, 2000; Whyte et al., 2001; Gobler et al., 2008; Imai and Kimura, 2008; Kudela et al., 2008; Mulholland et al., 2009; Park et al., 2013; Jeong et al., 2015). *C. polykrikoides* red tides have often resulted in the large-scale mortality of fish and/or shellfish in both coastal aqua-cages and aqua-tanks on land (Whyte et al., 2001; Fukuyo et al., 2002; Park et al., 2013; Lee et al., 2014). *C. polykrikoides* is known to kill finfish and shellfish in these restricted facilities by producing reactive

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http://dx.doi.org/10.1016/j.hal.2017.02.001
1568-9883/

oxygen species after clogging their gills (Kim et al., 1999, 2000; Tang and Gobler, 2009a,b; Dorantes-Aranda et al., 2010; Rountos et al., 2014; Griffith and Gobler, 2016). Furthermore, the accumulation of *Cochlodinium* cells on the bottom of land aqua-tanks depletes dissolved oxygen (Ryu et al., 1998; He, 2015). Thus, to prevent the death of finfish and/or shellfish in land-based aquaculture farms, it is important to remove *C. polykrikoides* cells in the influent seawater of land-based flow-through tanks.

Global land-based aquaculture has been extended for several decades (Bostock et al., 2010). Land-based aquaculture farms use seawater, which is pumped into the aqua-tanks (Bostock et al., 2010). When seawater is taken from nearby water columns, diverse microorganisms including *C. polykrikoides* enter the tanks. To minimize loss due to *Cochlodinium* red-tide patches in aqua-tanks, several protocols have been suggested to prevent the death of finfish and/or shellfish (NFRDI, 2013; Lee et al., 2014); (1) pumping deep water in which *Cochlodinium* concentrations are usually low and then filtering the water before use; (2) refraining from pumping and supplying water to aqua-tanks and instead supplying liquefied and solidified oxygen into the tanks to increase the concentration of dissolved oxygen; and (3) withholding feed from fish to minimize their oxygen consumption. Despite such efforts, economic losses due to *C. polykrikoides* in land-based aquaculture have been steadily incurred. Thus, to directly remove *C. polykrikoides* from influent seawaters, several methods such as chemical treatment, UV sterilization, and screen filtrations have been developed (Kang et al., 1998; Ryu et al., 1998; Kim, 2006). However, these physico-chemical methods may have adverse effects and thus biological methods may be safer in land-based aquaculture farms. Mass cultured protist grazers have been suggested as an effective biological method of controlling red tides because some protist grazers sometimes have considerable grazing impact on populations of red tide species (Stoecker et al., 2002; Jeong et al., 2003, 2008; Tillmann, 2004; Kamiyama et al., 2005; Kim, 2006). For example, the large naked ciliates *Strombidinopsis* spp. are known to effectively eliminate *Cochlodinium* cells and are able to divide twice per day after feeding on them (Jeong et al., 2008). Thus, this has been suggested as an effective method to control *Cochlodinium* populations in restricted waters. However, maintaining seed populations of these ciliates during non-*Cochlodinium* red-tide periods is not easy. Furthermore, the ciliates cannot easily be reused to eliminate *Cochlodinium* cells when subsequent red-tide patches occur 1–2 weeks of the ciliates eliminating all *Cochlodinium* cells in the first red-tide patches, because ciliates starve to death within a few days without prey. Thus, a method in which grazers can be maintained or remain available during non-red tide events is needed. Mixotrophic protists, which are able to feed on *Cochlodinium* and maintain their populations photosynthetically, may be ideal grazers.

The recently described mixotrophic dinoflagellate *Alexandrium pohangense* is known to grow well together with *C. polykrikoides* and can lyse *C. polykrikoides* cells (Lim et al., 2015a,b). Moreover, this species does not carry saxitoxin (STX)-related genes and it proved not to be toxic towards *Artemia* (Lim et al., 2015b; Kim et al., 2016). Thus, *A. pohangense* may be an ideal grazer for the removal of *C. polykrikoides* cells in aqua-tanks.

To develop an effective method of controlling *C. polykrikoides* populations in aqua-tanks using mass-cultured *A. pohangense*, the concentrations of *A. pohangense* and culture filtrates able to kill all *C. polykrikoides* cells were determined. *A. pohangense* cells or filtrates were added to *C. polykrikoides* cultures and field seawater samples from the waters off Tongyoung, Korea in 2015. Simultaneously, the abundances of protists in the collected waters with and without cultured *A. pohangense* cells or culture filtrates were determined. Furthermore, to investigate possible adverse effects, survival of the juvenile olive flounder *Paralichthys olivaceus* was determined after the juveniles were incubated with dense *A. pohangense* cells for 3 days. Finally, based on the results of the incubation experiments, the culture volume of dense *A. pohangense* cells over time following the addition of *C. polykrikoides* cells, and the volume of *C. polykrikoides* waters treatable with these cultures were calculated. This study provides a basis for the development of an effective biological method of controlling *C. polykrikoides* in land-based aqua-tanks.

## 2. Materials and methods

### 2.1. Preparation of experimental organisms

*Alexandrium pohangense* (GenBank Accession no. = LN811348) was originally isolated from plankton samples collected off the coast of Pohang, a city in southeastern South Korea, in September 2014. At that time, the water temperature and salinity were 23.3 °C and 31.1, respectively (Lim et al., 2015b). *A. pohangense* was grown mixotrophically with *Cochlodinium polykrikoides* (prey concentrations = 500–1000 cells $ml^{-1}$) at 20 °C under a 14:10 h light-dark (LD) cycle at 100 $\mu E\, m^{-2}\, s^{-1}$ provided by cool-white fluorescent lights (Lim et al., 2015a).

**Table 1**
Design of experiments investigating the effects of *Alexandrium pohangense* cells (culture containing cells) and cell-free culture filtrates on cultured (A) and natural (B) populations of *Cochlodinium polykrikoides*, and juveniles of the flounder *Paralichthys olivaceus* (C). The numbers in the potential predator and prey columns represent the initial densities.

A. Effects of *A. pohangense* cells and culture filtrates on cultured populations of *C. polykrikoides*

| Expt. | Species or filtrate | Density (cells $ml^{-1}$) | Species | Density |
|---|---|---|---|---|
| 1 | *Alexandrium pohangense* | 0, 100, 200, 400, 800 | *Cochlodinium polykrikoides* | 1000 |
| 2 | Filtrate of *A. pohangense* | Filtrates from a culture with a cell concentration of 0, 100, 200, 400, 800 | *Cochlodinium polykrikoides* | 1000 |

B. Effects of *A. pohangense* cells and culture filtrates on the natural populations of *Cochlodinium* (see text)

| Expt. | Species or filtrate | Density | Species | Density (cells $ml^{-1}$) |
|---|---|---|---|---|
| 3 | *Alexandrium pohangense* | 800 | Natural population of *Cohlodinium* | 1500 |
| 4 | Filtrate of *A. pohangense* | Filtrates from a culture with a cell concentration of 800 | Natural population of *Cohlodinium* | 1500 |

C. Effects of *A. pohangense* cells and culture filtrates on juveniles of *Paralichthys olivaceus*

| Expt. | Species or conditions | Density | No. of fish used | Length of fish (means ± SD) (cm) |
|---|---|---|---|---|
| 5 | *Alexandrium pohangense* | 2000 | 15 | 7.5 ± 1.7 |
| 5 | Filtrate of *A. pohangense* | 2000 | 15 | 8.1 ± 1.8 |
| 5 | Control | 0 | 15 | 9.0 ± 1.6 |