

OCCURRENCE OF *GYMNODINIUM CATENATUM* IN THE GULF OF THAILAND

Thaithaworn Lirdwitayaprasit^{1,*}, Darin Panuksubkasul¹, Yoshinobu Takata²,
Shigeru Sato², Masaaki Kodama² and Yasuwo Fukuyo³

¹Dept. of Marine Science, Chulalongkorn University, Phyathai Road, Bangkok 10330, Thailand

²School of Fisheries Sciences, Kitasato University, Sanriku, Ofunato,
Iwate, 022-0101 Japan

³Asian Natural Environmental Science Centre, the University of Tokyo,
Bunkyo-ku, Tokyo, 113-8657, Japan

*e-mail: lthaiha@chula.ac.th

ABSTRACT

A clonal culture of *Gymnodinium catenatum* was established from a plankton sample collected from Leamthan, Chonburi Province, in the Upper Gulf of Thailand. The culture was maintained at $28 \pm 1^\circ\text{C}$ under light intensity of $70 \mu\text{mol photons m}^{-2}\text{s}^{-1}$ with 12:12 L:D cycle. Cells were harvested at late exponential phase and extracted with 0.03 M acetic acid. The HPLC analysis on thus obtained extract revealed that toxins consist of N-sulfocarbamoyl toxins, C1 and C2, as major components. GTX1, GTX2, GTX3, GTX4 and dcSTX were also detected as minor components. The toxin profile of this clone is different from clones of *G. catenatum* found in Singapore which was dominated by the highly potent carbamate toxins, primarily GTX 1 and 4 with less amounts of GTX2, GTX3, neosaxitoxin, and saxitoxin. No N-sulfocarbamoyl, decarbaomyl, or deoxy-decarbamoyl toxins were detected.

Keywords: *Gymnodinium catenatum*, toxin profile, Gulf of Thailand

INTRODUCTION

Gymnodinium catenatum is the only unarmoured dinoflagellate known to produce PSP toxins. This chain-forming dinoflagellate was first described from the Gulf of California (Graham, 1943) and the outbreak of PSP caused by this species was first recorded in Spain (Fraga and Sanchez, 1985). Since then, the global distribution and PSP out breaks of *G. catenatum* have been well documented by a number of authors (Yuki and Yoshimatsu, 1987; Ikeda *et al.*, 1989; Hallegraeff *et al.*, 1989; Anderson *et al.*, 1989; La Barbera Sanchez, 1991; Holmes *et al.*, 2002; Gomez, 2003, Park *et al.*, 2004, Band-Schmidt, 2005). In Southeast Asian waters, the occurrences and the toxin profiles of this species were investigated in Philippines (Fukuyo *et al.*, 1993) and Singapore waters (Holmes *et al.*, 2002). As pointed out by Oshima and Hallegraeff (1993), the toxins profiles of *G. catenatum* could be used as a stable biochemical marker for this species while

Holmes *et al.*, (2002) proposed that the unique toxin profile of Singapore strains, in which carbamate toxins dominated, could be a useful signature to identify the potential spread of this strain from Singapore. Although Matsuoka and Fukuyo (1994) reported the motile stage of this species in the phytoplankton samples collected from the Gulf of Thailand but the toxin profiles of Thai strain has not been characterized yet. This preliminary study aimed to provide the toxin information of *G. catenatum* found in this region.

MATERIALS AND METHODS

Isolation and culture of *Gymnodinium catenatum*

Leamthan is a small sea side place located at the coastal area of Chonburi Province, the Upper Gulf of Thailand. This is the public area where the local people or tourists come for picnics, fishing and so on. Net phytoplankton samples were collected by a 20 μm phytoplankton net from the

platform of Leamthan in June, 2005. These samples were the materials for the students of marine production course to use for marine micro-organism observation. Several cells of *G. catenatum* were found in those concentrate samples. Capillary technique was used for isolated cells and inoculated into 24 wells of the multiwell plate containing 1 ml. of enriched seawater medium T.1 (Ogata, *et al.*, 1987). The clonal culture was maintained in the T.1 medium at 28 ± 1 °C. $70 \mu\text{mol photons m}^{-2}\text{s}^{-1}$ provided by white fluorescence lamps on 12:12 light and dark cycle. The taxonomic identification of Thai *G. catenatum* ascertained by comparing it to both of the original (Graham, 1943) and the recent detailed descriptions (Blackburn *et al.*, 1989).

Toxin profiles of *G. catenatum*

Cells of *G. catenatum* at the exponential growth phase were enumerated. The sampling cells of 5 ml. were taken from the culture flask and harvested by centrifugation ($1,000 \times g$, 10 min). The supernatant part was removed by pipetting carefully and then the pellet, containing 12,500 cells, was extracted by sonification with 0.03 N acetic acid. The toxin profiles of cell extracts were analyzed by post-column derivatization HPLC methods for PSP toxins (Oshima *et al.*, 1993). 100 μl of the extracted toxin was prepared from the sample and 10 μl was injected in to that mentioned HPLC system.

RESULTS

Isolation and culture of *G. catenatum*

Noctiluca red tide took place during collecting samples but not a severe case. The phytoplankton community in the net sample was composed mostly of *Noctiluca scintillans*, *Ceratium furca* and *Chaetoceros* spp. A few chains of four cells of *G. catenatum* were found in those samples and then were removed to the sterile filtered-seawater for isolation. A clonal culture of *G. catenatum* was established by isolating one of the chains and inoculating it into T.1 medium. The culture for toxin analysis was grown in 500 ml round bottom flask.

The single cells were observed in the old culture and the newly inoculated culture. They often form long chains of 2-32 cells at the exponential growth phase.

Toxin profiles

The results showed that toxins consisted of N-sulfocarbamoyl toxins, C1 and C2 as major components. GTX 1, GTX2, GTX3, GTX4 and dcSTX were also detected as minor components. The other toxins have not been detected.

DISCUSSION

The finding of high N-sulfocarbamoyl component, C1 and C2 in Thai *G. catenatum* was consistent with other studies which showed that N-sulfocarbamoyl toxins (C1-C4) were the major toxin component in this species (Anderson *et al.*, 1988; Fukuyo *et al.*, 1993; Oshima *et al.*, 1993; Park *et al.*, 2004). However, the recent studies in this species showed that some strains have a unique toxin profiles that differed from the above mentioned works. Holmes *et al.* (2002) showed that Singapore *G. catenatum* was dominated by the highly potent carbamate toxins, primarily GTX1 and 4 with less amounts of GTX2, GTX3, neosaxitoxin, and saxitoxin. No N-sulfocarbamoyl, decarbaomyl, or deoxy-decarbomyl toxins were detected. Band-Schmidt *et al.* (2006) showed that California strains were characterized by a high proportion of neosaxitoxin. These results implied that this species has evolved particular physiological responses to their environment that are reflected in the toxin profile, suggesting different populations. Although, Thai *G. catenatum* has high C1 and C2 that similar to Japan strains (Oshima *et al.*, 1993) and Philippines strain (Fukuyo *et al.*, 1993) but the minor component is quite different. This finding confirmed the toxin profile of Thai *G. catenatum* and can be used to distinguish from other populations.

CONCLUSION

A few cells of the PSP toxin producer dinoflagellate, *G. catenatum*, were found at the coastal area of Chonburi Province, the Upper Gulf of Thailand. The toxin profile was examined of which C1 and C2 were the major toxin component. The different in minor toxin components of each strain was probably due to the geographical habitat and might be used to distinguish from each other populations.

Acknowledgements. This work was partially supported by Graduate School of Chulalongkorn University and Multilateral Cooperative Research Program on Coastal Oceanography of JSPS.

REFERENCES

- Anderson, D.M., J.J. Sullivan and B. Reguera, 1989. Paralytic shellfish poisoning in Northwest Spain: The toxicity of the dinoflagellate *Gymnodinium catenatum*. *Toxicon*, 27(6): 663–674.
- Band-Schmidt, C.J., J. Bustillos-Guzmán, I. Gárate-Lizárraga, C.H. Lechuga-Devéze, K. Reinhardt and B. Luckas, 2005. Paralytic shellfish toxin profile in strains of the dinoflagellate *Gymnodinium catenatum* Graham and the scallop *Argopecten ventricosus* G.B. Sowerby II from Bah'ya Concepción, Gulf of California, Mexico *Harmful Algae*, 4: 21–31.
- Blackburn, S.I., G.M. Hallegraeff, and C.J. Bolch. 1989. Vegetative reproduction and sexual life cycle of the toxic dinoflagellate *Gymnodinium catenatum* from Tasmania, *Australia Journal of Phycology*, 25: 577–590.
- Fraga, S. and E.J. Sanchez. 1985. Toxic and potentially toxic dinoflagellates found in Galician rias (NW Spain). In: D. M. Anderson, A.W. White, and D. Baden. (eds.) *Toxic dinoflagellates*. Elsevier North Holland, New York: 51–54.
- Fukuyo, Y., M. Kodama, T. Ogata, T. Ishimaru, K. Matsuoka, T. Okaichi, A.M. Maala and J.A. Ordonez, 1993. Occurrence of *Gymnodinium catenatum* in Manila Bay, The Philippines. In: T.J. Smayda, and Y. Shimizu (eds): *Toxic Phytoplankton Blooms in the Sea*. Elsevier Science Publishers B.V.: 875–880.
- Gómez, F. 2003. The toxic dinoflagellate *Gymnodinium catenatum*: an invader in the Mediterranean Sea. *Acta Bot. Croat.*, 62 (2): 65–72.
- Graham, H.W. 1943. *Gymnodinium catenatum*, a new dinoflagellate from the Gulf of California. *Trans. Amer. Micros. Soc.*, 62 (3): 259–261.
- Hallegraeff, G.M., S.O. Stanley, J.C. Bolch and S.I. Blackburn, 1989. *Gymnodinium catenatum* blooms and shellfish toxicity in Southern Tasmania, Australia. In: T. Okaichi, D.M. Anderson, and T. Nemoto (eds.) *Red tides: biology, environmental science and toxicology*. Elsevier, New York: 77–80.
- Holmes, M.J., C.J.S. Bolch, D.H. Green, A.D. Cembella, and S.L. Ming Teo. 2002. Singapore isolates of the dinoflagellate *Gymnodinium catenatum* (Dinophyceae) produce a unique profile of paralytic shellfish poisoning toxins. *J. Phycol.*, 38: 96–106.
- Ikeda, T., S. Matsuno, S. Sato, T. Ogata, M. Kodama, Y. Fukuyo, and M. Takayama. 1989. First report on paralytic shellfish poisoning caused by *Gymnodinium catenatum* Graham in Japan. In: T. Okaichi, D.M. Anderson, and T. Nemoto, (eds.) *Red tides: biology, environmental science and toxicology*. Elsevier, New York: 411–414.
- La Barbera Sanchez, A. 1991. Mollusc toxicity caused by a red tide of *Gymnodinium catenatum*. *Red Tide Newsl*, 4 (2/3): 1–2.
- Matsuoka, K. and Y. Fukuyo. 1994. Geographical distribution of the toxic dinoflagellate *Gymnodinium catenatum* Graham in Japanese Coastal Waters. *Botanica Marina*, 37: 495–503.
- Ogata, T., T. Ishimaru and M. Kodama 1987. Effect of water temperature and light intensity on growth rate and toxicity change in *Protogonyaulax tamarensis*. *Mar. Biol.*, 95: 217–220.
- Oshima, Y., M. Hasegawa, T. Yasumoto, G.M. Hallegraeff and S. Blackburn. 1987. Dinoflagellate *Gymnodinium catenatum* as the source of paralytic shellfish toxins in Tasmanian shellfish. *Toxicon*, 25: 1105–1111.
- Oshima, Y., S.I. Blackburn and G.M. Hallegraeff. 1993. Comparative study on paralytic shellfish toxin profiles of the dinoflagellate *Gymnodinium catenatum* from three different countries. *Mar. Biol.*, 116: 471–476.
- Park, Tae-Gyu, Chang-Hoon Kim and Y. Oshima, 2004. Paralytic shellfish toxin profiles of different geographic populations of *Gymnodinium catenatum* (Dinophyceae) in Korean coastal waters. *Phycological Research.*, 52: 300–305.
- Yuki, K. and S. Yoshimatsu, 1987. Morphology of the athecate dinoflagellate *Gymnodinium eatenatum* in culture. *Bull. Plankton Soc. Japan*, 34: 109–117.