Harmful Algae 9 (2010) 180–185

Contents lists available at ScienceDirect

Harmful Algae

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

Temperature tolerance and expression of heat shock protein 70 in the toxic dinoflagellate *Alexandrium tamarense* (Dinophyceae)

Atsushi Kobiyama [a,*], Shohei Tanaka [a], Yutaka Kaneko [a], Po-Teen Lim [b], Takehiko Ogata [a]

[a] School of Marine Biosciences, Kitasato University, Sanriku, Ofunato, Iwate 022-0101, Japan
[b] Faculty of Resource Science and Technology, Universiti Malaysia Sarawak, 94300 Kota Samarahan, Sarawak, Malaysia

ARTICLE INFO

Article history:
Received 8 May 2009
Received in revised form 9 September 2009
Accepted 9 September 2009

Keywords:
Alexandrium tamarense
Dinoflagellate
Heat stress
Hsp70
Temperature tolerance

ABSTRACT

The aim of this study was to investigate the relationship between temperature tolerance and geographical origin in a species. Temperature tolerance and heat shock protein 70 (Hsp70) content were investigated in the toxic dinoflagellate *Alexandrium tamarense*, which was isolated from temperate and tropical areas. Results of treatment at 0–37 °C for 1 h revealed that 15 °C-cultured Japanese *A. tamarense* could survive treatment at 0–25 °C, whereas 30 and 37 °C treatment reduced the survival rate. Malaysian *A. tamarense* cultured at 25 °C survived at 30 °C; however, 37 °C and low temperature treatment reduced the survival rate. After acclimation of both strains at 20 °C, they were treated at 0, 4, 30, and 37 °C. The survival rate of Japanese *A. tamarense* at 30 °C was slightly increased compared to that of 15 °C-cultured cells. Treatment at 37 °C for 1 h showed no difference between acclimated and unacclimated cells of both strains. At 0 and 4 °C treatment, almost all cells of Japanese *A. tamarense* survived; however, the Malaysian cells were unable to survive. Both strains of *A. tamarense*, acclimated at 20 °C, were treated at 30 °C; change in the amount of Hsp70 was analyzed. Western blot analysis revealed that the induction of Hsp70 in the Japanese strain occurred more quickly than in the Malaysian strain. These results indicate that Hsp70 of *A. tamarense* is a heat stress-inducible protein and the response is different between strains.

1. Introduction

The dinoflagellate *Alexandrium tamarense* (Lebour) Balech is a marine phytoplankton that causes paralytic shellfish poisoning (PSP). PSP toxification of bivalves is a serious problem for fisheries and in food hygiene. Since *A. tamarense* is a harmful species and its toxicity and growth characteristics can be important information to work out a scheme for PSP, studies have been conducted to examine the relationship between environmental conditions and toxin production. Hamasaki et al. (2001) reported variability in toxicity of *A. tamarense* based on environmental conditions. They showed that cell toxicity in *A. tamarense* isolated from Hiroshima Bay increased in low salinity environments with high ammonium concentrations and low light intensity. Temperature is considered to be an important factor for occurrence of blooms and toxin production. Ogata et al. (1987) reported that the growth rate of *A. tamarense* declined and the amount of cellular toxin rose in response to a drop in water temperature. This finding is consistent with previous observations that the toxicity of natural cells of the temperate strain of *A. tamarense* at lower temperatures is greater than those in higher temperature waters (Ogata et al., 1982). In Ofunato Bay (North eastern Japan), *A. tamarense* appears within the temperature range of 5–14 °C (Fukuyo, 1982). On the other hand, in tropical areas, seawater temperature is consistently above 20 °C, and Malaysian strains have been maintained at 25 °C (Lim and Ogata, 2005). These Malaysian strains were morphologically identified as *A. tamarense*, although the nucleotide sequence of the nuclear ribosomal RNA gene was similar to that of *A. affine* (Leaw et al., 2005). *A. tamarense* has been found in various areas of the world, such as South America, South Africa, Australia, the Pacific Islands, India, Asia, and the Mediterranean (Lilly et al., 2007), thus indicating that *A. tamarense* can survive in a wide range of water temperatures. Although some characteristics of the relationship between temperature and growth in *A. tamarense* have been identified (Watras et al., 1982), the mechanism underlying the response to change in water temperature has not been examined.

Heat shock protein 70 (Hsp70) is an important molecule associated with heat stress response. It plays an important role in molecular chaperone and protein turnover during translation and maturation of proteins. Hsp70 prevents aggregation of denatured proteins and refolding of proteins denatured by heat stress (Sonna et al., 2002). It has a highly conserved sequence across all organismal kingdoms, and its expression is induced by heat stress

* Corresponding author. Tel.: +81 192 44 1928; fax: +81 192 44 2125.
E-mail address: kobiyama@kitasato-u.ac.jp (A. Kobiyama).

1568-9883/$ – see front matter © 2009 Elsevier B.V. All rights reserved.
doi:10.1016/j.hal.2009.09.002

at transcriptional level. The pattern of Hsp70 accumulation after heat stress has been studied in fish, mammalian cells, plants, and many other organisms (Wu et al., 1994; Theodorakis et al., 1999; Ojima et al., 2005). The *Hsp70* gene has also been identified in the microalgae *Chlorococcum littorale* (Beuf et al., 1999) and *Volvox carteri* (Cheng et al., 2006). Accumulation of *Hsp70* mRNA after heat treatment has been confirmed in these 2 microalgae. In dinoflagellates, Okamoto and Hastings (2003) isolated *Hsp70* from *Pyrocystis lunula* and demonstrated increase in mRNA levels after treatment with sodium nitrite. On the other hand, Miller-Morey and Van Dolah (2004) reported absence of the protein in *Karenia brevis*, which crossreacted with an anti Hsp70 antibody. Until date, there have been very few studies investigating heat stress-inducible expression using Western blot or RNA blot approaches, and the relationship between Hsp70 and heat stress in dinoflagellates has not been clearly identified.

In this study, among various environmental factors, we focused on water temperature tolerance. We specifically investigated differences in temperature tolerance of *A. tamarense* isolated from temperate and tropical areas and performed Western blot analysis to examine the induction pattern of Hsp70 after temperature treatment.

2. Material and methods

2.1. Cell culture

Two different clonal strains of *A. tamarense* were isolated from the temperate waters of Ofunato Bay in north Japan (OFAT0105-8; Kobiyama et al., 2007) and the tropical waters in Malaysia (AtPA01; Usup et al., 2002). These strains were maintained in 150 mL SWII medium (Iwasaki, 1961) at 15 and 25 °C, respectively, under constant irradiance of 110 μmol photon m^{-2} s^{-1} and 15:9 h light and dark cycle using a cool white fluorescent lamp. Light intensity was measured using the QSL2100 irradiance sensor (Biospherical Instruments, Inc., San Diego, CA). Cultures that reached a density of ca. 5000 cells mL^{-1} were subsequently used in temperature treatments. Furthermore, both strains were cultured at 20 °C for at least 2 months to provide 20 °C-acclimated cells.

2.2. Temperature treatment

For the temperature experiment, *A. tamarense* cells were collected using 10- or 20-μm nylon mesh filters attached to the bottom of microtubes. Cells were transferred to 24-well culture plates containing SWII medium preincubated at 0, 4, 15, 20, 25, 30, and 37 °C. After temperature treatment for 1 h, Japanese and Malaysian *A. tamarense* were incubated at 15 and 25 °C, respectively, for 12 h. A time-course experiment was performed using the same treatment method but with treatment times of 0, 5, 10, 30, 60, and 120 min. Furthermore, 20 °C-acclimated cells of both strains were used in the temperature treatment. After temperature treatment, cells were stained with fluorescein diacetate (FDA) to detect live and dead cells. The number of FDA-stained and unstained cells was counted under a fluorescence microscope and percentage survival was calculated. Survival rates among these treatments were statistically analyzed using analysis of variance and Tukey's test.

2.3. Western blot analysis

For Western blot analysis, the cells acclimated at 20°C were cultured in 300mL SWII medium and heated in water bath at 30°C for 0, 30, 60 and 120 min. The cells were frozen in liquid nitrogen immediately after temperature treatment and stored at −80°C. Frozen samples for protein extraction were suspended in 20 μL sample buffer [0.1 M Tris–HCl (pH 6.8), 4% (w/v) sodium dodecyl sulfate (SDS), 1.5 M 2-mercaptoethanol, and 20% (v/v) glycerol] and heated at 100 °C for 20 min. After centrifugation, the supernatants were used for SDS-polyacrylamide gel electrophoresis (SDS-PAGE) and Western blot analysis. Protein samples were run on 8% (w/v) polyacrylamide gels. Following electrophoresis, proteins were stained using the Silver stain II kit Wako (Wako Pure Chemical Industries Ltd., Tokyo, Japan). In Western blot analysis, proteins on other gels were transferred to Hybond-P membranes (Amersham Pharmacia Biotech, Piscataway, NJ) using a semidry blotter. The membranes were washed with wash buffer [0.1 M Tris–HCl (pH 7.5), 0.1 M NaCl, and 0.1% (v/v) Tween 20] for 5 min, and blocking was performed in wash buffer containing 1% (w/v) blocking reagent (Roche Molecular Biochemicals, Mannheim, Germany). The membranes were then reacted with a rabbit polyclonal anti-rat Hsp70/heat shock cognate protein 70 (Hsc70) antibody (Stressgen, Arbor, MI) for 1 h and washed 2 times in wash buffer. After washing, the membranes were reacted with horseradish peroxidase-conjugated anti-rabbit IgG for 1 h and then washed 2 times in wash buffer. Signals were detected using the enhanced chemiluminescence Plus Western blotting detection system (Amersham Pharmacia Biotech) according to the manufacturer's instructions. The blot was exposed to an X-ray film for 1 h at room temperature.

3. Results

In the temperature experiment, we observed no difference in survival rates in Japanese *A. tamarense* in the temperature range of 0–25 °C (Fig. 1A). However, treatment at 30 and 37 °C for 1 h resulted in cell damage and significant decrease in the survival rates of 15 °C-treated cells to 30 and 2%, respectively (Fig. 1). Although 30% of the cells initially survived treatment at 30 °C, there was no long-term survival of Japanese *A. tamarense* cells

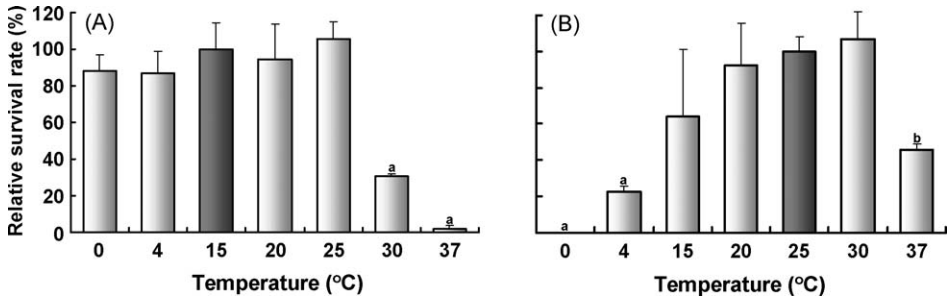

Fig. 1. The survival rates of Japanese and Malaysian *A. tamarense* following temperature treatment. Japanese *A. tamarense* (A) maintained at 15 °C and Malaysian *A. tamarense* (B) maintained at 25 °C were treated at 0, 4, 15, 20, 25, 30 and 37 °C for 1 h. The survival rates were calculated based on the number of FDA stained and unstained cells. The *y*-axis indicates the relative survival rate compared to that in the 15 °C- and 25 °C-treated cells of Japanese and Malaysian *A. tamarense*, respectively (dark grey bar). Data represent the means ± SD (n = 3). The bars with different letters indicate significant difference from the value in the 15 °C—(A) and 25 °C—(B) treated cells (a: $P < 0.01$, b: $P < 0.05$).