TOXIN PRODUCTION IN A MALAYSIAN ISOLATE OF THE TOXIC DINOFLAGELLATE *PYRODINIUM BAHAMENSE* VAR. *COMPRESSUM*

GIRES USUP[1], DAVID KULIS[2], DONALD M. ANDERSON[2]
[1]BU Marine Program, MBL, Woods Hole, MA 02543, U.S.A.
[2]Woods Hole Oceanographic Institution, Woods Hole, MA 02543, U.S.A.

Abstract

Toxin production of a Malaysian isolate of the toxic dinoflagellate *Pyrodinium bahamense* var. *compressum* was investigated at various stages of the batch culture growth cycle and under growth conditions affected by temperature, salinity and light intensity variations. Similar to what have been found in toxic *Alexandrium* and *Gymnodinium* species, toxin content in batch cultures of *Pyrodinium* peaked during mid-exponential phase, increased significantly as temperature decreased, and decreased significantly as light intensity decreased. *Pyrodinium* differed, however, from *Alexandrium* and *Gymnodinium* in that it produced only neosaxitoxin, saxitoxin, gonyautoxins V and VI, and decarbamoylsaxitoxin under all culture conditions investigated. Toxin content in this *Pyrodinium* isolate also increased significantly at its lowest salinity tolerance limit, a pattern never observed thus far in *Alexandrium* species.

Introduction

The thecate, chain-forming dinoflagellate *Pyrodinium bahamense* var. *compressum* has been known for many years as the causative organism of paralytic shellfish poisoning (PSP) in several countries in the tropical Pacific (Hallegraeff and Maclean, 1989; Maclean, 1983). Despite its obvious public health and economic importance, the species has been very little studied. This is mainly due to failure in establishing laboratory cultures of the species (Blackburn and Oshima, 1989). The only documented success in this respect was by Harada et al. (1982a, 1982b, 1983) who maintained cultures of the species from Palau for a few years. These cultures were used to elucidate the toxin composition of the species (Harada et al. 1982a, 1982b, 1983). Subsequently, the toxins present in shellfish and finfish from Southeast Asia contaminated by the dinoflagellate were analyzed (Oshima, 1989).

Recently, we were able to successfully establish laboratory cultures of a Malaysian isolate of *P. bahamense* var. *compressum*. We present here data obtained from studies on toxin production of the isolate and compare them to what has already been known on toxin production in other PSP toxin-producing species.

Materials and Methods

Pyrodinium bahamense var. *compressum* clone PbSA01 was cultured from plankton material collected from the west coast of Sabah, Malaysia in December 1991. The cultures are routinely maintained in modified ES medium of Provasoli (1968) at 29 °C under a 14:10 h L:D cycle (light intensity ~150 $\mu E\ m^{-2}\ s^{-1}$). Vineyard Sound seawater (salinity 30 PSU) was used as the medium base, except where stated otherwise. The same medium was used in all experiments described here. Details

of the medium are to be published elsewhere.

The first experiment examined toxin production over the course of normal batch culture. Cells were grown in 25 ml culture tubes under conditions described above. Growth was monitored by in vivo fluorescence measurements on a Turner Designs Model 10 fluorometer. At regular time intervals from the lag phase to stationary phase, groups of four tubes were harvested for toxin extraction in 0.05 N acetic acid. Temperature effects on toxin production were studied in a temperature gradient bar (Watras et al. 1982). Cells in 25 ml batch cultures were allowed to acclimate to each temperature through three transfers. Groups of four tubes at each temperature were harvested for toxin extraction at late-exponential phase. Salinity effects were studied in modified ES medium in which Sargasso Sea water (salinity 36 PSU) was used as the medium base. Medium salinities were adjusted using Milli-Q water (Millipore Corp., USA). The cells, grown at 29 °C under a 14:10 L:D cycle, were allowed to acclimate to each salinity over three transfers. Groups of four tubes at each salinity were harvested at mid-exponential phase for toxin extraction. Light intensity effects on toxin production were studied at 29 °C under continuous illumination. The amount of light reaching each tube was adjusted by the use of screens. The light intensity in each tube was measured with a Biospherical Instruments (San Diego, California) Model QSL-100P light meter. The cultures were harvested at late-exponential phase for toxin extraction. Cell harvests in all the experiments were made 6-8 hours into the light phase, well after cell division has been completed.

Samples were analyzed by the three step isocratic elution method of Oshima et al. (1989) for the HPLC determination of the saxitoxins. Toxin concentrations in the samples were determined from standards generously provided by Dr Y. Oshima. All the samples were ran twice for toxins. The cell density in each harvested sample was determined through cell counts made in a Sedgewick-Rafter slide at 200X magnification.

Results and Discussion

A typical growth profile of the isolate in nutrient-replete ES medium is shown in Fig. 1. The normal growth rate achieved was 0.3 - 0.4 divisions day^{-1}. Toxin content increased rapidly during early exponential phase and peaked at ca. 400 fmol cell^{-1} at mid-exponential phase (Fig. 2a). Toxin content then decreased just as rapidly as the cultures approached stationary phase. This is similar to what has been found in *Alexandrium* species, in which toxin content typically peaks during early or mid-exponential phase of growth in batch cultures (Proctor et al. 1975; Hall, 1982; Boyer et al. 1987; Anderson et al. 1990b; Kim et al. 1993). The toxins produced by this isolate of *P. bahamense* were NEO, STX, dcSTX, GTX5 and GTX6. No C toxins or GTX1 - GTX4 were detected under any of the culture conditions studied. This was similar to what were found in the Palau isolate (Harada et al. 1982a, 1982b, 1983) and in the contaminated shellfish and finfish samples from Southeast Asia (Oshima, 1989). These results suggest that this species may be genetically incapable of producing C toxins and GTX1 - GTX4. Analyses of additional P. bahamense isolates are clearly need.

Temperature had marked effects on toxin content of the isolate (Fig. 2b). There was a 3-fold increase in toxin content as temperature decreased from 30 °C
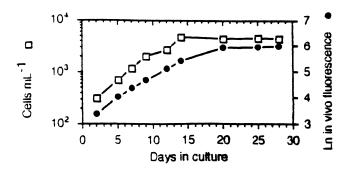
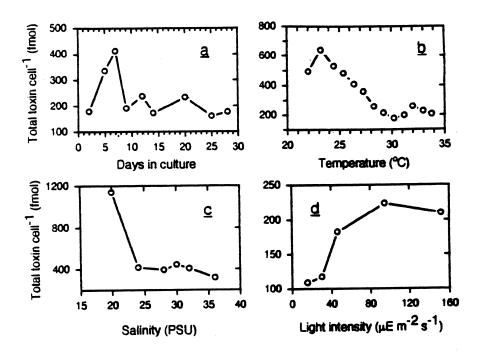Figure 1. Growth profile of *P. bahamense* var. *compressum* in batch culture.

Figure 2. Toxin content of *P. bahamense* var. *compressum*: (a) over the course of normal batch culture; under (b) temperature, (c) salinity, and (d) light intensity variations.

to 22 °C. The marked increase in toxin content at low culture temperatures was similar to the pattern observed in cultures of toxic *Alexandrium* species (Hall, 1982; Ogata et al. 1987; Anderson et al. 1990b). A study on *A. fundyense* by Anderson et al. (1990b) suggested that elevation in toxin content at low temperatures resulted from allocation of more cellular nitrogen to toxin synthesis and less to protein synthesis.

Salinity had less pronounced effects on toxin content. Total toxin content was relatively constant at ca. 400 fmol cell^{-1} over the salinity range of 24 to 36 PSU, but tripled to 1200 fmol cell^{-1} at 20 PSU (Fig. 2b). This was a major difference from what was found in *Alexandrium excavatum*, in which toxin content was found to increase with increasing salinity (White, 1978). A later study by Anderson et al. (1990b), however, showed that salinity did not affect toxin content in *Alexandrium fundyense*. There is a possibility that discrepancies observed in these studies may have resulted from different experimental protocols used.

Light intensity also affected toxin production in the isolate, with toxin content decreasing as light intensity decreased (Fig. 2c). Ogata et al. (1987) found a similar decrease in toxin content in *A. tamarense* with decreasing light intensity. It was suggested by Ogata et al. (1987) that low light intensities may result in reduced
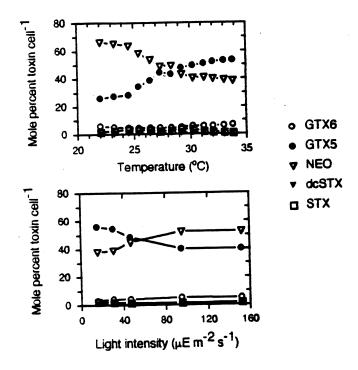

Figure 3. Toxin composition variation in P. bahamense due to temperature and light intensity variations.

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uptake of nitrate by the cells. This in turn may result in less *de novo* synthesis of amino acid(s) which may be precursors in toxin biosynthesis. It is also likely that in low light conditions, a large portion of the N pool is diverted to increased pigment synthesis.

Another aspect of toxin production affected by growth conditions is toxin composition (i.e the relative proportions of the toxins in a cell). For example, we show in Fig. 3 the relative abundance of individual toxins in cells studied in the temperature and light experiments. These data show that temperature and light variations can significantly alter the relative proportions of NEO and GTX5 in this isolate, while the relative abundance of the other three toxins remained constant. In *Alexandrium* species, there is some disagreement regarding toxin composition stability. While some studies have shown the composition in a particular isolate to be stable (Hall, 1982; Boyer et al. 1987; Cembella et al. 1987; Ogata et al. 1987; Kim et al. 1993), others have shown that the composition could change depending on growth conditions (Boczar et al. 1988; Anderson et al. 1990a). Recently, Oshima et al. (1993) also found the toxin composition in *Gymnodinium catenatum* isolates to be stable. This disagreement may be the result of the different growth conditions under which toxin composition was investigated.

This study has indicated that while the actual toxins produced by *Pyrodinium bahamense* var. *compressum* may be invariant, the total amount of these toxins in a cell (toxin content) and their relative abundance (toxin composition) will vary with growth conditions. It must be stressed however that similar studies on many other isolates from other regions where this species occur should be carried out before generalizations on toxin production by this important species could be made.

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