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Research review paper

Biotechnological significance of toxic marine dinoflagellates

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Abstract

Dinoflagellates are microalgae that are associated with the production of many marine toxins. These toxins poison fish, other wildlife and humans. Dinoflagellate-associated human poisonings include paralytic shellfish poisoning, diarrhetic shellfish poisoning, neurotoxic shellfish poisoning, and ciguatera fish poisoning. Dinoflagellate toxins and bioactives are of increasing interest because of their commercial impact, influence on safety of seafood, and potential medical and other applications. This review discusses biotechnological methods of identifying toxic dinoflagellates and detecting their toxins. Potential applications of the toxins are discussed. A lack of sufficient quantities of toxins for investigational purposes remains a significant limitation. Producing quantities of dinoflagellate bioactives requires an ability to mass culture them. Considerations relating to bioreactor culture of generally fragile and slow-growing dinoflagellates are discussed. Production and processing of dinoflagellates to extract bioactives, require attention to biosafety considerations as outlined in this review.
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Keywords: Algal toxins; Ciguatera fish poisoning; Diarrhetic shellfish poisoning; Dinoflagellates; Marine toxins; Neurotoxic shellfish poisoning; Paralytic shellfish poisoning

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Abbreviations: AOAC, Association of Official Analytical Chemists; ASP, Amnesic shellfish poisoning; AZAs, Azaspiracids; AZP, Azaspiracid shellfish poisoning; bFGF, Basic fibroblast growth factor; CFP, Ciguatera fish poisoning; CTXs, Ciguatoxins; DGGE, Denaturing gradient gel electrophoresis; DNA, Deoxyribonucleic acid; DSP, Diarrhetic shellfish poisoning; DTX, Dinophysis toxins; DTX*n*, DTXs (n=1–3); ELISA, Enzyme-linked immunosorbent assay; EU, European Union; FISH, Fluorescent in situ hybridization; GNTX2/3, Gonyautoxin 2/3; HABs, Harmful algal blooms; HPLC, High-performance liquid chromatography; IC_{50}, Concentration required to produce half the maximal inhibition; IOC, Intergovernmental Oceanographic Commission; LC-MS, Liquid chromatography coupled mass spectroscopy; MAbs, Monoclonal antibodies; MBA, Mouse bioassay; MTXs, Maitotoxins; NSP, Neurotoxic shellfish poisoning; PbTx, Brevetoxins; PbTx-*n*, Brevetoxin *n* (n=1–10); PSP, Paralytic shellfish poisoning; PTXs, Pectenotoxins; PTX2, Pectenotoxin-2; RIA, Radioimmunoassay; RNA, Ribonucleic acid; STXs, Saxitoxins; TT, Tetanus toxoid; TTH, *Tachypleus tridentatus* hemocyanin; TTX, Tetrodotoxin; YTXs, Yessotoxins; ZTs, Zooxanthellatoxins.

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1. Introduction

Phytoplanktons are responsible for a large proportion of the photosynthetically produced biomass that supports zooplankton and other higher lifeforms. Filter-feeding organisms such as bivalve shellfish and larvae of commercially important crustaceans rely almost completely on phytoplankton for food. Mass proliferations of phytoplankton are known as algal blooms. Such blooms contain high concentrations of algal biomass. Blooms occur typically in localized regions that are temporarily rich in inorganic nutrients such as nitrogen, phosphorus and iron. Algal blooms are more frequent in summers when the sunlight levels are high. Most algal blooms are harmless and can actually benefit aquaculture and wild fisheries; however, under exceptional circumstances, high-density blooms of normally beneficial algae can cause indiscriminate kill of fish and invertebrates through oxygen deprivation. In addition, nontoxic algal blooms can make finfish more vulnerable to disease (Rensel and Whyte, 2003).

Certain microalgae are toxic. Blooms of toxic algae are known as harmful algal blooms, or HABs. Effects of HABs on aquatic organisms have been reviewed by Landsberg (2002). HABs causing red discoloration of water are commonly known as "red tides". At least 90 species of marine microalgae are known to produce toxins. Of these species, 70 are dinoflagellates (Taxonomic Reference List of Toxic Plankton Algae of Intergovernmental Oceanographic Commission (IOC); http://ioc.unesco.org/hab/data.htm). Dinoflagellates occur widely in aquatic environments. Only about half of the dinoflagellate species are photosynthetic. Worldwide, marine algal toxins cause more than 60,000 poisoning events annually with an associated mortality rate of 1.5% (Gill et al., 2003). This review is focused on biotechnology aspects of dinoflagellates and their toxins. The latter are of potential medical and commercial significance. Bioactive compounds from nondinoflagellate phytoplanktons are reviewed elsewhere (Metting and Pyne, 1986; Schwartz et al., 1990; Borowitzka, 1995, 1999; Codd, 1995; Shimizu, 1996, 2003; Moore, 1996, 2005; Tringali, 1997; Tyagi et al., 1999; Lebeau and Robert, 2003a,b; Singh et al., 2005).

2. Harmful algal blooms (HABs)

HABs are a natural phenomena, but their frequency, geographic range and intensity appear to have increased since the 1970s and their economic impact is greater now than in the past. HABs appear to be stimulated by nutrient discharges in domestic, industrial and agricultural wastes (Lam and Ho, 1989). Such discharges alter ratios of key nutrients in coastal waters to favor development of HABs (Smayda, 1990; Riegman et al., 1992). Nitrogenous runoffs have been clearly linked with the formation of large phytoplankton blooms in the Gulf of California (Beman et al., 2005). The increased economic impact of HABs is probably linked with the increased consumption of seafood and growth in coastal populations (Hallegraef, 2003).

HABs can develop quite suddenly. Dormant cysts of most of the red tide dinoflagellate species survive in ocean sediments for years (Pfiester and Anderson, 1987). Under appropriate environmental conditions, the cysts germinate to produce HABs. Climatic changes that appear to be taking place may be a contributory factor to increased frequency of HABs. In past warmer geological periods, some of the HAB-associated dinoflagellates occurred over larger ranges than they do today, as reflected in the fossil records of the resting cysts. A warmer climate may favor their return to former ranges (Edwards and Richardson, 2004). MacLean (1989) presented strong circumstantial evidence for a coincidence between

blooms of the dinoflagellate *Pyrodinium bahamense* and El Niño–Southern oscillation climatological events. Examples of temporarily altered ranges of dinoflagellates include human shellfish poisonings in New Zealand that were associated with blooms of algal species similar to *Karenia brevis* (Jasperse, 1993) that is considered endemic to the Gulf of Mexico and the east coast of Florida. These blooms coincided with the unusual climatic conditions associated with an El Niño event. Other similar cases have been documented (Dale and Nordberg, 1993). Geographic distribution of certain dinoflagellate cysts has been discussed by Marret and Zonneveld (2003).

Transport of toxic dinoflagellates and their cysts in ship ballast waters appears to contribute to their spread to nonnative regions. Moving contaminated shellfish stocks from one region to another, is a further likely contributor. Bivalves can harbor viable dinoflagellate cells and sometimes can also contain resistant resting cysts (Scarratt et al., 1993; Schwinghamer et al., 1994).

Barely any effort is made currently to control marine HABs. Dinoflagellate-infecting viruses are potentially

Table 1
Some harmful algal blooms caused by marine dinoflagellates and their effects (Hallegraef, 2003; FAO, 2004; IOC Taxonomic Reference List of Toxic Plankton Algae, http://ioc.unesco.org/hab/data.htm)

Effects	Causative dinoflagellates (examples)	Main toxins	Main aquatic species containing toxins
Harmless water discolorations (under exceptional conditions in sheltered bays, blooms can grow dense enough to cause indiscriminate kills of fish and invertebrates through oxygen depletion)	*Akashiwo sanguinea*, *Gonlyaylax polygramma*, *Noctiluca scinilans*, *Scrippsiella trochoidea*		
Produce potent toxins that if ingested by humans, cause gastrointestinal and neurological illnesses, such as[1]:			
Paralytic shellfish poisoning (PSP)	*Alexandrium catenella*, *A. cohorticula*, *A. fundyense*, *A. fraterculus*, *A. leei*, *A. minutum*, *A. tamarense*, *A. andersonii*, *A. ostenfeldii*, *A. tamiyavanichii*, *Gymnodinium catenatum*, *Pyrodinium bahamense* var. *compressum*	Saxitoxins (STXs)	Clams, mussels, oysters, cockles, gastropods, scallops, whelks, lobsters, copepods, crabs, fish
Diarrhetic shellfish poisoning (DSP)	*Dinophysis acuta*, *D. caudate*, *D. fortii*, *D. norvegica*, *D. mitra*, *D. rotundata*, *D. sacculus*, *D. fortii*, *D. miles*, *D. norvegica*, *tripos*, *Prorocentrum lima*, *P. arenarium*, *P. belizeanum*, *P. cassubicum*, *P. concavum*, *P. faustiae*, *P. hoffmannianum*, *P. maculosum*, *Protoceratium reticulatum*, *Coolia* sp., *Protoperidium oceanicum*, *P. pellucidum*, *Phalacroma rotundatum*	Okadaic acid, dinophysis toxins (DTXs), yessotoxins (YTXs) and pectenotoxins (PTXs)	Mussels, scallops, clams, gastropods
Ciguatera fish poisoning (CFP)	*Gambierdiscus toxicus*, *Prorocentrum* spp., *P. lima*, *P. concavum*, *P. hoffmannianum*, *P. mexicanum*, *P. rhathytum*, *Gymnodinium sangieneum*, *Gonyaulax polyedra*, *Ostreopsis* spp., *O. lenticularis*, *O. siamensis*	Ciguatoxins (CTXs), maitotoxins (MTXs), palytoxin, gambierol	Fish, snail, shrimps, crabs
Neurotoxic shellfish poisoning (NSP)	*Karenia brevis*, *K. papilonacea*, *K. sellformis*, *K. bicuneiformis*, *K. Concordia*, *Procentrum borbonicum?*, *Gymnodinium breve*	Brevetoxins (PbTxs)	Oyster, clams, mussels, cockles, whelks
Azaspiracid shellfish poisoning (AZP)	[2]*Protoperidinium crassipes*	Azaspiracids (AZAs)	Mussels, oyster
Non-toxic to humans but harmful to fish and invertebrates (especially in intensive aquaculture systems) by damaging or clogging their gills	*Karenia mikimotoi*, *K. brevisulkata*, *Karlodinium micrum*		

[1]Amnesic shellfish poisoning (ASP) is not included because the causative toxin is produced by a diatom and not by a dinoflagellate.
[2]The ultimate origin of azaspiracids is the dinoflagellate *Protoperidinium crassipes* (James et al., 2003).

useful for controlling dinoflagellate blooms. Such viruses have been reviewed by Nagasaki et al. (2006).

3. Health impact of dinoflagellates

Human diseases associated with exposure to marine dinoflagellate toxins are well known. These diseases include paralytic shellfish poisoning (PSP), diarrhetic shellfish poisoning (DSP), ciguatera fish poisoning (CFP), neurotoxic shellfish poisoning (NSP), and azaspiracid shellfish poisoning (AZP). Clinical characteristics, geographic distribution, treatment and epidemiology of these ailments have been documented (Clark et al., 1999; CDC, 2001; Fleming et al., 2001; Backer et al., 2003). Symptoms occur generally as a consequence of consumption of contaminated seafood and direct human exposure to HABs.

Ciguatera fish poisoning is the most common marine toxin disease worldwide. The primary toxin involved appears to be ciguatoxin. Ciguatera poisoning is usually diagnosed on clinical grounds. Toxin may be detected in serum, plasma, and urine of patients. Ciguatera poisoning can also affect fish populations, as the larval survivability in finfish is affected (Edmunds, 1999).

Table 2
Some recent patents and patent applications relating to toxic dinoflagellates and/or their biotoxins

Title	Reference	Year
Biomarkers for toxic algae	US 7,109,297	2006
Cigua-dart method for detection of ciguatera toxins	US 7,015,045	2006
Water soluble toxin produced by *Pfiesteria* species	US 2006/0111557	2006
Stinging cells expressing an exogenous polynucleotide encoding a therapeutic, diagnostic or a cosmetic agent and methods compositions and devices utilizing such stinging cells or capsules derive therefrom for delivering the therapeutic, diagnostic or cosmetic agent into a tissue	US 2006/0039897	2006
Use of sodium channel blockers and their analogues for the treatment of nicotine dependency	WO 2006/084765	2006
Dinoflagellate karlotoxins, methods of isolation and uses thereof	US 2005/209104	2005
Method for the production of dinoflagellate cultures	US 2005/070008	2005
Novel method for wholly synthesizing of ciguatoxin ctx3c derivative	US 2005/0107622	2005
Polyether brevetoxin derivatives as a treatment for neurotoxic shellfish poisoning and ciguatera fish poisoning	WO 2005/027903	2005
Quantitative method for detecting yessotoxins in fish products based on the activation caused by the toxin in cellular phosphodiesterase and therapeutic usefulness of said activation	WO 2005/012543	2005
Brevetoxin derivative, process for producing the same and method of detecting shellfish neurotoxin using the same	WO 2005/051956	2005
Polyether brevetoxin derivatives as a treatment for neurotoxic shellfish poisoning and ciguatera fish poisoning	WO 2005/027903	2005
Polyether brevetoxin derivatives as a treatment for cystic fibrosis, mucociliary dysfunction, and pulmonary diseases	WO 2005/028482	2005
Sandwich assay kits for detecting shigatoxin ctx3c	US 2005/148041	2005
Novel method for wholly synthesizing of ciguatoxin ctx3c derivative	US 2005/107622	2005
Membrane immunobead assay for the detection of ciguatoxin and related polyether marine toxins	US 6,770,490	2004
The detection and identification of saxiphilins using saxitoxin–biotin conjugates	WO 2004/072640	2004
Analgesic composition and method	US 2004/214842	2004
Anti-ciguatoxin monoclonal antibody	US 2004/059096	2004

Table 1 summarizes the algal species, toxins and seafood that are generally associated with various types of human poisonings. In addition to the commonly found toxins noted in Table 1, many other toxins have been isolated from microalgae but their effects and significance remain unknown (Daranas et al., 2001).

No vaccines exist to protect against poisonings by dinoflagellate toxins, but attempts are being made to produce suitable vaccines. For example, Xu et al. (2005a,b) tested in mice a vaccine against tetrodotoxin (TTX). The vaccine used *Tachypleus tridentatus* hemocyanin (TTH) and tetanus toxoid (TT) as carrier proteins to form the artificial antigen TTX–TTH and TTX–TT. The TTH–TTX vaccine proved better than the TTX–TT in protecting mice against TTX administered orally. This demonstrated for the first time that experimental vaccines can effectively protect animals against repeated ingestion of marine biotoxins. Similar approaches are likely to prove useful for developing vaccines against other marine toxins.

Seafood species can adapt to tolerate high levels of certain algal toxins. For example, softshell clams (*Mya arenaria*) from areas exposed to red tides are more resistant to PSP toxins and accumulate toxins at greater rates than sensitive clams from unexposed areas (Bricelj et al., 2005). Phenomena such as this can increase risk to humans. In addition, toxins undergo various biotransformations in the organisms in which they accumulate (Shimizu and Yoshioka, 1981; Sullivan et al., 1983; Oshima et al., 1990; Oshima, 1995; Sakamoto et al., 2000; Sato et al., 2000; Sekiguchi et al., 2001). These modified toxins may have effects that have been barely studied in humans. In addition to the commonly known toxins, dinoflagellates produce some of the largest and most complex polyketides that have been identified. These compounds have a diverse range of biological activities, including cytotoxic, antitumor, antibiotic, antifungal, immunosuppressant, and neurotoxic activities (Wright and Cembella, 1998). Polyketide synthase genes appear to be responsible for production of most dinoflagellate toxins (Berry et al., 2002; Snyder et al., 2003).

In view of their diverse range of bioactivities and emerging biotechnological significance, dinoflagellate toxins are attracting increasing interest. This is reflected in the numerous recent patents and patent applications relating to these toxins (Table 2). Origins, pharmacology and biosynthesis of some of these bioactives have been discussed by others (Rein and Borrone, 1999; Daranas et al., 2001; Shimizu, 2003; Moore, 2005; Rein and Snyder, 2006). Occurrence of paralytic shellfish toxins in tropical oceans has been reviewed by Llewellyn et al. (2006).

4. Dinoflagellate biotoxins

4.1. Potential applications

Marine toxins are used in medical studies in attempts to understand their modes of action (Dechraoui and Ramsdell, 2003) and assess therapeutic potential of toxins and their analogs. Saxitoxin and tetrodotoxin (TTX), a marine toxin found in the pufferfish (Nishikawa et al., 2003) and related to saxitoxins, are considered safe, effective, long-acting topical anesthetics (Schwartz et al., 1998; Duncan et al., 2001; Kohane et al., 2003). TTX has been found to curb narcotic cravings in laboratory animals and is consequently being used in drug dependence research. Tetrodin™, a drug derived from TTX, is being developed by WEX Pharmaceuticals Inc. (www.wex-pharma.com) for withdrawal treatment of heroin addiction.

Okadaic acid (Fig. 1) is a marine biotoxin that is of value in medical research as it has been linked to multiple health risks and has proved useful in understanding many cellular processes (Fernandez et al., 2002; Bujalance et al., 2003). Tumor promoting and cytotoxic activities of okadaic acid are of potential interest (Fujiki and Suganuma, 1999; Islam et al., 2002). Okadaic acid is considered a model potent neurotoxin

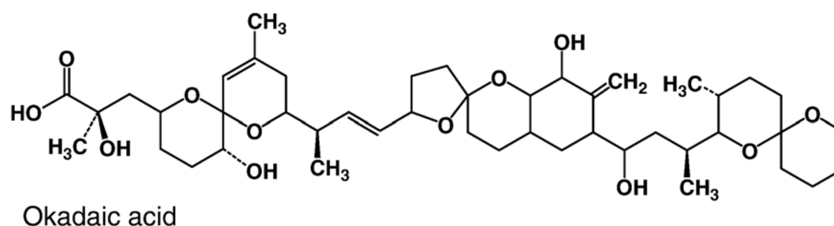

Fig. 1. Okadaic acid. Structure courtesy of Dr. F.S. Fry, United States Food and Drug Administration.

for analyzing the therapeutic effects of atypical antipsychotic drugs in treatment of cognitive impairment and neuropathological changes of schizophrenia and other neurodegenerative diseases (He et al., 2005). Because of its activity as inhibitor of protein phosphatase 2A, okadaic acid is used in studies to elucidate mechanisms by which conjugated linoleic acids might act as anti-tumor agents on breast cancer cells (Liu and Sidell, 2005).

Amphidinolides and colopsinols are two groups of more than twenty structurally unique macrolides that are produced by marine dinoflagellates of the genus *Amphidinium* (Kobayashi et al., 2003). These toxins have potent antitumor properties. Extremely limited availability of these compounds has prevented detailed biological studies. A lack of supply has hampered progress toward elucidation of molecular structures of many of these compounds (Kobayashi and Tsuda, 2004). Amphidinolides have shown strong cytotoxicity towards murine lymphoma L1210 and human epidermoid carcinoma KB cells in vitro. A related compound, caribenolide I, has shown strong cytotoxicity against human colon tumor cell line HCT 116 and its drug-resistant variant HCT 116/VM 46 (Daranas et al., 2001). Caribenolide is active against murine tumor P388 in vivo (Kobayashi and Ishibashi, 1997).

Gonyautoxins are paralytic toxins produced by *Amphidinium* dinoflagellates. Gonyautoxins have been used for anal sphincter infiltrations in clinical practice (Garrido et al., 2005). Goniodomin-A, an antifungal polyether macrolide produced by the dinoflagellate *Goniodoma pseudogoniaulax* (Murakami et al., 1988), has been shown to inhibit angiogenesis by inhibiting endothelial cell migration and basic fibroblast growth factor (bFGF)-induced tube formation (Abe et al., 2002). Goniodomin-A is active in vivo (Abe et al., 2002). Gymnocin-A isolated from red tide dinoflagellate *Gymnodinium mikimotoi* is weakly toxic in fish, but is cytotoxic to P388 mouse leukemia cells (Satake et al., 2002).

Yessotoxins (YTXs) (Fig. 2) are a group of marine toxins that are produced by dinoflagellates of the genera *Protoceratium* and *Gonyaulax* (Takahashi et al., 1996). Multiple analogs of yessotoxins have been identified in *P. reticulatum* (Miles et al., 2005; Souto et al., 2005; Bowden, 2006). YTXs cause selective disruption of the E-cadherin–catenin system in epithelial cells, to potentially compromise the tumor suppressive functions of E-cadherin (Ronzitti et al., 2004). Yessotoxins are further reviewed by Bowden (2006).

Konishi et al. (2004) reported potent cytotoxic metabolites in the supernatant of the cultured dinoflagellate *Protoceratium* cf. *reticulatum*. The four equally active glycoside polyether principles in the extract were named protoceratins I, II, III and IV. These compounds had mean IC_{50} values of less than 0.0005 μM against human cancer cell lines and demonstrated some cell-line selectivity.

Dinophysis species produce pectenotoxins (PTXs) (Fig. 3) (Miles et al., 2006). PTXs are potently cytotoxic against several human cancer cell lines (Zhou et al., 1994; Jung et al., 1995). Pectenotoxin-2 (PTX2), an actin inhibitor, has been suggested as a candidate potent chemotherapeutic agent against p53-deficient tumors (Chae et al., 2005).

Zooxanthellatoxins (ZTs) A, B, and Cs are polyhydroxypolyenes with potent vasoconstrictive activity. These compounds have been isolated from the cultured dinoflagellate *Symbiodinium* sp. (Onodera et al., 2005). The same dinoflagellate genus is known to produce amphoteric iminium metabolites symbioimine and neosymbioimine. Symbioimine is a potential antiresorptive

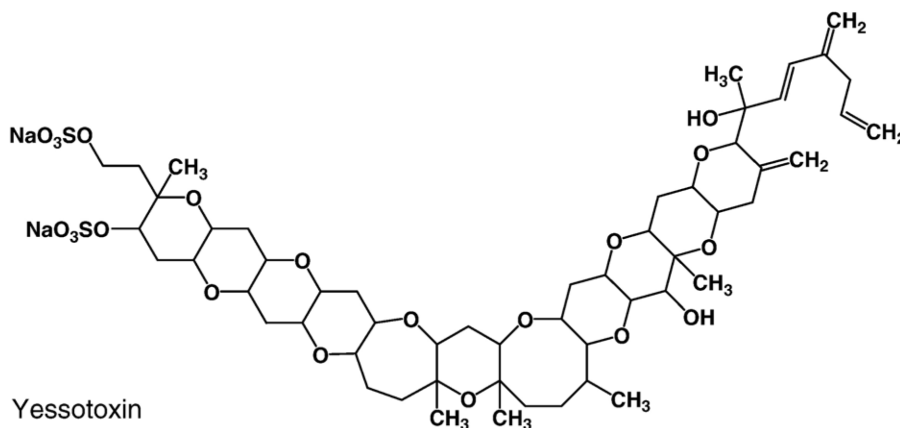

Fig. 2. Yessotoxin. Structure courtesy of Dr. F.S. Fry, United States Food and Drug Administration.

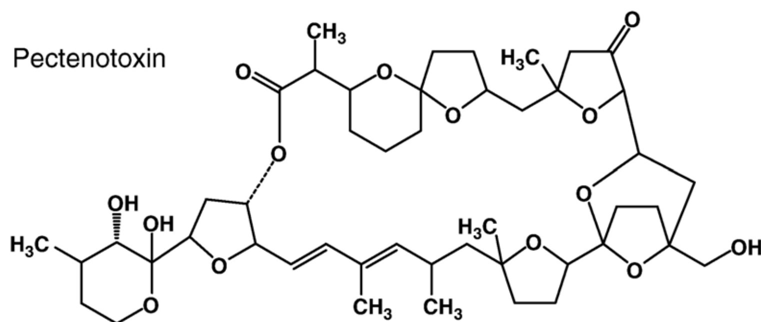

Fig. 3. Pectenotoxin. Structure courtesy of Dr. F.S. Fry, United States Food and Drug Administration.

drug for prevention and treatment of osteoporosis in postmenopausal women (Kita et al., 2005). In addition, symbioimine may be useful in developing new nonsteroid anti-inflammatory drugs for treatment of cyclooxygenase-2-associated diseases (Kita et al., 2005).

A new type of bioactive ceramide, symbioramide, was isolated from the laboratory-cultured dinoflagellate *Symbiodium* sp. by Kobayashi et al. (1988). Symbioramide exhibited antileukemic activity against L-1210 murine leukemia cells in vitro (Kobayashi, 1989).

Potent antifungal agents, gambieric acids A–D, were isolated by Yasumoto and co-workers from a culture of the marine dinoflagellate *Gambierdiscus toxicus* (GIII strain) (Nagai et al., 1992a,b). Gambieric acids display significant activity against filamentous fungi but are inactive against yeasts. Gambieric acids are up to 2000-fold more active against some fungi than is amphotericin B. Gambieric acids are also cytotoxic, but they do not possess the significant neurotoxicity that is associated with other large marine fused-polyether toxins such as brevetoxins, ciguatoxins, yessotoxins, and maitotoxins (Nagai et al., 1993).

Paradoxical thermal dysthesia is an unusual dysfunction of the thermoregulatory system that occurs in humans ingesting certain algal toxins. Work is being done in mice for assessing the thermoregulatory mechanisms affected by marine algal toxins such as maitotoxin and brevetoxin. Work such as this should lead to improved methods for treating victims of ciguatera and other poisonings (Gordon and Ramsdell, 2005). Maitotoxin is a powerful activator of voltage-insensitive Ca^{2+} channels and it stimulates synthesis and secretion of the nerve growth factor (Obara et al., 1999).

4.2. Detection of toxins

Mouse bioassay (AOAC, 1980) remains the accepted regulatory method for detection of and quantification of many marine toxins in suspect samples. The assay involves intraperitoneally injecting white mice with extracts of toxic seafood. Mouse bioassay is expensive and lacks specificity. Consequently other rapid and inexpensive methods are being established to replace the mouse bioassay. These methods are the focus here.

Antibody-based immunoassays are potentially useful for accurate, sensitive and routine determinations of marine toxins (Lewis, 2001). These assays can be inexpensive and portable. Antibody-based assays typically take the form an enzyme-linked immunosorbent assay (ELISA) or radioimmunoassay (RIA). Unfortunately, relatively few antibody-based assays are available because pure toxins in sufficient quantities are rarely accessible (Hirama, 2005) for preparing anti-toxin antibodies and validating the assays. In addition, a poor supply of relevant radiolabeled compounds and cross-reactivity of antibodies with nontarget molecules, pose problems. Other cumbersome and expensive physicochemical procedures such as LC-MS and HPLC have been developed for detection and quantification of many toxins (Doucette et al., 1996; Morton and Tindall, 1996; Fremy et al., 1999; Puech et al., 1999; Pierce and Kirkpatrick, 2001; Quilliam, 2003; Samdal et al., 2005).

In the European Union (EU), physicochemical testing procedures are acceptable for all marine biotoxins as an alternative to testing in animals, so long as they have been validated in compliance with an internationally accepted protocol. Animal testing is used nonetheless, as not all physicochemical protocols have been validated because of a lack of reference materials. In the event of discrepancy between different test methods, the mouse bioassay remains the EU reference standard for okadaic acid, dinophysis toxins, yessotoxins, pectenotoxins and azaspiracids.

Many of the initial symptoms of marine toxin poisoning in humans tend to be nonspecific, hence poisoning is hard to diagnose. Methods are needed for verifying human exposure to toxins. Biological diagnostic methods for use in humans are mostly experimental, or

nonexistent (Backer et al., 2003). Radioimmunoassay (RIA) has been used for determining brevetoxin (Fig. 4) in blood of aquatic animals that have been exposed to *Karenia brevis* (Woofter et al., 2005). Similar technologies can be extended for detecting toxicologically relevant levels of biotoxins in human bodyfluids. Naar et al. (2002) reported an ELISA for detecting brevetoxins in mammalian bodyfluids. A receptor binding assay and HPLC may be used to diagnose paralytic shellfish poisoning caused by saxitoxin (Doucette et al., 1996).

Here we focus on immunodetection techniques that are useful in the field for establishing contamination with toxins involved in paralytic shellfish poisoning (PSP), diarrhetic shellfish poisoning (DSP), neurotoxic shellfish poisoning (NSP), and ciguatera fish poisoning (CFP). The techniques discussed in the following sections have been developed primarily for use with extracts of seafood and are not intended for diagnostic purposes in human patients. Analytical methods for algal toxins have been further reviewed by others (Fremy et al., 1999; Garthwaite, 2000; Van Dolah and Ramsdell, 2001; Pierce and Kirkpatrick, 2001; Mackintosh et al., 2002; Metcalf and Codd, 2003; Quilliam, 2003; Samdal et al., 2005).

4.2.1. Paralytic shellfish poisoning (PSP)

Paralytic shellfish poisoning (PSP) is associated with saxitoxins (STXs). An integrated ELISA screening system for screening extracts for ASP, NSP, PSP and DSP toxins (including yessotoxin) has been described (Garthwaite, 2000; Garthwaite et al., 2001). Such a system is suitable only for identifying suspect shellfish samples for subsequent analysis by methods that have been approved by international regulatory authorities. Kawatsu et al. (2002) developed a direct competitive enzyme immunoassay based on a gonyautoxin 2/3 (GNTX2/3)-specific monoclonal antibody and a saxitoxin–horseradish peroxidase conjugate. GNTX2/3, dc-GNTX2/3, C1/2, GNTX1/4, STX and neoSTX were detectable at concentrations lower than the regulatory limit.

An ELISA test kit for detecting saxitoxin in shellfish is commercially available (RIDASCREEN® Saxitoxin and RIDASCREEN® FAST Saxitoxin; r-Biopharm AG). Possible cross-reactivities with decarbamoyl saxitoxin, gonyautoxins and neosaxitoxin have been reported by the manufacturer.

A commercial rapid test kit (MIST Alert™; Jellett Rapid Testing Ltd., www.jellett.ca) is available for detection of PSP toxins in shellfish. This test is accepted by the United States Food and Drug Administration for use in the US National Shellfish Sanitation Program. In an assessment of MIST Alert™ reported by Mackintosh et al. (2002), the kit detected toxin in all samples that had the European Union tolerance level of 8 μg saxitoxin equivalents per kilogram of shellfish flesh as determined by the mouse bioassay. Importantly, the kit could be readily used and accurately interpreted by individuals with no technical or scientific background (Mackintosh et al., 2002). Clearly, MIST Alert™ is suitable for initial screening for PSP toxins and eliminates the need for routine use of the mouse bioassay. Similar conclusions were reached by Inami et al. (2004) concerning the use of commercial in vitro immunodetection methods.

In view of the cross-reactivities that occur with immunoassays and the potential for a lack of response to all toxins in the PSP group, usefulness of these assays is likely to remain limited unless they can be rigorously validated in accordance with internationally accepted procedures.

The marine toxin domoic acid (Fig. 5) is associated with amnesic shellfish poisoning (ASP), but is not a dinoflagellate toxin. Domoic acid is produced by certain diatoms. An ELISA test (Biosense ELISA test;

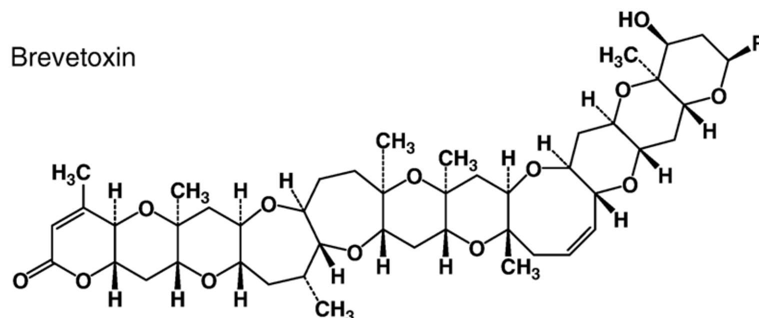

Fig. 4. Brevetoxins (R = $-CH_2C(=CH_2)CHO$ in PbTx-2; R = $-CH_2C(=CH_2)CH_2OH$ in PbTx-3; R = $-CH_2COCH_2Cl$ in PbTx-8). Structure courtesy of Dr. F.S. Fry, United States Food and Drug Administration.

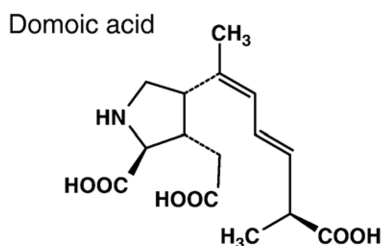

Fig. 5. Domoic acid. Structure courtesy of Dr. F.S. Fry, United States Food and Drug Administration.

www.biosense.com) for domoic acid in shellfish has been approved by the Association of Official Analytical Chemists (AOAC; www.aoac.org), suggesting that reliable ELISA-type assays may be developed for other marine toxins. Unlike most dinoflagellate toxins, domoic acid is a relatively small molecule.

4.2.2. Diarrhetic shellfish poisoning (DSP)

Diarrhetic shellfish poisoning (DSP) is associated with polycyclic ether toxins okadaic acid, dinophysistoxin-1 (DTX1) (Fig. 6), and pectenotoxins (PTX) (Bowden, 2006). Yessotoxin (YTX) (Fig. 2) is classified as a DSP toxin because it was first isolated in 1987 from scallops that were linked to a DSP incident, but pharmacological activity of YTXs is quite different from those of DSP toxins (Bowden, 2006).

The DSP-Check® ELISA test kit (r-Biopharm AG; www.r-biopharm.com) has been used worldwide for screening for okadaic acid and its derivative DTX1 in seafood. A detection limit of 10 µg per kg sample is claimed. The monoclonal antibody used in the kit cross-reacts with DTX1 at a level comparable to okadaic acid but PTXs and YTXs are not reactive (Cembella et al., 2003). DSP-Check® test is known to underestimate total okadaic acid in extracts that contain both okadaic acid and methylokadaic acid (Morton and Tindall, 1996).

A new rapid field test kit to screen for DSP toxins has been developed by Jellett Rapid Testing Ltd. (Laycock et al., 2006). This antibody-based kit detects presence of okadaic acid and some of its analogs in about 30 min. Potentially, immunoaffinity chromatography of mixed toxin samples may be used as a cleanup step prior to immunodetection of specific toxins. Anti-okadaic acid monoclonal antibodies used on immunoaffinity columns have shown cross-reactivity with DTX1 and DTX2 (Puech et al., 1999).

Briggs et al. (2004) produced polyclonal antibodies for developing a cELISA for detecting yessotoxins. Cross-reactivity studies indicated that the antibodies had broad reactivity and that binding to yessotoxin analogs was strongly affected by changes to the A-ring and, to a lesser extent, the K-ring regions of the toxin molecule (Briggs et al., 2004). Samdal et al. (2005) have compared the ELISA methodology for screening shellfish samples for YTXs with data obtained by LC-MS analysis of extracts. The results of ELISA were 3–13 times higher than LC-MS, probably because the antibodies reacted to YTX analogs that were not included in the LC-MS analysis. This discrepancy notwithstanding, there was a good correlation between ELISA and LC-MS data (Samdal et al., 2005). Samples measuring less than 4 mg/kg by ELISA were below the current EU regulatory limit of 1 mg/kg by LC-MS (Samdal et al., 2005). Use of ELISA was recommended as a screening tool, with samples measuring less than 4 mg YTX per kilogram by this method being considered YTX-negative.

4.2.3. Neurotoxic shellfish poisoning (NSP)

Neurotoxic shellfish poisoning (NSP) is caused by brevetoxins (PbTxs). A competitive radioimmunoassay (RIA) for detecting brevetoxins PbTx-2 and PbTx-3 was developed by Trainer and Baden (1991) using bovine serum albumin-linked PbTx-3 as the antigen. The antiserum was produced in goats. The RIA technique for PbTx is based on the competitive displacement of ^{3}H-PbTx-3 from complexation with the antibody. Both PbTx-2 and PbTx-3 were detected with roughly equal responses. However, oxidized PbTx-2, which is not toxic in either the fish or mouse bioassay, also displaced PbTx-3 in RIA, indicating a lack of a high specificity (Trainer and Baden, 1991). Woofter et al. (2005)

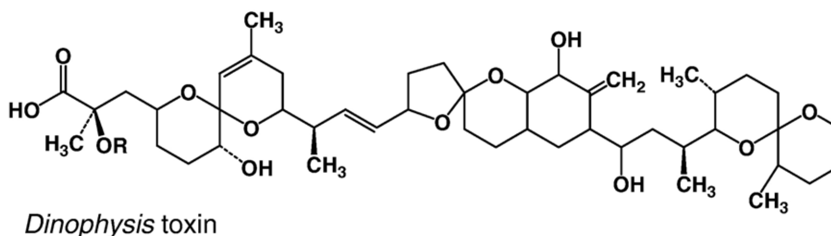

Fig. 6. *Dinophysis* toxin (R=H in DTX1; R=–COOH in DTX3). Structure courtesy of Dr. F.S. Fry, United States Food and Drug Administration.

successfully used a radioimmunoassay (RIA) for detecting various levels of brevetoxin PbTx-3 in blood of aquatic animals that had been exposed to a *Karenia brevis* culture.

Naar et al. (2001) produced and characterized mice polyclonal and monoclonal antibodies (MAbs) specific for PbTx-2 type toxins. PbTx-3–carrier-conjugates prepared at the nanomolar level in a reversed micellar medium were used to generate the antibodies. These apparently were the first MAbs produced against PbTxs. A competitive ELISA for detecting brevetoxins in seawater, mammalian bodyfluids and shellfish extracts has been reported (Naar et al., 2002).

4.2.4. Ciguatera fish poisoning (CFP)

Ciguatoxins (CTXs) (Nicholson and Lewis, 2006) are the primary toxins responsible for ciguatera fish poisoning. CFP is associated with consumption of contaminated reef fish such as barracuda, grouper, and snapper. The disease is often not properly diagnosed and is therefore grossly under reported. Toxins may be detected in bodyfluids of patients by an enzyme-linked immunosorbent assay or high-performance liquid chromatography (HPLC) (Adaeda, 2001). Ciguatera poisoning has been reviewed extensively (Lehane, 2000; Lehane and Lewis, 2000; Lewis, 2001; Hokama and Yoshikawa-Ebesu, 2001).

Hokama and coworkers developed several immunochemical tests for detecting ciguatoxin (CTX) (Fig. 7) and related polyethers (Hokama et al., 1988; Hokama and Yoshikawa-Ebesu, 2001). An anti-ciguatoxin monoclonal antibody was prepared using natural CTX, but cross-reactivity with other toxins was observed.

A rapid commercial Cigua-Check™ immunodiagnostic test (http://cigua.oceanit.com) for detecting ciguatoxin in seafood is available. This test is based on binding of antibodies to the CTX polyether skeleton and okadaic acid. A recent study compared the Cigua-Check™ test kit with the well-established mouse bioassay (Wong et al., 2005). Although the number of samples used was small, substantial discrepancies were found between the validated mouse bioassay and the rapid commercial immunoassay. The rapid test is useful for rapid general screening to select positive samples for further analysis by a more established assay.

4.3. Reference standards

Validation and calibration of toxin assays requires reference standards of toxins. Reference materials also help in establishing identity of unknown samples of toxins. Very few of the marine biotoxins are available commercially (Quilliam, 2003). Small quantities of purified dinoflagellate biotoxins can be purchased from suppliers such as Sigma-Aldrich (www.sigmaaldrich.com), Gentaur Molecular Products (www.gentaur.com). The Lab Depot (www.labdepotinc.com), ScienceLab (www.sciencelab.com), PKC Pharmaceuticals (www.lclabs.com), Tocris (www.tocris.com), Wako Pure Chemical Industries (www.wako-chem.co.jp), and VWR International (www.vwrsp.com). A given product can vary a great deal in price, depending on claimed purity and supplier. Some products are available only from a single source. Prices range from several hundred Euros per mg to more than €30,000 per mg. These biotoxins have been sourced from an extremely small number of species, namely *Prorocentrum concavum*, *Ptychodiscus brevis* (*Gymnodinium breve*), *Gambierdiscus toxicus*, *Palythoa caribaeorum*, and *Protogonyalaux* sp. In many cases, the credibility of the stated quantity and purity are questionable and the available materials are not suitable for use as reference standards in quantitative analysis (Quilliam, 2003).

In principle, reference standards could be produced by chemical synthesis, but de novo synthesis of most dinoflagellate toxins is complicated, when feasible. The existing total syntheses typically require more than 100 steps. Development of more practical synthetic routes remains a challenge (Inoue, 2004). Certified reference standards of some marine biotoxins are available from

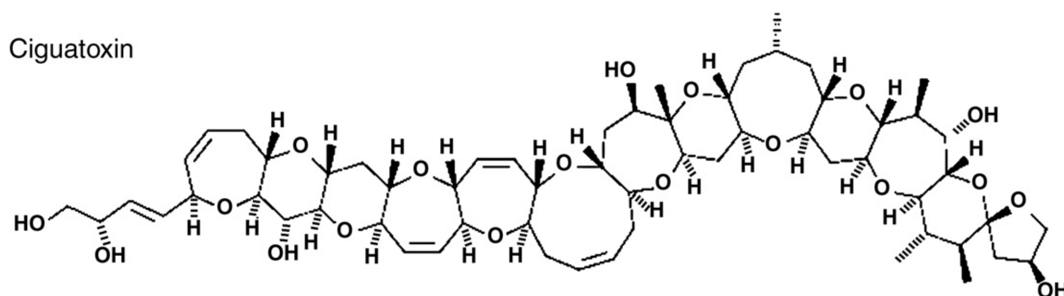

Fig. 7. Ciguatoxin.

the National Research Council of Canada's Certified Reference Materials Program (www.imb.nrc.ca/crmp/). The problem of inexpensively supplying quantities of toxins for various investigational purposes remains intractable.

5. Detection and identification of HAB species

Taxonomic classification of dinoflagellate has been discussed by Taylor (1987) and Adl et al. (2005). Taxonomy of harmful dinoflagellates has been reviewed by Taylor et al. (2003). Evolutionary aspects of dinoflagellates have been reviewed by Hackett et al. (2004) and Saldarriaga et al. (2004). Here the focus is on identification of harmful dinoflagellates using highly specific molecular probes.

Strategies for molecular detection and characterization of HAB species have been reviewed by Litaker and Tester (2002) and Rublee et al. (2005). Molecular methods include conventional PCR, real-time PCR, denaturing gradient gel electrophoresis (DGGE), fluorescent fragment detection PCR, fluorescent in situ hybridization (FISH), lectins, antibodies and ribosomal RNA (rRNA)-targeted DNA probes. Lectins are glycoproteins that bind noncovalently to specific sugar residues on the surface of a cell. Fluorescently labeled lectins with different binding specificities have been used to differentiate among different HAB species, including different serotypes of the same species (Costas et al., 1993; Costas and Rodas, 1994; Cho et al., 2001; Cho, 2003). Although, lectin probes are promising, they do have limitations. For example, lectin-binding profiles may be altered as cells go through different stages of the division cycle or experience different environmental conditions (Costas et al., 1993; Rhodes et al., 1995; Alvarez et al., 1998). Using cultures of *Scrippsiella lachrymose*, Kremp and Anderson (2004) recently confirmed that glycoconjugate composition of dinoflagellate cells can vary with their physiological state.

Antibodies are commonly used for detection and identification of HAB species (Anderson, 1995; Alvarez et al., 1998; Scholin and Anderson, 1998; Peperzak et al., 2000; Cho and Costas, 2004), but only a few antibodies are available commercially. Antibodies bind to specific peptides, glycoproteins, and toxins associated with HAB agents. Polyclonal antibodies are sometimes sufficient for detecting the species of interest, but they may exhibit cross-reactivity as some antigens may be common to different HAB species (Mendoza et al., 1995). Monoclonal antibodies (MAbs) are more specific and can be produced in large batches for binding to specific antigens.

Rublee et al. (2001) used DNA-based probes to assess geographic distribution of *Pfiesteria* species. Hosoi-Tanabe and Sako (2005a,b) used FISH and real-time PCR to identify and enumerate cells of *Alexandrium tamarense* and *A. catenella* in natural plankton assemblages. Takahashi et al. (2005) used FISH for assessing plastid density and activity of dinoflagellates. Wang et al. (2005) used PCR and DGGE for identifying species and establishing population complexity of dinoflagellates.

Fiber optic microarrays that use oligonucleotide probes specific for ribosomal RNA (rRNA) of target HAB species, have been described for simultaneous detection of multiple species (Ahn et al., 2006). Automatic image analysis of dinoflagellates is another emerging technology that can potentially help in rapid morphologically-based identification of species (Culverhouse et al., 2006).

6. Bioreactor culture of dinoflagellates

Many bioactive compounds have been discovered in dinoflagellates and other marine organisms (Metting and Pyne, 1986; Schwartz et al., 1990; Borowitzka, 1995, 1999; Tringali, 1997; Codd, 1995; Shimizu, 1996, 2003; Moore, 1996, 2005; Tyagi et al., 1999; Proksch et al., 2003; Lebeau and Robert, 2003a,b; Belarbi et al., 2003; Singh et al., 2005; Walker et al., 2005; Dittmann and Wiegand, 2006), but few of these have led to commercial products. This is because in many cases insufficient bioactive material has been available for investigational purposes (Rouhi, 1995; Belarbi et al., 2003). Producing useful quantities of bioactive materials from dinoflagellates for biomedical, toxicological and chemical research requires an ability to mass culture these microorganisms.

Although production of large quantities of nondinoflagellate microalgae in photobioreactors has proved extremely successful (Molina Grima et al., 1999, 2000, 2001, 2003), culturing dinoflagellates poses new problems. Dinoflagellates have substantially lower growth rates compared with typical microalgae. The causes for this have been speculated on (Tang, 1996), but not clearly established. Low growth rates are unlikely to be related to efficiency of light harvesting because the photosynthetic capacity per unit of chlorophyll *a* in dinoflagellates is not significantly different from that in diatoms. Because of the low biomass concentrations that are typically attained in dinoflagellate cultures, the concentrations of toxins in the broth tend to be of the order of micrograms per liter. Consequently, extremely large volumes of culture

broth are needed to produce minuscule amounts of toxins.

Small-scale turbulence is known to affect the growth rate and morphology of dinoflagellates (Berdalet and Estrada, 1993; Sullivan and Swift, 2003; Sullivan et al., 2003). Inhibition of dinoflagellate growth by agitation, shaking, aeration and stirring has been reported in laboratory cultures (White, 1976; Pollingher and Zemel, 1981; Berdalet, 1992). Controlled daily exposure to laminar shear flow has inhibited growth of some dinoflagellates (Thomas and Gibson, 1990, 1995; Juhl et al., 2000). Field data confirming a negative correlation between dinoflagellate abundance and high intensity of winds and waves have been known for some time (Pollingher and Zemel, 1981; Berman and Shteinman, 1998; Stoecker et al., 2006). The shear stress levels that dinoflagellates may withstand are generally one or two orders of magnitude lower than the levels that generally damage most animal and plant cells (Namdev and Dunlop, 1995; Joshi et al., 1996; Chisti, 1999, 2000, 2001; Juhl et al., 2000).

The shear stress threshold of some dinoflagellates is even lower than that of erythrocytes (0.029 $N \cdot m^{-2}$) (Chisti, 2000). For example, a continuous laminar shear stress level of only 0.0044 $N \cdot m^{-2}$ (equivalent a shear rate of 2.2 s^{-1}) has proved lethal to the dinoflagellate *Gonyaulax polyedra* Stein, a producer of yessotoxins (Thomas and Gibson, 1990, 1995; Juhl et al., 2000). Like animal and plant cells (Chisti, 1999, 2001), *G. polyedra* responds differently to laminar and turbulent shear stresses. The observed response depends also on the intensity, duration and frequency of exposure to shear field. Growth phase during which exposure occurs, prevailing irradiance level, and light–dark cycling also appear to influence shear tolerance. Other dinoflagellate species behave similarly (Juhl et al., 2001; Juhl and Latz, 2002). Shear stress levels can influence the production of dinoflagellate toxins (Juhl et al., 2001).

In bioreactors, average shear rates range from 0.2 to 1000 s^{-1} in airlift and bubble column devices. In commonly used stirred tank bioreactors, shear rates can range from 2 to 2×10^5 s^{-1}. In spinner flasks that are commonly used to culture highly fragile animal cells in the laboratory, an average shear stress value of 0.8 $N \cdot m^{-2}$ that occurs at a relatively low 150 rpm agitation speed may be lethal for dinoflagellates. In addition to hydrodynamic fluid shear forces, effects associated with the rupture of gas bubbles at the surface of the broth, bubble coalescence and breakup in the fluid, and bubble formation at the gas sparger, can contribute to cell damage in a bioreactor (Contreras Gómez et al., 1998; Contreras et al., 1999; Chisti, 1999, 2000, 2001; García Camacho et al., 2000, 2001; Sánchez Mirón et al., 2003). How these factors might affect dinoflagellates, remains unknown. Fragility of dinoflagellates notwithstanding, successful strategies have been developed for large scale culture of highly shear-sensitive cells (Chisti, 1999, 2000, 2001; García Camacho et al., 2001; Sánchez Mirón et al., 2003; Voisard et al., 2003; Mazzuca Sobczuk et al., 2006). Some of these strategies can be adapted for use with dinoflagellates.

Photobioreactor engineering problems of dinoflagellate culture notwithstanding, dinoflagellates can potentially contribute to improved understanding of shear stresses and other turbulence phenomena in bioreactors. For example, many morphologically diverse dinoflagellates are bioluminescent (e.g. *Lingulodium polyedrum*, *Ceratocorys horrida*, *Pyrocystis fusiformis*, *G. polyedra*) and turbulence appears to stimulate bioluminescence. Dinoflagellate bioluminescence is easily visualized and quantified (Latz et al., 2004a). This provides a powerful tool for flow visualization under conditions not amenable to conventional methods (Latz et al., 1995, 2004a; Rohr et al., 1997, 1998; Blaser et al., 2002). Bioluminescence stimulation and suppression under different conditions of turbulence may assist in establishing mechanisms of cell damage in bioreactors (Chen et al., 2003). Potentially, dinoflagellates can be used to map regions of high shear in biomedical devices such as prosthetic heart valves (Yoganathan et al., 2000). Bioluminescence response of dinoflagellates to developed and developing turbulent flow in pipes has been discussed (Latz et al., 2004b; von Dassow et al., 2005).

Dinoflagellates appear to exhibit complex circadian systems. For example, in nature, actively swimming *G. polyedra* cells aggregate in the upper layer of the ocean during daylight and photosynthesize. During the night they sink to deeper layers where nutrient (nitrate) concentrations are higher (Roenneberg and Merrow, 2002). Gradients in nutrient levels in the water column appear to affect vertical migrations (Doblin et al., 2006). How essential are these rhythms to cellular metabolism in a photobioreactor that would normally be always rich in inorganic nutrients, remains to be elucidated. Other issues that need addressing to enable mass cultivation of dinoflagellates are: the development of suitable media for cell growth and production of toxins; identification of optimal temperature, pH and oxygen tolerance; understanding of irradiance-photosynthesis behavior; elucidation of possible triggers for synthesis of toxins; and engineering of photobioreactors for large scale

culture. For example, in controlled cultures of the dinoflagellate *Alexandrium catenella*, temperature has been shown to influence the saxitoxin content of the cells (Navarro et al., 2006). The effect of environmental factors on growth of *K. brevis* has been discussed (Magana and Villareal, 2006). In cultures of *A. tamarense*, nutritional supplementation has influenced toxin productivity (Wang and Hsieh, 2002) and a two-step culture methodology has been found to benefit toxin production (Hu et al., 2006).

Biosynthesis of some microbial products is suppressed as the product accumulates in the culture broth. Examples of such products include many secondary metabolites (Casas López et al., 2004) and products such as ethanol (Minier and Goma, 1982). Synthesis is suppressed either because a high concentration of the product is toxic to the producing microorganism, or the biosynthetic pathway is subject to feed-back inhibition of one of the enzymes. Extractive fermentations in which the product is removed continuously as it is made, can be used to greatly increase the productivity of such self-inhibited fermentations (Minier and Goma, 1982; Chisti and Moo-Young, 1996; Zijlstra et al., 1998; Banik et al., 2003). This approach has been used in producing β-carotene from the microalga *Dunaliella salina* (Hejazi and Wijffels, 2004) and hydrocarbons from the green alga *Botryococcus braunii* (Banerjee et al., 2002). Use of extractive fermentation principle has been proposed for producing microalgal neurotoxins (Hejazi and Wijffels, 2004). Whether extractive production is possible with dinoflagellates, remains unproved. Economically feasible methods remain to be established for extraction and purification of toxins.

Production of paralytic shellfish toxins in cultured dinoflagellates has been reviewed (Hsieh et al., 2001). Production of yessotoxins in laboratory cultures has been discussed by Paz et al. (2004). Effects of organic nutrient additives on growth and gymnodimine production in cultures of the dinoflagellate *Karenia selliformis* have been discussed by Mountfort et al. (2006). The maximum value of the specific growth rate observed was 0.23 d^{-1} (Mountfort et al., 2006). Toxin production in cultures of *A. tamarense* has been discussed (Wang et al., 2002; Wang and Hsieh, 2002).

7. Biosafety considerations

Dinoflagellate toxins can be extremely toxic in minute quantities. A high level of attention to safety is therefore necessary in producing dinoflagellates and their toxins. Safety issues become further accentuated as the scale of operation increases. A comprehensive safety management plan is necessary for any facility producing or processing biotoxins. Unlike the case with many toxic chemicals, no acceptable exposure limits have been established for biotoxins. Often, toxicological data on toxins are quite limited. Information on chronic exposure to subsymptomatic doses is virtually non-existent. No commercially available devices exist for routine monitoring of personnel at risk of exposure.

Dinoflagellates and their toxins are hazardous if inhaled or ingested. Marine toxins are odorless, tasteless and are not destroyed by cooking or autoclaving. Marine toxins do not vaporize, but airborne toxic dust is easily generated during processing (Chisti, 1998). Work with toxins requires personnel that have been trained in relevant good working practices; suitably designed facilities with engineered controls for risk mitigation; use of personnel protective equipment; exposure monitoring; and emergency response plans. Waste from processing facilities must be assessed for contamination with toxins and suitably inactivated. Specific procedures are required for deactivation of specific toxins (Wannemacher, 1989). Ciguatoxin and maitotoxin are heat and acid stable. Saxitoxins can be inactivated by a 60-min exposure to 10% sodium hypochlorite.

General biosafety considerations in production and handling bioactive substances have been discussed by Chisti (1998). Specific information relating to handling of marine toxins is reviewed by Johnson et al. (2001) and Richmond and McKinney (1999). Further information is available from the American Biological Safety Association (www.absa.org).

8. Concluding remarks

Dinoflagellate toxins and bioactives are potentially useful in many applications. Dinoflagellate bioactives are inaccessible in large quantities and this severely limits research in potential applications of these compounds. Some of the toxins that are available in small amounts are quite expensive. Chemical synthesis of most dinoflagellate toxins is complex and expensive. Gaining access to toxins requires an ability to mass culture dinoflagellates. These microorganisms appear to be extremely sensitive to hydrodynamic shear forces and pose new challenges in photobioreactor engineering. Bioprocess engineering studies for enabling economic use of dinoflagellates as cell factories are only just beginning. This paucity of knowledge notwithstanding, a track record of achievements in culturing fragile cells of higher animals, plants and

microalgae suggests that commercial mass culture of dinoflagellates will be feasible in the future.

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