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The Biosynthesis of Polyketide Metabolites by Dinoflagellates

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I. Introduction

Flagellated species of algae account for 90% of harmful algal blooms (HAB), and in this group approximately 75% are dinoflagellates (Smayda, 1997). More importantly, the geographic expansion of HAB outbreaks has been attributed to 10–12 species of these toxic dinoflagellates (Hallegraeff, 1993). Dinoflagellates are members of the eukaryotic subgroup *Alveolates*, along with apicomplexans and ciliates. They are biflagellate protists (unicellular eukaryotes) that possess numerous eukaryotic traits with some irregularities (Table I). Some dinoflagellates produce secondary metabolites of unparalleled complexity. Some may have therapeutic potential, but many are highly toxic.

Marine algal toxins have been grouped based on the six human illnesses: azaspiracid poisoning (AZP), amnesic shellfish poisoning (ASP), ciguatera fish poisoning (CFP), diarrhetic shellfish poisoning (DSP), neurotoxic shellfish poisoning (NSP), and paralytic shellfish poisoning (PSP). Table II summarizes the illnesses, their sources, poisoning symptoms, and causative toxins (Fig. 1). Four of the six illnesses are caused by dinoflagellate-derived polyketide toxins. The two exceptions are domoic acid, a kainic acid analog produced by diatoms in the *Pseudo-nitzschia* genus (Ramsey *et al.*, 1998), and the saxitoxins, a group of cyclic perhydropurine compounds produced by *Moraxella* (an *Alexandrium tamarenses* intracellular bacteria) (Kodama *et al.*, 1990), cyanobacteria (Ferreira *et al.*, 2001), and species of *Alexandrium* (Hold *et al.*, 2001).

The majority of dinoflagellate toxins are polyketide in origin. Poly-ketides are biosynthesized via the sequential Claisen condensations of small carboxylic acid subunits in a fashion reminiscent of fatty acid biosynthesis. Polyketide synthases (PKSs) have been traditionally classified into three types. However, many variants have recently emerged that do not fit this classification scheme, leading some to suggest that it should be abandoned entirely (Muller, 2004; Shen, 2003). Based on their size, functionalities, and complex structures, one would predict that dinoflagellate-derived polyketides are biosynthesized by Type I modular PKSs. These enzymes are composed of multiple enzymatic domains on the same polypeptide chain. Thus, they are large, multifunctional, and can generate complex structures. Typical structures produced by Type I PKSs are polyenes, macrocycles (including macrolides and nonmacrolides), and polyethers, whereas Type II PKSs produce aromatic polyketides, in general.

Our knowledge of polyketide biosynthesis in dinoflagellates is derived entirely from stable isotope incorporation experiments, which have confirmed the head-to-tail assembly of acetate units with some irregularities. To date, not a single gene has been linked to dinoflagellate polyketide biosynthesis. However, PKS genes have been localized by fluorescence *in situ* hybridization (FISH) to the toxic dinoflagellate *Karenia brevis* (Snyder *et al.*, 2005). This is the first indication that dinoflagellates carry their own suite of resident PKS genes. Additionally, PKS-encoding genes have been identified in a dinoflagellate expressed sequence tags (ESTs) library (*vide infra*) (Lidie *et al.*, 2005). The motivation to identify genes related to polyketide biosynthesis in dinoflagellates is twofold. First, the manipulation of PKS associate

genes could provide unprecedented bio-synthetic capability to the growing field of combinatorial biosynthesis (Rodriguez and McDaniel, 2001). Second, an understanding of regulation of polyketide biosynthetic genes may present novel and improved strategies for predicting and monitoring toxic blooms.

Molecular genetic studies of dinoflagellates are very limited. While a survey of Genbank for dinoflagellate genes returns more than 20,000, most of them are related to photosynthesis, protein synthesis (i.e., rRNA), or located in the chloroplast or mitochondrial genomes, or are from EST libraries. Very little has been done in terms of finding a targeted gene from a dinoflagellate nuclear genome. There are many obstacles that will need to be addressed before these types of studies of dinoflagellates become more commonplace. These obstacles have been reviewed in the past (Plumley, 1997) and will be briefly mentioned here in Section IV. Also included, is a survey of strategies that others have used for the study of functional genes in dinoflagellates that may, in the future, be applied to dinoflagellate polyketide toxin biosynthesis.

II. Recently Discovered Dinoflagellate Polyketides

Numerous dinoflagellate polyketide variants have been identified over the past 5 years. However, this section is not meant to be an exhaustive review of new structures. The reader is directed to the many excellent reviews of dinoflagellate toxins for a more comprehensive account (Balmer-Hanchey *et al.*, 2003; Holmes and Lewis, 2002; Kobayashi and Tsuda, 2004; Kobayashi *et al.*, 2003; Rein and Borrone, 1999; Shimizu, 2003). We have chosen to focus on new toxins identified since our last review of the field (Rein and Borrone, 1999), as well as structural refinements to known compounds. This section is further limited to polyketides isolated either from laboratory cultures of dinoflagellates or from wild samples or plankton tows. Many analogs of previously identified dinoflagellate-derived polyketides have been isolated from shellfish or marine invertebrates. However, it is not clear that these analogs are produced *de novo* by the dinoflagellates themselves or if they are shell-fish metabolites. Admittedly, it is not even certain that some of the variants isolated from laboratory cultures of dinoflagellates are not bacterial metabolites, as most of the dinoflagellates in question have not been grown axenically. We will also not cover the pharmacology of marine toxins as this is beyond the scope of this review and would be best covered separately. Again, the reader is directed to recent reviews on the subject (Rein and Borrone, 1999; Yasumoto, 2001).

Only a handful of truly novel dinoflagellate polyketides, with respect to backbone structure, have been discovered since we last reviewed this field. The vast majority of new polyketides identified are structural variants of known compounds. Novel structures include azaspiracid, brevenal, prorocentxin, and a number of new amphidinolides.

A. FROM *PROTOPERIDINIUM CRASSIPES*

In 1995, several people in the Netherlands became ill after eating mussels, which had been farmed in Killarney Harbor in Ireland. The causative toxin, named azaspiracid, was first isolated from 20 kg of mussel tissue (Satake *et al.*, 1998). It was later found in isolated cells of what had previously been believed to be a nontoxic dinoflagellate, *Protoperidinium crassipes*. Two hundred cells were hand picked from plankton samples for LC-MS analysis. Two structural analogs, AZA2 and AZA3, the 8-methyl and 22-desmethyl analogs respectively (Fig. 1) (James *et al.*, 2003a; Ofuji *et al.*, 1999), were also associated with this dinoflagellate. Numerous hydroxyl derivatives have also been found, but all from shellfish (James *et al.*, 2003b). After the total synthesis of azaspiracid-1 was completed by the Nicolaou group, the structure was revised to that shown in Fig. 1 (Nicolaou *et al.*, 2004).

B. FROM *KARENIA BREVIS*

Brevenal has been isolated from cultures of *Karenia brevis*, the Florida red tide organism (Fig. 2) (Bourdelaïs *et al.*, 2005). It is roughly half the size of the brevetoxins, having only 4 fused ether rings compared to 10 for brevetoxin A and 11 for brevetoxin B. The structure of brevenal is distinct from, but somewhat reminiscent of, the hemi-brevetoxins that were reported by Shimizu in 1989 (Prasad and Shimizu, 1989; Shimizu *et al.*, 1989). Brevenal appears not to share the sodium channel activity of the brevetoxins, but surprisingly acts as a brevetoxin antagonist (Bourdelaïs *et al.*, 2004). Several new side-chain variants of brevetoxin B have been isolated from *K. brevis* (Baden *et al.*, 2005). These include PbTx-11 (propenyl), PbTx-12 (2-oxo-hexyl), and PbTx-tbm (no side chain) (Fig. 1). Also reported were the dimethyl acetal and hemiacetal of PbTx-2. However, these are more likely to be artifacts of the isolation process, which includes a chromatography step on silica gel with acetic acid and methanol mobile phase, rather than toxin variants produced by the dinoflagellate.

C. FROM *PROROCENTRUM* AND *DINOPHYSIS*

Prorocentin (Fig. 2) was isolated from cultures of the dinoflagellate *Prorocentrum lima* (Lu *et al.*, 2005). The C/D spiroketal fused ring system of prorocentin is highly reminiscent of the A/B ring system of okadaic acid (OA). Because it was isolated from an OA-producing strain of *P. lima*, the authors suggested that the two polyketides share a common biogenic pathway.

In addition to prorocentin, a number of variants of OA and dinophysis toxins have been identified. Several new ester derivatives of OA have been described (Fig. 1) (Fernandez *et al.*, 2003; Suarez-Gomez *et al.*, 2001, 2005). In addition, two isomers of OA, DTX-2b, and -2c, have been observed in HPLC-MS analysis of phytoplankton extracts (Draisci *et al.*, 1998). These compounds have the same molecular mass and fragmentation pattern as OA, yet have different HPLC retention times. The authors suggest that these are spiroketal epimers of OA.

Two isomers of pectenotoxin, PTX-12 (36R and 36S) having an *exo* methylene at the terminal pyran ring as opposed to a methyl group, were isolated from *Dinophysis acuta*, *D. acuminata*, and *D. norvegica* (Fig. 2) (Miles *et al.*, 2004c). It had been believed that the hydrolysis of the PTXs was an enzymatic process in the digestive glands of shellfish; however PTX-2-seco acid and 7-epi-PTX-2-seco acid were also isolated from *Dinophysis acuta* (James *et al.*, 1999).

D. FROM *PROTOCERATIUM RETICULATUM*

Three glycosylated derivatives of homoyessotoxins were isolated from culture media of *Protoceratium reticulatum*. These compounds possessed mono-, di-, and tri- β -arabinofuranose moieties at the 32-hydroxyl group. They were named protoceratin 3, 2, and 4 respectively (Konishi *et al.*, 2004). These represent the first example of glycosylated polyether ladder toxins from a dinoflagellate, however, the polyether ladder prymnesins from haptophyte *Prymnesium parvum* are also glycosidic (Igarashi *et al.*, 1999). Later, the corresponding mono arabinofuranose derivative of yessotoxin, glycoyessotoxin A, was isolated from cell mass of *Protoceratium reticulatum* cultures (Fig. 2) (Souto *et al.*, 2005).

As part of a program to evaluate yessotoxin profiles in shellfish and phytoplankton and to evaluate the pharmacology of analogs, several new analogs were identified from shellfish and from cultures of *Protoceratium reticulatum*. At least seven new YTX analogs were isolated and characterized from *P. reticulatum* (Fig. 2). These include, 41a-homoyessotoxin, 9-methyl-41a-homoyessotoxin, nor-ring A-yessotoxin (not shown) (Miles *et al.*, 2004b), a 1, 3-enone isomer of heptanor -41-oxo-YTX (Miles *et al.*, 2004a), (44-*R,S*)-44, 55-dihydroxyessotoxin (Finch *et al.*, 2005), and the first examples of YTXs having an amide side chain (not shown) (Miles *et al.*, 2005).

E. FROM AMPHIDINIUM SP

Symbiotic dinoflagellates of the genus *Amphidinium* continue to be a rich source of polyketides (Fig. 3). The amphidinolides are an expanding group of cytotoxic macrolides (Kobayashi and Tsuda, 2004). Two variants of amphidinolide B (B4 and B5) were reported in 2005 (Tsuda *et al.*, 2005). Two other amphidinolide series share the same carbon skeleton as the amphidinolide B series. The G and H series differ from the B series in that they have a C26 hydroxyl. The B and H series are lactonized at the C25 hydroxyl, whereas the G-series is lactonized at the C26-hydroxyl. New amphidinolides in these series include amphidinolide G2, G3, H2, H3, H4, and H5 (Kobayashi *et al.*, 2002). Within these three series, variations are seen in the configurations at C16, C18, and C22, and the level of saturation of the C6–C7 bond.

The amphidinolide T series was first reported in 2000 (Tsuda *et al.*, 2000). Since then, four additional members were discovered (T2–T5) (Kobayashi *et al.*, 2001; Kubota *et al.*, 2001a). All are 19-membered ring macrolides possessing a furan ring that spans C7–C10. This series varies in the oxidation state and configuration at C12. Additionally, amphidinolide T2 has an extra carbon and hydroxyl group on its side chain.

A number of novel amphidinolides, U, V, W, X, and Y were recently described (Fig. 3 and Fig. 5) (Kubota *et al.*, 2000a; Shimbo *et al.*, 2002; Tsuda *et al.*, 1999, 2003a,b). While these structures are unique, some do share similarities with previously characterized amphidinolides. For instance, C7 through C29 and C1–C8 of amphidinolide U correspond to C12–C34 and C1–C8 of amphidinolides C and A, respectively. C9–C16 of amphidinolide W corresponds to C6–C13 of amphidinolide H. These similarities suggest a common biogenic origin for several of the amphidinolide series. Amphidinolides X and Y are entirely unique but related to one another. The oxidative cleavage of the C6–C7 bond of amphidinolide Y with lead tetraacetate yields amphidinolide X. This suggests that amphidinolide Y is certainly a biogenic precursor to amphidinolide X.

Other notable developments within the amphidinolides include the isolation of an acetylated version of amphidinolide C (C2) (Kubota *et al.*, 2004), the total syntheses of amphidinolides A (Trost and Harrington, 2004) and W (Ghosh and Gong, 2004), which led to the revision of these structures, and the determination of the absolute configuration of amphidinolides G and H (Kobayashi *et al.*, 2000).

In addition to cytotoxic macrolides, dinoflagellates of the genus *Amphidinium* produce several classes of polyhydroxy polyenes. These include the amphidinols, luteophanols, lingshuiol, and colopsinols. The amphidinols, luteophanols, and lingshuiol are closely related with two pyran rings in the central portion of the chain, giving them a hair-pinlike conformation, which is believed to contribute to their membrane disrupting activities (Echigoya *et al.*, 2005). Currently, there are seven known amphidinols. They are identical in the central portion of the molecules and vary in the level of saturation, oxidation, and substitution of the side chains (Echigoya *et al.*, 2005). Amphidinol 7 is unique among them in that it possesses a truncated (by 10 carbons over AM 1) polyene chain (Fig. 4) (Morsy *et al.*, 2005). The absolute configuration of AM 3 was established by analysis of C–H spin coupling constants (Murata *et al.*, 1999). The polyhydroxylated lingshuiol A and B were isolated from an epiphytic species of *Amphidinium* (Huang *et al.*, 2004). These are identical to the previously isolated amphidinol 2 from C29–C65 except that amphidinol 2 is saturated from C58–C61. The C1–C28 portion of lingshuiol is distinct from amphidinol 2. Finally, colopsinols A–D are similar to the amphidinols except that they have a single pyran ring at the terminus of the polyketide chain (Fig. 4) (Kubota *et al.*, 1999, 2000b). The colopsinols vary in the extent of glycosylation.

F. OTHER

Like many dinoflagellate toxins, spirolides were first isolated from shellfish in 1995 and presumed to be of dinoflagellate origin on the basis of their structures (Fig. 4). In 2001, the source of these compounds was identified as *Alexandrium ostenfeldii* (Cembella *et al.*, 1999). Since then, spirolides A and C, and 13-desmethyl C and D have been characterized from this organism (Hu *et al.*, 2001; Sleno *et al.*, 2004). The relative configurations of the stereocenters of the spirolides were established in 2001 by extensive ROESY and NOESY NMR analysis (Falk *et al.*, 2001). New gymnodimine isomers, gymnodimine B and C, were isolated from *Karenia selliformis* (Fig. 4) (Miles *et al.*, 2000, 2003). The absolute configuration of gambieric acid from *Gambierdiscus toxicus* was determined by a combination of Mosher analysis, NMR, and chiral HPLC (Morohashi *et al.*, 2000).

III. Stable Isotope Incorporation Experiments

The first biosynthetic studies of dinoflagellate-derived polyketides were performed on the brevetoxins (Chou and Shimizu, 1987; Lee *et al.*, 1989). The incorporation of [$1\text{-}^{13}\text{C}$], [$2\text{-}^{13}\text{C}$], and [$1, 2\text{-}^{13}\text{C}$] labeled acetate revealed a head-to-tail arrangement of acetate units over much of the carbon skeleton confirming the polyketide origins of these compounds. However, some unusual patterns of incorporation were observed. Most notable was the frequent deletions of C1 of acetate leaving subunits of c-m-m, c-m-m-m, and c-m-m-m(m). These patterns have been repeated in every dinoflagellate derived polyketide studied to date.

A. AMPHIDINOLIDES

In terms of biosynthetic experiments, the most extensively studied group of dinoflagellate-derived polyketides are the amphidinolides. Stable isotope incorporation experiments have been performed on amphidinolide B, C, H, J, T1, W, X, and Y (Fig. 5) (Kobayashi *et al.*, 1995, 2001; Kubota *et al.*, 2001b; Sato *et al.*, 2000; Tsuda *et al.*, 2001, 2002, 2003a,b). These studies have revealed that all carbons of the amphidinolides, including pendent methyl groups, which are more typically derived from S-adenosylmethionine (SAM) or propionate incorporation, are derived from acetate. This is in contrast to the brevetoxins, where some of the pendent methyl groups were labeled with S-adenosyl methionine.

The biosynthetic subunits, which were observed in the brevetoxins, are also apparent in the amphidinolides. The c-m-m fragment is observed in amphidinolides C, J, T1, W, and X. The c-m-m-m fragment is observed in amphidinolides H and T1, and the c-m-m-m(m) fragment is observed in amphidinolide Y. However, the C1 deletions appear to be even more frequent in the amphidinolides. The frequent C1 deletions and incorporation of pendent methyl groups has led to the identification of a wider variety of fragments than observed in prior studies. A c-m-m (m) fragment appears in amphidinolides B, J, and Y. A c-m-m-m fragment appears in amphidinolides B. A c-m-m(m)-m(m) fragment appears in amphidinolides C and H, and a c-m-m(m)-m-m(m) fragment appears in amphidinolide C. Also noteworthy is the appearance of an m(m)-m fragment in amphidinolides B, H, T1, and W. This pattern appears at the termination of the polyketide chain of these macrolides.

The biosynthetic origin of these fragments has been the subject of speculation for many years. Shimizu and Nakanishi invoked the incorporation of citric acid cycle intermediates to account for C1 deletions in the brevetoxins (Chou and Shimizu, 1987). Incorporation of atypical subunits like succinate and ketoglutarate would require a truly novel PKS. On the other hand, Wright proposed a Favorskii-like rearrangement in the construction of OA (Wright *et al.*, 1996). Later, Rawlings (1999) suggested a Tiffeneau-Demyanov rearrangement of an α , β -epoxy ketone. A comparison of the intensity ratios (labeled/unlabeled) of the different types of acetate methyls for amphidinolide B indicate that these ratios are similar whether the carbon

is incorporated as part of an intact acetate, a cleaved acetate, or as a pendent methyl or methylene at either a C1 or C2 of an acetate subunit (Tsuda *et al.*, 2001). This would seem to imply that the labeled precursors are not diluted in the metabolic pool prior to incorporation and are incorporated as intact acetate units and modified only after incorporation into the growing polyketide chain. This hypothesis is not consistent with the incorporation of citric acid cycle intermediates but is more consistent with Wright's Favorskii rearrangement hypothesis. Further support for this hypothesis is the Favorskii type rearrangement observed during the biosynthesis of enterocin and the wailupemycins by a marine bacterium, *Streptomyces maritimus* (Xiang *et al.*, 2004). In these metabolites one carbon derived from C1 of acetate is extruded from the parent polyketide chain but is retained as a pendent carboxyl group. The *encM* gene, found in the enterocin gene cluster, codes for a flavin-dependent oxygenase (EncM) and was demonstrated to be solely responsible for the Favorskii rearrangement.

While common in dinoflagellate-derived polyketides, the incorporation of C2 of acetate as a pendent methyl group is relatively rare among other organisms. Some bacterial polyketides have shown this same pattern. In the biosynthesis of curacin A (Chang *et al.*, 2004) and jamaicamide (Edwards *et al.*, 2004), the incorporation of C2 of acetate is performed by an HMG-CoA synthetase-like enzyme via an aldol condensation followed by a decarboxylation. The pathways for the antibiotic TA (Paitan *et al.*, 1999), mupirocin (El-Sayed *et al.*, 2003), leinamycin (Cheng *et al.*, 2003), and diffidin believed to be made by PksX (Albertini *et al.*, 1995) also contain HMG-CoA synthase-like genes. These genes show high homology to each other and lower homology to HMG-CoA synthase genes associated with terpene biosynthesis. In these examples, the growing polyketide chain is modified at a carbonyl from C1 of acetate. What is unusual about dinoflagellate-derived polyketides is the incorporation of the acetate methyl group at carbon derived from C2 of acetate. Most of the pendent methyl groups of the amphidinolides are incorporated at C1 of acetate. However, 14 of the 36 methyl groups of amphidinolide B, C, H, J, T1, W, and Y are appended to C2 of acetate. It may be noteworthy that an acetate-derived pendent methyl group never appears at C2 of an *intact* acetate unit among the amphidinolides, or any other dinoflagellate-derived polyketide. Seven pendent methyl groups are present in the brevetoxins. Four of those are at C2 of an intact acetate unit; however those four methyl groups are derived exclusively from S-adenosyl methionine. Similarly, six pendent methyl groups and one methylene are present in yessotoxin. The single methyl group that is positioned at C2 of an intact acetate unit is derived from S-adenosyl methionine (Satake, 2000).

A comparison of labeling patterns for amphidinolides B and H revealed the surprising conclusion that they do not share identical incorporation patterns even though their carbon skeletons are identical. Stable isotope incorporation experiments revealed different patterns of incorporation for carbons 16–19. For amphidinolide B, the pattern is cm–cm, whereas the pattern for amphidinolide H is m–cm–c. While they share a common carbon framework, these two macrolides are not from the same strain of *Amphidinium* (Fig. 5) (Sato *et al.*, 2000; Tsuda *et al.*, 2001).

B. AMPHIDINOLS

A stable isotope incorporation study has been reported for amphidinols 2, 3, and 4 (Fig. 5) (Houdai *et al.*, 2001). Amphidinols 3 and 4 showed identical patterns of carbon incorporation. These polyhydroxy polyenes appear to be more polyketide-like in their construction than the amphidinolides having as many as nine contiguous acetate units. Also present are three c–m–m and one c–m–m(m). Unlike the amphidinolides, acetate was not incorporated at all positions. Carbons 1 and 2 remained unlabeled by either [1-¹³C], [2-¹³C]. This and the presence of vicinal hydroxyls at C1 and C2 suggest that the starter unit for this polyketide may be glycolic acid,

which is also the starter unit for OA and the ester portion of DTX-4 (Needham *et al.*, 1994, 1995). Although amphidinols 2 and 4 share an identical carbon backbone, their acetate incorporation patterns were not the same. The first C1 deletion occurs between carbons 11 and 12 in amphidinol 2 and between carbons 21 and 22 in amphidinol 4. Thus the labeling patterns are reversed between C12 and C21 and restored at C22. AM2 has methyl substituents, derived from C2 of acetate at C17 and C19.

C. DINOPHYSIS TOXINS

Stable isotope incorporation experiments have been performed on DTX-5a and -5b (Fig. 5) (Macpherson *et al.*, 2003). Incorporation patterns of the OA portion of these two analogs were revealed to be identical to DTX-4 (Needham *et al.*, 1995; Wright *et al.*, 1996). DTX-5a and -5b have very similar side chains, the only difference being the number of carbons in the diol portion of the ester side chain. This portion of DTX-5b is identical to DTX-4. However, DTX-5a has one fewer carbon in this region. Earlier experiments with DTX-4 indicated that this polyketide was constructed of contiguous acetate units and no C1 deletions were observed (Needham *et al.*, 1995; Wright *et al.*, 1996). Further, the ester portion of this side chain was introduced by the Baeyer-Villiger oxidation of a continuous polyketide chain. One bond coupling between carbons at 8' and 1'' of DTX-4 revealed that these two carbons originated from the same acetate unit. A similar pattern was observed for this region of DTX-5b. This was not the case for DTX-5a. Whereas the ester carbonyl (C1'') of DTX-4 and -5b is derived from C1 of acetate, in DTX-5a it is derived from C2 of acetate. Further, labeling studies revealed that this carbon was not coupled to the adjacent carbon suggesting a deletion of C1 of acetate. The amide containing regions of DTX-5a and -5b are identical, with the Baeyer-Villiger oxidation occurring exactly 14 carbons and 1 nitrogen from the sulfated end in both. Two important conclusions were drawn from this observation. First that the Baeyer-Villiger oxidation is highly regioselective and must have occurred after the C1 deletion in order to place the ester exactly the same distance from the sulfated end of both analogs. Second, the Favorskii-like deletion of C1 of acetate must be an integral part of the polyketide chain assembly process. Another interesting observation is the incorporation of a single glycine at carbons 8'' and 9'' and at the nitrogen of DTX-5a and -5b. This suggests that the side chain of DTX-5a and -5b is constructed from a mixed polyketide synthase/nonribosomal peptide synthetase type enzyme. This is the first and only demonstration of the incorporation of an amino acid into a dinoflagellate derived polyketide.

D. YESSOTOXIN

Finally, stable isotope incorporation studies were reported for yessotoxin (Fig. 5) (Satake, 2000). Like all other dinoflagellate-derived polyketides, extensive deletions of C1 of acetate were observed. Yessotoxin is composed of three intact c-m units, eight c-m-m, three c-m-m-(m) m, and one c-m-m(m)-m(m) and one isolated m. Six pendent methyl groups and one methylene are present in YTX with only one at C19 derived from S-adenosyl methionine.

IV. Polyketide Biosynthesis at the Molecular Level

The identification of the genes for dinoflagellate polyketide biosynthesis is an attractive goal for a variety of reasons. However, there have been no reports of a characterized secondary metabolic pathway from a dinoflagellate. Furthermore, results of this nature may be slow in coming because of the obstacles presented by this group of organisms. This can include the ability (or inability) to culture the organism of interest. For example, *Dinophysis* species have not been maintained in long-term culture. For planktonic dinoflagellates adequate culturing is invariably in liquid medium. To date, the transition to solid media has not been overcome for most species. This seemingly straightforward step has a significant impact on classical methods of gene isolation. Typically, chemical or UV treatment is employed to create mutants. Since

dinoflagellates usually have large genomes, 10^4 – 10^7 mutants would have to be generated to have a high-statistical probability of creating a single cell with a *pks*[−] genotype (Plumley, 1997). The mutagenized cells are subsequently plated on agar-solidified medium, although this step could theoretically be carried out in liquid medium. Regardless, the most difficult obstacle is distinguishing desired mutagenized cells from wild type cells. In primary metabolic studies, the surviving mutants typically display a distinctive phenotype (e.g., growth on a selection medium). In the case of secondary metabolite production (i.e., polyketide biosynthesis), the identification of a mutant phenotype would require the screening of individual mutants by a single polyketide metabolite assay (e.g., HPLC or ELISA) or single *PKS* gene assay (e.g., PCR).

The most closely related organism to dinoflagellates proven to have a Type I *PKS* is *Cryptosporidium parvum*, an apicomplexan. Initially, a Type I fatty acid synthase (FAS) (CpFAS1; Genbank Accession AAC99407) was found in *C. parvum* by screening a *HindIII* and *EcoRI* genomic DNA library with a FAS amplicon from *C. parvum* (Zhu *et al.*, 2000). The CpFAS1 ORF spans 25 kb and contains a starter module, three complete modules, and a terminal reductase. The starter module contains a loading domain, which has sequence similarity to ATP-dependent CoA ligases, and an acyl carrier protein (ACP). Each of the three modules has a full set of enzymatic domains (i.e., AT, KS, KR, DH, ER, and ACP) with the expected, conserved functional motifs. Surprisingly, the terminal domain is not a TE, but instead it has high-sequence similarity to a yeast α amino adipate reductase. This suggests *C. parvum* releases the tethered product by a nonhydrolytic mechanism like yeasts and fungi (Schweizer *et al.*, 1986). The CpFAS1 gene architecture resembles an FAS because the full set of domains are present in each module. However, the presence of multiple modules is suggestive of a *PKS* and the putative CpFAS1 is actually more similar to *PKS*s in a phylogenetic analysis (Snyder *et al.*, 2005). A genome sequencing survey project of *C. parvum* made possible the discovery of the first Type I *PKS* (CpPKS1) in any protist (Zhu *et al.*, 2002). The CpPKS1 gene spans an intronless 40 kb and contains seven *PKS* modules. The loading module and terminal reductase are similar to those found in CpFAS1. Only two AT domains are found within the entire gene leading to three possibilities: (1) the use of an AT acting *in trans*, (2) the two CpPKS1 AT domains have multiple roles, or (3) the KS domains in the AT-lacking modules accept the acyl moieties directly. A portion of CpPKS1 was created synthetically, and polyclonal chicken antibodies were raised against the peptide. The anti-CpPKS1 antibodies localized presumably to the native CpPKS1 protein in *C. parvum* sporozoites. This was observed by immunofluorescence microscopy.

Despite the obstacles that still need to be overcome when dealing with dinoflagellates, advances have been made in the identification of putative polyketide synthase genes from marine dinoflagellates (Snyder *et al.*, 2003). Degenerate primers for the ketosynthase domain of Type I *PKS*s and Type II *PKS*s were tested against nine strains (representing six genera and seven species) of dinoflagellates by PCR and RT-PCR. Seven of the nine strains yielded products that were homologous with known and putative Type I *PKS*s. In each case, the presence of a *PKS* gene was correlated with the presence of bacteria in the cultures as identified by amplification of the bacterial 16S rRNA gene. Clearly, the origin of the amplified *PKS*s remained debatable, and this was addressed from several angles. Southern hybridization was used to demonstrate the presence of highly methylated DNA extracted from dinoflagellate cultures by the ineffectiveness of methylation-sensitive restriction enzymes. Furthermore, some of the *PKS* amplicons hybridized to the highly methylated (i.e., dinoflagellate) DNA for some species. Overall, an amino acid phylogenetic comparison of the *PKS* amplicons showed a general dispersion rather than a grouping with clades of bacterial or fungal Type I *PKS*s. Likewise, the *PKS*s amplified from dinoflagellate cultures did not form a clade either, with the exception of three *PKS*s from *K. brevis* (Wilson). These three *K. brevis* *PKS*s also grouped with the CpFAS1 ketosynthases, and recall the CpFAS1 is very similar to *PKS*s in the National

Center for Biotechnology Information (NCBI) database. However, *K. brevis* has not been grown axenically. The associated bacteria might be the source of the toxins or the PKS genes.

A more detailed analysis was undertaken to confirm the origin of the three *C. parvum*-like PKSs from *K. brevis*. A PCR survey using sequence-specific primers against five strains (six isolates) of *K. brevis* revealed all three of these PKSs in all of the isolates. Furthermore, these PKS-encoding genes were localized to *K. brevis* by a combination of flow cytometry/PCR and FISH. A *K. brevis* culture was subjected to flow-cytometric cell sorting based on size and chlorophyll autofluorescence. Dinoflagellate cells were successfully sorted from bacterial cells; however, bacterial cells were not successfully separated from dinoflagellate cells. This was confirmed by the presence (or absence) of 18S and 16S rRNA genes in a PCR assay. Sequence-specific PCR (same as done in the *K. brevis* survey) indicated all three PKS genes were present in the dinoflagellate fraction, but only one of these three was also present in the bacterial fraction. Whole-cell FISH confirmed the presence of the same two PKSs not found in the bacterial fraction by labeling to the *K. brevis* cells and not to associated bacteria. The third PKS found in the bacterial fraction labeled a bacteria-sized coccoid-like particle and *K. brevis* to a lesser extent. Thus, two genes localized exclusively to *K. brevis* cells while a third localized to both *K. brevis* and associated bacteria. While these genes have not yet been linked to toxin production, the work described the first definitive evidence of resident PKS genes in any dinoflagellate.

Polyether ladders, such as the brevetoxins and yessotoxin, are entirely unique to dinoflagellates. There is, therefore, no precedent to look to for clues as to the processes that would be required for their biosynthesis. The closest analogy would probably be the polyether metabolite monensin, which is biosynthesized by the soil-borne *Streptomyces cinnamonensis*. This nonladder polyether provided the first example of a sequenced Type I PKS polyether gene cluster (Leadlay *et al.*, 2001). The only other nonladder polyether with a sequenced Type I PKS gene cluster is nanchangmycin (Sun *et al.*, 2003). The biosynthetic process for both of these polyethers includes ketoreductases and dehydratases for the formation of the backbone carbon-carbon double bonds. The alkenes are proposed to be epoxidized and undergo a polyepoxide cyclization to form the ether linkages (Fig. 6). The identification of a putative flavin-linked epoxidase and epoxide hydrolases (for cyclization) in these gene clusters support this hypothesis. In the case of monensin, deletions of these genes abolished production of the polyether (Oliynyk *et al.*, 2003). The carbon backbone of brevetoxin is predicted to be a mostly all *trans* polyene that is epoxidized and undergoes a similar polyepoxide cyclization (Fig. 6) (Lee *et al.*, 1989). Evidence supporting this hypothesis in another dinoflagellate polyketide has been shown by ^{18}O incorporation from molecular oxygen into rings D and E of dinophysistoxins, suggesting a β -epoxidation intermediate (Murata *et al.*, 1998).

V. Current Methods for Isolating Dinoflagellate Genes

Since most dinoflagellate genomes are large, the discovery of novel genes has mostly been achieved through partial sequencing of cDNA libraries, (i.e., ESTs). This approach identifies thousands of genes and is not hindered by genome size or introns. Recently, about 7000 ESTs were sequenced yielding 5280 unique gene clusters from *K. brevis* (Wilson) (Lidie *et al.*, 2005). Only 1556 (29%) of these genes showed similarity to previously identified genes using a cutoff of $P < 10^{-4}$ in the BLASTx search algorithm. The genes that met this criteria were involved in metabolism (23%), signal transduction (20%), transcription/translation (15%), and structure/cytoskeleton (11%). This library was submitted to two databases, Genbank at NCBI (www.ncbi.nlm.nih.gov) and Marine Genomics (www.marinegenomics.org). In the latter, the sequences can be searched based on gene ontology. Searches for PKS-related genes are shown in Table III. Search terms were polyketide, ketosynthase, transacylase, acyl-carrier protein, and thioesterase. Other PKS-related search queries that did not return any sequences were

acyltransferase (yet transacylase did), ketoreductase, enoylreductase, and dehydratase. Curiously the PKS genes found previously were not identified in the EST database.

One of the greater purposes for the creation of the *K. brevis* EST library is the development of a DNA microarray (Lidie *et al.*, 2005). When optimized and normalized, this tool enables genomewide investigations into the mechanisms that regulate the growth and toxicity of *K. brevis* by comparing expression levels under different environmental conditions. Clearly, an EST library is an effective and informative method for the acquisition of new sequences; however, it requires a substantial amount of resources. The remainder of this chapter focuses on techniques used to identify specific genes.

Despite being part of the enormous *Gonyaulax polyedra* (= *Lingulodinium polyedrum*) genome, the full-length luciferase mRNA was one of the first targeted genes to be characterized from any dinoflagellate (Bae and Hastings, 1994). Two regions of the mRNA were found using an antibody versus luciferase and a cDNA expression library, and the full mRNA was found by Northern hybridization. Later, the structure and organization of the *luciferase* gene was characterized by a combination of Southern analysis, inverse PCR, the 5' rapid amplification of cDNA ends (RACE), and an RNase protection assay (Li and Hastings, 1998).

Functional assignments for novel genes can only be made indirectly when they are identified by degenerate-oligonucleotide-primed PCR or mass sequencing of genomes or cDNAs. Heterologous functional complementation of auxotrophic yeast allows both the isolation and functional confirmation of genes. Essentially, a prototrophic phenotype in an auxotrophic organism is restored by transformation with a gene or gene library derived from another organism. This technique has been used in the case of *Cryptocodinium cohnii* (Lippmeier *et al.*, 2002). The study used a mutant *Saccharomyces cerevisiae* deficient in its ability to biosynthesize adenine. The auxotrophic *S. cerevisiae* was transformed with a *C. cohnii* cDNA library that was cloned into a yeast expression vector, pFL-61. Of 90,000 primary transformants, only one was able to cure adenine auxotrophy. Specifically, the *ade2* phenotype was restored by a 2468 bp complementing cDNA (AY032657). Complementation of phenotypes showing deficiencies in leucine and tryptophan biosynthesis were also attempted but not observed.

Functional complementation was also used to isolate and confirm the function of a cyclin from *G. polyhedra* (Bertomeu and Morse, 2004). Cyclins control the timing and location of activated cyclin-dependent kinases (CDKs), and CDK activity is necessary for the cell's entry into the M and S phases of mitosis. The mechanics of cell division in dinoflagellates, particularly the harmful species, is important for understanding cell proliferation to high levels (i.e., blooms). In addition, the cell cycle in *Gonyaulax* is linked to the circadian clock. The experimental design used yeast, harboring mutations in either G1- or M-phase cyclins. They also had a distinct Gal1 promoter inducible cyclin. Thus, media containing galactose was necessary for wild-type survival in the absence of complementation. The transformation was performed with a *G. polyhedra* cDNA library that was cloned into a modified pYES2 yeast expression vector. The selectable marker for this vector is *URA3*, which confers the ability to synthesize uracil. Thus, transformants were grown in the absence of uracil, which allowed for successful transformation selection. Also, galactose was not present in the media, which prevented the growth of nontransformants.

Essentially, two unique sequences were identified from the 136 transformants. One of the sequences, GpCyc1, showed a low similarity (~30%) to cyclins from other organisms, which is typical for the similarity of cyclins from different groups. However, GpCyc1 showed a high similarity (77%) to an EST sequence from *Alexandrium*. This line of support along with GpCyc1 having a typical *Gonyaulax* GC content (66.5%) and codon usage suggests it

originated from the dinoflagellate. While this method is effective for yeast lacking a critical gene for primary metabolism or cell division, its application to polyketide biosynthesis (i.e., secondary metabolism) may be limited.

Differential display (DD) is a molecular tool used to analyze differences between complex genomes at the level of gene expression. This allows the analysis of differentially expressed genes in eukaryotes. Two or more sets of differentially expressed mRNAs to be compared are used as templates to generate the corresponding cDNAs. The mRNAs are reverse transcribed using a degenerate oligo (dT) primer with the general sequence 5' T_nVN ($n = 11-12$). Thus, there are 12 permutations of the last two 3' bases, and any particular primer (e.g., 5' T_{11}CA) will recognize $\{1/12\}$ of the total mRNA population. A portion of the cDNAs is then amplified in a PCR reaction containing one random decamer primer, the respective modified oligo (dT) primer, and [$\alpha\text{-P}^{32}$]dATP. Following the amplification, the products are separated by polyacryl-amide gel electrophoresis, and the bands are visualized by autoradiography. Bands of desired size are excised and reamplified with the same pair of primers. Finally, the products are sequenced. Usually, the purpose is to identify bands unique to one physiological state (i.e., genes correlated with growth phase or environmental stresses). This could also be applied to two strains of the same organism with the goal of identifying genes contributing to a characteristic of one of the two strains.

In this regard, DD has been applied to three toxic and three nontoxic strains of *Alexandrium tamarense* (Taroncher-Oldenburg and Anderson, 2000). However, the inter- and intragenetic variabilities were high. Thus, there were no shared expressed genes in all three toxic strains that were also not present in the nontoxic strains. More success was achieved using synchronized cultures of *A. fundyense*. In this case, the DD patterns were identical for G₁, S, and G₂ phases, which permitted the identification of differentially expressed bands. Saxitoxin has been shown to accumulate during a discrete time period in the G₁ phase of the cell cycle (Taroncher-Oldenburg *et al.*, 1997). Thus, the focus was to identify genes expressed or suppressed in early G₁ when compared with the remainder of the cell cycle. Three genes met the criteria, and these coded for S-adenosylhomocysteine hydrolase, methionine aminopeptidase, and a histone-like protein. Both S-adenosylhomocysteine hydrolase (in SAM regulation) and methionine aminopeptidase (in protein synthesis processing) theoretically could play a secondary role in saxitoxin biosynthesis (e.g., regulation), but they probably do not contribute directly.

VI. Conclusions

Stable isotope feeding studies early on in dinoflagellate biochemical investigations firmly established a polyketide origin for many metabolites. The next step will be linking a gene to toxin production in a dinoflagellate. The advances in molecular biology will help to identify gene clusters from these organisms, establish the role of these genes and understand how they are regulated under varying environmental conditions. Growing use of techniques like flow-cytometry, functional complementation, and differential display will contribute to characterizing novel dinoflagellate genes. However, the genomewide approaches will lead to the identification of gene clusters. Eventually there will come a time when genome sequencing will become practical for these complex microbes. Presently, there is growing support for an effort to sequence a *Symbiodinium* genome. In combination with genomic data, DNA microarrays will help to understand the role the environment plays on the expression of polyketide biosynthetic genes and hopefully how it can be influenced to protect and enhance human health. The demand for novel polyketides in uses like medicinals, as molecular tools and in understanding toxin biosynthesis will continue to drive research in this field.

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REFERENCES

- Albertini AM, Caramori T, Scoffone F, Scotti C, Galizzi A. Sequence around the 159.degree. region of the *Bacillus subtilis* genome: The pksX locus spans 33.6 kb. *Microbiology* (Reading, United Kingdom) 1995;141:299–309.
- Baden DG, Bourdelais AJ, Jacocks H, Michelliza S, Naar J. Natural and derivative brevetoxins: Historical background, multiplicity, and effects. *Environ. Health Perspect* 2005;113:621–625. [PubMed: 15866774]
- Bae YM, Hastings JW. Cloning, sequencing and expression of dinoflagellate luciferase DNA from a marine alga *Gonyaulax polyedra*. *Biochim. Biophys. Acta* 1994;1219:449–456. [PubMed: 7918642]
- Balmer-Hanchey EL, Jaykus L, Green DP, McClellan-Green P. Marine biotoxins of algal origin and seafood safety. *J. Aquat. Food Prod. Technol* 2003;12:29–54.
- Bertomeu T, Morse D. Isolation of a dinoflagellate mitotic cyclin by functional complementation in yeast. *Biochem. Biophys. Res. Commun* 2004;323:1172–1183. [PubMed: 15451420]
- Bhaud Y, Guillebault D, Lennon J, Defacque H, Soyer-Gobillard MO, Moreau H. Morphology and behaviour of dinoflagellate chromosomes during the cell cycle and mitosis. *J. Cell Sci* 2000;113:1231–1239. [PubMed: 10704374]
- Bourdelais AJ, Campbell S, Jacocks H, Naar J, Wright JL, Carsi J, Baden DG. Brevenal is a natural inhibitor of brevetoxin action in sodium channel receptor binding assays. *Cell. Mol. Neurobiol* 2004;24:553–563. [PubMed: 15233378]
- Bourdelais AJ, Jacocks HM, Wright JL, Bigwarfe PM Jr, Baden DG. A new polyether ladder compound produced by the dinoflagellate *Karenia brevis*. *J. Nat. Prod* 2005;68:2–6. [PubMed: 15679307]
- Cembella AD, Lewis NI, Quilliam MA. Spirolide composition of micro-extracted pooled cells isolated from natural plankton assemblages and from cultures of the dinoflagellate *Alexandrium ostenfeldii*. *Nat. Toxins* 1999;7:197–206. [PubMed: 10945482]
- Chang Z, Sitachitta N, Rossi JV, Roberts MA, Platt PM, Jia J, Sherman DH, Gerwick WH. Biosynthetic pathway and gene cluster analysis of curacin A, an antitubulin natural product from the tropical marine cyanobacterium *Lyngbya majuscula*. *J. Nat. Prod* 2004;67:1356–1367. [PubMed: 15332855]
- Cheng Y, Tang G, Shen B. Type I polyketide synthase requiring a discrete acyltransferase for polyketide biosynthesis. *Proc. Natl. Acad. Sci. USA* 2003;100:3149–3154. [PubMed: 12598647]
- Chou HN, Shimizu Y. Biosynthesis of brevetoxins. Evidence for the mixed origin of the backbone carbon chain and possible involvement of dicarboxylic acids. *J. Am. Chem. Soc* 1987;109:2184–2185.
- Draisci R, Giannetti L, Lucentini L, Marchiafava C, James KJ, Bishop AG, Healy BM, Kelly SS. Isolation of a new okadaic acid analogue from phytoplankton implicated in diarrhetic shellfish poisoning. *J. Chromatogr. A* 1998;798:137–145. [PubMed: 9542135]
- Echigoya R, Rhodes L, Oshima Y, Satake M. The structures of five new antifungal and hemolytic amphidinol analogs from *Amphidinium carterae* collected in New Zealand. *Harmful Algae* 2005;4:383–389.
- Edwards DJ, Marquez BL, Nogle LM, McPhail K, Goeger DE, Roberts MA, Gerwick WH. Structure and biosynthesis of the Jamaicamides, new mixed polyketide-peptide neurotoxins from the marine cyanobacterium *Lyngbya majuscula*. *Chem. Biol* 2004;11:817–833. [PubMed: 15217615]
- El-Sayed AK, Hothersall J, Cooper SM, Stephens E, Simpson TJ, Thomas CM. Characterization of the mupirocin biosynthesis gene cluster from *Pseudomonas fluorescens* NCIMB 10586. *Chem. Biol* 2003;10:419–430. [PubMed: 12770824]
- Falk M, Burton IW, Hu T, Walter JA, Wright JLC. Assignment of the relative stereochemistry of the spirolides, macrocyclic toxins isolated from shellfish and from the cultured dinoflagellate *Alexandrium ostenfeldii*. *Tetrahedron* 2001;57:8659–8665.
- Fernandez JJ, Suarez-Gomez B, Souto ML, Norte M. Identification of new okadaic acid derivatives from laboratory cultures of *Prorocentrum lima*. *J. Nat. Prod* 2003;66:1294–1296. [PubMed: 14510622]

- Ferreira FM, Franco Soler JM, Fidalgo ML, Fernandez-Vila P. PSP toxins from *Aphanizomenon flos-aquae* (cyanobacteria) collected in the Crestuma-Lever reservoir (Douro river, northern Portugal). *Toxicon* 2001;39:757–761. [PubMed: 11137533]
- Finch SC, Wilkins AL, Hawkes AD, Jensen DJ, Mackenzie AL, Beuzenberg V, Quilliam MA, Olseng CD, Samdal IA, Aasen J, Selwood AI, Cooney JM, et al. Isolation and identification of (44-R,S)-44,55-dihydroxyessotoxin from *Protoceratium reticulatum*, and its occurrence in extracts of shellfish from New Zealand, Norway and Canada. *Toxicon* 2005;46:160–170. [PubMed: 15975615]
- Ghosh AK, Gong G. Total synthesis and structural revision of (+)-amphi-dinolide W. *J. Am. Chem. Soc* 2004;126:3704–3705. [PubMed: 15038710]
- Guillebault D, Sasorith S, Derelle E, Wurtz JM, Lozano JC, Bingham S, Tora L, Moreau H. A new class of transcription initiation factors, intermediate between TATA box-binding proteins (TBPs) and TBP-like factors (TLFs), is present in the marine unicellular organism, the dinoflagellate *Cryptocodinium cohnii*. *J. Biol. Chem* 2002;277:40881–40886. [PubMed: 12154093]
- Hallegraeff GM. A review of harmful algal blooms and their apparent global increase. *Phycologia* 1993;32:79–99.
- Herzog M, Maroteaux L. Dinoflagellate 17S rRNA sequence inferred from the gene sequence: Evolutionary implications. *Proc. Natl. Acad. Sci. USA* 1986;83:8644–8648. [PubMed: 16578795]
- Hold GL, Smith EA, Birkbeck TH, Gallacher S. Comparison of paralytic shellfish toxin (PST) production by the dinoflagellates *Alexandrium lusitanicum* NEPCC 253 and *Alexandrium tamarense* NEPCC 407 in the presence and absence of bacteria. *FEMS Microbiol. Ecol* 2001;36:223–234. [PubMed: 11451527]
- Holmes, MJ.; Lewis, R. Toxin-producing dinoflagellates. In: Menez, editor. *Perspectives in Molecular Toxicology*. Chichester: Wiley and Sons; 2002. p. 39–65.
- Houdai T, Matsuoka S, Murata M, Satake M, Ota S, Oshima Y, Rhodes LL. Acetate labeling patterns of dinoflagellate polyketides, amphidinols 2, 3 and 4. *Tetrahedron* 2001;57:5551–5555.
- Hu T, Burton IW, Cembella AD, Curtis JM, Quilliam MA, Walter JA, Wright JL. Characterization of spirolides a, c, and 13-desmethyl c, new marine toxins isolated from toxic plankton and contaminated shellfish. *J. Nat. Prod* 2001;64:308–312. [PubMed: 11277745]
- Huang X, Zhao D, Guo Y, Wu H, Trivellone E, Cimino G. Lingshuiols A and B, two new polyhydroxy compounds from the Chinese marine dinoflagellate *Amphidinium* sp. *Tetrahedron Lett* 2004;45:5501–5504.
- Igarashi T, Satake M, Yasumoto T. Structures and partial stereochemical assignments for prymnesin-1 and prymnesin-2: Potent hemolytic and ichthyotoxic glycosides isolated from the red tide alga *Prymnesium parvum*. *J. Am. Chem. Soc* 1999;121:8499–8511.
- James KJ, Bishop AG, Draisci R, Palleschi L, Marchiafava C, Ferretti E, Satake M, Yasumoto T. Liquid chromatographic methods for the isolation and identification of new pectenotoxin-2 analogues from marine phytoplankton and shellfish. *J. Chromatogr. A* 1999;844:53–65. [PubMed: 10399323]
- James KJ, Moroney C, Roden C, Satake M, Yasumoto T, Lehane M, Furey A. Ubiquitous “benign” alga emerges as the cause of shellfish contamination responsible for the human toxic syndrome, azaspiracid poisoning. *Toxicon* 2003a;41:145–151. [PubMed: 12565733]
- James KJ, Sierra MD, Lehane M, Brana Magdalena A, Furey A. Detection of five new hydroxyl analogues of azaspiracids in shellfish using multiple tandem mass spectrometry. *Toxicon* 2003b;41:277–283. [PubMed: 12565749]
- Kobayashi J, Tsuda M. Amphidinolides, bioactive macrolides from symbiotic marine dinoflagellates. *Nat. Prod. Rep* 2004;21:77–93. [PubMed: 15039836]
- Kobayashi J, Takahashi M, Ishibashi M. Biosynthetic studies of amphidinolide J: Explanation of the generation of the unusual odd-numbered macrocyclic lactone. *J. Chem. Soc. Chem. Comm* 1995:1639–1640.
- Kobayashi J, Shimbo K, Sato M, Shiro M, Tsuda M. Absolute stereo-chemistry of amphidinolides G and H. *Org. Lett* 2000;2:2805–2807. [PubMed: 10964370]
- Kobayashi J, Kubota T, Endo T, Tsuda M. Amphidinolides T2, T3, and T4, new 19-membered macrolides from the dinoflagellate *Amphidinium* sp. and the biosynthesis of amphidinolide T1. *J. Org. Chem* 2001;66:134–142. [PubMed: 11429890]

- Kobayashi J, Shimbo K, Sato M, Tsuda M. Amphidinolides H2–H5, G2, and G3, new cytotoxic 26- and 27-membered macrolides from dinoflagellate *Amphidinium* sp. J. Org. Chem 2002;67:6585–6592. [PubMed: 12227785]
- Kobayashi J, Shimbo K, Kubota T, Tsuda M. Bioactive macrolides and polyketides from marine dinoflagellates. Pure Appl. Chem 2003;75:337–342.
- Kodama M, Ogata T, Sakamoto S, Sato S, Honda T, Miwatani T. Production of paralytic shellfish toxins by a bacterium *Moraxella* sp. isolated from *Protogonyaulax tamarensis*. Toxicon 1990;28:707–714. [PubMed: 2402764]
- Konishi M, Yang X, Li B, Fairchild CR, Shimizu Y. Highly cytotoxic metabolites from the culture supernatant of the temperate dinoflagellate *Protoceratium* cf. *reticulatum*. J. Nat. Prod 2004;67:1309–1313. [PubMed: 15332847]
- Kubota T, Tsuda M, Takahashi M, Ishibashi M, Naoki H, Kobayashi J. Colopsinols B and C, new long chain polyhydroxy compounds from cultured marine dinoflagellate *Amphidinium* sp. J. Chem. Soc. Perk. T 1999;1:3483–3487.
- Kubota T, Tsuda M, Kobayashi J. Amphidinolide V, novel 14-membered macrolide from marine dinoflagellate *Amphidinium* sp. Tetrahedron Lett 2000a;41:713–716.
- Kubota T, Tsuda M, Takahashi M, Ishibashi M, Oka S, Kobayashi J. Colopsinols D and E, new polyhydroxyl linear carbon chain compounds from marine dinoflagellate *Amphidinium* sp. Chem. Pharm. Bull 2000b;48:1447–1451. [PubMed: 11045448]
- Kubota T, Endo T, Tsuda M, Shiro M, Kobayashi J. Amphidinolide T5, a new 19-membered macrolide from a dinoflagellate and X-ray structure of amphidinolide T1. Tetrahedron 2001a;57:6175–6179.
- Kubota T, Tsuda M, Kobayashi J. Biosynthetic study of amphidinolide C. Tetrahedron 2001b;57:5975–5977.
- Kubota T, Sakuma Y, Tsuda M, Kobayashi J. Amphidinolide C2, new macrolide from marine dinoflagellate *Amphidinium* species. Mar. Drugs 2004;2:83–87.
- Leadlay PF, Staunton J, Oliynyk M, Bisang C, Cortes J, Frost E, Hughes-Thomas ZA, Jones MA, Kendrew SG, Lester JB, Long PF, McArthur HAI, et al. Engineering of complex polyketide biosynthesis insights from sequencing of the monensin biosynthetic gene cluster. J. Ind. Microbiol. Biotechnol 2001;27:360–367. [PubMed: 11774001]
- Lee MS, Qin G, Nakanishi K, Zagorski MG. Biosynthetic studies of brevetoxins, potent neurotoxins produced by the dinoflagellate *Gymnodinium breve*. J. Am. Chem. Soc 1989;111:6234–6241.
- Li L, Hastings JW. The structure and organization of the luciferase gene in the photosynthetic dinoflagellate *Gonyaulax polyedra*. Plant Mol. Biol 1998;36:275–284. [PubMed: 9484439]
- Lidie KB, Ryan JC, Barbier M, Van Dolah FM. Gene expression in Florida red tide dinoflagellate *Karenia brevis*: Analysis of an expressed sequence tag library and development of DNA microarray. Mar. Biotechnol 2005;7:1–14. [PubMed: 15806334]
- Lippmeier JC, Brown AM, Apt KE. Isolation of algal genes by functional complementation of yeast. J. Phycol 2002;38:529–533.
- Lu CK, Chou HN, Lee CK, Lee TH. Prorocentin, a new polyketide from the marine dinoflagellate *Prorocentrum lima*. Org. Lett 2005;7:3893–3896. [PubMed: 16119925]
- Macpherson GR, Burton IW, LeBlanc P, Walter JA, Wright JLC. Studies of the biosynthesis of DTX-5a and DTX-5b by the dinoflagellate *Prorocentrum maculosum*: Regiospecificity of the putative Baeyer-Villigerase and insertion of a single amino acid in a polyketide chain. J. Org. Chem 2003;68:1659–1664. [PubMed: 12608776]
- Maruyama T. Extraction model of the longitudinal flagellum of *Ceratium tripos* (Dinoflagellida): Reactivation of flagellar retraction. J. Cell Sci 1985;75:313–328. [PubMed: 3850092]
- Miles CO, Wilkins AL, Stirling DJ, MacKenzie AL. New analogue of gymnodimine from a *Gymnodinium* species. J. Agric. Food Chem 2000;48:1373–1376. [PubMed: 10775400]
- Miles CO, Wilkins AL, Stirling DJ, MacKenzie AL. Gymnodimine C, an isomer of gymnodimine B, from *Karenia selliformis*. J. Agric. Food Chem 2003;51:4838–4840. [PubMed: 14705921]
- Miles CO, Wilkins AL, Hawkes AD, Selwood A, Jensen DJ, Aasen J, Munday R, Samdal IA, Briggs LR, Beuzenberg V, MacKenzie AL. Isolation of a 1,3-enone isomer of heptanor-41-oxoyessotoxin from *Protoceratium reticulatum* cultures. Toxicon 2004a;44:325–336. [PubMed: 15321705]

- Miles CO, Wilkins AL, Jensen DJ, Cooney JM, Quilliam MA, Aasen J, MacKenzie AL. Isolation of 41a-homoyessotoxin and the identification of 9-methyl-41a-homoyessotoxin and nor-ring A-yessotoxin from *Protoceratium reticulatum*. Chem. Res. Toxicol 2004b;17:1414–1422. [PubMed: 15540939]
- Miles CO, Wilkins AL, Samdal IA, Sandvik M, Petersen D, Quilliam MA, Naustvoll LJ, Rundberget T, Torgersen T, Hovgaard P, Jensen DJ, Cooney JM. A novel pectenotoxin, PTX-12, in *Dinophysis* spp. and shell-fish from Norway. Chem. Res. Toxicol 2004c;17:1423–1433. [PubMed: 15540940]
- Miles CO, Wilkins AL, Hawkes AD, Selwood AI, Jensen DJ, Munday R, Cooney JM, Beuzenberg V. Polyhydroxylated amide analogs of yessotoxin from *Protoceratium reticulatum*. Toxicon 2005;45:61–71. [PubMed: 15581684]
- Moreno Diaz de la Espina S, Alverca E, Cuadrado A, Franca S. Organization of the genome and gene expression in a nuclear environment lacking histones and nucleosomes: The amazing dinoflagellates. Eur. J. Cell Biol 2005;84:137–149. [PubMed: 15819396]
- Morohashi A, Satake M, Nagai H, Oshima Y, Yasumoto T. The absolute configuration of gambieric acids A–D, potent antifungal polyethers, isolated from the marine dinoflagellate *Gambierdiscus toxicus*. Tetrahedron 2000;56:8995–9001.
- Morsy N, Matsuoka S, Houdai T, Matsumori N, Adachi S, Murata M, Iwashita T, Fujita T. Isolation and structure elucidation of a new amphidinol with a truncated polyhydroxyl chain from *Amphidinium klebsii*. Tetrahedron 2005;61:8606–8610.
- Muller R. Don't classify polyketide synthases. Chem. Biol 2004;11:4–6. [PubMed: 15112984]
- Murata M, Izumikawa M, Tachibana K, Fujita T, Naoki H. Labeling pattern of okadaic acid from 18O2 and [18O2] acetate elucidated by collisionally induced dissociation tandem mass spectrometry. J. Am. Chem. Soc 1998;120:147–151.
- Murata M, Matsuoka S, Matsumori N, Paul GK, Tachibana K. Absolute configuration of amphidinol 3, the first complete structure determination from amphidinol homologues: Application of a new configuration analysis based on carbon-hydrogen spin-coupling constants. J. Am. Chem. Soc 1999;121:870–871.
- Needham J, McLachlan JL, Walter JA, Wright JLC. Biosynthetic origin of C-37 and C-38 in the polyether toxins okadaic acid and dinophysistoxin-1. J. Chem. Soc. Chem. Comm 1994;22:2599–2600.
- Needham J, Hu T, McLachlan JL, Walter JA, Wright JLC. Biosynthetic studies of the DSP toxin DTX-4 and an okadaic acid diol ester. J. Chem. Soc. Chem. Comm 1995;16:1623–1624.
- Nicolaou KC, Koftis TV, Vyskocil S, Petrovic G, Ling T, Yamada YM, Tang W, Frederick MO. Structural revision and total synthesis of azaspiracid-1, part 2: Definition of the ABCD domain and total synthesis. Angew. Chem. Int. Ed. Engl 2004;43:4318–4324. [PubMed: 15368381]
- Ofuji K, Satake M, McMahon T, Silke J, James KJ, Naoki H, Oshima Y, Yasumoto T. Two analogs of azaspiracid isolated from mussels, *Mytilus edulis*, involved in human intoxication in Ireland. Nat. Toxins 1999;7:99–102. [PubMed: 10647511]
- Oliynyk M, Stark CB, Bhatt A, Jones MA, Hughes-Thomas ZA, Wilkinson C, Oliynyk Z, Demydchuk Y, Staunton J, Leadlay PF. Analysis of the biosynthetic gene cluster for the polyether antibiotic monensin in *Streptomyces cinnamomensis* and evidence for the role of monB and monC genes in oxidative cyclization. Mol. Microbiol 2003;49:1179–1190. [PubMed: 12940979]
- Paitan Y, Orr E, Ron EZ, Rosenberg E. Genetic and functional analysis of genes required for the post-modification of the polyketide antibiotic TA of *Myxococcus xanthus*. Microbiology (Reading, United Kingdom) 1999;145:3059–3067.
- Plumley FG. Marine algal toxins: Biochemistry genetics, and molecular biology. Limnol. Oceanogr 1997;42:1252–1264.
- Prasad AVK, Shimizu Y. The structure of hemibrevetoxin-B: A new type of toxin in the Gulf of Mexico red tide organism. J. Am. Chem. Soc 1989;111:6476–6477.
- Rae PMM. Hydroxymethyluracil in eukaryote DNA: A natural feature of the *Pyrrophyta* (Dinoflagellates). Science (Wash.) 1976;194:1062–1064. [PubMed: 988637]
- Ramsey UP, Douglas DJ, Walter JA, Wright JL. Biosynthesis of domoic acid by the diatom *Pseudo-nitzschia multiseriata*. Nat. Toxins 1998;6:137–146. [PubMed: 10223629]
- Rawlings BJ. Biosynthesis of polyketides (other than actinomycete macrolides). Nat. Prod. Rep 1999;16:425–484. [PubMed: 10467738]

- Rein KS, Borrone J. Polyketides from dinoflagellates: Origins, pharmacology and biosynthesis. *Comp. Biochem. Physiol. B Biochem. Mol. Biol* 1999;124:117–131. [PubMed: 10584296]
- Rizzo PJ. Those amazing dinoflagellate chromosomes. *Cell Res* 2003;13:215–217. [PubMed: 12974611]
- Rodriguez E, McDaniel R. Combinatorial biosynthesis of antimicrobials and other natural products. *Curr. Opin. Microbiol* 2001;4:526–534. [PubMed: 11587928]
- Santos SR, Coffroth MA. Molecular genetic evidence that dinoflagellates belonging to the genus *Symbiodinium* Freudenthal are haploid. *Biol. Bull* 2003;204:10–20. [PubMed: 12588740]
- Satake M. Biosynthesis of the marine polyether toxin, yessotoxin. *Tennen Yuki Kagobutsu Toronkai Koen Yoshishu* 2000;42:259–264.
- Satake M, Ofuji K, Naoki H, James KJ, Furey A, McMahon T, Silke J, Yasumoto T. Azaspiracid, a new marine toxin having unique spiro ring assemblies, isolated from Irish mussels, *Mytilus edulis*. *J. Am. Chem. Soc* 1998;120:9967–9968.
- Sato M, Shimbo K, Tsuda M, Kobayashi J. Biosynthetic studies of amphidinolides G and H: Unusual labeling patterns in feeding experiments with ¹³C-labeled acetates. *Tetrahedron Lett* 2000;41:503–506.
- Schweizer M, Roberts LM, Holtke HJ, Takabayashi K, Hollerer E, Hoffmann B, Muller G, Kottig H, Schweizer E. The pentafunctional FAS1 gene of yeast: Its nucleotide sequence and order of the catalytic domains. *Mol. Gen. Genet* 1986;203:479–486. [PubMed: 3528750]
- Shen B. Polyketide biosynthesis beyond the type I, II and III polyketide synthase paradigms. *Curr. Opin. Chem. Biol* 2003;7:285–295. [PubMed: 12714063]
- Shimbo K, Tsuda M, Izui N, Kobayashi J. Amphidinolide W, a new 12-membered macrolide from dinoflagellate *Amphidinium* sp. *J. Org. Chem* 2002;67:1020–1023. [PubMed: 11856056]
- Shimizu Y. Microalgal metabolites. *Curr. Opin. Microbiol* 2003;6:236–243. [PubMed: 12831899]
- Shimizu Y, Gupta S, Masuda K, Maranda L, Walker CK, Wang R. Dinoflagellate and other microalgal toxins: Chemistry and biochemistry. *Pure Appl. Chem* 1989;61:513–516.
- Sleno L, Windust AJ, Volmer DA. Structural study of spirolide marine toxins by mass spectrometry. Part I. Fragmentation pathways of 13-desmethyl spirolide C by collision-induced dissociation and infrared multiphoton dissociation mass spectrometry. *Anal. Bioanal. Chem* 2004;378:969–976. [PubMed: 14647954]
- Smayda TJ. What is a bloom? A commentary. *Limnol. Oceanogr* 1997;42:1132–1136.
- Snyder RV, Gibbs PDL, Palacios A, Abiy L, Dickey R, Lopez JV, Rein KS. Polyketide synthase genes from marine dinoflagellates. *Mar. Biotechnol* 2003;5:1–12. [PubMed: 12925913]
- Snyder RV, Guerrero MA, Sinigalliano CD, Winshell J, Perez R, Lopez JV, Rein KS. Localization of polyketide synthase encoding genes to the toxic dinoflagellate *Karenia brevis*. *Phytochemistry* 2005;66:1767–1780. [PubMed: 16051286]
- Souto ML, Fernandez JJ, Franco JM, Paz B, Gil LV, Norte M. Glycoyessotoxin a, a new yessotoxin derivative from cultures of *Protoceratium reticulatum*. *J. Nat. Prod* 2005;68:420–422. [PubMed: 15787449]
- Spector, DL. Dinoflagellates: An introduction. In: Spector, DL., editor. *Dinoflagellates*. London: Academic Press; 1984. p. 1–15.
- Suarez-Gomez B, Souto ML, Norte M, Fernandez JJ. Isolation and structural determination of DTX-6, a new okadaic acid derivative. *J. Nat. Prod* 2001;64:1363–1364. [PubMed: 11678670]
- Suarez-Gomez B, Souto ML, Cruz PG, Fernandez JJ, Norte M. New targets in diarrhetic shellfish poisoning control. *J. Nat. Prod* 2005;68:596–599. [PubMed: 15844958]
- Sun Y, Zhou X, Dong H, Tu G, Wang M, Wang B, Deng Z. A complete gene cluster from *Streptomyces nanchangensis* NS3226 encoding biosynthesis of the polyether ionophore nanchangmycin. *Chem. Biol* 2003;10:431–441. [PubMed: 12770825]
- Taroncher-Oldenburg G, Anderson DM. Identification and characterization of three differentially expressed genes, encoding S-adenosylhomocysteine hydrolase, methionine aminopeptidase, and a histone-like protein, in the toxic dinoflagellate *Alexandrium fundyense*. *Appl. Environ. Microbiol* 2000;66:2105–2112. [PubMed: 10788388]
- Taroncher-Oldenburg G, Kulis DM, Anderson DM. Toxin variability during the cell cycle of the dinoflagellate *Alexandrium fundyense*. *Limnol. Oceanogr* 1997;42:1178–1188.

- Ten Lohuis MR, Miller DJ. Hypermethylation at CPG-motifs in the dinoflagellates *Amphidinium carterae* (Dinophyceae) and *Symbiodinium microadriaticum* (Dinophyceae): Evidence from restriction analyses, 5-azacytidine and ethionine treatment. *J. Phycol* 1998;34:152–159.
- Trost BM, Harrington PE. Structure elucidation of (+)-amphidinolide a by total synthesis and NMR chemical shift analysis. *J. Am. Chem. Soc* 2004;126:5028–5029. [PubMed: 15099060]
- Tsuda M, Endo T, Kobayashi J. Amphidinolide U, novel 20-membered macrolide from marine dinoflagellate *Amphidinium* sp. *Tetrahedron* 1999;55:14565–14570.
- Tsuda M, Endo T, Kobayashi J. Amphidinolide T, novel 19-membered macrolide from marine dinoflagellate *Amphidinium* sp. *J. Org. Chem* 2000;65:1349–1352. [PubMed: 10814095]
- Tsuda M, Kubota T, Sakuma Y, Kobayashi J. Biosynthetic study of amphidinolide B. *Chemical & Pharmaceutical Bulletin* 2001;49:1366–1367.
- Tsuda M, Izui N, Sato M, Kobayashi J. Biosynthetic study of amphidinolide W. *Chem. Pharm. Bull* 2002;50:976–977. [PubMed: 12130859]
- Tsuda M, Izui N, Shimbo K, Sato M, Fukushi E, Kawabata J, Katsumata K, Horiguchi T, Kobayashi J. Amphidinolide X, a novel 16-membered macrodiolide from dinoflagellate *Amphidinium* sp. *J. Org. Chem* 2003a;68:5339–5345. [PubMed: 12816496]
- Tsuda M, Izui N, Shimbo K, Sato M, Fukushi E, Kawabata J, Kobayashi J. Amphidinolide Y, a novel 17-membered macrolide from dinoflagellate *Amphidinium* sp.: Plausible biogenetic precursor of amphidinolide X. *J. Org. Chem* 2003b;68:9109–9112. [PubMed: 14604389]
- Tsuda M, Kariya Y, Iwamoto R, Fukushi E, Kawabata J, Kobayashi J. Amphidinolides B4 and B5, potent cytotoxic 26-membered macrolides from dinoflagellate *Amphidinium* species. *Marine Drugs* 2005;3:1–8.
- Withers, N. Dinoflagellate sterols. In: Shever, PJ., editor. *Marine Natural Products*. Vol. vol. 5. New York: Academic Press; 1983. p. 87-130.
- Wright JLC, Hu T, McLachlan JL, Needham J, Walter JA. Biosynthesis of DTX-4: Confirmation of a polyketide pathway, proof of a Baeyer-Villiger oxidation step, and evidence for an unusual carbon deletion process. *J. Am. Chem. Soc* 1996;118:8757–8758.
- Xiang L, Kalaitzis JA, Moore BS. EncM, a versatile enterocin biosynthetic enzyme involved in Favorskii oxidative rearrangement, aldol condensation, and heterocycle-forming reactions. *Proc. Natl. Acad. Sci. USA* 2004;101:15609–15614. [PubMed: 15505225]
- Yasumoto T. The chemistry and biological function of natural marine toxins. *Chem. Rec* 2001;1:228–242. [PubMed: 11895121]
- Zhu G, Marchewka MJ, Woods KM, Upton SJ, Keithly JS. Molecular analysis of a Type I fatty acid synthase in *Cryptosporidium parvum*. *Mol. biochem. parasitol* 2000;105:253–260. [PubMed: 10693747]
- Zhu G, LaGier MJ, Stejskal F, Millership JJ, Cai X, Keithly JS. *Cryptosporidium parvum*: The first protist known to encode a putative polyketide synthase. *Gene* 2002;298:79–89. [PubMed: 12406578]

FIG. 1. Marine algal toxins responsible for classified human illnesses. Also shown are ester derivatives of okadaic acid.

FIG. 2.
Novel polyketides from dinoflagellates. See text for details.

FIG. 3.
 New amphidinolides from *Amphidinium* species.

FIG. 4.
Amphidinol 7, lingshuiol, colopsinols B and C, spiorides A–D, and gymnodimine B and C.

FIG. 5.

Labeling pattern of polyketides from stable isotope feeding experiments including amphidinolides, amphidinols, okadaic acid and dinophys toxins, and yessotoxin. Legend: g = glycolate, c = C1 acetate, m = C2 acetate, Me represents methyl from S-adenosyl methionine, bold lines represent intact acetates, unlabeled positions represent C2 of acetate unless otherwise noted. The red subunit originated from glycine. For amphidinol 3, only C1 labeled acetate was fed as a precursor, the origin of other positions was not determined. * Glycolate feeding experiments were not conducted for these polyketides, but source is presumed to be glycolate by analogy with DTX-4.

FIG. 6. Proposed mechanism for brevetoxin production and monensin biosynthesis. Both polyenes are epoxidized and undergo cyclization.

TABLE I

Shared characteristics of most dinoflagellates

Characteristic	References
Relatively large amount of DNA	Spector, 1984
Unusual sterols	Withers, 1983
Hypermethylated DNA	Ten Lohuis and Miller, 1998
Hydroxymethyluracil substituted for thymine	Rae, 1976
Most are uninucleates, but some binucleates exist	Rizzo, 2003
Permanently condensed chromosomes	Rizzo, 2003
Lack histones and nucleosomes	Moreno Diaz <i>et al.</i> , 2005
Possess a vegetative haploid nuclear phase	Santos and Coffroth, 2003
Typical eukaryotic nuclear envelope, but it remains intact during mitosis	Bhaud <i>et al.</i> , 2000
Typical eukaryotic 9 + 2 axoneme flagella	Maruyama, 1985
Membrane-bound organelles (endoplasmic reticula, Golgi apparatus, mitochondria, and chloroplasts)	Spector, 1984
Percentages of repetitive DNA (50–60%) consistent with higher eukaryotic genomes	Moreno Diaz <i>et al.</i> , 2005
Typical eukaryotic cell cycle	Moreno Diaz <i>et al.</i> , 2005
Heavy (28S) and light (17S) rRNA that is structurally similar to higher eukaryotes	Herzog and Maroteaux, 1986
Lack the TATA-box (typical eukaryotic promoter)	Guillebault <i>et al.</i> , 2002

TABLE II

Algal-related human poisoning syndromes and Causative agents

Syndrome	Toxins	Symptoms	Causative organisms	Route of acquisition
Azaspic acid poisoning (AZP)	Azaspic acids	Acute gastroenteritis	<i>Protophycidium crassipes</i>	Eating tainted shellfish
Amnesic shellfish poisoning (ASP)	Domoic acid	Gastroenteritis followed by neurologic manifestations leading to amnesia, coma, and death in severe cases	<i>Pseudo-nitzschia multiseries</i> ;	Eating tainted shellfish
Ciguatera fish poisoning (CFP)	Ciguatoxin, maitotoxin	Acute gastroenteritis followed by paresthesias and other neurologic symptoms	<i>Gambierdiscus toxicus</i>	Eating predacious reef fish (e.g., barracuda)
Diarrhetic shellfish poisoning (DSP)	Okadaic acid dinophy-sistoxins	Acute gastroenteritis	<i>Prorocentrum lima</i> <i>Dinophysis acuta</i> <i>D. fortii</i> <i>D. norvegica</i>	Eating tainted shellfish
Neurotoxic shellfish poisoning (NSP)	Brevetoxins	Neurologic symptoms	<i>Karenia brevis</i> <i>K. cf brevis</i> (New Zealand) Raphidophytes	Eating tainted shellfish
Paralytic shellfish poisoning (PSP)	Saxitoxins	Acute paresthesias and other neurologic manifestations; may progress rapidly to respiratory paralysis	<i>Alexandrium catenella</i> <i>A. minutum</i> <i>A. tamarense</i> <i>Gymnodinium catenatum</i> <i>Pyrodinium bahamense</i>	Eating tainted shellfish

TABLE III
PKS-Related Ests from K. brevis and their translated BLAST best match in the Genbank NR Database*

ESTMGID #	EST accession #	Translated BLAST best match	% identity/% positive	Score/e value	Proposed function (active site)
4	CO059032	Malonyl CoA-ACP transacylase [<i>Arabidopsis thaliana</i>]	39/56	112/1e-23	Acyltransferase
2796	CO063201	Acetyl-CoA acyltransferase, [<i>Bos taurus</i>]	70/83	186/7e-46	Acyltransferase
1008	CO517375	Type I polyketide synthase [<i>Mycobacterium avium</i>]	35/53	108/2e-22	Ketosynthase (DTECCSA)
4332	CO064722	Type I polyketide synthase [<i>Nostoc punctiforme</i>]	37/54	71.6/8e-12	Ketosynthase (DTACSAS)
5361	CO059138	Type I polyketide synthase [<i>Anabaena variabilis</i>]	37/55	135/1e-30	Ketosynthase (DTACSAS)
6736	CO060493	Type I polyketide synthase [<i>Nostoc</i> sp. <i>PCC 7120</i>]	42/57	157/5e-37	Ketosynthase (DTACSAS)
4718	CO065100	Type I polyketide synthase [<i>Desulfovibrio desulfuricans</i>]	33/51	58.9/1e-07	Ketosynthase (N-C termini boundary)
6371	CO060132	Type I polyketide synthase [<i>Streptomyces rochei</i>]	30/47	63.5/7e-09	Ketosynthase (N-C termini boundary)
4674	CO065056	Acyl-CoA thioesterase [<i>Strongylocentrotus purpuratus</i>]	48/67	108/7e-23	Thioesterase
6862	CO060619	Acyl-CoA thioesterase [<i>Strongylocentrotus purpuratus</i>]	48/67	107/2e-22	Thioesterase

* The listed EST accession numbers correspond to the EST's MGID (marine genomics identification number).