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(54) Title: DETECTION OF DINOFLAGELLATES

(57) Abstract: Provided herein is technology relating to detecting saxitoxin-producing dinoflagellates and particularly, but not exclusively, to methods and compositions for detecting dinoflagellates by amplification of the sxtG gene.



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DETECTION OF DINOFLAGELLATES

FIELD OF TECHNOLOGY

Provided herein is technology relating to detecting saxitoxin-producing
5 dinoflagellates and particularly, but not exclusively, to methods and
compositions for detecting dinoflagellates by amplification of the *sxtG* gene.

BACKGROUND

Saxitoxin (STX), commonly known as paralytic shellfish poison (PSP), and its
10 derivatives, are environmental neurotoxins that can cause severe symptoms
upon consumption of vector species (see, e.g., Deeds JR, et al. (2008), “Non-
traditional vectors for paralytic shellfish poisoning”, *Marine Drugs* **6**: 308-348;
Wiese M, et al. (2010) “Neurotoxic Alkaloids: Saxitoxin and Its Analogs”, *Marine
Drugs* **8**: 2185-2211). These toxic compounds accumulate in shellfish and in other
15 marine organisms that feed on dinoflagellates. Ingestion of these saxitoxin-
infested vector species by humans may cause paralytic shellfish poisoning, which
is a sickness that can result in paralysis and, in severe cases, death. All but one
of the classical seafood poisoning syndromes—paralytic (PSP), diarrhetic (DSP),
neurotoxic (NSP), azaspiracid shellfish poisoning (AZP), and ciguatera fish
20 poisoning (CFP)—are caused by toxins produced by eukaryotic marine
dinoflagellates. An estimated 2000 cases of human paralytic shellfish poisoning,
with a mortality rate of 15%, occur globally each year. The costs of monitoring
and mitigation of STX have led to an annual economic loss from harmful
plankton blooms calculated at US \$895 million.

25 Conventional detection of dinoflagellates comprises regular collection of
water samples and manual analysis to detect the presence of potentially toxic
dinoflagellate species. If dinoflagellates are present in sufficient numbers, the
aquaculture area affected (e.g., a mussel bed) is closed. In addition, chemical
methods (e.g., HPLC and LCMS) are often used to measure the amount of
30 saxitoxin in affected tissues (e.g., mussel tissue). Sometimes a mouse bioassay is
used.

SUMMARY

Provided herein are technologies related to improved detection of dinoflagellates, in particular saxitoxin-producing dinoflagellates. During the development of the technologies provided herein, the *sxtG* gene was identified and sequenced. These new isolated genes and gene sequences provided a basis for the development of PCR targets in *sxtG* and appropriate primers for the amplification of the *sxtG* gene. These findings and technologies form the basis of an improved test for dinoflagellate detection. The *sxtG* gene is an addition to the *sxtA* gene previously identified. As such, in some embodiments, a double assay based on detecting both *sxtA* and *sxtG* provides increased accuracy in identification of STX producing organisms present in environmental samples.

Accordingly, provided herein is technology related to a method for detecting a saxitoxin-producing dinoflagellate in a sample, the method comprising obtaining a sample for use in the method, and analyzing the sample for the presence or absence of one or more of a dinoflagellate saxitoxin G polynucleotide or a polypeptide encoded by said polynucleotide, wherein the presence of said polynucleotide or polypeptide indicates the presence of a saxitoxin-producing dinoflagellate in the sample and wherein the absence of said polynucleotide or polypeptide indicates the absence of a saxitoxin-producing dinoflagellate in the sample. In some embodiments, the polynucleotide comprises a saxitoxin G nucleotide sequence selected from those set forth in any one of SEQ ID NOs: 12–13, or a fragment or variant of any one of those sequences. In some embodiments, the method further comprises analyzing the sample for the presence or absence of one or more of a dinoflagellate saxitoxin A polynucleotide or a polypeptide encoded by said polynucleotide.

For example, some embodiments provide a method wherein said analyzing comprises amplification of polynucleotides from the sample by polymerase chain reaction. In some embodiments, the polymerase chain reaction utilises one or more primers comprising a sequence set forth in SEQ ID NOs: 1–9, or a fragment or variant of any of these sequences. In some embodiments, the polypeptide comprises a saxitoxin G amino acid sequence, or a fragment or variant thereof. In addition, in some embodiments, the saxitoxin producing dinoflagellate is from

the genus *Alexandrium*, *Pyrodinium*, or *Gymnodinium*. In more specific embodiments, the saxitoxin-producing dinoflagellate is selected from the group consisting of *A. catenella*, *A. fundyense*, *A. lusitanicum*, *A. minutum*, *A. ostenfeldii*, *A. tamarense*, *G. catenatum*, and *P. bahamense var compressum*.

5 Embodiments of the technology also comprise kits for the detection of a saxitoxin-producing dinoflagellate in a sample or for determining the absence of a saxitoxin-producing dinoflagellate in a sample, the kits comprising at least one agent for detecting the presence of a dinoflagellate saxitoxin G polynucleotide or a polypeptide encoded by said polynucleotide. In some embodiments, the agent
10 binds specifically to a polynucleotide comprising a saxitoxin G nucleotide sequence selected from those set forth in any one of SEQ ID NOs: 12–13, or a fragment or variant of any one of those sequences. In some embodiments, the agent binds specifically to a polynucleotide encoding a saxitoxin G peptide sequence or a fragment thereof. Additional embodiments further comprise at
15 least one agent for detecting the presence of a dinoflagellate saxitoxin A polynucleotide or a polypeptide encoded by said polynucleotide. For example, in some embodiments, the agent is a primer, a probe, or an antibody. Specific embodiments provide an agent that is a primer comprising a sequence set forth in SEQ ID NOs: 1–9, or a fragment or variant of any of these sequences. For
20 example, in some embodiments, the agent binds specifically to a saxitoxin G amino acid sequence, or a fragment or variant thereof.

 The technology encompasses an isolated polynucleotide comprising a sequence set forth in SEQ ID NO: 12 or 13, or a variant or fragment thereof and an isolated polynucleotide comprising the sequence set forth in any one of SEQ
25 ID NOS: 1–9, or a variant or fragment of any one of those sequences. In addition, embodiments include an isolated polypeptide encoded by these polynucleotides.

 Additional embodiments will be apparent to persons skilled in the relevant art based on the teachings contained herein.

30 BRIEF DESCRIPTION OF THE DRAWINGS

 These and other features, aspects, and advantages of the present technology will become better understood with regard to the following drawings:

Figure 1 is a sequence comprising *sxtG* from *A. fundyense*.

Figure 2 is a sequence comprising *sxtG* from *A. minutum*.

It is to be understood that the figures are not necessarily drawn to scale, nor are the objects in the figures necessarily drawn to scale in relationship to one another. The figures are depictions that are intended to bring clarity and understanding to various embodiments of apparatuses, systems, and methods disclosed herein. Wherever possible, the same reference numbers will be used throughout the drawings to refer to the same or like parts. Moreover, it should be appreciated that the drawings are not intended to limit the scope of the present teachings in any way.

DETAILED DESCRIPTION

Provided herein is technology relating to detecting saxitoxin-producing dinoflagellates and particularly, but not exclusively, to methods and compositions for detecting dinoflagellates by amplification of the *sxtG* gene.

The section headings used herein are for organizational purposes only and are not to be construed as limiting the described subject matter in any way.

In this detailed description of the various embodiments, for purposes of explanation, numerous specific details are set forth to provide a thorough understanding of the embodiments disclosed. One skilled in the art will appreciate, however, that these various embodiments may be practiced with or without these specific details. In other instances, structures and devices are shown in block diagram form. Furthermore, one skilled in the art can readily appreciate that the specific sequences in which methods are presented and performed are illustrative and it is contemplated that the sequences can be varied and still remain within the spirit and scope of the various embodiments disclosed herein.

All literature and similar materials cited in this application, including but not limited to, patents, patent applications, articles, books, treatises, and internet web pages are expressly incorporated by reference in their entirety for any purpose. Unless defined otherwise, all technical and scientific terms used herein have the same meaning as is commonly understood by one of ordinary

skill in the art to which the various embodiments described herein belongs. When definitions of terms in incorporated references appear to differ from the definitions provided in the present teachings, the definition provided in the present teachings shall control.

5 It will be appreciated that there is an implied “about” prior to the temperatures, concentrations, times, etc. discussed in the present teachings, such that slight and insubstantial deviations are within the scope of the present teachings. In this application, the use of the singular includes the plural unless specifically stated otherwise. Also, the use of “comprise”, “comprises”,
10 “comprising”, “contain”, “contains”, “containing”, “include”, “includes”, and “including” are not intended to be limiting. It is to be understood that both the foregoing general description and the following detailed description are exemplary and explanatory only and are not restrictive of the present teachings.

Further, unless otherwise required by context, singular terms shall
15 include pluralities and plural terms shall include the singular. Generally, nomenclatures utilized in connection with, and techniques of, cell and tissue culture, molecular biology, and protein and oligo- or polynucleotide chemistry and hybridization described herein are those well known and commonly used in the art. Standard techniques are used, for example, for nucleic acid purification
20 and preparation, chemical analysis, recombinant nucleic acid, and oligonucleotide synthesis. Enzymatic reactions and purification techniques are performed according to manufacturer’s specifications or as commonly accomplished in the art or as described herein. The techniques and procedures described herein are generally performed according to conventional methods well
25 known in the art and as described in various general and more specific references that are cited and discussed throughout the instant specification. See, e.g., Sambrook et al., *Molecular Cloning: A Laboratory Manual* (Third ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. 2000). The nomenclatures utilized in connection with, and the laboratory procedures and
30 techniques described herein are those well known and commonly used in the art.

Definitions

To facilitate an understanding of the present technology, a number of terms and phrases are defined below. Additional definitions are set forth throughout the detailed description.

5 Throughout the specification and claims, the following terms take the meanings explicitly associated herein, unless the context clearly dictates otherwise. The phrase “in one embodiment” as used herein does not necessarily refer to the same embodiment, though it may. Furthermore, the phrase “in another embodiment” as used herein does not necessarily refer to a different
10 embodiment, although it may. Thus, as described below, various embodiments of the technology may be readily combined, without departing from the scope or spirit of the technology.

 In addition, as used herein, the term “or” is an inclusive “or” operator and is equivalent to the term “and/or” unless the context clearly dictates otherwise.

15 The term “based on” is not exclusive and allows for being based on additional factors not described, unless the context clearly dictates otherwise. In addition, throughout the specification, the meaning of “a”, “an”, and “the” include plural references. The meaning of “in” includes “in” and “on.”

 As used herein, the term “saxitoxin” encompasses pure saxitoxin and
20 analogs of thereof, non-limiting examples of which include neosaxitoxin (neoSTX), gonyautoxins (GTX), decarbamoylsaxitoxin (dcSTX), non-sulfated analogs, mono-sulfated analogs, disulfated analogs, decarbamoylated analogs and hydrophobic analogs.

 As used herein, “STX” refers to a saxitoxin.

25 As used herein, “SXT” refers to a gene product (e.g., a polypeptide) of an *sxt* gene such as *sxtG* and/or *sxtA*.

 A polynucleotide “fragment” as contemplated herein is a polynucleotide molecule that is a constituent of a polynucleotide of the technology or variant thereof. Fragments of a polynucleotide do not necessarily need to encode
30 polypeptides which retain biological activity although this is not excluded from being the case. In certain embodiments the fragment may be useful as a hybridization probe or PCR primer. The fragment may be derived by cleaving a

polynucleotide of the technology or alternatively may be synthesized by some other means, for example by chemical synthesis. A polynucleotide fragment as contemplated herein may be less than about 5000 nucleotides in length, less than about 4500 nucleotides in length, or less than about 4000, 3500, 3000, 2500, 2000, 1500, 1000, 750, 500, 400, 300, 250, 200, 150, 100, 75, 50, 25 or 15 nucleotides in length. Additionally or alternatively, a polynucleotide fragment as contemplated herein may be more than about 15 nucleotides in length, more than about 25 nucleotides in length, or more than about 50, 75, 100, 150, 200, 250, 300, 400, 500, 750, 1000, 1500, 2000, 2500, 3000, 3500 or 4000 nucleotides in length. Additionally or alternatively, a polynucleotide fragment as contemplated herein may be between about 25 and about 50 nucleotides in length, between about 25 and about 75 nucleotides in length, or between about 25 and about 100, 100 and 250, 100 and 500, 250 and 500, 100 and 1000, 500 and 2000, 1000 and 2000 nucleotides in length. Polynucleotide fragments of the technology comprise fragments of the *sxtG* gene.

A polypeptide "fragment" as contemplated herein is a polypeptide molecule is a constituent of a polypeptide of the technology or variant thereof. Typically the fragment possesses qualitative biological activity in common with the polypeptide of which it is a constituent though this is not necessarily required. A polypeptide fragment as contemplated herein may be less than about 1500 amino acid residues in length, less than about 1400 amino acid residues in length, or less than about 1300, 1200, 1100, 1000, 900, 800, 700, 600, 500, 400, 300, 250, 200, 150, 100, 75, 50, 25 or 15 amino acid residues in length. Additionally or alternatively, a polypeptide fragment as contemplated herein may be more than about 15 amino acid residues in length, more than about 25 amino acid residues in length, or more than about 50, 75, 100, 150, 200, 250, 300, 400, 500, 600, 700, 800, 900, 1000, 1100, 1200, or 1300 amino acid residues in length. Additionally or alternatively, a polypeptide fragment as contemplated herein may be between about 15 and about 25 amino acid residues in length, between about 15 and about 50 amino acid residues in length, or between about 15 and 75, 15 and 100, 15 and 150, 25 and 50, 25 and 100, 50 and 100, 50 and

150, 100 and 200, 100 and 250, 100 and 300, 100 and 500, 500 and 750, 500 and 1000, or 1000 and 1300 amino acid residues in length.

Polypeptide fragments of the technology comprise fragments of the *sxtG* protein or the saxitoxin protein.

5

Embodiments of the technology

Disclosed herein are dinoflagellate saxitoxin polynucleotide and polypeptide sequences (“polynucleotides of the technology” and “polypeptides of the technology”, respectively). Polynucleotides of the technology may be
 10 deoxyribonucleic acids (DNA), ribonucleic acids (RNA), or complementary deoxyribonucleic acids (cDNA). In certain embodiments, the sequences are saxitoxin G gene (*sxtG*) polynucleotide sequences or saxitoxin G polypeptide (SXTG) sequences.

In some embodiments, the polynucleotide and polypeptide sequences are
 15 from saxitoxin producing dinoflagellates. For example, the polynucleotide and polypeptide sequences may be from dinoflagellates of the order *Gonyaulacales* or *Gymnodiniales*. Preferably, the dinoflagellates are of the genus *Alexandrium* (formerly *Gonyaulax*), *Pyrodinium*, or *Gymnodinium*.

20 **sxtG**

The biosynthetic pathway and genes synthesizing STX have been reported in numerous freshwater cyanobacterial species (Mihali T, et al. (2009)

“Characterisation of the paralytic shellfish toxin biosynthesis gene clusters in *Anabaena circinalis* AWQC131C and *Aphanizomenon* sp. NH-5”, *BMC*

25 *Biochemistry* 10: 8; Moustafa A, et al. (2009) “Origin of Saxitoxin Biosynthetic Genes in Cyanobacteria”, *PlosOne* 4; Kellmann R, et al. (2008) “Biosynthetic intermediate analysis and functional homology reveal a saxitoxin gene cluster in cyanobacteria”, *Applied and Environmental Microbiology* 74: 4044-4053; Stucken K, et al. (2010) “The Smallest Known Genomes of Multicellular and Toxic

30 Cyanobacteria: Comparison, Minimal Gene Sets for Linked Traits and the Evolutionary Implications”, *PlosOne* 5; Mihali TK, et al. (2011) “A Putative Gene Cluster from a *Lyngbya wollei* Bloom that Encodes Paralytic Shellfish Toxin

Biosynthesis”, *PlosOne* 6). For example, *sxtA*, the starting gene of saxitoxin synthesis, was recently identified in dinoflagellates (e.g., *Gymnodinium catenatum* and multiple *Alexandrium* species) (Stüken A, et al. (2011) “Discovery of Nuclear-Encoded Genes for the Neurotoxin Saxitoxin in Dinoflagellates”, *PlosOne* 6: e20096). The technology provided herein provides *sxtG*, the second core gene in the STX synthesis pathway. By comparison to its cyanobacterial homologs, *sxtG* was predicted to encode an amidinotransferase domain. In cyanobacteria, the product of *sxtA* is the substrate for the amidinotransferase *sxtG*. *SxtG* is proposed to incorporate an amidino group from a second arginine molecule into the STX intermediate. In contrast to the bacterial transcripts the *sxtG* transcript possessed a eukaryotic polyA-tail at the 3' end and the dinoflagellate spliced-leader sequence at the 5' end. The result demonstrates that both the primary and secondary STX-pathway genes are encoded in the nuclear genome of dinoflagellates (Stüken, supra). This is further supported with the GC content of *sxtG* being ~20% higher for the dinoflagellates (with a GC of ~64%) than for the STX-producing cyanobacteria species.

In contrast to *sxtA*, the presence of *sxtG* is not specific to STX production. The *sxtG* amidinotransferase was present and transcribed in all tested *Alexandrium* species, including those where *sxtA* and STX synthesis were undetectable (Stüken A, et al. (2011) “Discovery of Nuclear-Encoded Genes for the Neurotoxin Saxitoxin in Dinoflagellates” *PlosOne* 6: e20096; Orr RJS, et al. (2011) “Improved phylogenetic resolution of toxic and non-toxic *Alexandrium* strains using a concatenated rDNA approach”, *Harmful Algae* 10: 676-688). However, external to the *Alexandrium* genus and *Gymnodinium catenatum*, and in congruence with *sxtA*, *sxtG* was not detected. Twenty-two species from five orders are seemingly devoid of these genes (Fig. 2). Further, only significant hits to *Alexandrium* were produced upon blasting multiple databases within Genbank with the *sxtG* query. While *sxtG* is not exclusive to toxic species, it is present in all *Alexandrium* species and it is absent from non-PSP dinoflagellate genera.

The phylogenetic inference of *sxtG* shows that dinoflagellate *sxtG* sequences form a fully supported clade. Several slightly different sequences for

some of the strains were distributed within the clade. The branching pattern within the clade is unclear, a result of high sequence conservation between species. Even at the nucleotide level sequences are highly invariable, limiting *sxtG* as a phylogenetic marker. Thus it was not possible to determine if the evolution of *sxtG* mirrors that of *sxtA* or even the *Alexandrium* genus (see, e.g., Stüken A, et al. (2011) "Discovery of Nuclear-Encoded Genes for the Neurotoxin Saxitoxin in Dinoflagellates", *PlosOne* 6: e20096; Orr RJS, et al. (2011) "Improved phylogenetic resolution of toxic and non-toxic *Alexandrium* strains using a concatenated rDNA approach", *Harmful Algae* 10: 676-688).

Variants of polynucleotides of the technology and polypeptides of the technology, and fragments thereof, are also provided herein. A "variant" as contemplated herein refers to a substantially similar sequence. In general, two sequences are "substantially similar" if the two sequences have a specified percentage of amino acid residues or nucleotides that are the same (percentage of "sequence identity"), over a specified region, or, when not specified, over the entire sequence. Accordingly, a "variant" of a polynucleotide and polypeptide sequence disclosed herein may share at least 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 83%, 85%, 88%, 90%, 93%, 95%, 96%, 97%, 98% or 99% sequence identity with the reference sequence.

In general, polypeptide sequence variants possess qualitative biological activity in common. Polynucleotide sequence variants generally encode polypeptides which generally possess qualitative biological activity in common. Also included within the meaning of the term "variant" are homologues of polynucleotides of the technology and polypeptides of the technology. A polynucleotide homologue is typically from a different dinoflagellate species but sharing substantially the same biological function or activity as the corresponding polynucleotide disclosed herein. A polypeptide homologue is typically from a different dinoflagellate species but sharing substantially the same biological function or activity as the corresponding polypeptide disclosed herein. The term "variant" also includes analogues of the polypeptides of the technology. A polypeptide "analogue" is a polypeptide which is a derivative of a polypeptide of the technology, which derivative comprises addition, deletion,

substitution of one or more amino acids, such that the polypeptide retains substantially the same function. The term “conservative amino acid substitution” refers to a substitution or replacement of one amino acid for another amino acid with similar properties within a polypeptide chain (primary sequence of a
5 protein).

For example, the substitution of the charged amino acid glutamic acid (Glu) for the similarly charged amino acid aspartic acid (Asp) would be a conservative amino acid substitution.

Typically, polynucleotides of the technology and polypeptides of the
10 technology are “isolated”. It will be understood that the term “isolated” in this context means that the polynucleotide or polypeptide has been removed from or is not associated with some or all of the other components with which it would be found in its natural state. For example, an “isolated” polynucleotide may be removed from other polynucleotides of a larger polynucleotide sequence, or may
15 be removed from natural components such as unrelated polynucleotides. Likewise, an “isolated” polypeptide may be removed from other polypeptides of a larger polypeptide sequence, or may be removed from natural components such as unrelated polypeptides. For the sake of clarity, an “isolated” polynucleotide of polypeptide also includes a polynucleotide or polypeptide which has not been
20 taken from nature but rather has been prepared de novo, such as chemically synthesised and/or prepared by recombinant methods. As described herein an isolated polypeptide of the technology may be included as a component part of a longer polypeptide or fusion protein.

In certain embodiments, polynucleotides of the technology may be cloned
25 into a vector. The vector may comprise, for example, a DNA, RNA or complementary DNA (cDNA) sequence. The vector may be a plasmid vector, a viral vector, or any other suitable vehicle adapted for the insertion of foreign sequences, their introduction into cells and the expression of the introduced sequences. Typically the vector is an expression vector and may include
30 expression control and processing sequences such as a promoter, an enhancer, ribosome binding sites, polyadenylation signals and transcription termination sequences. The technology also contemplates host cells transformed by such

vectors. For example, the polynucleotides of the technology may be cloned into a vector which is transformed into a bacterial host cell, for example *E. coli*.

Methods for the construction of vectors and their transformation into host cells are generally known in the art, and described in standard texts such as, for

5 example, Sambrook et al. (1989) *Molecular Cloning: A Laboratory Manual*, 2nd ed., Cold Spring Harbor Laboratory Press, Plainview, New York; and Ausubel et al. (Eds) *Current Protocols in Molecular Biology* (2007), John Wiley and Sons, Inc.

10 **Probes, primers and antibodies**

Polynucleotides of the technology include derivatives and fragments thereof for use as primers and probes. The derivatives and fragments may be in the form of oligonucleotides. Oligonucleotides are short stretches of nucleotide residues suitable for use in nucleic acid amplification reactions such as PCR,

15 typically being at least about 5 nucleotides to about 80 nucleotides in length, more typically about 10 nucleotides in length to about 50 nucleotides in length, and even more typically about 15 nucleotides in length to about 30 nucleotides in length.

Probes are nucleotide sequences of variable length, for example between
20 about 10 nucleotides and several thousand nucleotides, for use in detection of homologous sequences, typically by hybridization. Hybridization probes may be genomic DNA fragments, cDNA fragments, RNA fragments, or other oligonucleotides.

Methods for the design and/or production of nucleotide probes and/or
25 primers are known in the art, and described in standard texts such as Sambrook et al. (1989) *Molecular Cloning: A Laboratory Manual*, 2nd ed., Cold Spring Harbor Laboratory Press, Plainview, New York; and publications such as Itakura et al. (1984) *Annu. Rev. Biochem.* 53:323; Innis et al. (Eds) (1990) *PCR Protocols: A Guide to Methods and Applications*, Academic Press, New York;
30 Innis and Gelfand, (Eds) (1995) *PCR Strategies*, Academic Press, New York; and Innis and Gelfand, (Eds) (1999) *PCR Methods Manual*, Academic Press, New York.

Polynucleotide primers and probes may be prepared, for example, by chemical synthesis techniques such as the phosphodiester and phosphotriester methods (see for example Narang et al. (1979) *Meth. Enzymol.* 68:90; Brown et al. (1979) *Meth. Enzymol.* 68:109; and U.S. Patent No. 4356270), and the
5 diethylphosphoramidite method (see Beaucage et al. (1981) *Tetrahedron Letters*, 22:1859-1862). Polynucleotides of the technology, including the aforementioned probes and primers, may be labeled by incorporation of a marker to facilitate their detection. Techniques for labelling and detecting nucleic acids are described, for example, in standard texts such as Ausubel et al. (Eds) *Current*
10 *Protocols in Molecular Biology* (2007), John Wiley and Sons, Inc.. Non-limiting Examples of suitable markers include fluorescent molecules (e.g. acetylaminofluorene, 5-bromodeoxyuridine, digoxigenin, and fluorescein) and radioactive isotopes (e.g. ^{32}P , ^{35}S , ^3H , ^{33}P). Detection of the marker may be achieved, for example, by chemical, photochemical, immunochemical,
15 biochemical, or spectroscopic techniques.

The probes and primers may be used, for example, to detect or isolate dinoflagellates in a sample of interest. In certain embodiments, the probes and primers may be used to detect STX-producing dinoflagellates in a sample of interest. Additionally or alternatively, the probes or primers may be used to
20 isolate corresponding sequences in other organisms including, for example, other dinoflagellate species. Methods such as the polymerase chain reaction (PCR), hybridization, and the like can be used to identify such sequences based on their sequence homology to the sequences set forth herein. Sequences that are selected based on their sequence identity to the entire sequences set forth herein or to
25 fragments thereof are encompassed by the embodiments. Such sequences include sequences that are orthologs of the disclosed sequences. The term "orthologs" refers to genes derived from a common ancestral gene and which are found in different species as a result of speciation. Genes found in different species are considered orthologs when their nucleotide sequences and/or their encoded
30 protein sequences share substantial identity as defined elsewhere herein. Functions of orthologs are often highly conserved among species.

In hybridization techniques, all or part of a known nucleotide sequence is used to generate a probe that selectively hybridizes to other corresponding nucleic acid sequences present in a given sample. The hybridization probes may be genomic DNA fragments, cDNA fragments, RNA fragments, or other oligonucleotides, and may be labelled with a detectable marker. Thus, for example, probes for hybridization can be made by labelling synthetic oligonucleotides based on the sequences of the technology. The level of homology (sequence identity) between probe and the target sequence will largely be determined by the stringency of hybridization conditions. In particular the nucleotide sequence used as a probe may hybridize to a homologue or other variant of a polynucleotide disclosed herein under conditions of low stringency, medium stringency or high stringency. There are numerous conditions and factors, well known to those skilled in the art, which may be employed to alter the stringency of hybridization such as, for example, the length and nature (DNA, RNA, base composition) of the nucleic acid to be hybridized to a specified nucleic acid; concentration of salts and other components, such as the presence or absence of formamide, dextran sulfate, polyethylene glycol etc; and altering the temperature of the hybridization and/or washing steps.

Under a PCR approach, oligonucleotide primers can be designed for use in PCR reactions to amplify corresponding DNA sequences from cDNA or genomic DNA extracted from any organism of interest. Methods for designing PCR primers and PCR cloning are generally known in the art. Known methods of PCR include, but are not limited to, methods using paired primers, nested primers, single specific primers, degenerate primers, gene-specific primers, vector-specific primers, partially-mismatched primers, and the like. The skilled addressee will recognise that the primers described herein for use in PCR or RT-PCR may also be used as probes for the detection of dinoflagellate *sxt* gene sequences.

Also contemplated by the technology are antibodies which are capable of binding specifically to polypeptides of the technology. The antibodies may be used to qualitatively or quantitatively detect and analyse one or more STX polypeptides (e.g., toxin) in a given sample and/or one or more SXT polypeptides (e.g., a product of the *sxtG* gene and/or a product of the *sxtA* gene) in a sample.

By "binding specifically" it will be understood that the antibody is capable of binding to the target polypeptide or fragment thereof with a higher affinity than it binds to an unrelated protein. For example, the antibody may bind to the polypeptide or fragment thereof with a binding constant in the range of at least about 10^{-4} M to about 10^{-10} M. Preferably the binding constant is at least about 10^{-5} M, or at least about 10^{-6} M. More preferably the binding constant is at least about 10^{-7} M, at least about 10^{-8} M, or at least about 10^{-9} M or more. In the context of the present technology, reference to an antibody specific to a particular polypeptide includes, e.g., an antibody that is specific to a fragment of the polypeptide.

Antibodies of the technology may exist in a variety of forms including, for example, as a whole antibody, or as an antibody fragment, or other immunologically active fragment thereof, such as complementarity determining regions. Similarly, the antibody may exist as an antibody fragment having functional antigen-binding domains, that is, heavy and light chain variable domains. Also, the antibody fragment may exist in a form selected from the group consisting of, but not limited to: Fv, F_{ab}, F(ab)₂, scFv (single chain Fv), dAb (single domain antibody), chimeric antibodies, bi-specific antibodies, diabodies and triabodies.

An antibody "fragment" may be produced by modification of a whole antibody or by synthesis of the desired antibody fragment. Methods of generating antibodies, including antibody fragments, are known in the art and include, for example, synthesis by recombinant DNA technology. The skilled addressee will be aware of methods of synthesising antibodies, such as those described in, for example, US Patent No. 5296348 and standard texts such as Ausubel et al. (Eds) Current Protocols in Molecular Biology (2007), John Wiley and Sons, Inc.

Preferably, antibodies are prepared from discrete regions or fragments of the SxtG or SXT polypeptide of interest. An antigenic portion of a polypeptide of interest may be of any appropriate length, such as from about 5 to about 15 amino acids. Preferably, an antigenic portion contains at least about 5, 6, 7, 8, 9, 10, 11, 12, 13 or 14 amino acid residues.

Antibodies that specifically bind to a polypeptide of the technology can be prepared, for example, using purified SXT polypeptides of the technology, or purified *sxt* polynucleotide sequences of the technology that encode SXT polypeptides of the technology using any suitable methods known in the art. For example, a monoclonal antibody, typically containing F_{ab} portions, may be prepared using hybridoma technology described in Harlow and Lane (Eds) Antibodies - A Laboratory Manual, (1988), Cold Spring Harbor Laboratory, N.Y; Coligan, Current Protocols in Immunology (1991); Goding, Monoclonal Antibodies: Principles and Practice (1986) 2nd ed; and Kohler & Milstein, (1975) Nature 256: 495-497. Such techniques include, but are not limited to, antibody preparation by selection of antibodies from libraries of recombinant antibodies in phage or similar vectors, as well as preparation of polyclonal and monoclonal antibodies by immunizing rabbits or mice (see, for example, Huse et al. (1989) Science 246: 1275-1281; Ward et al. (1989) Nature 341: 544-546).

It will also be understood that antibodies of the technology include humanised antibodies, chimeric antibodies and fully human antibodies. An antibody of the technology may be a bi-specific antibody, having binding specificity to more than one antigen or epitope. For example, the antibody may have specificity for one or more STX polypeptides or fragments thereof, and additionally have binding specificity for another antigen. Methods for the preparation of humanised antibodies, chimeric antibodies, fully human antibodies, and bispecific antibodies are known in the art and include, for example, those described in United States Patent No. 6995243.

Generally, a sample potentially comprising an STX polypeptide of the technology can be contacted with an antibody that specifically binds the STX polypeptide or fragment thereof. Optionally, the antibody can be fixed to a solid support to facilitate washing and subsequent isolation of the complex, prior to contacting the antibody with a sample. Examples of solid supports include, for example, microtitre plates, beads, ticks, or microbeads. Antibodies can also be attached to a ProteinChip array or a probe substrate as described above.

Detectable labels for the identification of antibodies bound to polypeptides of the technology include, but are not limited to, fluorochromes, fluorescent dyes,

radiolabels, enzymes such as horse radish peroxide, alkaline phosphatase and others commonly used in the art, and colorimetric labels including colloidal gold or coloured glass or plastic beads. Alternatively, the antibody can be detected using an indirect assay, wherein, for example, a second, labelled antibody is used to detect bound polypeptide-specific antibody.

Methods for detecting the presence of, or measuring the amount of, an antibody-marker complex include, for example, detection of fluorescence, chemiluminescence, luminescence, absorbance, birefringence, transmittance, reflectance, or refractive index such as surface plasmon resonance, ellipsometry, a resonant mirror method, a grating coupler wave guide method, or interferometry. Radio frequency methods include multipolar resonance spectroscopy. Electrochemical methods include amperometry and voltametry methods. Optical methods include imaging methods and non-imaging methods and microscopy.

Useful assays for detecting the presence of, or measuring the amount of, an antibody-marker complex include, include, for example, enzyme-linked immunosorbent assay (ELISA), a radioimmune assay (RIA), or a Western blot assay. Such methods are described in, for example, Stites & Terr, (Eds) (1991) Clinical Immunology, 7th ed; and Asai, (Ed) (1993) Methods in Cell Biology: Antibodies in Cell Biology, volume 37.

Methods for detecting dinoflagellates

The technology provides methods for the detection and/or isolation of polynucleotides of the technology and/or polypeptides of the technology (“methods of the technology”).

In one embodiment the technology provides a method for detecting a dinoflagellate in a sample. The method comprises obtaining a sample for use in the method, and detecting the presence of a polynucleotide of the technology and/or a polypeptide of the technology, or a fragment or variant thereof in the sample. The presence of the polynucleotide, polypeptide, or variant or fragment thereof in the sample is indicative of dinoflagellates in the sample.

The present inventors have determined that the *sxtG* gene is present in saxitoxin-producing dinoflagellates and that absence of *sxtG* indicates dinoflagellates that do not produce saxitoxin. Accordingly, in another embodiment the technology provides a method for detecting a saxitoxin-producing dinoflagellate in a sample. The method comprises obtaining a sample for use in the method, and detecting the presence of a polynucleotide of the technology and/or a polypeptide of the technology, or a fragment or variant thereof in the sample. The presence of the polynucleotide, polypeptide, or variant or fragment thereof in the sample is indicative of a saxitoxin-producing dinoflagellate in the sample.

In another embodiment the technology provides a method for determining an absence of saxitoxin-producing dinoflagellates in a sample. The method comprises obtaining a sample for use in the method, and determining an absence of a polynucleotide of the technology and/or a polypeptide of the technology, or a fragment or variant thereof in the sample. The absence of the polynucleotide, polypeptide, or variant or fragment thereof in the sample is indicative that saxitoxin-producing dinoflagellates are not present in the sample.

In the context of the methods of the technology (including those referred to in the paragraphs immediately above), the polynucleotide sequence may be a saxitoxin A gene (*sxtA*) sequence. The *sxtA* polynucleotide sequence may comprise any one or more *sxtA* gene catalytic domain(s) (e.g., the *sxtA1*, *sxtA2*, *sxtA3*, or *sxtA4* catalytic domain(s)), or fragment(s) thereof. Preferably, the *sxtA* polynucleotide sequence comprises an *sxtA1* and/or a *sxtA4* domain, or fragment(s) thereof. More preferably, the *sxtA* polynucleotide sequence comprises an *sxtA4* domain, or fragment(s) thereof. The polypeptide sequence may be saxitoxin A polypeptide (SXTA) sequence.

In the context of the methods of the technology (including those referred to in the paragraphs immediately above), the polynucleotide sequence may be a saxitoxin G gene (*sxtG*) sequence. The *sxtG* polynucleotide sequence may comprise any one or more *sxtG* gene catalytic domain(s) or fragment(s) thereof. In some embodiments, the nucleotide sequence corresponds to the sequence provided in Figure 1 (e.g., SEQ ID NOs: 12 and/or 13).

In some embodiments, the technology comprises detecting both *sxtA* and *sxtG* sequences, e.g., using the primers in Table 1 (e.g., SEQ ID NOs: 1–9) and the primers defined as having the sequences defined by SEQ ID NOs: 10 and 11.

In the context of the methods of the technology (including those referred to in the paragraphs above), the polypeptide sequence may be a saxitoxin A protein (SXTA) sequence. The SXTA polypeptide sequence may comprise any one or more SXTA protein catalytic domain(s) (e.g., the SXTA1, SXTA2, SXTA3, or SXTA4 catalytic domain(s)), or fragment(s) thereof. Preferably, the SXTA polypeptide sequence comprises an SXTA1 or an SXTA4 domain, or fragment(s) thereof. More preferably, the SXTA polypeptide sequence comprises an SXTA4 domain, or fragment(s) thereof.

In the context of the methods of the technology (including those referred to in the paragraphs above), the polypeptide sequence may be a saxitoxin G protein (SXTG) sequence. The SXTG polypeptide sequence may comprise any one or more SXTG protein catalytic domain(s) or fragment(s) thereof.

Dinoflagellates detected in a sample or determined to be absent from a sample using the methods of the technology may be saxitoxin-producing dinoflagellates. Without imposing any particular limitation, the dinoflagellates may be from the order Gonyaulacales or Gymnodiniales. The dinoflagellates may be from the genus *Alexandrium* (formerly *Gonyaulax*), *Pyrodinium*, or *Gymnodinium*. Suitable examples of *Alexandrium* species include *A. catenella* (e.g. strains ACCC01, ACSH02, ACTRA02 and CCMP1493), *A. fundyense* (e.g. strains CCMP1719 and CCMP1979), *A. lusitanicum*, *A. minutum* (e.g. strains CCMP1888, CCMP113, ALSP01, ALSP02 and AMD16/AMAD16), *A. ostenfeldii*, and *A. tamarense* (e.g. strains CCMP1771, ATBB01, ATEB01, ATCJ33 and ATNWB01). Suitable examples of *Gymnodinium* species include *G. catenatum* (e.g. strains GCTRA01 and CS-395). Suitable examples of *Pyrodinium* species include *P. bahamense var compressum*.

A sample for use in the methods of the technology may be “obtained” by any means. For example, the sample may be obtained by removing it from a naturally-occurring state (e.g. a sample from a lake, ocean or river), or, by

removing it from a “non-natural” state (e.g. a culture in a laboratory setting, dam, reservoir, tank etc.).

A sample for use in the methods of the technology may be suspected of comprising one or more dinoflagellates, or one or more saxitoxin-producing
5 dinoflagellates. The sample may be a comparative or control sample, for example, a sample comprising a known concentration or density of dinoflagellates or saxitoxin-producing dinoflagellates or a sample comprising one or more known species or strains of dinoflagellates or saxitoxin-producing
10 dinoflagellates. The sample may be derived from any source. For example, a sample may be an environmental sample. The environmental sample may be derived from, for example, saltwater, freshwater, a river, a lake, an ocean, or coastal waters. The environmental sample may be derived from a dinoflagellate bloom.

Alternatively, the sample may be derived from a laboratory source, such
15 as a culture, or a commercial source. Alternatively, the sample may be derived from a biological source such as, for example, tissue or biological fluid. The sample may be modified from its original state, for example, by purification, dilution or the addition of any other component or components.

In certain embodiments, a sample tested using the methods of the
20 technology may provide information regarding the presence or absence of saxitoxin in animals populating the source of the sample. For example, the sample may be tested to determine the presence or absence of saxitoxin in animal seafoods such as, for example, fish (e.g., pufferfish) and in particular shellfish (e.g. mussels, clams, oysters, scallops and the like).

Polynucleotides and polypeptides for use in methods of the technology may
25 be isolated (e.g., extracted) from microorganisms either in mixed culture or as individual species or genus isolates. Accordingly, the microorganisms of a sample may be cultured prior to extraction or the extraction may be performed directly on a given sample. Suitable methods for the isolation (e.g., extraction) and
30 purification of polynucleotides and polypeptides for analysis using methods of the technology are generally known in the art and are described, for example, in standard texts such as Ausubel (Eds) Current Protocols in Molecular Biology

(2007), John Wiley and Sons, Inc; Coligan et al. (Eds) Current Protocols in Protein Science (2007), John Wiley and Sons, Inc; Walker, (Ed) (1988) New Protein Techniques: Methods in Molecular Biology, Humana Press, Clifton, N.J; and Scopes, R. K. (1987) Protein Purification: Principles and Practice, 3rd. Ed.,
5 Springer-Verlag, New York, N.Y.. Additional methods are described in Neilan (1995) Appl. Environ. Microbiol. 61:2286-2291. Suitable polypeptide purification techniques suitable for use in the methods of the technology include, but are not limited to, reverse-phase chromatography, hydrophobic interaction
10 chromatography, centrifugation, gel filtration, ammonium sulfate precipitation, and ion exchange. In alternative embodiments, methods of the technology may be performed without isolating nucleic acids and/or polypeptides from the sample.

Detecting the presence (or determining the absence) of polynucleotides of the technology and/or polypeptides of the technology in a given sample may be
15 performed using any suitable technique. Suitable techniques may typically involve the use of a primer, probe or antibody specific for any one or more polynucleotides of the technology or any one or more polypeptides of the technology. Suitable techniques include, for example, the polymerase chain reaction (PCR) and related variations of this technique (e.g. quantitative PCR),
20 antibody based assays such as ELISA, western blotting, flow cytometry, fluorescent microscopy, and the like. These and other suitable techniques are generally known in the art and are described, for example, in standard texts such as Coligan et al. (Eds) Current Protocols in Protein Science (2007), John Wiley and Sons, Inc; Walker, (Ed) (1988) New Protein Techniques: Methods in
25 Molecular Biology, Humana Press, Clifton, N.J; and Scopes (1987) Protein Purification: Principles and Practice, 3rd. Ed.,
Springer-Verlag, New York, N.Y..

In preferred embodiments, detecting the presence (or determining the absence) of polynucleotides of the technology in a given sample is achieved by
30 amplification of nucleic acids extracted from a sample of interest by polymerase chain reaction using primers that hybridise specifically to the polynucleotide sequence, and detecting the amplified sequence. Under the PCR approach,

oligonucleotide primers can be designed for use in PCR reactions to amplify polynucleotides of the technology such as, for example, RNA (e.g. mRNA), DNA and/or cDNA polynucleotides. Suitable methods of PCR include, but are not limited to, those using paired primers, nested primers, single specific primers, 5 degenerate primers, gene-specific primers, vector-specific primers, partially-mismatched primers, and the like. Methods for designing PCR and RT-PCR primers are generally known in the art and are disclosed, for example, in standard texts such as Ausubel et al. (Eds) *Current Protocols in Molecular Biology* (2007), John Wiley and Sons, Inc; Maniatis et al. *Molecular Cloning* 10 (1982), 280-281; Innis et al. (Eds) (1990) *PCR Protocols: A Guide to Methods and Applications* (Academic Press, New York); Innis and Gelfand, (Eds) (1995) *PCR Strategies* (Academic Press, New York); Innis and Gelfand, (Eds) (1999) *PCR Methods Manual* (Academic Press, New York); and Sambrook et al. (1989) *Molecular Cloning: A Laboratory Manual* (2nd ed., Cold Spring Harbor 15 Laboratory Press, Plainview, New York).

The skilled addressee will readily appreciate that various parameters of PCR and RT-PCR procedures may be altered without affecting the ability to obtain the desired product. For example, the salt concentration may be varied or the time and/or temperature of one or more of the denaturation, annealing and 20 extension steps may be varied. Similarly, the amount of DNA, cDNA, or RNA template may also be varied depending on the amount of nucleic acid available or the optimal amount of template required for efficient amplification. The primers for use in the methods and kits of the present technology are typically oligonucleotides typically being at least about nucleotides to about 80 nucleotides 25 in length, more typically about 10 nucleotides in length to about 50 nucleotides in length, and even more typically about 15 nucleotides in length to about 30 nucleotides in length.

The skilled addressee will recognise that primers of the technology may be useful for a number of different applications, including but not limited to, PCR, 30 RT-PCR, and as probes for the detection of polynucleotides of the technology. Such primers can be prepared by any suitable method, including, for example, direct chemical synthesis or cloning and restriction of appropriate sequences. Not

all bases in the primer need reflect the sequence of the template molecule to which the primer will hybridize. The primer need only contain sufficient complementary bases to enable the primer to hybridize to the template. A primer may also include mismatch bases at one or more positions, being bases that are
5 not complementary to bases in the template, but rather are designed to incorporate changes into the DNA upon base extension or amplification. A primer may include additional bases, for example in the form of a restriction enzyme recognition sequence at the 5' end, to facilitate cloning of the amplified DNA.

10 The methods of the technology involve detecting the presence (or determining the absence) of polynucleotides of the technology and/or polypeptides of the technology in a given sample. As noted above, the sequences may comprise saxitoxin A sequences including any one or more of the saxitoxin A1, A2, A3 or A4 catalytic domain sequences (or fragment(s) thereof). The
15 sequences may comprise saxitoxin G sequences. The sequences may comprise a sequence comprising both *sxtA* and *sxtG*, e.g., a *sxtA/sxtG* sequence.

The skilled addressee will recognise that any primer(s) capable of the amplifying a polynucleotide of the technology, any probe capable of detecting a polynucleotide of the technology, or any antibody capable of detecting a
20 polypeptide of the technology, may be used when performing the methods of the technology. In preferred embodiments, the primers, probes and antibodies bind specifically to any one or more of the saxitoxin G sequences referred to in the preceding paragraph (e.g., paragraph directly above).

By "binding specifically" it will be understood that the primer, probe or
25 antibody is capable of binding to the target sequence with a higher affinity than it binds to an unrelated sequence. Accordingly, when exposed to a plurality of different but equally accessible sequences as potential binding partners, the primer, probe or antibody specific for a target sequence will selectively bind to the target sequence and other alternative potential binding partners will remain
30 substantially unbound by the primer, probe or antibody. In general, a primer, probe or antibody specific for a target sequence will preferentially bind to the target sequence at least 10-fold, preferably 50-fold, more preferably 100-fold, and

most preferably greater than 100-fold more frequently than to other potential sequences that are not target sequences. A primer, probe or antibody specific for a target sequence may be capable of binding to non-target sequences at a weak, yet detectable level. This is commonly known as background binding and is readily discernible from specific binding, for example, by use of an appropriate control.

Suitable primers and probes may bind specifically to any fragment of a saxitoxin G polynucleotide sequence. Suitable antibodies may bind specifically to a fragment of a saxitoxin G polypeptide sequence encoded by such polynucleotide sequences. By way of non-limiting example only, suitable primers and probes may bind specifically to a fragment of the saxitoxin G polynucleotide sequence defined by SEQ ID NOs: 12 and/or 13 as provided herein. Suitable antibodies may bind specifically to a fragment of a saxitoxin G polypeptide sequence encoded by such polynucleotide sequences.

In some embodiments, the methods of the technology may involve detecting the presence (or determining the absence) of polynucleotides of the technology in a sample using PCR amplification. Suitable oligonucleotide primer pairs for the PCR amplification of saxitoxin G polynucleotide sequences may be capable of amplifying any one or more domain(s) of the *sxtG* gene, or fragments(s) thereof. By way of non-limiting example only, suitable primer pairs for this purpose may comprise primers provided in Table 1. The skilled addressee will recognise that the exemplified primers are not intended to limit the region of the saxitoxin G gene amplified or the methods of the technology in general. The skilled addressee will also recognise that the technology is not limited to the use of the specific primers exemplified, and alternative primer sequences may also be used, provided the primers are designed appropriately so as to enable the amplification of saxitoxin polynucleotide sequences, preferably saxitoxin G polynucleotide sequences, and in some embodiments, both *sxtG* and *sxtA* sequences.

That is, in some embodiments, technology involves detecting the presence (or determining the absence) of *sxtA* polynucleotides of the invention in a sample using PCR amplification. Suitable oligonucleotide primer pairs for the PCR

amplification of saxitoxin A polynucleotide sequences may be capable of amplifying any one or more regions, e.g., one or more catalytic domain(s), of the *sxtA* gene, or fragments(s) thereof. Preferably, the primers amplify a sequence comprising a saxitoxin A4 catalytic domain polynucleotide sequence, or a
5 fragment thereof. By way of non-limiting example only, a suitable primer pair for this purpose may comprise a first primer comprising the polynucleotide sequence defined as

CTGAGCAAGGCGTTCAATTC (SEQ ID NO: 10),

10 or a fragment or variant thereof, and/or a second primer comprising the polynucleotide sequence defined as

TACAGATMGGCCCTGTGARC (SEQ ID NO: 11),

15 or a fragment or variant thereof. The skilled addressee will recognise that the exemplified primers are not intended to limit the region of the saxitoxin A gene amplified or the methods of the invention in general. The skilled addressee will also recognise that the invention is not limited to the use of the specific primers
20 exemplified, and alternative primer sequences may also be used, provided the primers are designed appropriately so as to enable the amplification of saxitoxin (e.g., *sxtA* and/or *sxtG*) polynucleotide sequences. For amplification of *sxtA* sequences, it is preferred to amplify saxitoxin A1 and/or A4 domain polynucleotide sequences.

25 In other embodiments, the methods of the technology may involve detecting the presence (or determining the absence) of polynucleotides of the technology in a sample by the use of suitable probes. Probes of the technology are based on *sxt* polynucleotide sequences of the technology. Probes are nucleotide sequences of variable length, for example between about 10 nucleotides and
30 several thousand nucleotides, for use in detection of homologous sequences, typically by hybridization. Hybridization probes of the technology may be genomic DNA fragments, cDNA fragments, RNA fragments, or other

oligonucleotides. Probes of the technology may be labelled by incorporation of a marker to facilitate their detection. Examples of suitable markers include fluorescent molecules (e.g. acetylaminofluorene, 5-bromodeoxyuridine, digoxigenin, fluorescein) and radioactive isotopes (e.g. ^{32}P , ^{35}S , ^3H , ^{33}P). Detection of the marker may be achieved, for example, by chemical, photochemical, immunochemical, biochemical, or spectroscopic techniques. Methods for the design and/or production of nucleotide probes are generally known in the art, and are described, for example, in standard texts such as Robinson et al. (Eds) Current Protocols in Cytometry (2007), John Wiley and Sons, Inc; Ausubel et al. (Eds) Current Protocols in Molecular Biology (2007), John Wiley and Sons, Inc; Sambrook et al. (1989) Molecular Cloning: A Laboratory Manual (2nd ed., Cold Spring Harbor Laboratory Press, Plainview, New York; and Maniatis et al. (1982) Molecular Cloning, 280-281.

In other embodiments, the methods of the technology may involve detecting the presence (or determining the absence) of polypeptides of the technology in a sample using antibodies. The antibodies may be used to qualitatively or quantitatively to detect and analyse one or more SXT polypeptides of the technology in a given sample. The antibodies may be conjugated to a fluorochrome allowing detection, for example, by flow cytometry, immunohistochemistry or other means known in the art. Alternatively, the antibody may be bound to a substrate allowing colorimetric or chemiluminescent detection. The technology also contemplates the use of secondary antibodies capable of binding to one or more antibodies capable of binding specifically to a polypeptide of the technology.

Kits for detecting dinoflagellates

The technology also provides kits for the detection and/or isolation of polynucleotides of the technology and/or polypeptides of the technology ("kits of the technology").

In certain embodiments the kits are used for detecting a dinoflagellate in a sample. In other embodiments the kits are used for detecting a saxitoxin-producing dinoflagellate in a sample. In other embodiments the kits are used for

determining an absence of saxitoxin producing dinoflagellates in a sample. In general, the kits of the technology comprise at least one agent for detecting the presence of one or more polynucleotides of the technology and/or one or more polypeptides of the technology, and/or variants or fragments thereof (see
5 description in the section above entitled “Polynucleotides and polypeptides”). Any agent suitable for this purpose may be included in the kits. Non-limiting examples of suitable agents include primers, probes and antibodies such as those described above in the sections entitled “Probes, primers and antibodies” and “Methods for detecting dinoflagellates”.

10 In preferred embodiments, the kits are for use in the methods of the technology (see description in the section above entitled “Methods for detecting dinoflagellates”). In some embodiments the technology provides a kit for the detection of a dinoflagellate in a sample, the kit comprising at least one agent for detecting in the sample the presence of one or more polynucleotides of the
15 technology, and/or one or more polypeptides of the technology, and/or a variant or fragment of either. Preferably, the dinoflagellate is a saxitoxin-producing dinoflagellate.

In other embodiments the technology provides a kit for determining the absence of a dinoflagellate in a sample, the kit comprising at least one agent for
20 determining in the sample the absence of one or more polynucleotides of the technology, and/or one or more polypeptides of the technology, and/or a variant or fragment of either. Preferably, the dinoflagellate is a saxitoxin-producing dinoflagellate.

In general, kits of the technology may comprise any number of additional
25 components. By way of non-limiting example the additional components may include components for collecting and/or storing samples, reagents for cell culture, reference samples, buffers, labels, and/or written instructions for performing method(s) of the technology. Dinoflagellates detected in a sample or determined to be absent from a sample using kits of the technology may be
30 saxitoxin-producing dinoflagellates. Without imposing any particular limitation, the dinoflagellates may be from the order Gonyaulacales or Gymnodiniales. The dinoflagellates may be from the genus *Alexandrium* (formerly *Gonyaulax*),

Pyrodinium, or *Gymnodinium*. Suitable examples of *Alexandrium* species include *A. catenella* (e.g. strains ACCC01, ACSH02, ACTRA02 and CCMP1493), *A. fundyense* (e.g. strains CCMP1719 and CCMP1979), *A. lusitanicum*, *A. minutum* (e.g. strains CCMP1888, CCMP113, ALSP01, ALSP02 and AMD16/AMAD16), *A.*
 5 *ostenfeldii*, and *A. tamarense* (e.g. strains CCMP1771, ATBB01, ATEB01, ATCJ33 and 15 ATNWB01). Suitable examples of *Gymnodinium* species include *G. catenatum* (e.g. strains GCTRA01 and CS-395). Suitable examples of *Pyrodinium* species include *P. bahamense var compressum*. It will be appreciated by persons skilled in the art that numerous variations and/or
 10 modifications may be made to the technology as described in the specific embodiments without departing from the spirit or scope of the technology as broadly described. The present embodiments are, therefore, to be considered in all respects as illustrative and not restrictive.

Although the disclosure herein refers to certain illustrated embodiments,
 15 it is to be understood that these embodiments are presented by way of example and not by way of limitation.

Examples

Methods

Strains and culture conditions.

During the development of the technologies provided herein, several species of dinoflagellates were cultured and tested. The dinoflagellate species/strains used in this study included *Adenoides eludens* CCMP1891, *Alexandrium affine* CCMP112, *Alexandrium andersoni* CCMP2222, *Alexandrium catenella*
 25 CCMP1493, *Alexandrium fundyense* CCMP1719, *Alexandrium insuetum* CCMP2082, *Alexandrium minutum* CCMP113, *Alexandrium tamarense* CCMP1771, *Amphidinium carteri* UIO081, *Amphidinium massartii* CS-259, *Amphidinium mootonorum* CAWD161, *Azadinium spinosum* RCC2538, *Ceratium longipes* CCMP1770, *Coolia monotis*, *Gamberdiscus australes*
 30 CAWD148, *Gymnodinium aureolum* SCCAP K-1561, *Heterocapsa triquetra* RCC2540, *Karlodinium veneficum* RCC2539, *Lepidodinium chlorophorum* RCC2537, *Lingulodinium polyedrum* CCMP1931, *Pentaporphosodium dalei*

SCCAP K-1100, *Prorocentrum lima* CS-869, *Prorocentrum micans* UIO292, *Prorocentrum minimum* UIO085, *Protoceratium reticulatum*, *Pyrocystis noctiluca* CCMP732, *Scrippsiella trochoideae* BS-46, and *Thecadinium kofoidii* SCCAP K-1504. These strains were grown in L1 media (see, e.g., Guillard RRL and Hargraves PE (1993), “Stichochrysis immobilis is a diatom, not a chrysophyte”, *Phycologia* 32: 234-236) at 16–25°C, and *Polarella glacialis* CCMP2088 was grown at 5°C. All strains were grown with a 12 hour/12 hour light-dark photoperiod and a photon irradiance of ~100 mmol photons m⁻² s⁻¹. Strains were not maintained axenic. Culture identity was confirmed with a 18S PCR using the NSF83 and 1528R primers (see, e.g., Hendriks L, et al. (1989), “The Nucleotide-Sequence of the Small Ribosomal-Subunit RNA of the Yeast *Candida albicans* and the Evolutionary Position of the Fungi among the Eukaryotes”, *Systematic and Applied Microbiology* 12: 223-229.)

sxtG transcripts: identification, PCR amplification, sequencing and assembly

Putative *sxtG* was identified from two previously published *Alexandrium* 454 libraries (*A. fundyense* CCMP1719 and *A. minutum* CCMP113) deposited in the NCBI Sequence Read Archive (SRA) under the respective accessions SRX040427 and SRX040428 (see, e.g., Stüken A, et al. (2011), “Discovery of Nuclear-Encoded Genes for the Neurotoxin Saxitoxin in Dinoflagellates”, *PlosOne* 6: e20096). Candidate hits (E-value < 0.1) were identified using a custom BLAST against the cyanobacterial SxtG amino acid sequence, before being re-assembled with MacClade v4.07 (Sinauer Associates). Resulting contigs were BLASTed against the non-redundant EST and SRA databases at NCBI. Orthologous *Alexandrium* EST (Accessions: EX463008, CK786100, and CK782453) and SRA (Accession: SRX111568) sequences were identified and aligned with all *sxtG* sequences before designing primers (as discussed below).

The *sxtG* transcript was amplified by various primer methods (see Table 1 for primer sequences). First, the primer pair sxtG160F and sxtG1005R and negative amplifications were used (1 µl product as template) in a nested system with the primer pair sxtG203F and sxtG1005R. Additionally, a nested system using the Zhang dinoSL (Zhang H, et al. (2007), “Spliced leader RNA trans-

splicing in dinoflagellates", *PNAS* 104: 4618-4623) and AUAP (Invitrogen) primer pair followed by sxtG160F and sxtG1005R was utilized. The 5' end of the transcript, including the spliced leader sequence was amplified using the dinoSL primer and the sxtg660R primer. The 3' end was amplified using the sxtg203F and the AUAP adaptor primer. *SxtG* presence was checked for all dinoflagellates (genomic and transcript) with the primer pair sxtG203F and sxtG660R as well as with the nested system, sxtG203F+sxtG1005R / sxtG160F+sxtG1005R. PCR was performed with 3% DMSO with the following conditions: an initial 5 minute 95°C denaturing before 35 cycles of (1) 30 seconds at 94°C (denaturing); (2) 30 seconds at 62°C (annealing); and (3) 1-2 minutes at 72°C (extension), with a final 10-minute extension at the same temperature. All other PCR parameters were standard as described below. The 5' and 3' end products were cloned with TOPO TA (Invitrogen) before being sequenced with the M13 forward and reverse primers. All other PCR products were directly sequenced with respective forward and reverse primer, as well as several internal primers covering the entire transcript. Sequences were assembled as discussed below.

Table 1–Primers designed specifically for this study

Primer name	Primer direction	Primer sequence 5'-3'	SEQ ID NO:
Sxt (160F)	F	TCCGGCGACTACGAGTTC	1
Sxt (203F)	F	GGGCCGTGAAGGATTACCTGA	2
Sxt (203R)	R	TCAGGTAATCCTTCACGGCCC	3
Sxt (218R)	R	GTTTATGCCGTCGCGCTTCAGGT	4
Sxt (660F)	F	CATGGAGTCGATGGTGAGCAAC	5
Sxt (660R)	R	GTTGCTCACCATCGACTCCATG	6
Sxt (760F)	F	CTGGACTCGMACACGATAATGA	7
Sxt (960F)	F	CGAGTCCTACGGCTACAAGC	8
Sxt (1005R)	R	ATCGGCTTGTAAGCCGTAGGACTC	9

DNA and RNA isolation, cDNA synthesis, PCR amplification, sequencing and assembly

Genomic DNA and Total RNA was isolated from 20 ml of culture in the exponential growth phase, centrifuged for 2 minutes at 12,000 × *g*, washed with PBS, and bead-beaten on dry ice with the FastPrep-24 from Medinor (20 seconds, speed 4) using 1.4-mm beads (Medinor). Then, the samples were processed with the Invitrogen ChargeSwitch gDNA plant kit (Invitrogen) and Invitrogen ChargeSwitch TotalRNA cell kit (Invitrogen) in accordance with supplied

protocol. Total RNA from *Gymnodinium catenatum* (CCMP1937) was kindly donated by Johannes Hagström. First strand cDNA was synthesized with the Invitrogen 3' RACE system (Invitrogen) following the high-GC protocol and utilizing the (AP) adapter primer. DNA, RNA, and cDNA quality was checked
5 with a NanoDrop spectrophotometer (ThermoScientific).

Using PCR, template was amplified using Qiagen HotStarTaq *Plus* polymerase (Qiagen) in the presence of 10% BSA in a MJ Research PTC-200 Thermo Cycler (MJ Research). PCR products were gel excised using Promega Wizard SV Gel and PCR Clean-Up System (Promega) before direct sequencing
10 with an ABI3730 DNA analyzer (Applied Biosystems). Primers used in this study have been designed using Primacade (*Bioinformatics* 21: 1263-1264). Melting temperature (T_M) was calculated using OligoCalc (*Nucleic Acids Research* 35: W43-W46). Sequences were quality checked and assembled using the Phred/Phrap/Consed (*Genome Research* 8: 195-202) package under default
15 settings. Additional manual editing was performed in MacCladev4.07. *Sxt41/4* PCR were performed as described (see Stücken, supra).

Bioinformatics

All sequences generated in this study plus dinoflagellate orthologous sequences
20 in the NCBI nr nucleotide and EST databases for each gene were downloaded and separated into their respective datasets. For analysis of ribosomal RNA gene sequence, the three rDNA genes (18S, 5.8S, and 28S) were separately aligned using MAFFT v6 Q-INS-I model (Kiryu H, et al. (2007), "Robust prediction of consensus secondary structures using averaged base pairing probability
25 matrices", *Bioinformatics* 23: 434-441; Hofacker IL, et al. (2002), "Secondary structure prediction for aligned RNA sequences", *Journal of Molecular Biology* 319: 1059-1066; Katoh K and Toh H (2008), "Improved accuracy of multiple ncRNA alignment by incorporating structural information into a MAFFT-based framework", *BMC Bioinformatics* 9: 13) considering secondary RNA structure
30 (default parameters used). For phylogenetic analysis, the outgroup taxon (Apicomplexa) was established from previous dinoflagellate phylogenies (Hoppenrath M and Leander BS (2010), "Dinoflagellate Phylogeny as Inferred

from Heat Shock Protein 90 and Ribosomal Gene Sequences”, *PlosOne* 5; Zhang H, et al. (2007), “A three-gene dinoflagellate phylogeny suggests monophyly of prorocentrales and a basal position for *Amphidinium* and *Heterocapsa*”, *Journal of Molecular Evolution* 65: 463-474; Shalchian-Tabrizi K, et al. (2006),

- 5 “Combined heat shock protein 90 and ribosomal RNA sequence phylogeny supports multiple replacements of dinoflagellate plastids”, *Journal of Eukaryotic Microbiology* 53: 217-224), as well as global eukaryotic phylogenies that concur in placing this as the closest extant relative to the dinoflagellates (Minge MA, et al. (2009), “Evolutionary position of breviate amoebae and the primary eukaryote
- 10 divergence”, *Proceedings of the Royal Society B-Biological Sciences* 276: 597-604; Burki F, et al (2008), “Phylogenomics reveals a new ‘megagroup’ including most photosynthetic eukaryotes”, *Biology Letters* 4: 366-369).

SxtG analyses and Phylogenetic inference

- 15 Dinoflagellate *sxtG* transcript structure was determined by aligning the translated sequences to cyanobacteria *sxtG* and/or using conserved domains searches (Punta M, et al. (2012), “The Pfam protein families database” *Nucleic Acids Research* 40: D290-D301). Open reading frames (ORF) were predicted using OrfPredictor (Min XJ, et al., “OrfPredictor: predicting protein-coding
- 20 regions in EST-derived sequences”, *Nucleic Acids Research* 33: W677-W680). Catalytic and substrate-binding residues of *sxtG* from cyanobacteria have been previously determined (Kellmann R, et al. (2008) “Biosynthetic intermediate analysis and functional homology reveal a saxitoxin gene cluster in
- 25 cyanobacteria”, *Applied and Environmental Microbiology* 74: 4044-4053). The dinoflagellate *sxtG* amino acid sequences were aligned using MAFFT v6 L-INS-I model (Kato H and Toh H (2008) “Improved accuracy of multiple ncRNA alignment by incorporating structural information into a MAFFT-based
- 30 framework”, *BMC Bioinformatics* 9: 13) to orthologous cyanobacteria *sxtG* sequences, in addition to a selection of closely related NCBI nr Blastp hits. Resulting alignments were checked manually and poorly aligned positions excluded using MacClade v4.07. ProtTest v2.4 (*Bioinformatics* 21: 2104-2105) determined LG as the optimal evolutionary model for all inferred alignments.

Maximum Likelihood (ML) analyses were performed with RAxML-VI-HPCv7.2.6, PROTCATLG model with 25 rate categories (Stamatakis A (2006) "RAxML-VI-HPC: Maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models", *Bioinformatics* **22**: 2688-2690). Bayesian analyses were performed with Phylobayes v3.3b (Lartillot N and Philippe H (2004) "A Bayesian mixture model for across-site heterogeneities in the amino-acid replacement process", *Molecular Biology and Evolution* **21**: 1095-1109; Lartillot N and Philippe H (2006) "Computing Bayes factors using thermodynamic integration", *Systematic Biology* **55**: 195-207; Lartillot N, et al. (2007) "Suppression of long-branch attraction artefacts in the animal phylogeny using a site-heterogeneous model", *BMC Evolutionary Biology* **7**: 14) under the CATLG model with a free number of mixing categories and a discrete across site variation under 4 categories. Trees were inferred when the largest maximum difference between the bipartitions (chains) was <0.1.

sxtG sequences

PCR amplification was used to amplify *sxtG* and *sxtA* from DNA template prepared from *A. affine* (CCMP112), *A. andersoni* (CCMP2222), *A. catenella* (CCMP1493), *A. insuetum* (CCMP2082), *A. tamarense* (CCMP1771), *A. carteri* (UIO081), *C. longipes* (CCMP1770), *C. monotis*, *G. catenatum* (CCMP1937), *H. triquetra* (RCC2540), *K. veneficum* (RCC2539), *L. polyedrum* (CCMP1931), *P. lima* (CS-869), *P. micans* (UIO292), *P. minimum* (UIO085), *P. noctiluca* (CCMP732), and *P. reticulatum*.

sxtG was successfully amplified from 8 species and 2 orders and undetected for 22 species in 5 orders (Table 2). *sxtA* (1/4) was not detected in an additional 21 species and 2 orders to those already reported (Table 2) (see, e.g., Stüken A, et al. (2011) "Discovery of Nuclear-Encoded Genes for the Neurotoxin Saxitoxin in Dinoflagellates", *PlosOne* **6**: e20096; previously reported results noted with an asterisk *)

Table 2–List of dinoflagellates used in this study

amplification of *sxtA1*, *sxtA4* and *sxtG* noted by:
 “n.d.”, signifying not detected, or “+”, signifying a successfully amplified sequence

Species/Taxon	<i>sxtA</i> (1/4)	<i>sxtG</i>
<i>Adenoides eludens</i> CCMP1891	n.d.	n.d.
<i>Alexandrium affine</i> CCMP112	n.d.*	+
<i>Alexandrium andersonii</i> CCMP2222	n.d.*	+
<i>Alexandrium catenella</i> CCMP1493	+	+
<i>Alexandrium fundyense</i> CCMP1719	+	+
<i>Alexandrium insuetum</i> CCMP2082	n.d.	+
<i>Alexandrium minutum</i> CCMP113	+	+
<i>Alexandrium tamarense</i> CCMP1771	+	+
<i>Amphidinium carteri</i> UIO081	n.d.	n.d.
<i>Amphidinium mootonorum</i> CAWD161	n.d.	n.d.
<i>Amphidinium massartii</i> CS-259	n.d.*	n.d.
<i>Azadinium spinosum</i> RCC2538	n.d.	n.d.
<i>Ceratium longipes</i> CCMP1770	n.d.	n.d.
<i>Coolia monotis</i>	n.d.	
<i>Gambierdiscus australes</i> CAWD148	n.d.	n.d.
<i>Gymnodinium aureolum</i> SCCAP K-1561	n.d.	n.d.
<i>Gymnodinium catenatum</i> CCMP1937	+	+
<i>Heterocapsa triquetra</i> RCC2540	n.d.	n.d.
<i>Karlodinium veneficum</i> RCC2539	n.d.	n.d.
<i>Lepidodinium chlorophorum</i> RCC2537	n.d.	n.d.
<i>Lingulodinium polyedrum</i> CCMP1931	n.d.	n.d.
<i>Pentapharsodinium dalei</i> SCCAP K-1100	n.d.	n.d.
<i>Polarella glacialis</i> CCMP2088	n.d.	n.d.
<i>Prorocentrum lima</i> CS-869	n.d.*	n.d.
<i>Prorocentrum micans</i> UIO292	n.d.	n.d.
<i>Prorocentrum minimum</i> UIO085	n.d.	n.d.
<i>Protoceratium reticulatum</i>	n.d.	n.d.
<i>Pyrocystis noctiluca</i> CCMP732	n.d.	n.d.
<i>Scrippsiella trochoideae</i> BS-46	n.d.	n.d.
<i>Thecadinium kofoidii</i> SCCAP K-1504	n.d.	n.d.

5 Identification of *sxtG*

Searching the unassembled 454 cDNA library reads with the cyanobacterial *sxtG* amidinotransferase gene resulted in 88 hits for *A. fundyense* CCM1719 and 67 hits for *A. minutum* CCMP113. After pooling and re-assembling the sequences a full *sxtG* ORF was obtained for both species, though a dinoflagellate spliced leader sequence and polyA-tail were lacking from the contigs. Additionally, one *A. fundyense* and three *A. minutum* homologous contigs were also obtained and predicted to be amidinotransferase genes. Sequences of the *A. fundyense* CCMP1719 and the *A. minutum* CCMP113 *sxtG* cDNAs are provided in Figures 1 and 2, respectively.

The structure of *sxtG*

RACE experiments resulted in full-length *sxtG* transcripts for both *A. fundyense* CCMP1719 and *A. minutum* CCMP113. This included dinoflagellate spliced-
 5 leader sequence at the 5' end and a eukaryotic poly-A tail at the 3' end. The transcripts were 1283 bp and 1276 bp in length, respectively, excluding the poly-A tails. Additionally, the *A. tamarensis* SRA contig (SRX111568) contained 9 bp of the 22 bp dinoSL sequence. Conserved domain searches identified *sxtG* as an amidinotransferase. The ORF was predicted to be 375 amino acids in length. The
 10 1125 bp ORF contig of *A. fundyense* CCMP1719 had 5 single nucleotide polymorphisms (SNPs) all of which are non-synonymous. In comparison the same region of *A. minutum* CCMP113 lacked a single SNP.

The phylogeny of *sxtG*

The *sxtG* primers designed in this study (Table 1) amplified an 881 bp sequence from seven *Alexandrium* species (both STX producers and non-producers) and *Gymnodinium catenatum* (Table 2). No *sxtG* PCR products were amplified for 23
 15 non-STX-producing dinoflagellates (Table 2). In addition, no putative *sxtG* sequence external to *Alexandrium* was identified by BLAST against the NCBI non-redundant, EST and SRA databases.
 20

The phylogenetic inference of *sxtG* shows that dinoflagellate *sxtG* sequences form a fully supported clade. Though, the branching pattern within the clade is unclear as a result of high sequence conservation between species. The planctomycete *Gemmata obscuriglobus* is the unsupported sister to the
 25 dinoflagellate *sxtG* clade. The proteobacterium *Beggiatoa* further excludes the dinoflagellate sequences from a sister relationship with a fully supported cyanobacterial *sxtG* clade. The four previous clades form a weakly supported (61/0.66) group with a cluster of proteobacteria species. This is further included in a moderately supported (76/0.98) monophyly with an additional proteobacteria
 30 clade, constituting the cluster defined amidinotransferase 1. The amidinotransferase 1 clade is excluded from Opisthokonta (100/1.00) and amidinotransferase 2 (92/0.99). The amidinotransferase 2 clade harbors the

additional dinoflagellate amidinotransferase sequences. This fully supported clade forms a weak (53/0.82) grouping to Actinobacteria and Cyanobacteria AoaA sequences.

All publications and patents mentioned in the above specification are
5 herein incorporated by reference in their entirety for all purposes. Various
modifications and variations of the described compositions, methods, and uses of
the technology will be apparent to those skilled in the art without departing from
the scope and spirit of the technology as described. Although the technology has
been described in connection with specific exemplary embodiments, it should be
10 understood that the technology as claimed should not be unduly limited to such
specific embodiments. Indeed, various modifications of the described modes for
carrying out the technology that are obvious to those skilled in pharmacology,
biochemistry, medical science, or related fields are intended to be within the
scope of the following claims.

15

CLAIMS

We claim:

- 5 1. A method for detecting a saxitoxin-producing dinoflagellate in a sample, the method comprising:
- a) providing a sample, and
- b) analyzing the sample for the presence or absence of one or more of a
10 dinoflagellate saxitoxin G polynucleotide or a polypeptide encoded by said polynucleotide,
- wherein the presence of said polynucleotide or polypeptide indicates the presence of a saxitoxin-producing dinoflagellate in the sample and wherein the absence of said polynucleotide or polypeptide indicates the absence of a saxitoxin-producing dinoflagellate in the sample.
- 15 2. The method according to claim 1, wherein the polynucleotide comprises a saxitoxin G nucleotide sequence selected from those set forth in any one of SEQ ID NOs: 12–13, or a fragment or variant of any one of those sequences.
- 20 3. The method according to claim 1 or 2, further comprising analyzing the sample for the presence or absence of one or more of a dinoflagellate saxitoxin A polynucleotide or a polypeptide encoded by said polynucleotide.
- 25 4. The method according to any of claims 1 to 3, wherein said analyzing comprises amplification of polynucleotides from the sample by polymerase chain reaction.
- 30 5. The method according to claim 4, wherein said polymerase chain reaction utilizes one or more primers comprising a sequence set forth in SEQ ID NOs: 1–9, or a fragment or variant of any of these sequences.

6. The method according to any of claims 1 to 5, wherein the polypeptide comprises a saxitoxin G amino acid sequence, or a fragment or variant thereof.

5

7. The method according to any of claims 1 to 6, wherein the saxitoxin producing dinoflagellate is from the genus *Alexandrium*, *Pyrodinium*, or *Gymnodinium*.

- 10 8. The method according to claim 7, wherein the saxitoxin-producing dinoflagellate is selected from the group consisting of *A. catenella*, *A. fundyense*, *A. lusitanicum*, *A. minutum*, *A. ostenfeldii*, *A. tamarense*, *G. catenatum*, and *P. bahamense* var *compressum*.

- 15 9. A kit for the detection of a saxitoxin-producing dinoflagellate in a sample or for determining the absence of a saxitoxin-producing dinoflagellate in a sample, the kit comprising at least one agent for detecting the presence of a dinoflagellate saxitoxin G polynucleotide or a polypeptide encoded by said polynucleotide.

20

10. The kit according to claim 9, wherein the agent binds specifically to a polynucleotide comprising a saxitoxin G nucleotide sequence selected from those set forth in any one of SEQ ID NOs: 12–13, or a fragment or variant of any one of those sequences.

25

11. The kit according to claim 9 or 10, wherein the agent binds specifically to a polynucleotide encoding a saxitoxin G peptide sequence or a fragment thereof.

- 30 12. The kit according to claim 11 further comprising at least one agent for detecting the presence of a dinoflagellate saxitoxin A polynucleotide or a polypeptide encoded by said polynucleotide

13. The kit according to any of claims 9 to 12, wherein the agent is a primer, a probe, or an antibody.
- 5 14. The kit according to claim 13, wherein the agent is a primer comprising a sequence set forth in SEQ ID NOs: 1–9, or a fragment or variant of any of these sequences.
- 10 15. The kit according to claim 13, wherein the agent binds specifically to a saxitoxin G amino acid sequence, or a fragment or variant thereof.
16. An isolated polynucleotide comprising a sequence set forth in SEQ ID NO: 12 or 13, or a variant or fragment thereof.
- 15 17. An isolated polynucleotide comprising the sequence set forth in any one of SEQ ID NOS: 1–9, or a variant or fragment of any one of those sequences.
18. An isolated polypeptide encoded by a polynucleotide according to claim 16.
- 20 19. Use of an agent selected from a saxitoxin G polynucleotide or a polypeptide encoded by said polynucleotide or combinations thereof for detecting the presence of a dinoflagellate.
- 25 20. Use according to claim 19, wherein the agent binds specifically to a polynucleotide comprising a saxitoxin G nucleotide sequence selected from those set forth in any one of SEQ ID NOs: 12–13, or a fragment or variant of any one of those sequences.
- 30 21. Use according to claim 19 or 20, wherein the agent binds specifically to a polynucleotide encoding a saxitoxin G peptide sequence or a fragment thereof.

22. Use according to claim 21 further comprising at least one agent for detecting the presence of a dinoflagellate saxitoxin A polynucleotide or a polypeptide encoded by said polynucleotide
- 5 23. Use according to any of claims 19 to 22, wherein the agent is a primer, a probe, or an antibody.
24. Use according to claim 23, wherein the agent is a primer comprising a sequence set forth in SEQ ID NOs: 1–9, or a fragment or variant of any of
10 these sequences.
25. Use according to claim 23, wherein the agent binds specifically to a saxitoxin G amino acid sequence, or a fragment or variant thereof.

>A_fundyense_CCMP1719_cDNA_sxtG (SEQ ID NO: 12)
accgtagccaTTTTGGCTCAAGCCAttctggcTCAAGGTTGAGCAGTGCTCGCCGCGGCCGCTGGGGTTCCTT
GGAcCCCTCCGCCCgCCATGTGGAACAATGACgTCGGCAGGCTGCGGaCCGTCTGTATCGGGAGCGTGCGCAGG
GCTGGCAGTCGCAGGACATGGACGAGAATAGCTGGGGGCGCGCCTACTGCAGGAAGATGGCCTCGCCCTCCGGCG
ACTACGAGTTCATGCACAGCCTGCCGAtCTATGACTTCTACAAGAGGGTCGAGGGCGCGAGGAGCGAGCTCCGGG
CCGTGAAGGATTACCTGAAGCGCGACGGCATAAACGTGCTCGCCATCGACGAGCCACAGCTCGCCAAGGTGACGC
CGCAGCCGGGGATCTACCCCGGCAGCGAGTGCCCTCCCGTTCAACGCCGCAAACGTGCGGGACCCCTTCATCATGC
TCGGCAAGACGCTGGTCGTAGCCGGTCGACGCGCgCGGGCACGACGCACATCCCAGACTGGTACATGCCGCTGC
TCAGGGACTTCTGCGGCAAGCAGGGGCTTCGCGTCTTCGAGAGCCCGGCGGTACCCCGCACCTGCAGCTTGCCA
AGCCTGGTCTCTGGGAGGACCCCGACGAGATGCACAGGTACATCCATACGGACGGGAACGGCTACAACGGGAGGA
TCTACGAGGCCTTCCAGCAGGAGGACCCGATCTTCGAGCCCGCCGACATGTTCCGCACCGACGGGAGGCGCGTCT
TCTGCATGGAGTCGATGGTGAGCAACGGCGTCTACCACGAGTGGTTCGCGAGGGAGTTCCCGAGGTGGAGTTCG
TCCCCTTCAGGCGGTACTTCTACTCCGAGGTGGACGTGCCGATGCACTCGGACGCCAGCTACGTCACGCTGGACT
CGCACACGATAATGATGGCGCCCGAGCAGATGCCCGACCTGAGACGATCCGGAAGGTCCAGGAGCGCTACCGCA
TCCTGATCCCCCGCGCTCCGACCTGCCCAACCCGACGTGCGCGCGCTACCATTTGAACACGCTCTCGCTGGACG
AGAAGCGGATGCTGGTCAACGCGAAGGAGAAGACGATGATCAAGTGGCTCGAGTCTACGGCTACAAGCCGATCC
CGATGGAGATATGCAACATGAACTTCTGCGGCGGCGGCTTCCACTGCACGTCCCTGGACCTGGAACGCGACGACA
TCGCCGCCCCCCCCGGCGTGATGCCGCAGGAGGTCCAGAGCATGCCCGGCTTCTCTTTTGATGCAAACAAAAAAA
AAAAAAAAAAAAAAAAAAAA

Figure 1

>A_minutum_CCMP113_cDNA_sxtG (SEQ ID NO: 13)
tccgtagccatTTTTGCTCAAGGTTTGCAGTGCTCGCCGTCGCCGCTGGGGTTCCCCGGACCCCTCCACCCGCT
ATGTGCAACAATGACGTCGGCAGGCTGCGGACCGTCCTGATCGGGAGCGTCGGCGAGGGCTGGCAGTCGCAGGAC
ATGGACGATAATAGCTGGGGGCGCGCCTACTGCAAGAAGATGGCCTCGCCCTCCGGCGACTACGAGTTCATGCAC
AGCCTGCCGATCTACGACTTCTACAAGAGGGTCCAGGGCGCGAGGAGCGAGCTCCGGGCCGTGAAGGATTACCTG
AAGCGCGACGGCATAAACGTGCTCGAAATCGACGAGCCACAGCTCGCCAAGGTGACGCCGAGCCGGGGATCTAC
CCCGACAGCGAGTGCCTCCCGTTCAACGCCGCGAACGTGCGGGACCCCTTCATCATGCTCGGCAAGACTCTGGTC
GTCAGCCGGTCGACGCGCGcAGGCACGACGCACATCCCAGATTGGTACATGCCGCTGCTCAGGGACTTTTGCGGC
AAGCAGGGGCTTCGCGTCTCGAGAGCCCGGTTGTACCCCCACATCTGCAGCTTGCCAAGCCTGGTCTTTGGGAG
GACCCCGACGAGATGCACAGATACATCCATACGGACGGGAACGGCTACAATGGGAGGATCTACGAGGCCTTCCAG
CAGGAGGACCCGATCTTCGAGCCCGCCGACATGTTCCGCACCGACGGGAGGCGCGTCTTCTGCATGGAGTCGATG
GTGAGCAACGGCGCCTACCACGAGTGGTTCCGGAGGGAGTTCCCGGAGGTGGAGTTCGTCCCTTCAGGCGGTAT
TTCTACTCCGAGGTGGACGTGCCGATGCACTCGGATGCCAGCTACGTACGCTGGACTCGAACACGATAATGATG
GCGCCCGAGCAGATGCCCGACCCCGAGACGATCCGGAAGGTCCAGGAGCGCTACCGCATCCTGATCCCCCGCGC
TCCGACCTGCCCAACCCGACGTGCGCGCGCTACCACCTGAACACGCTCTCGCTGGACGAGAAGCGGATGTTGGTC
AACGCGCAGGAGAAGACGATGATCAAGTGGCTCGAGTCTACGGCTACAAGCCGATCCCGATGGAGATATGCAAC
ATGAAC TTCTGCGGCGCGGGCTTCCATTGCACGTCCCTGGACCTGGAGCGCGACGACATCGCCGCCCCCGGCG
TGATGCCACAAGGGGGTCCAGGGCATGCCCGGCTTCGTTC TTGATGCAAACCAACTTCACAAAAAAAAAAAAAA
AAAAAAAAAAAAAAAAAAAA

Figure 2