DETECTION OF SAXITOXIN-PRODUCING DINOFLAGELLATES

~~Incorporation by Cross-Reference~~Priority Claim

This application claims priority from United States of America Provisional Patent Application No. 61/486,633 filed on 16 May 2011~~, the entire content of which is incorporated herein by cross-reference~~.

Technical Field

The invention generally relates to the field of saxitoxins and the identification of microorganisms capable of producing them. More specifically, the invention relates to the identification of genes encoding saxitoxin in dinoflagellates, and methods for the specific detection of dinoflagellates that are producers of saxitoxins.

Background

Saxitoxin (STX) is a potent neurotoxin that occurs in aquatic environments worldwide and has significant economic, environmental and human health impacts. Ingestion of vector species can lead to paralytic shellfish poisoning, a severe human illness that may lead to paralysis and death. 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. In freshwater environments, STX is predominantly produced prokaryotic cyanobacteria. However, in marine environments eukaryotic dinoflagellates have been associated with the presence of STX. Despite the association of a number of dinoflagellate species with STX production, the genetic basis for the production of STX in these microorganisms remains elusive.

There is a need for methods to detect the presence (or absence) of STX-producing dinoflagellates in marine samples.

Summary of the Invention

A number of studies have unsuccessfully attempted to identify STX pathway genes and/or enzymes in dinoflagellates by enzymatic characterisation, PCR approaches, *in silico* analyses of expressed sequence tag (EST) libraries, or the use of other publicly available nucleotide sequences. In addition, several previous studies have suggested that STX may not in fact be produced by dinoflagellates but instead by co-cultured bacteria.

The present inventors have determined that dinoflagellates are in fact producers of STX and have identified genes responsible for STX production in these microorganisms. The identification of genes responsible for STX production provides a basis for numerous

Definitions

As used in this application, the singular form "a", "an" and "the" include plural references unless the context clearly dictates otherwise. For example, the term "a dinoflagellate" also includes a plurality of dinoflagellates.

As used herein, the term "comprising" means "including". Variations of the word "comprising", such as "comprise" and "comprises," have correspondingly varied meanings. Thus, for example, a polynucleotide "comprising" a sequence encoding a protein may consist exclusively of that sequence or may include one or more additional sequences.

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

Any description of prior art documents herein, or statements herein derived from or based on those documents, is not an admission that the documents or derived statements are part of the common general knowledge of the relevant art.

~~For the purposes of description all documents and GenBank sequences referred to herein are incorporated by reference in their entirety.~~

Detailed Description

The present inventors have determined that dinoflagellates are producers of saxitoxin (STX) and have identified genes responsible for STX production in these microorganisms. Based on this discovery, the inventors have developed an assay capable of discerning between STX-producing dinoflagellate species and dinoflagellate species which do not produce STX.

Accordingly, certain aspects of the invention relate to the provision of STX polynucleotide and polypeptide sequences present in dinoflagellates.

Also provided are methods for the detection of STX-producing dinoflagellates in a given sample based on detecting the presence (or absence) of one or more sequences of the invention in the sample.

Also provided are kits for the detection of STX-producing dinoflagellates in a given sample comprising agent(s) for detecting the presence (or absence) of one or more sequences of the invention in the sample.

Polynucleotides and polypeptides

Disclosed herein are dinoflagellate saxitoxin polynucleotide and polypeptide sequences ("polynucleotides of the invention" and "polypeptides of the invention",

respectively). Polynucleotides of the invention may be deoxyribonucleic acids (DNA), ribonucleic acids (RNA) or complementary deoxyribonucleic acids (cDNA).

In certain embodiments, the sequences are saxitoxin A gene (*sxtA*) polynucleotide sequences or saxitoxin A polypeptide (STXA) sequences.

The *sxtA* polynucleotide sequences may comprise any one or more *sxtA* gene catalytic domain(s) (i.e. the *sxtA1*, *sxtA2*, *sxtA3*, or *sxtA4* catalytic domain(s)), or fragment(s) thereof. ~~By way of non-limiting example only, T~~the *sxtA1* sequence may be defined by nucleotides 160-1821 of the polynucleotide sequence set forth in GenBank accession number JF343238 (SEQ ID NO: 1) or nucleotides 277-2022 of the polynucleotide sequence set forth in GenBank accession number JF343239 (SEQ ID NO: 3). The *sxtA2* sequence may be defined by nucleotides 1837-2415 of the polynucleotide sequence set forth in GenBank accession number JF343238 (SEQ ID NO: 1) or nucleotides 2038-2604 of the polynucleotide sequence set forth in GenBank accession number JF343239 (SEQ ID NO: 3). The *sxtA3* sequence may be defined by nucleotides 2479-2694 of the polynucleotide sequence set forth in GenBank accession number JF343238 (SEQ ID NO: 1) or nucleotides 2722-2949 of the polynucleotide sequence set forth in GenBank accession number JF343239 (SEQ ID NO: 3). The *sxtA4* sequence may be defined by nucleotides 3115-4121 of the polynucleotide sequence set forth in GenBank accession number JF343239 (SEQ ID NO: 3) or nucleotides 3597-3721 of the polynucleotide sequence set forth in GenBank accession number JF343239 (SEQ ID NO: 3).

Other ~~non-limiting~~ examples of *sxtA* gene polynucleotide sequences of the invention include those provided in GenBank accession numbers JF343238 and JF343239 (SEQ ID NO: 1 and SEQ ID NO: 3).

The STXA polypeptide sequences may comprise any one or more STX protein catalytic domain(s) (i.e. the STXA1, STXA2, STXA3, or STXA4 catalytic domain(s)) or fragment(s) thereof. ~~By way of non-limiting example only, t~~The STXA1 sequence may be defined by amino acid residues 1-554 of the polypeptide sequence set forth in GenBank accession number JF343238 (SEQ ID NO: 2) or amino acid residues 1-582 of the polypeptide sequence set forth in GenBank accession number JF343239 (SEQ ID NO: 4). The STXA2 sequence may be defined by amino acid residues 560-752 of the polypeptide sequence set forth in GenBank accession number JF343238 (SEQ ID NO: 2) or amino acid residues 588-776 of the polypeptide sequence set forth in GenBank accession number JF343239 (SEQ ID NO: 4). The STXA3 sequence may be defined by amino acid residues 774-845 of the polypeptide sequence set forth in GenBank accession number JF343238 (SEQ ID NO: 2) or amino acid residues 816-891 of the polypeptide sequence set forth in GenBank accession number JF343239 (SEQ ID NO: 4). The STXA4 sequence

may be defined by amino acid residues 947-1281 of the polypeptide sequence set forth in GenBank accession number JF343239 (SEQ ID NO: 4).

Other ~~non-limiting~~ examples of STX polypetide sequences of the invention include those provided in GenBank accession numbers JF343238 and JF343239 (SEQ ID NO: 2 and SEQ ID NO: 4).

Preferably, the polynucleotide and polypeptide sequences are 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*.

~~Non-limiting e~~Examples of preferred *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). ~~Non-limiting~~ eExamples of preferred *Gymnodinium* species include *G. catenatum* (e.g. strains GCTRA01 and CS-395). ~~Non-limiting e~~Examples of preferred *Pyrodinium* species include *P. bahamense var compressum*.

Fragments of both polynucleotides of the invention and polypeptides of the invention are also provided herein.

A polynucleotide "fragment" as contemplated herein is a polynucleotide molecule that is a constituent of a polynucleotide of the invention or variant thereof. Fragments of a polynucleotide do not necessarily need to encode 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 invention 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 invention comprise fragments of the *sxtA* gene. For example, polynucleotide fragments of the invention may comprise any one or more of the *sxtA1*, *sxtA2*, *sxtA3*, or *sxtA4* catalytic domain(s), or fragment(s) thereof. Polypeptide fragments of the invention comprise fragments of the STX protein. For example, polynucleotide fragments of the invention may comprise any one or more of the STXA1, STXA2, STXA3, or STXA4 catalytic domain(s), or fragment(s) thereof.

Specific ~~and non-limiting~~ examples of *sxtA* gene polynucleotide sequence fragments of the invention include those provided in GenBank accession numbers JF343240 - JF343432 (SEQ ID NO: 5 - SEQ ID NO: 197), and those set forth in SEQ ID NOs: 224-227, 230-242 and 247.

Additional specific ~~and non-limiting~~ examples of *sxtA* gene polynucleotide sequence fragments of the invention include those defined by nucleotides 3115-4121 and nucleotides 3597-3721 of the polynucleotide sequence set forth in GenBank accession number JF343238 (SEQ ID NO: 3), and fragments and variants thereof.

Specific ~~and non-limiting~~ examples of *sxtA1* catalytic domain sequences include those set out in SEQ ID NOs: 224-227 and 247.

Specific ~~and non-limiting~~ examples of *sxtA4* catalytic domain sequences include those set out in SEQ ID NOs: 230-242.

A polypeptide "fragment" as contemplated herein is a polypeptide molecule is a constituent of a polypeptide of the invention or variant thereof. Typically the fragment possesses qualitative biological activity in common with the polypeptide of which it is a constituent or 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.

Specific ~~and non-limiting~~ examples of STXA polypeptide sequence fragments of the invention include those defined by amino acid residues X-Y of the polypeptide sequence set forth in GenBank accession number JF343248 (SEQ ID NO: 3), those defined by amino acid residues 947-1281 of the polypeptide sequence set forth in

Methods for the design and/or production of nucleotide probes and/or 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; 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 diethylphosphoramidite method (see Beaucage et al. (1981) Tetrahedron Letters, 22:1859-1862).

Polynucleotides of the invention, including the aforementioned probes and primers, may be labelled 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 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. 32P, 35S, 3H, 33P). Detection of the marker may be achieved, for example, by chemical, photochemical, immunochemical, 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 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 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 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

prior to extraction or the extraction may be performed directly on a given sample. Suitable methods for the isolation (i.e. extraction) and purification of polynucleotides and polypeptides for analysis using methods of the invention 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., 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 invention include, ~~but are not limited to,~~ reverse-phase chromatography, hydrophobic interaction chromatography, centrifugation, gel filtration, ammonium sulfate precipitation, and ion exchange.

In alternative embodiments, methods of the invention may be performed without isolating nucleic acids and/or polypeptides from the sample.

Detecting the presence (or determining the absence) of polynucleotides of the invention and/or polypeptides of the invention in a given sample may be 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 invention or any one or more polypeptides of the invention. Suitable techniques include, for example, the polymerase chain reaction (PCR) and related variations of this technique (e.g. quantitative PCR), 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 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 invention in a given sample is achieved by 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 invention 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, degenerate primers, gene-specific primers, vector-specific primers, partially-mismatched primers, and the like. Methods for designing PCR and RT-PCR primers are generally

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

By "binding specifically" it will be understood that the primer, probe or 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 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.

In preferred embodiments, the primers, probes or antibodies bind specifically to a saxitoxin A1 or A4 catalytic domain polynucleotide or polypeptide sequence, or a fragment thereof. More preferably the primers, probes or antibodies bind specifically to a saxitoxin A4 catalytic domain polynucleotide or polypeptide sequence, or a fragment thereof.

Suitable primers and probes may bind specifically to any fragment of a saxitoxin A1 catalytic domain polynucleotide sequence. Suitable antibodies may bind specifically to a fragment of a saxitoxin A1 catalytic domain polypeptide sequence encoded by such polynucleotide sequences.

Suitable primers and probes may bind specifically to any fragment of a saxitoxin A4 catalytic domain polynucleotide sequence. ~~By way of non-limiting example only,~~ suitable primers and probes may bind specifically to a fragment of the saxitoxin A4 catalytic domain polynucleotide sequence defined by nucleotides 3115-4121 of the polynucleotide sequence set forth in GenBank accession number JF343239 (SEQ ID NO: 3). Suitable antibodies may bind specifically to a fragment of a saxitoxin A4 catalytic domain polypeptide sequence encoded by such polynucleotide sequences.

In some embodiments, the methods of the invention may involve detecting the presence (or determining the absence) of 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 catalytic domain(s) of the *sxt* gene, or fragments(s) thereof. Preferably, the primers amplify a sequence comprising a saxitoxin A4 catalytic domain polynucleotide sequence, or a fragment thereof. ~~By way of non-limiting example only, a~~A suitable primer pair for this purpose may comprise a first primer comprising the polynucleotide sequence of SEQ ID NO: 198, or a fragment or variant thereof, and/or a second primer comprising the polynucleotide sequence of SEQ ID NO: 199, or a fragment or variant thereof. Other ~~non-limiting~~ examples of suitable primer pairs include those set forth in SEQ ID NOs: 200-211, 220-223, 228-229, and 243-244 (including fragments and variants of these primer pair sequences).

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 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 A polynucleotide sequences, and more preferably saxitoxin A1 and/or A4 domain polynucleotide sequences.

In other embodiments, the methods of the invention may involve detecting the presence (or determining the absence) of polynucleotides of the invention in a sample by the use of suitable probes. Probes of the invention are based on *sxt* polynucleotide sequences of the invention. Probes are nucleotide sequences of variable length, for example between about 10 nucleotides and several thousand nucleotides, for use in detection of homologous sequences, typically by hybridization. Hybridization probes of the invention may be genomic DNA fragments, cDNA fragments, RNA fragments, or other oligonucleotides. Probes of the invention 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. 32P, 35S, 3H, 33P). 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 invention may involve detecting the presence (or determining the absence) of polypeptides of the invention in a sample using

antibodies. The antibodies may be used to qualitatively or quantitatively detect and analyse one or more STX polypeptides of the invention in a given sample. The antibodies may be conjugated to a fluorochrome allowing detection, for example, by flow cytometry, immunohistochemisty or other means known in the art. Alternatively, the antibody may be bound to a substrate allowing colorimetric or chemiluminescent detection. The invention also contemplates the use of secondary antibodies capable of binding to one or more antibodies capable of binding specifically to a polypeptide of the invention.

Kits for detecting dinoflagellates

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

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 invention comprise at least one agent for detecting the presence of one or more polynucleotides of the invention and/or one or more polypeptides of the invention, and/or variants or fragments thereof (see description in the section above entitled "Polynucleotides and polypeptides"). Any agent suitable for this purpose may be included in the kits. ~~Non-limiting e~~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".

In preferred embodiments, the kits are for use in the methods of the invention (see description in the section above entitled "Methods for detecting dinoflagellates").

In some embodiments the invention 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 invention, and/or one or more polypeptides of the invention, and/or a variant or fragment of either. Preferably, the dinoflagellate is a saxitoxin-producing dinoflagellate.

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

In general, kits of the invention may comprise any number of additional components. ~~By way of non-limiting example t~~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 invention.

Dinoflagellates detected in a sample or determined to be absent from a sample using kits of the invention 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*.

It will be appreciated by persons skilled in the art that numerous variations and/or modifications may be made to the invention as described in the specific embodiments without departing from the ~~spirit or~~ scope of the invention as broadly described. ~~The present embodiments are, therefore, to be considered in all respects as illustrative and not restrictive~~.

Examples

The invention will now be described with reference to specific examples~~, which should not be construed as in any way limiting~~.

Example 1: Identification of Nuclear-Encoded Genes for the Neurotoxin Saxitoxin in Dinoflagellates

Materials and Methods

- *Culturing and toxin measurements*

Saxitoxin-producing and non-producing dinoflagellate cultures were obtained from various culture collections (Table 1).

Table 1. List of dinoflagellate strains used in this study, their production of STX and whether *sxtA1* and *sxtA4* fragments were amplified from their genomic DNA.

Order Genus Species	Strain	STX	PCR sxtA1	PCR sxtA4
Gonyaulacales				
Alexandrium				

CLAIMS:

1. 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 of one or more of a dinoflagellate saxitoxin A catalytic domain polynucleotide or a polypeptide encoded by said polynucleotide;

wherein:

the saxitoxin catalytic domain is a saxitoxin A4 catalytic domain; and

the presence of said polynucleotide or polypeptide indicates the presence of a saxitoxin-producing dinoflagellate in the sample.

2. A method for determining the absence of 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 of one or more of a dinoflagellate saxitoxin A catalytic domain polynucleotide or a polypeptide encoded by said polynucleotide;

wherein:

the saxitoxin catalytic domain is a saxitoxin A4 catalytic domain; and

the absence of said polynucleotide or polypeptide indicates the absence of a saxitoxin-producing dinoflagellate in the sample.

3. The method according to claim 1 or claim 2, wherein the saxitoxin A4 catalytic domain sequence comprises nucleotides 3115-4121 of the polynucleotide sequence set forth in SEQ ID NO: 3, nucleotides 3597-3721 of the polynucleotide sequence set forth in SEQ ID NO: 3.

4. The method according to claim 1 or claim 2, wherein said analyzing comprises amplification of polynucleotides from the sample by polymerase chain reaction.

5. The method according to claim 4, wherein said polymerase chain reaction utilises one or more primers consisting of a sequence set forth in any one of SEQ ID

NOs: 198-199.

6. The method according to claim 1 or claim 2, wherein the polypeptide comprises a saxitoxin A4 catalytic domain amino acid sequence as set forth in residues 947-1281 of SEQ ID NO: 4.

7. The method according to claim 1 or claim 2, wherein the saxitoxin-producing dinoflagellate is from the Alexandrium, Pyrodinium or Gymnodinium genus.

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.

9. A kit for the detection of a saxitoxin-producing dinoflagellate in a sample, the kit comprising at least one agent for detecting the presence of a dinoflagellate saxitoxin A catalytic domain polynucleotide or a polypeptide encoded by said polynucleotide, wherein the saxitoxin catalytic domain is a saxitoxin A4 catalytic domain.

10. A kit for the 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 A catalytic domain polynucleotide or a polypeptide encoded by said polynucleotide, wherein the saxitoxin catalytic domain is a saxitoxin A4 catalytic domain.

11. The kit according to claim 9 or claim 10, wherein the saxitoxin A4 catalytic domain sequence comprises nucleotides 3115-4121 of the polynucleotide sequence set forth in SEQ ID NO: 3, nucleotides 3597-3721 of the polynucleotide sequence set forth in SEQ ID NO: 3.

12. The kit according to claim 9 or claim 10, wherein the agent is a primer, probe or antibody.

13. The kit according to claim 9 or claim 10, wherein the agent is a primer consisting of a sequence set forth in any one of SEQ ID NOs: 198-199.

14. The kit according to claim 9 or claim 10, wherein the agent binds specifically to a saxitoxin A4 catalytic domain amino acid sequence as set forth in residues 847-1281 of SEQ ID NO: 4.

15. A primer pair for detecting a saxitoxin-producing dinoflagellate in a sample, wherein said primer pair consists of:

a first primer consisting of the polynucleotide sequence of SEQ ID NO: 198 and a second primer consisting of the polynucleotide sequence of SEQ ID NO: 199.

CLAIMS:

1. 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 of one or more of a dinoflagellate saxitoxin A catalytic domain polynucleotide or a polypeptide encoded by said polynucleotide;

wherein:

the saxitoxin catalytic domain is selected from: saxitoxin A4 catalytic domain, or a fragment of a saxitoxin A4 catalytic domain; and

the presence of said polynucleotide or polypeptide indicates the presence of a saxitoxin-producing dinoflagellate in the sample.

2. A method for determining the absence of 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 of one or more of a dinoflagellate saxitoxin A catalytic domain polynucleotide or a polypeptide encoded by said polynucleotide;

wherein:

the saxitoxin catalytic domain is selected from: saxitoxin A4 catalytic domain, or a fragment of a saxitoxin A4 catalytic domain; and

the absence of said polynucleotide or polypeptide indicates the absence of a saxitoxin-producing dinoflagellate in the sample.

3. The method according to claim 1 or claim 2, wherein the polynucleotide comprises a saxitoxin A nucleotide sequence selected from those set forth in any one of SEQ ID NOS: 230-242 or a fragment of any one of those sequences.

4. The method according to claim 1 or claim 2, wherein the saxitoxin A4 catalytic domain sequence or fragment thereof comprises nucleotides 3115-4121 of the polynucleotide sequence set forth in SEQ ID NO: 3, nucleotides 3597-3721 of the polynucleotide sequence set forth in SEQ ID NO: 3, or a fragment of either sequence.

5. The method according to claim 1 or claim 2, wherein said analyzing comprises amplification of polynucleotides from the sample by polymerase chain

reaction.

6. The method according to claim 5, wherein said polymerase chain reaction utilises one or more primers consisting of a sequence set forth in any one of SEQ ID NOs: 198-199, 228-229, and 243-244.

7. The method according to claim 1 or claim 2, wherein the polypeptide comprises a saxitoxin A4 catalytic domain amino acid sequence as set forth in residues 947-1281 of SEQ ID NO: 4, or a fragment thereof.

8. The method according to claim 1 or claim 2, wherein the saxitoxin-producing dinoflagellate is from the Alexandrium, Pyrodinium or Gymnodinium genus.

9. The method according to claim 8, 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.

10. A kit for the detection of a saxitoxin-producing dinoflagellate in a sample, the kit comprising at least one agent for detecting the presence of a dinoflagellate saxitoxin A catalytic domain polynucleotide or a polypeptide encoded by said polynucleotide, wherein the saxitoxin catalytic domain is selected from: saxitoxin A4 catalytic domain, or a fragment of a saxitoxin A4 catalytic domain.

11. A kit for the 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 A catalytic domain polynucleotide or a polypeptide encoded by said polynucleotide, wherein the saxitoxin catalytic domain is selected from: saxitoxin A4 catalytic domain, or a fragment of a saxitoxin A4 catalytic domain.

12. The kit according to claim 10 or claim 11, wherein the agent binds specifically to a polynucleotide comprising a saxitoxin A nucleotide sequence selected from those set forth in any one of SEQ ID NOS: 230-242 or a fragment of any one of those sequences.

13. The kit according to claim 10 or claim 11, wherein the saxitoxin A4 catalytic

domain sequence or fragment thereof comprises nucleotides 3115-4121 of the polynucleotide sequence set forth in SEQ ID NO: 3, nucleotides 3597-3721 of the polynucleotide sequence set forth in SEQ ID NO: 3, or a fragment of either sequence.

14. The kit according to claim 10 or claim 11, wherein the agent is a primer, probe or antibody.

15. The kit according to claim 10 or claim 11, wherein the agent is a primer consisting of a sequence set forth in any one of SEQ ID NOs: 198-199, 228-229, and 243-244.

16. The kit according to claim 10 or claim 11, wherein the agent binds specifically to a saxitoxin A4 catalytic domain amino acid sequence as set forth in residues 847-1281 of SEQ ID NO: 4, or a fragment thereof.

17. A primer pair for detecting a saxitoxin-producing dinoflagellate in a sample, wherein said primer pair consists of:

a first primer consisting of the polynucleotide sequence of SEQ ID NO: 198 and a second primer consisting of the polynucleotide sequence of SEQ ID NO: 199.