DETECTION OF SAXITOXIN-PRODUCING DINOFLAGELLATES

Priority Claim

This application claims priority from United States of America Provisional Patent Application No. 61/486,633 filed on 16 May 2011.

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

molecular tests to detect STX-producing dinoflagellates in both freshwater and marine environments.

In one aspect, there is provided 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.

In ~~a first aspect~~one example, the ~~invention~~disclosure provides 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 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.

In another aspect, there is provided 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.

In ~~a second aspect~~one example, the ~~invention~~disclosure provides 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 polynucleotide or a polypeptide encoded by said polynucleotide,

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

In one ~~embodiment of the first or second aspect~~example, the polynucleotide comprises a saxitoxin A nucleotide sequence selected from those set forth in any one of SEQ ID NOS: 5-197, 224-227, 230-242 and 247 or a fragment or variant of any one of those sequences.

In ~~one embodiment of the first or second aspect~~another example, the polynucleotide comprises a saxitoxin A1 catalytic domain sequence, a saxitoxin A4 catalytic domain sequence, or a fragment thereof.

In ~~one embodiment of the first or second aspect~~a further example, 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 or variant of either sequence.

In ~~one embodiment of the first or second aspect~~yet a further example, the saxitoxin A4 catalytic domain sequence or fragment thereof consists of 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 or variant of either sequence.

In one ~~embodiment of the first or second aspect~~example, the saxitoxin A1 catalytic domain sequence comprises nucleotides 160-1821 of the polynucleotide sequence set forth in SEQ ID NO: 1, nucleotides 277-2022 of the polynucleotide sequence set forth in SEQ ID NO: 3, or a fragment or variant of either sequence.

In ~~one embodiment of the first or second aspect~~another example, the saxitoxin A1 catalytic domain sequence consists of nucleotides 160-1821 of the polynucleotide sequence set forth in SEQ ID NO: 1, nucleotides 277-2022 of the polynucleotide sequence set forth in SEQ ID NO: 3, or a fragment or variant of either sequence.

In ~~one embodiment of the first or second aspect~~yet another example, the analyzing comprises amplification of polynucleotides from the sample by polymerase chain reaction.

In one ~~embodiment of the first or second aspect~~example, the polymerase chain reaction utilises one or more primers comprising a sequence set forth in SEQ ID NO: 198 or SEQ ID NO: 199, or a fragment or variant of either sequence.

In ~~one embodiment of the first or second aspect~~another example, the polymerase chain reaction utilises one or more primers consisting of a sequence set forth in SEQ ID NO: 198 or SEQ ID NO: 199, or a fragment or variant of either sequence.

In ~~one embodiment of the first or second aspect~~a further example, the polymerase chain reaction utilises one or more primers comprising or consisting of a sequence set forth in any one of SEQ ID NOs: 198-199, 200-211, 220-223, 228-229, and 243-244, or a fragment or variant of any one of those sequences.

In ~~one embodiment of the first or second aspect~~yet a further example, the polypeptide comprises a saxitoxin A amino acid sequence set forth in SEQ ID NO: 2 or SEQ ID NO: 4, or a fragment or variant of either sequence.

In one ~~embodiment of the first or second aspect~~example, the polypeptide consists of a saxitoxin A amino acid sequence set forth in SEQ ID NO: 2 or SEQ ID NO: 4, or a fragment or variant of either sequence.

In ~~one embodiment of the first or second aspect~~another example, the saxitoxin-producing dinoflagellate is from the *Alexandrium*, *Pyrodinium* or *Gymnodinium* genus.

In ~~one embodiment of the first or second aspect~~a further example, 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*.

In ~~one embodiment of the first or second aspect~~yet a further example, said analysing is performed using a primer pair of the eleventh or twelfth aspect.

In another aspect, there is provided 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.

In ~~a third aspect~~one example, the ~~invention~~disclosure provides 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 polynucleotide or a polypeptide encoded by said polynucleotide.

In yet another aspect, there is provided 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.

In ~~a fourth aspect~~one example, the ~~invention~~disclosure provides a kit 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 A polynucleotide or a polypeptide encoded by said polynucleotide.

In one ~~embodiment of the third or fourth aspect~~example, 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: 5-197, 224-227, 230-242 and 247 or a fragment or variant of any one of those sequences.

In one ~~embodiment of the third or fourth aspect~~example, the agent binds specifically to a polynucleotide comprising a saxitoxin A1 catalytic domain sequence, a saxitoxin A4 catalytic domain sequence, or a fragment thereof.

In ~~one embodiment of the third or fourth aspect~~another example, 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 or variant of either sequence.

In ~~one embodiment of the third or fourth aspect~~a further example, the saxitoxin A4 catalytic domain sequence or fragment thereof consists of 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 or variant of either sequence.

In ~~one embodiment of the third or fourth aspect~~yet a further example, the saxitoxin A1 catalytic domain sequence comprises nucleotides 160-1821 of the polynucleotide sequence set forth in SEQ ID NO: 1, nucleotides 277-2022 of the polynucleotide sequence set forth in SEQ ID NO: 3, or a fragment or variant of either sequence.

In one ~~embodiment of the third or fourth aspect~~example, the saxitoxin A1 catalytic domain sequence consists of nucleotides 160-1821 of the polynucleotide sequence set forth in SEQ ID NO: 1, nucleotides 277-2022 of the polynucleotide sequence set forth in SEQ ID NO: 3, or a fragment or variant of either sequence.

In ~~one embodiment of the third or fourth aspect~~another example, the agent is a primer, probe or antibody.

In ~~one embodiment of the third or fourth aspect~~a further example, the agent is a primer comprising a sequence set forth in SEQ ID NO: 198 or SEQ ID NO: 199, or a fragment or variant of either sequence.

In ~~one embodiment of the third or fourth aspect~~yet a further example, the agent is a primer consisting of a sequence set forth in SEQ ID NO: 198 or SEQ ID NO: 199, or a fragment or variant of either sequence.

In one ~~embodiment of the third or fourth aspect~~example, the agent is a primer comprising or consisting of a sequence set forth in any one of SEQ ID NOs: 198-199, 200-211, 220-223, 228-229, and 243-244, or a fragment or variant of any one of those sequences.

In ~~one embodiment of the third or fourth aspect~~another example, the agent binds specifically to a saxitoxin A amino acid sequence set forth in SEQ ID NO: 2 or SEQ ID NO: 4, or a fragment or variant of either sequence.

In ~~one embodiment of the third or fourth aspect~~yet another example, the kit comprises two agents, wherein the two agents are a primer pair of the eleventh or twelfth aspect.

In ~~one embodiment of the first, second, third or fourth aspect~~a further example, the sample is an environmental sample.

In one ~~embodiment of the first, second, third or fourth aspect~~example, the sample is a saltwater sample.

In ~~one embodiment of the first, second, third or fourth aspect~~another example, the sample is a freshwater sample.

In ~~one embodiment of the first, second, third or fourth aspect~~yet another example, the sample is a marine sample.

In ~~a fifth aspect~~one example, the ~~invention~~disclosure provides an isolated polynucleotide comprising the sequence set forth in SEQ ID NO: 1, or a variant or fragment thereof.

In one ~~embodiment of the fifth aspect~~example, the isolated polynucleotide consists of the sequence set forth in SEQ ID NO: 1, or a variant or fragment thereof.

In ~~a sixth aspect~~one example, the ~~invention~~disclosure provides an isolated polynucleotide comprising the sequence set forth in SEQ ID NO: 3, or a variant or fragment thereof.

In one ~~embodiment of the sixth aspect~~example, the isolated polynucleotide consists of the sequence set forth in SEQ ID NO: 3, or a variant or fragment thereof.

In ~~a seventh aspect~~one example, the ~~invention~~disclosure provides an isolated polynucleotide comprising the sequence set forth in any one of SEQ ID NOS: 5-197, 224-227, 230-242 and 247 or a variant or fragment of any one of those sequences.

In one ~~embodiment of the seventh aspect~~example, the isolated polynucleotide consists of the sequence set forth in any one of SEQ ID NOS: 5-197, 224-227, 230-242 and 247 or a variant or fragment of any one of those sequences.

In ~~an eighth aspect~~one example, the ~~invention~~disclosure provides an isolated polypeptide encoded by any one of the polynucleotides disclosed herein~~according to the fifth, sixth or seventh aspect~~.

In ~~a ninth aspect~~one example, the ~~invention~~disclosure provides a primer or probe that binds specifically to a polynucleotide disclosed herein~~according to the fifth, sixth or seventh aspect~~.

In ~~a tenth aspect~~another example, the ~~invention~~disclosure provides an antibody that binds specifically to a polypeptide disclosed herein~~according to the eighth aspect~~.

In one aspect, there is provided 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.

In ~~an eleventh aspect~~one example, the ~~invention~~disclosure provides an primer pair for detecting a saxitoxin-producing dinoflagellate in a sample, wherein said primer pair comprises a first primer comprising the polynucleotide sequence of SEQ ID NO: 198, or a fragment or variant thereof, and a second primer comprising the polynucleotide sequence of SEQ ID NO: 199, or a fragment or variant thereof.

In one ~~embodiment of the eleventh aspect~~example, the primer pair comprises a first primer consisting of the polynucleotide sequence of SEQ ID NO: 198, or a fragment or variant thereof, and a second primer consisting of the polynucleotide sequence of SEQ ID NO: 199, or a fragment or variant thereof.

In ~~a twelfth aspect~~one example, the the ~~invention~~disclosure provides a primer pair for detecting a saxitoxin-producing dinoflagellate in a sample, wherein said primer pair comprises: