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# Gene Expression in the Biosynthesis of Paralytic Shellfish Poisoning (PSP) Toxins in Dinoflagellates: A Mini Review

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## ABSTRACT

Some dinoflagellates are known to synthesize saxitoxin (STX), a potent neurotoxin that causes severe paralytic shellfish poisoning (PSP). In addition, several freshwater species of cyanobacteria also synthesize the same toxin with the same biosynthetic pathway and genes responsible. This review focuses on the gene expression involved in the biosynthesis pathway of PSP toxins in dinoflagellates. The expression of the PSP biosynthetic genes have been identified in certain cyanobacteria and the dinoflagellate *Alexandrium* sp. with eight genes involved *viz*. *sxtA, sxtB, sxtD, sxtG, sxtH/T, sxtI, sxtS* and *sxtU*. s*xtA*, a unique starting gene, and *sxtG*, the second "core" gene appearing in the biosynthesis of PSP toxins are found in both cyanobacteria and *Alexandrium* sp. Three theories have been proposed to explain the origin of PSP toxin in dinoflagellates: I) the genes are produced by bacteria associated with the dinoflagellates, II) independent evolution III) horizontal gene transfer between cyanobacteria and dinoflagellates. Useful information regarding the expression and function of genes involved in the STX biosynthesis pathway provides an understanding of toxin production and possible mitigation and public health management of STX poisoning.



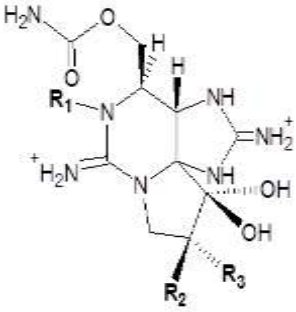
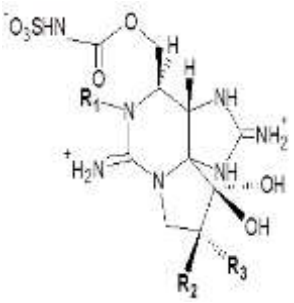
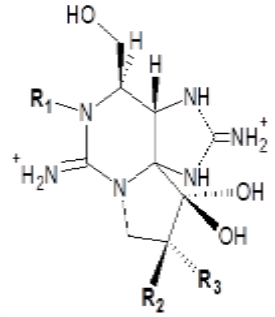
## Introduction

Dinoflagellates are an important group of unicellular algae that contribute to primary production in marine and freshwater ecosystems. Certain species from this group of organisms are known to produce toxic compounds that cause paralytic shellfish poisoning (PSP) after consumption of contaminated shellfish which results in severe impacts to public health and to aquaculture industries. Less than 100 species of dinoflagellates have been identified as being capable of synthesizing saxitoxin (STX) and its other derivatives, also known paralytic shellfish toxins (PSTs). These species producing PSTs are known as harmful algal blooms (HAB) species.

In general, PSTs are classified into three main groups according to the different substitutions at the side group moieties from STX, the parent compound (Table 1). STX, neosaxitoxin (neoSTX) and gonyautoxins (GTX1-4) under the carbamoyl group are known to be the most toxic derivatives. Whereas, the derivatives from *N*-sulfocarbamoyl group *viz*. GTX5-6 and C1-C4 are grouped as

intermediate toxin. Followed by the weak toxin derivatives from decarbamoyl group, consists of dcSTX, dcNEO, dcGTX1-4.

**Table 1.** Molecular structure of paralytic shellfish poisoning toxins

| Group | Toxin | Molecular structure | R1 | R2 | R3 | Toxicity level |
|---|---|---|---|---|---|---|
| Carbamoyl | STX |  | H | H | H | High |
| | NEO | | OH | H | H | |
| | GTX1 | | OH | H | $OSO_3^-$ | |
| | GTX2 | | H | H | $OSO_3^-$ | |
| | GTX3 | | H | $OSO_3^-$ | H | |
| | GTX4 | | OH | $OSO_3^-$ | H | |
| N-sulfocarbamoyl | GTX5 |  | H | H | H | Intermediate |
| | GTX6 | | OH | H | H | |
| | C1 | | H | H | $OSO_3^-$ | |
| | C2 | | H | $OSO_3^-$ | H | |
| | C3 | | OH | H | $OSO_3^-$ | |
| | C4 | | OH | $OSO_3^-$ | H | |
| Decarbamoyl | dcSTX |  | H | H | H | Low |
| | dcNEO | | OH | H | H | |
| | dcGTX1 | | OH | H | $OSO_3^-$ | |
| | dcGTX2 | | H | H | $OSO_3^-$ | |
| | dcGTX3 | | H | $OSO_3^-$ | H | |
| | dcGTX4 | | OH | $OSO_3^-$ | H | |

There are a few species from two different groups of dinoflagellates and cyanobacteria known to be able in synthesizing these PSTs. This includes the prokaryotic cyanobacteria, *Anabaena* (STX, GTX2, GTX3, GTX5, dcGTX2, dcGTX3, C1 and C2) (Humpage *et al.*, 1994), *Cylindrospermopsis* (STX, neoSTX, GTX2 and GTX3) (Lagos *et al.*, 1999), *Aphanizomenon* (GTX1, GTX3, GTX4 and Cs) (Filipa *et al.*, 2001) and *Lyngbya* (dcSTX, dcGTX2 and dcGTX3) (Onodera *et al.*, 1997). Meanwhile, in the eukaryotic dinoflagellates, studies by Usup *et al*. (2006) showed that *Alexandrium minutum* isolated from Malaysia produced only GTX 1-4 toxins. However, different toxin

compositions from the same species have been detected by Chang *et al.* (1997) from the New Zealand isolates (NEO, STX and GTX 1-4) and Hansen *et al.* (2003) from Denmark isolates (C1, C2, GTX2, 3 and STX). It is shown that same species but isolated from different geographical area vary in toxin compositions. Other studies on *Pyrodinium bahamense* showed another strong supporting data to this in which isolates from Malaysia (NEO, STX and GTX5) significantly different from Guatemala isolates (STX, NEO, GTX2, GTX3 and GTX4). The findings showed that the species under the same group or different group produces different types of PSTs. (Oshima *et al.,* 1993; Usup *et al.,* 1994; Usup *et al.*, 2012).

From the toxicological perspective, PSTs contribute to the neurotoxic mechanism as blocking agents of voltage-gated sodium channels that inhibit the permeability of sodium ions by binding closely to the opening of sodium channel (Faber, 2012). PSTs attack the nervous system which can lead to paralysis in humans, however it does not affect the gastro-intestinal tract. The development of the illness is extremely rapid and at high toxin concentration, death may occur within less than 15 minutes. Due to the toxicity effect, PSTs are listed in Schedule 1 of the Chemical Weapons Convention (CWC) by the Organization for the Prohibition of Chemical Weapons (OPCW, 2008; Wang, 2008). Understanding of the biosynthesis mechanism of PSTs where the genes are involved is important to resolve the proposed theories of the origins of PSTs. The 3 theories are: 1) that the genes are produced by bacteria associated with the dinoflagellates, 2) independent evolution 3) horizontal gene transfer (HGT) between cyanobacteria and dinoflagellates (Orr *et al.,* 2013). This paper describes the gene expression studies in the biosynthesis of PSTs in dinoflagellate species.

*PST biosynthesis genes in dinoflagellates*

In dinoflagellates, the genes involved in PSTs synthesis still remain unclear, whether co-cultured bacteria, HGT event from the ancestral cyanobacteria or the dinoflagellate genome itself are involved during STX synthesis. There are reports that the pathway of biosynthesis is believed to be the same between dinoflagellate and cyanobacteria (Shimizu, 1993; Kellmann *et al.,* 2008). The extremely large genomes of the dinoflagellates, which are up to 60 times the size of the human haploid genome that possess 3-245 Gb of DNA makes genomic studies on dinoflagellates very challenging (Lin, 2011). The dinoflagellate genes have specific characteristics such as high frequency of repeated copies, monocistronic transcripts and consist of eukaryotic polyadenylated (polyA) tail and the spliced leader sequences adding to the challenges particularly to identify genes associated with toxin production. Recently, the advance of next generation sequencing technologies particularly using high-throughput sequencing platform, have opened opportunities to study the toxin-related genes in dinoflagellates. As a consequence, PSTs biosynthesis studies have been proposed by using previously data generated from dinoflagellates (Morozova & Marra, 2008).

A study on the transcriptome of two dinoflagellate strains, *A. fundyense* and *A. minutum* (Stuken *et al.* 2011), by sequencing >1.2 x $10^6$ mRNA transcripts showed some unique features of dinoflagellates, with the characterization of the starting gene of STX biosynthesis, *sxtA*. The study

showed that the starting gene involved during STX bioynthesis in *Alexandrium* spp. is similar transcribed in cyanobacteria. However, the *sxtA* in dinoflagellates were monocistronic which means encoding the same domains but differs in transcript structure. The difference was the consistently higher GC content compared to cyanobacteria *sxtA* genes, transcripts occurred in multiple copies and consists of typical dinoflagellate spliced leader sequences and eukaryotic polyA tails. The two different transcripts with different length were found in *A. fundyense* where the shorter transcripts only encode *sxtA*1-3 and were missing *sxtA*4, the terminal aminotransferase domain. While, in the longer transcripts all four *sxtA*1-4 domains are available which is the only similarity found in cyanobacteria (Stuken *et al.,* 2011).

These differences between the dinoflagellates and cyanobacteria shows that the dinoflagellate carry their own STX genes which are required for STX synthesis and does not originate from co-cultured bacteria as proposed in the theories. However, these bacteria may still play an important role in modulating STX biosynthesis in dinoflagellates (Hold *et al.,* 2001; Palacios *et al.,* 2006). Another finding by Murray *et al.* (2011), where *sxtA*4 was also detected in a non-toxic dinoflagellate strain, *A. tamarense,* adds further confusion to the studies on PST biosynthesis.

In addition, the *sxtG* gene in the STX biosynthesis pathway is also sequenced in the genome from both toxin and non-toxic dinoflagellates species (Orr *et al.,* 2013). In cyanobacteria, the product of the starting gene, *sxtA* is the substrate for the amidinotransferase *sxtG* which is involved during STX biosynthesis. Thus, this *sxtG* became the second "core" gene in the STX biosynthesis. The findings of the *sxtG* gene were originally identified by Stuken *et al.* (2011) where a typical dinoflagellate structure was similar to the cyanobacteria *sxtG* genes. Consistent with *sxtA*, *sxtG* gene was also monocistronic and contains poly (A) tails and dinoflagellate spliced leader sequences. These results show that both starting gene and the second core gene of the STX biosynthesis are located in the nuclear genome of dinoflagellates. The GC content of *sxtG* was also higher, ~ 20% compared to cyanobacteria *sxtG*, suggesting that the gene has been modified prior to being introduced into dinoflagellates. The genomic sequence of *sxtG* also vary in length between different *Alexandrium* species which are useful in identifying strains within species or to differentiate between species of *Alexandrium.*

Recent study by Hii *et al.* (2016) on transcriptional responses of the *sxt* genes involved in STX biosynthesis to nutrient conditions provide another new supporting data. A total of 143,051 unigenes assembled from *A. minutum* transcriptomic library revealed that the core genes (*sxtA4* and *sxtG*) and putative gene (*sxtI*) was differentially expressed. However, the first core gene in STX biosynthesis, *sxtA1* was not detected. This is consistent with Perini *et al.,* (2014) finding where expression of *sxtA1* in *A. minutum* was absent in phosphorus (P) and nitrogen (N) limited conditions. Besides, the finding showed that *sxtA4* and *sxtG* genes was up-regulated in P-depleted, nitrate-grown cultures and in excess ammonium-grown cultures. In contrast, *sxtI* gene was down-regulated in ammonium-grown cultures and highly induced only in the P-depleted, nitrate-grown cultures.

Meanwhile, the third important gene which encodes a cytidine deaminase enzyme in the STX biosynthesis pathway in cyanobacteria is *sxtB*. The protein homolog encoded by *stxB* was found in the *Alexandrium pacificum* transcriptome. From studies by John *et al.* (2014) and Wang *et al.* (2014) all the three genes, *sxtA, sxtG* and *sxtB* were found to be involved in the starting step of STX biosynthesis in *A. fundyense* and *A. pacificum* which are phylogenetically close to the cyanobacteria. Other genes involved in STX biosynthesis viz. *sxtD, sxtS, sxtU, sxtH/T, sxt I, sxtL, sxtN, sxtX, sxtF/M* and *sxtP*, were also identified in the *A. pacificum* transcriptome study. In cyanobacteria, a total of 14 genes (*sxtA-sxtI, sxtP-sxtS* and *sxtU*) are commonly found in the STX biosynthesis pathway (Kellmann *et al.*, 2008). Based on these various studies, it is still unclear whether the STX biosynthesis pathway is the same between dinoflagellates and cyanobacteria. To date, no *sxt* genes have been detected from other genera of dinoflagellate other than the *Alexandrium* genus and *Gymnodinium cetenatum* (Orr *et al.*, 2013; Hii *et al.*, 2016). Therefore, an analysis of *sxt* genes from other dinoflagellate, *Pyrodinium bahamense* var. *compressum* transcript will provide useful information. However, cultures of this species are currently not publicly available and the only report of this species comprising the two genes are presented by Hackett *et al*. (2013) though the sequences which are yet to be made available in the GenBank.

*Evolution of the origin of PST biosynthesis*

Based on the unique and complex biosynthesis process of STX between both cyanobacteria and dinoflagellates, it is interesting to note if there are evolutionary relationships between the two phyla since there are similarities in the genes involved in STX biosynthesis. This is consistent with the initial three theories proposed whether co-cultured bacteria toxin evolved independently in the genome or through HGT event.

In dinoflagellates, the toxin production was suggested to be due to the bacteria associated with dinoflagellates, since there are many other microorganisms as epiphytes or endosymbionts which co-exist (Doucette, 1995; Silva, 1990; Gallacher *et al.*, 1997). But, this theory has been abandoned since the findings do not support it and more toxin related genes and homologs are identified in the dinoflagellates. However, the fact that toxin are synthesized in dinoflagellate itself, the co-existing bacteria still play a role in the various physiological aspects in dinoflagellate.

Another theory is that toxin was synthesized independently in both cyanobacteria and dinoflagellates and converged in their evolutionary history. However, convergence of gene usually occurs within phyla of lineages. For example studies by Stuken *et al.* (2011) and Orr *et al*. (2013) showed that the domain structures of the genes, *sxtA* and *sxtG* have been identified in both cyanobacteria and dinoflagellates. If convergent evolution occurred, this will result in the lack of sequence homology, however, multiple *sxt* homologues with high sequence identity were found from the studies. Besides, this theory also suggested that convergence evolved from environmental pressure to the organisms as a survival strategy. To date, there is still limited evidence to support this theory.

From the theories, the most accepted by most researchers is that dinoflagellate gaining the genes is associated with the toxin biosynthesis via HGT event from its ancestral bacteria, cyanobacteria. This is supported by the discovery of *sxtA* transcripts in dinoflagellates which are phylogenetically close to the cyanobacteria *sxtA* sequence (Stuken *et al.,* 2011). Another evidence is through phylogenetic analysis, where the second core gene, *sxtG,* in dinoflagellates is also found to be similar to its ancestral bacteria, actinobacterial and cyanobacteria (Orr *et al.,* 2013). It is, therefore, concluded that previous findings show that the initial three genes, *sxtA, sxtG* and *sxtB* have primary origins from proteobacteria before being transferred to cyanobacteria and further to dinoflagellates via HGT event (Wang, 2008; Shimizu, 1996; Faber, 2012; Chen *et al.,* 2015). But, it is believe the toxin genes are modified significantly during the evolution process in results with a higher GC content, monocistronic, multiple copies, typical dinoflagellates spliced-leader sequences and eukaryotic poly (A) tails (Stuken *et al.,* 2011; Orr *et al.,* 2013).

## Conclusion

The various perspectives of gene expression in the biosynthesis of PSP toxin in dinoflagellates can provide deeper knowledge and understanding about the evolutionary and the origins of toxin-related genes in dinoflagellates. Information on the genetic regulation of *sxt* gene expression will provide fundamental information involved within the STX biosynthesis pathway between dinoflagellates and cyanobacteria. In addition, the different environmental conditions that influence the toxin production in dinoflagellates at the same time will also provide further insights into the ecophysiological aspects of the toxins. Lastly, the works and results presented in this review present more opportunities for more studies on transcriptomic and proteomic of dinoflagellates due to the limited genomic information available on dinoflagellates.

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