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Paralytic shellfish toxin biosynthesis in cyanobacteria and dinoflagellates: A molecular overview

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ABSTRACT

Paralytic shellfish toxins (PSTs) are a group of water soluble neurotoxic alkaloids produced by two different kingdoms of life, prokaryotic cyanobacteria and eukaryotic dinoflagellates. Owing to the wide distribution of these organisms, these toxic secondary metabolites account for paralytic shellfish poisonings around the world. On the other hand, their specific binding to voltage-gated sodium channels makes these toxins potentially useful in pharmacological and toxicological applications. Much effort has been devoted to the biosynthetic mechanism of PSTs, and gene clusters encoding 26 proteins involved in PST biosynthesis have been unveiled in several cyanobacterial species. Functional analysis of toxin genes indicates that PST biosynthesis in cyanobacteria is a complex process including biosynthesis, regulation, modification and export. However, less is known about the toxin biosynthesis in dinoflagellates owing to our poor understanding of the massive genome and unique chromosomal characteristics [1]. So far, few genes involved in PST biosynthesis have been identified from dinoflagellates. Moreover, the proteins involved in PST production are far from being totally explored. Thus, the origin and evolution of PST biosynthesis in these two kingdoms are still controversial. In this review, we summarize the recent progress on the characterization of genes and proteins involved in PST biosynthesis in cyanobacteria and dinoflagellates, and discuss the standing evolutionary hypotheses concerning the origin of toxin biosynthesis as well as future perspectives in PST biosynthesis.

Scientific question: Paralytic shellfish toxins (PSTs) are a group of potent neurotoxins which specifically block voltage-gated sodium channels in excitable cells and result in paralytic shellfish poisonings (PSPs) around the world. Two different kingdoms of life, cyanobacteria and dinoflagellates are able to produce PSTs. However, in contrast with cyanobacteria, our understanding of PST biosynthesis in dinoflagellates is extremely limited owing to their unique features. The origin and evolution of PST biosynthesis in these two kingdoms are still controversial.

Technical significance: High-throughput omics technologies, such as genomics, transcriptomics and proteomics provide powerful tools for the study of PST biosynthesis in cyanobacteria and dinoflagellates, and have shown their powerful potential with regard to revealing genes and proteins involved in PST biosynthesis in two kingdoms.

Scientific significance: This review summarizes the recent progress in PST biosynthesis in cyanobacteria and dinoflagellates with focusing on the novel insights from omics technologies, and discusses the evolutionary relationship of toxin biosynthesis genes between these two kingdoms.

1. Introduction

Paralytic shellfish toxins (PSTs) are a group of potent neurotoxins which specifically block voltage-gated sodium channels in excitable cells and result in paralytic shellfish poisonings (PSPs) around the world. PSTs result in about 2000 cases annually with a human mortality rate of 15%, and they have become a worldwide environmental and health problem, attracting global concern [2,3].

Two kingdoms of life are able to produce PSTs: the prokaryotic cyanobacteria such as *Anabaena*, *Cylindrospermopsis*, *Aphanizomenon* and *Lyngbya* [4–7], and the eukaryotic dinoflagellates, *Alexandrium*, *Gymnodinium* and *Pyrodinium* [8–12]. The toxin profiles and contents vary in dinoflagellates and cyanobacteria within different species in the same genera, and within different geographical strains of the same species.

So far, a total of 57 natural analogs are reported which have similar structures but which are different mainly in the substitution at the side group moieties (R1–R4) on the parent compound saxitoxin (STX; Table 1) [13]. Based on the variation of the side group moieties, the PSTs can be classified as non-sulfated (STX, neoSTX), mono-sulfated

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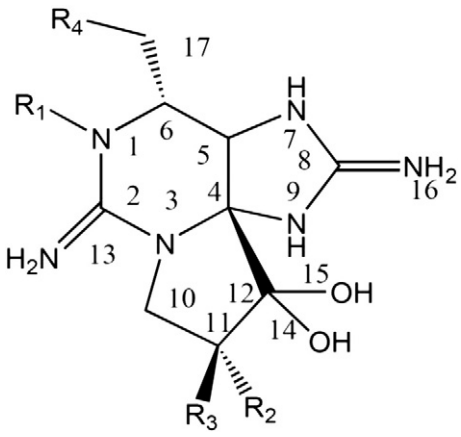
Table 1
Structure and toxicity of various paralytic shellfish poisoning toxins. 'R' represents the variable moieties. Toxicity values in mouse units (MUs), where 1 MU is the amount of toxin required to kill a mouse in 20 min. '-' represents no related information.



	Toxin	R1	R2	R3	R4	Toxicity (MU·μmol^{-1})
Non-sulfated	STX	H	H	H	$OCONH_2$	2483
	neoSTX	OH	H	H	$OCONH_2$	2295
Mono-sulfated	GTX1	OH	H	OSO_3^-	$OCONH_2$	2468
	GTX2	H	H	OSO_3^-	$OCONH_2$	892
	GTX3	H	OSO_3^-	H	$OCONH_2$	1584
	GTX4	OH	OSO_3^-	H	$OCONH_2$	1803
	GTX5	H	H	H	$OCONHSO_3^-$	160
	GTX6	OH	H	H	$OCONHSO_3^-$	-
Di-sulfated	C1	H	H	OSO_3^-	$OCONHSO_3^-$	15
	C2	H	OSO_3^-	H	$OCONHSO_3^-$	239
	C3	OH	H	OSO_3^-	$OCONHSO_3^-$	33
	C4	OH	OSO_3^-	H	$OCONHSO_3^-$	143
Decarbamoylated	dcSTX	H	H	H	OH	1274
	dcneoSTX	OH	H	H	OH	-
	dcGTX1	OH	H	OSO_3^-	OH	-
	dcGTX2	H	H	OSO_3^-	OH	1617
	dcGTX3	H	OSO_3^-	H	OH	1872
	dcGTX4	OH	OSO_3^-	H	OH	-
Deoxy-decarbamoylated	doSTX	H	H	H	H	-
	doGTX1	OH	H	OSO_3^-	H	-
	doGTX2	H	H	OSO_3^-	H	-
	doGTX3	H	OSO_3^-	H	H	-

(GTX1-6), di-sulfated (C1-4), decarbamylated (dcSTX, dcneoSTX, dcGTXs1-4) and deoxy-decarbamoylated (doSTX, doGTXs1-3) [13,14]. In addition, the structural variation determines the toxicity of the PSTs, which varies by two orders of magnitude, ranging from 15 MU·μmol^{-1} of C1 toxin to 2483 MU·μmol^{-1} of STX [14–16].

The mechanism of PST toxicity has been clarified: PSTs function as blocking agents that inhibit the temporary permeability of sodium ions by occupying sites near the outer opening of the voltage-gated Na^+ channel in a 1:1 high affinity specific receptor binding [17]. The symptoms of PSP include numbness of the extremities, difficulty in breathing and serious to complete paralysis or death [18,19]. Owing to the severe toxicity and damage to human beings or other animals, PSTs are listed as a Schedule I chemical intoxicant by the Organization for the Prohibition of Chemical Weapons [20]. However, on the other hand, because of their specific ability to induce anesthesia through interaction with site 1 of the voltage-gated Na^+ channel, PSTs show potential applications in pharmacology and toxicology [13,21–27].

The severe toxicity and potential pharmaceutical applications described above highlight the need to understand the biosynthesis of PSTs in these two kingdoms of life. This review summarizes the recent advances in PST biosynthesis in cyanobacteria and dinoflagellates, focusing on the novel insights from 'omics' technology (Table 2), and discusses the evolutionary relationship of toxin biosynthesis genes between these two kingdoms. Moreover, challenges and future perspectives in the study of PST biosynthesis are also discussed.

2. PST biosynthesis genes in the two kingdoms

2.1. PST biosynthesis genes in cyanobacteria

Much effort has been devoted in recent years to delineate the biosynthesis pathway of PST in cyanobacteria, and toxin gene clusters in five cyanobacteria species have been characterized [28–31]. Based on these toxin biosynthesis genes, the pathway of PST biosynthesis is well elucidated in the cyanobacteria (Fig. 1).

Previous work based on radioisotope tracing experiments in cyanobacteria suggests that the skeleton of PSTs is built from arginine, acetate (via acetyl-CoA) and methionine methyl (via S-adenosylmethionine), involving a rare Claisen condensation using presumably similar biochemical reactions [32–34]. However, the exploration of genes involved in PST biosynthesis has been at a standstill until recently, when a breakthrough was made in the identification of the toxin gene cluster in a cyanobacterium, *Cylindrospermopsis raciborskii* T3 [28]. The gene cluster spans 35 kb, encoding 31 open reading frames (ORFs) and is assigned to 26 proteins. Subsequently, the gene clusters from another four cyanobacterial species were characterized [29–31]. Although the gene profiles differ a little among these species, all the clusters contain a core set of genes for PST biosynthesis.

Based on their functions in the biosynthetic pathway, 26 toxin-related genes are classified into three groups (Fig. 2). The first group includes eight genes (*sxtA*, *sxtG*, *sxtB*, *sxtD*, *sxtS*, *sxtU*, *sxtH*/*T* and *sxtI*) which are directly involved in the biosynthesis of STX and three