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Sodium channel blocking (SCB) activity and transformation of paralytic shellfish toxins (PST) by dinoflagellate-associated bacteria

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ABSTRACT: Bacteria associated with toxic algae in culture have been implicated in the enhancement of algal toxin production and in auto-toxigenesis, with taxon specificity of the bacteria suggested to be important in their association with the algae. In this study, bacteria isolated from toxic and non-toxic dinoflagellates have been examined for both their potential to produce sodium channel blocking (SCB) toxins, as ascertained by the mouse neuroblastoma assay, and their ability to biotransform paralytic shellfish toxins (PST) using high-performance liquid chromatography (HPLC). Toxigenic bacteria were found to belong to the α- and γ-Proteobacteria. Bacteria capable of SCB activity were present in PST-producing *Alexandrium tamarense* and *A. lusitanicum* cultures, while the bacterial flora isolated from a non-toxic *A. tamarense* strain did not appear to demonstrate this trait. A disparate population of the bacterial flora associated with non-PST-producing *Scrippsiella trochoidea* also demonstrated SCB activity. Some bacteria from all the dinoflagellates examined were capable of transforming PST, with possible mechanisms including oxidase activity with transformation of gonyautoxins GTX 2/3 to GTX 1/4, reductive elimination as demonstrated by the transformation of GTX 1/4 to GTX 2/3, and further unknown pathways. Noticeably, there was little overlap between bacteria demonstrating SCB activity and those that were able to modify PST, indicating that biosynthesis of SCB toxins and catabolism of PST may not be related in these bacterial isolates.

KEY WORDS: Dinoflagellates · Bacteria · Sodium channel blocking toxins · Mouse neuroblastoma assay · Paralytic shellfish toxins · HPLC · Biotransformations

INTRODUCTION

It has been suggested that in some toxic algal cultures both intracellular and free-living bacteria are either directly or indirectly associated with phycotoxin production, although to date no clear picture has emerged (Kodama et al. 1988, Kodama 1990, Danzter & Levin 1997, Doucette & Powell 1998). Questions of how or why either organism produces these toxins have also not been fully answered. A key issue for several researchers has been the autonomous bacterial production of paralytic shellfish toxins (PST) (see Gallacher & Smith 1999), which in humans can cause severe poisoning via the consumption of contaminated shellfish, which filter-feed on algae, and hence may ingest toxic dinoflagellates (Hall et al. 1990).

Mechanisms of toxin dynamics in bacterial-algal interactions have been largely neglected. Early studies claimed that bacteria from toxic dinoflagellates could confer toxicity to non-toxic algae species and attributed this to the presence of intracellular bacteria (Silva 1982). In some cases taxon specificity has been demonstrated to be important to the association of toxigenic bacteria with toxic algae. For example, Doucette & Powell (1998) reported that *Alexandrium lusitanicum* produced less toxin when the bacterial flora was removed, but that toxin levels could be restored upon

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addition of a bacterium considered to be a PST producer and an original member of the dinoflagellate's bacterial consortium. Addition of bacteria alien to the algae did not, however, produce any change in toxicity.

Evidence for bacterial production of PST has mainly been based on the chromatographic behaviour of these compounds and their sodium channel blocking (SCB) activity (Gallacher & Smith 1999). Nevertheless, important steps in confirming synthesis of PST by bacteria have been made using capillary electrophoresis-mass spectrometry (Gallacher et al. 1997).

Bacterial biotransformations of PST are another potential mechanism by which these organisms may influence dinoflagellate toxicity. PST consist of saxitoxin (STX) and at least 22 derivatives (e.g. carbamate toxins including STX, neosaxitoxin (NEO) and the gonyautoxins [GTX 1, 2, 3 and 4], *N*-sulfocarbomoyl toxins consisting of GTX 5 and C toxins and decarbomyl toxins (dcGTX 2 and 3). Each derivative has its own potency, with some being more toxic than others (Shimizu 1993). Early reports by Kotaki et al. (1985a,b) demonstrated that marine bacteria were capable of inter-converting these neurotoxins, and more recently it has been demonstrated that bacteria isolated from a variety of shellfish possess the capacity to carry out side chain modifications of PST (Smith et al. 2001).

Previous phylogenetic analysis of cultivable bacteria associated with PST-producing dinoflagellates (*Alexandrium lusitanicum* NEPCC 253, *A. tamarense* NEPCC 407) and non-toxic dinoflagellates (*A. tamarense* PCC 173, *Scrippsiella trochoidea* NEPCC 15) placed these bacteria into 25 phyletic groups. These were limited to 2 bacterial phyla, the Proteobacteria (α- and γ-) and Cytophaga-Flavobacter-Bacteroides (CFB) (Hold et al. 2001). In this study, selected bacterial isolates from each phyletic group have been assessed for their ability both to produce SCB toxins and to biotransform a range of PST analogues. SCB activity of the bacteria was determined using the mouse neuroblastoma assay (Gallacher et al. 1997), while bacteria were incubated with various toxin analogues and the resulting derivative(s) detected by high-performance liquid chromatography (HPLC) to establish their capacity to transform PST.

MATERIALS AND METHODS

Bacterial cultures. Bacteria used in this investigation were first isolated from PST-producing *Alexandrium lusitanicum* NEPCC 253 (= *A. minutum*), a toxic and non-toxic strain of *A. tamarense* (NEPCC 407 and PCC 173, respectively) and non-toxic *Scrippsiella trochoidea* NEPCC 15 by Hold et al. (2001). The bacteria were isolated over the growth cycle of the dinoflagellates in batch culture (early and late exponential and stationary phases) and characterised by sequence determination of their16S rRNA gene (Hold et al. 2001). The bacterial strains used in this study are given in Table 1. All isolates were stored in marine broth (Difco) plus 10% glycerol at –70°C prior to investigation.

Detection of SCB activity using the mouse neuroblastoma (MNB) assay. Bacterial strains were cultured for 18 h in 30 ml of marine broth (20°C; 120 oscillations min^{-1}). Supernatants and cells were separated by centrifugation at 10 000 × *g* for 20 min. Supernatant (20 ml) from each isolate was stored at 4°C until processed by the MNB assay as previously detailed (Gallacher et al. 1997).

HPLC analysis of PST transformations. A mixture of 24 h bacterial cultures grown in marine broth (196 µl) and individual toxin standards (4 µl) were incubated in an orbital shaker (20°C; 120 oscillations min^{-1}) for 0, 12, 24, 48 and 72 h. Final concentrations of the individual toxins were (ng $µl^{-1}$): GTX 1, 1.48; GTX 4, 0.64;

Table 1. Dinoflagellate-associated bacterial strains. *Bacterial isolates were obtained from: ALUS_253, *Alexandrium lusitanicum* NEPCC 253; ATAM_407, *A. tamarense* NEPCC 407; ATAM173a, *A. tamarense* PCC 173; SCRIPPS, *Scrippsiella trochoidea* NEPCC 15

Bacterial isolate	GenBank accession no.
α-Proteobacteria	
ALUS253_18 (= ATAM407_61)	AF359525
ALUS253_59 (= ALUS253_43)	AF359526
ATAM407_2983 (= ATAM407_62)	AF359529
SCRIPPS_732	AF359534
ATAM407_2976 (= ATAM407_56)	AF359535
ATAM173a_51	AF359538
ATAM173a_9	AF359544
ALUS253_27 (= ALUS253_28	AF359524
ALUS253_25	AF359524
ATAM407_68	AF359528
SCRIPPS_101	AF359537
SCRIPPS_738 (= SCRIPPS_94)	AF359545
SCRIPPS_739	AF359546
SCRIPPS_423	AF359547
SCRIPPS_426	AF359549
SCRIPPS_733	Not fully sequenced
γ-Proteobacteria	
SCRIPPS_740	AF359550
ATAM407_77 (= ATAM407_18)	AF359529
ATAM173a_5	AF359542
ATAM173a_36	AF359543
ATAM407_2035	AF359552
Cytophaga-Flavobacter-Bacteroides	
ATAM173a_3	AF359540
SCRIPPS_413	AF359548
ATAM173a_6	AF359541
ALUS253_6	AF359551

GTX 2, 2.40; GTX 3, 0.58; GTX 5, 2.22; STX, 2.80; NEO, 2.80. A sample (2 µl) of each culture was spread onto marine agar after 72 h to ensure that cultures were still viable. Controls sampled over the same time period consisted of toxin standards in marine broth in the absence of bacteria. Controls and spent media from the bacterial cultures were subsequently analysed for residual substrates and their products by HPLC, applying the isocratic methods of Franco & Fernández-Vila (1993), with modifications detailed in Smith et al. (2001).

RESULTS AND DISCUSSION

Production of SCB toxins

The MNB assay used in this study to ascertain whether bacteria were toxigenic is highly specific for SCB toxins and is based on the pharmacological actions of these compounds (Gallacher & Birkbeck 1992). However, although highly sensitive, it does not differentiate which of the spectra of PST is present. Nor does it distinguish PST from another group of SCB toxins, the tetrodotoxins, which are widely accepted as being produced by bacteria (Matsui et al. 1989, Gallacher & Birkbeck 1992, Lee et al. 2000). Nevertheless, the MNB assay has been used to infer PST toxicity in marine bacteria by a number of researchers (see Doucette et al. 1998, Gallacher & Smith 1999). Hence, while the evidence for PST production by bacteria is compelling, in the absence of confirmatory mass spectrometric data bacteria in this study giving a positive result by the MBA are currently only considered to produce SCB toxins.

Seven out of the 25 bacterial strains investigated in this study were found to produce SCB toxins when grown in marine broth (Table 2). Of these toxigenic bacteria, ALUS253_59 and ATAM407_2976 were closely related with the *Roseobacter* phylogenetic lineage within the α subclass of the Proteobacteria. Strain ATAM407_2976 demonstrates greater than 99% 16S rRNA sequence homology with bacterial isolate 667-2, which was previously isolated from a weakly toxic culture of *Alexandrium affine* (Hold et al. 2001) and is reported as capable of synthesising multiple PST derivatives (Gallacher et al. 1997). It is interesting to note that Rausch de Traubenberg (1993), Lafay et al. (1995), Prokic et al. (1998), Alavi et al. (2001) and Vásquez et al. (2001) have all isolated and characterised bacteria as belonging to the *Roseobacter* clade (including the *Sulfitobacter* genera) from dinoflagellate cultures. Although the bacteria isolated by Prokic et al. (1998) were not found to be toxic *per se*, Rausch de Traubenberg (1993) suggested that strains of *Roseobacter algicola* (= *Ruegeria algicola*) synthesise the phycotoxin okadaic acid, and Vásquez et al. (2001) reported SCB activity in a *Sulfitobacter* strain.

Other bacteria within the α-Proteobacteria demonstrating SCB activity were ALUS253_25, SCRIPPS_423, SCRIPPS_426 and SCRIPPS_733 (Table 2). Isolate ALUS253_25 is closely associated with the PTB strains (Groben et al. 2000, Hold et al. 2001). These strains include PTB1, originally isolated from a highly toxic

Table 2. Dinoflagellate-associated bacteria demonstrating sodium channel blocking (SCB) activity.

Bacterial isolate	SCB activity activity (nM)	Nearest neighbour (GenBank accession no.)	16S rRNA sequence similarity (%)[a]
α-Proteobacteria (*Roseobacter* clade)			
ALUS253_59	35	*Roseovarius tolerans* (Y11551)	99.6
ATAM407_2976	34	*Roseobacter gallaeciensis* (Y13244)	98.9
		Isol. 667-2 (AJ294353) isolated from *Alexandrium affine* NEPCC 667	99.9
α-Proteobacteria			
ALUS253_25	39	*Stappia stellulata* (D88525)	98.6
		Isol. PTB1 (Y10913) isolated from highly toxic Japanese *Alexandrium tamarense*	99.7
		Clone isol. PTB5 (AJ000646)	99.4
SCRIPPS_423	64	*Hyphomonnas oceanitis* (AF082797)	90.9
SCRIPPS_426	39	*Caulobacter vibroides* (AJ227754)	94.5
SCRIPPS_733	72	Unknown (not fully sequenced)	–
γ-Proteobacteria			
ATAM407_77	84	*Alteromonas macleodii* (X82145)	98.9
		Isol. 407-2 (AJ294360), 2c3 (AJ294361), 253-19 (AJ294362) isolated from toxic *Alexandrium* sp. cultures	98.3

[a]Data adapted from Hold et al. (2001)

Alexandrium tamarense clone (Ogata et al. 1990, Kopp et al. 1997) and reported to be capable of producing PST as defined by HPLC (Doucette & Trick 1995). It is thought that further strains within the same bacterial lineage may differ in their abilities to produce such toxins. For example, PTB 6 isolated from a weakly toxic *A. tamarense* culture is reported to be capable of PST production, while PTB 7 isolated from a non-toxic *A. tamarense* culture does not appear to demonstrate this function (Doucette & Trick 1995). SCRIPPS_423 and SCRIPPS_426 are distantly related to the *Hyphomonas* and *Caulobacter* genera respectively (Hold et al. 2001). To our knowledge this is this first case of SCB activity being observed in such bacteria.

Within the γ-Proteobacteria, SCB toxicity was only detected in strain ATAM407_77, which is closely related to *Altermonas macleodii* (Hold et al. 2001). Other toxin-producing bacteria within this genus include the phylogenetically characterised bacteria 407-2, 2c3 and 253-19 isolated from toxic *Alexandrium* spp. (Gallacher et al. 1997, Hold et al. 2001) and 6SMI isolated from seawater and identified using carbon utilisation profiles (Levasseur et al. 1996). Within the γ-Proteobacteria, other bacteria reported to show SCB activity include *Acinetobacter* and *Pseudomonas* spp. (Levasseur et al. 1996), and bacteria isolated from shellfish by Juntongjin et al. (1993, 1996) and identified using G + C DNA content as belonging to the *Vibrio* and *Pseudomonas* genera. Franca et al. (1996) also reported that *Pseudomonas* spp. produce PST. However, in this study strain SCRIPPS_740 is known to be closely related to *Pseudomonas stutzeri* (Hold et al. 2001) and did not demonstrate SCB toxin production. All 4 strains tested within the CFB phyla were negative for SCB activity, even though SCB activity has previously been reported in a *Flavobacterium* sp. (Juntongjin et al. 1996).

From this and previous studies it would appear that several closely related bacteria originating from dinoflagellate cultures and distinctly different marine environments have the ability to synthesise SCB toxins. SCB toxin production by bacteria appears to span across species in different phylogenetic lineages, suggesting that this property is not uncommon among marine bacteria. However, direct association of these bacteria with eukaryotic organisms does not appear to be a prerequisite for toxin production. These observations pose the question of the possible function of such toxins in bacteria. PST have variously been linked to ideas of chemical defence in order to protect an environmental niche, to provide nitrogen reserves, to act in cell signalling or to aid osmotic regulation (Gallacher et al. 1997, Cembella 1998, Johnston et al. in press). However, PST have not been directly linked to these by any mechanistic evidence and the metabolic functions of the SCB toxins detected in this study, the reasons for their production, and their physiological and ecological significance remain unresolved.

Bacteria-producing SCB compounds were isolated from *Alexandrium tamarense* NEPCC 407 and *A. lusitanicum* at all growth phases examined, suggesting that toxigenic bacteria may contribute to PST levels in these cultures. Interestingly, bacteria isolated from non-toxic *A. tamarense* PCC 173 did not demonstrate SCB activity under the conditions used in this investigation. Toxigenic bacteria were, however, isolated from non-PST-producing *Scrippsiella trochoidea*, raising questions on the identity of the SCB compounds being produced and highlighting that further detailed spectral analysis is required to more clearly define the SCB compounds produced by these bacteria. The toxigenic bacterial strains isolated from *S. trochoidea* were not closely related with SCB-active bacteria isolated from the toxic *Alexandrium* cultures (Hold et al. 2001). What may be of interest, therefore, are possible bacterial species-specific associations with harmful algal species in nature. Future investigations in this area may contribute to our understanding of variations in the toxicity of individual algae.

PST biotransformations by dinoflagellate-associated bacteria

Studies conducted by Kotaki (1989), Kotaki et al. (1985a,b) and more recently by Smith et al. (2001) suggested that bacterial isolates from shellfish were capable of transforming PST. Bacterial transformations of PST are further potential mechanisms by which these organisms may influence dinoflagellate and shellfish toxicity. Therefore, this study further aimed to determine if PST could be utilised and/or modified by the algal-associated bacteria. With this aim, the bacteria were incubated with PST standards in marine broth and toxin profiles monitored over time using HPLC. As such, 5 out of the 25 bacterial strains investigated were found to transform PST (Table 3).

Quantitative assessment of control samples containing marine broth and PST standards (GTX 1/4, GTX 2/3 and GTX 5) but no bacteria showed that at least 98% of these toxins were recovered at all the time points investigated (Fig. 1). STX and NEO were also completely recovered from the marine broth in control samples. When the bacterial strains were grown with the addition of STX and NEO, reduction in the concentration of these toxins was again not observed throughout the incubation period (data not shown). It was previously reported that bacteria associated with shellfish were unable to transform or utilise these PST analogues under similar growth conditions (Smith et al. 2001).

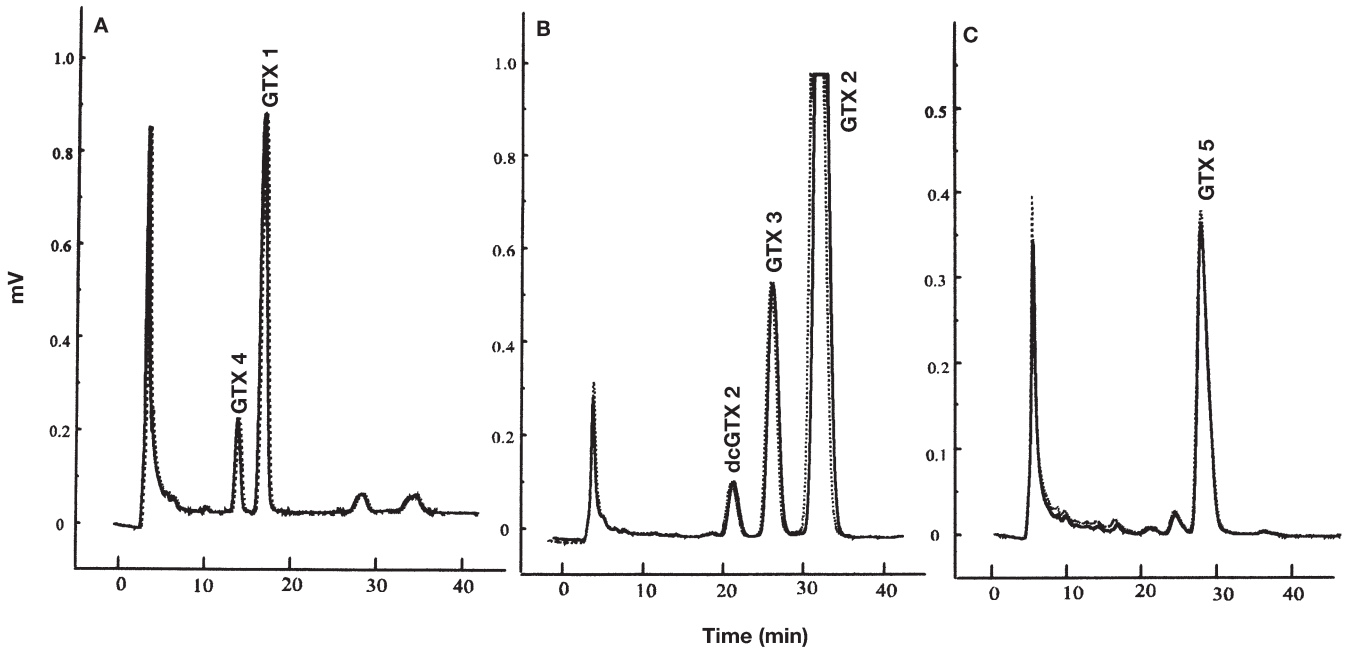

Fig. 1. Chromatogram showing paralytic shellfish toxin (PST) standards in marine broth: Marine broth spiked with (A) GTX 1/4; (B) GTX2/3; (C) GTX 5. Samples shown were taken at 0 h (black line) and 72 h (dotted line)

HPLC analysis of bacterial supernatants grown with GTX standards showed that bacterial strains within the α-Proteobacteria were capable of utilising and/or transforming these toxins (Table 3, Figs. 2, 3 & 4). As with the SCB-active bacteria, PST-transforming bacteria were found to be associated with both toxic and non-toxic dinoflagellates throughout the dinoflagellates' growth phase. Predominantly, of the isolates examined, the ability to transform PST was a trait of bacteria within the *Roseobacter* clade, with isolated strains being most closely related to *Ruegria algicola*, *Sulfitobacter pontiacus* and *Antarctobacter heliothermus* (Table 3). *Roseobacter* clade isolates are known to exhibit diverse modes of metabolism with bacterial strains from coastal and oceanic environments reported as able to both utilise organic and inorganic sulphur compounds and

Table 3. Dinoflagellate-associated bacteria capable of transforming paralytic shellfish toxins (PST). U: unknown metabolite(s)

Bacterial isolate	PST transformations	Nearest neighbour (GenBank accession no.)[a]	16S rRNA sequence similarity (%)[a]
α-Proteobacteria (*Roseobacter* clade)			
ALUS253_18	GTX 5 → U	*Ruegria algicola* (X78313)	94.5
		Isol. 253-13 (AJ294351) isolated from weakly toxic dinoflagellate culture *Alexandrium lusitanicum* NEPCC 253	99.9
		Env.PRLISY03 (Y15348) gene clone recovered from *Prorocentrum lima*	99.7
SCRIPPS_732	GTX 2/3 → U GTX 1/4 → GTX 2/3 + dcGTX 2	*Sulfitobacter pontiacus* (Y13155)	99.8
ATAM173a_51	GTX 2/3 → U	*Antarctobacter heliothermus* (Y11552)	99.6
		Isol. 667-12 (AJ294356) isolated from *Alexandrium affine* NEPCC 667	99.9
α-Proteobacteria			
ALUS253_ 27	GTX 2/3 + dcGTX 2 → GTX 1/4 GTX 1/4 → GTX 2/3 + dcGTX 2	*Ahrensia kielense* (D88524)	98.9
SCRIPPS_426	GTX 2/3 → GTX 1/4 + U	*Caulobacter vibroides* (AJ227754)	94.5

[a]Data adapted from Hold et al. (2001)

transform lignin (Gonzalez et al. 1997, 1999). It is therefore perhaps not surprising that *Roseobacter*-related strains demonstrate PST-transforming properties. Further, bacteria not within this clade, which demonstrated PST transformations, were associated with *Ahrensia kielense* and *Caulobacter* spp. (Hold et al. 2001).

SCRIPPS_426 (*Caulobacter* sp.) detected in *Scrippsiella trochoidea* cultures in the exponential growth phase was capable of both SCB toxin production and PST transformation under the conditions described (Tables 2 & 3). However, this was not a common trait among the bacterial isolates. As SCB activity is not evidence of PST production, additional investigations using alternative growth conditions and testing regimes will be required before conclusions on the catabolism and biosynthesis of PST can be made.

Bacterial isolates ALUS253_27, SCRIPPS_426, SCRIPPS_732 and ATAM173a_51 all appeared to utilise GTX 2 and 3, but in different ways (Table 3, Fig. 2). ALUS253_27 (*Ahrensia* sp.) and SCRIPPS_426 appeared to transform GTX 2/3 to GTX 1/4 (Fig. 2A,B), suggesting oxidase activity with the conversion of 1-NH to 1-NOH (Smith et al. 2001). Oshima (1995) reportedly detected oxidase activity in an extract obtained from *Alexandrium tamarense*, but the transformations of GTX 2/3 to GTX 1/4 have not previously been shown to occur in bacteria. In our chromatograms it appears that dcGTX 2 (ca. 20 min retention time), a minor contaminant of GTX 2/3 standards, was also degraded in cultures of ALUS253_27 and SCRIPPS_732 (Fig. 2A,C). SCRIPPS_732 (*Sulfitobacter* sp.) also showed rapid utilisation of GTX 2 (100% after 48 h, data not shown), with only a slight decrease in the amount of GTX 3 observed (Fig. 2C).

The *Antarctobacter*-related strain ATAM173a_51 and SCRIPPS_426 demonstrated utilisation of GTX 2/3 with concomitant increases in several unidentified compounds (Table 3, Fig. 2B,D). These compounds had retention times of ca. 4, 5, 10 and 18 min and were observed after 24 h. dcGTX 3 is known to elute prior to dcGTX 2 and this compound may account for the peak observed at 18 min (Fig. 2D). It has previously been suggested that bacterial activity may result in the decarbamoylation of GTX 3 (at N-21) to dcGTX 3 (Smith et al. 2001), while increases in the initial compounds may involve the sulfation of GTX 2/3 giving rise to C toxins (Smith et al. 2001). In animals and microorganisms, sulfotransferases play an important role in the detoxification of bioactive compounds (Roy 1981) and it is possible that bacterial sulfotransferases have the capacity to sulfate STX analogues, transforming them into less toxic sulfocarbamoyl derivatives.

Following incubation of ALUS253_27, and SCRIPPS_732 with GTX 1 and 4 reductive elimination of the N1 hydroxyl (OH) moiety was observed as demonstrated by the de novo appearance of GTX 2 and 3 (Fig.

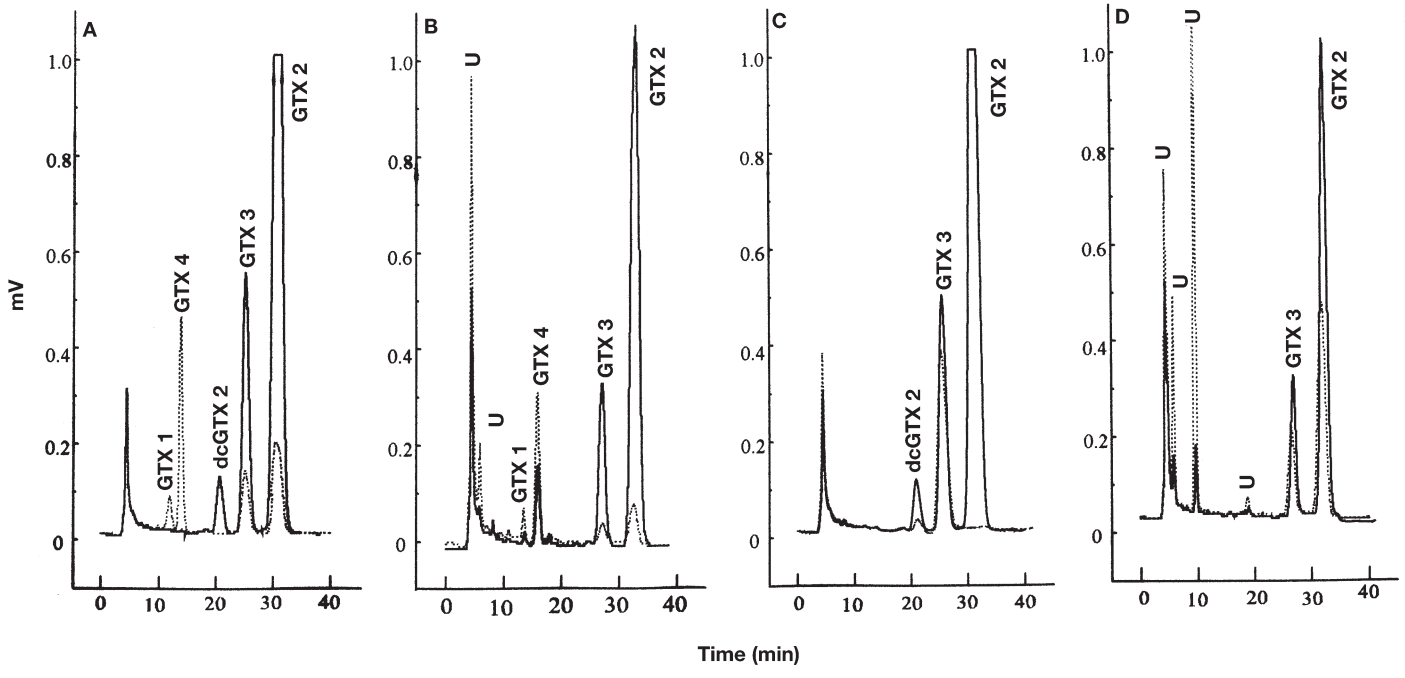

Fig. 2. Chromatogram showing utilisation of GTX2/3 by dinoflagellate-associated bacteria: (A) ALUS253_27, (B) SCRIPPS_426, (C) SCRIPPS_732, (D) ATAM173a_51. Samples shown were taken at 0 h (black line) and 72 h (dotted line). U: unknown metabolites

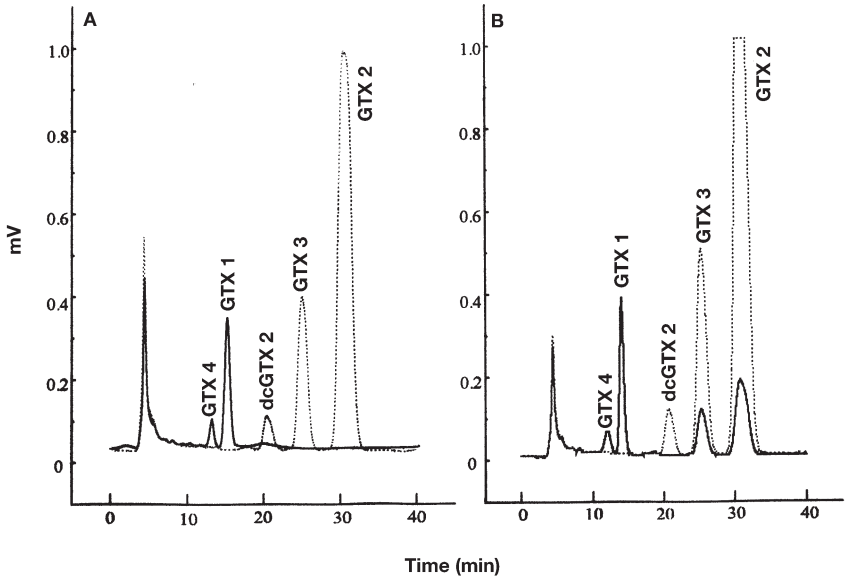

Fig. 3. Chromatogram showing utilisation of GTX1/4 by dinoflagellate-associated bacteria: (A) ALUS253_27, (B) SCRIPPS_732. Samples shown were taken at 0 h (black line) and 72 h (dotted line)

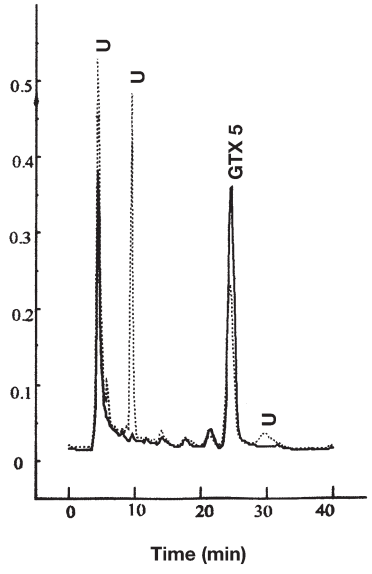

Fig. 4. Chromatogram showing utilisation of GTX 5 by ALUS253_18. Samples shown were taken at 0 h (black line) and 72 h (dotted line). U: unknown metabolites

3). This biotransformation mechanism has also been suggested to occur in cultures of bacteria isolated from shellfish (Kotaki et al. 1985a,b, Smith et al. 2001), although natural reductants, such as glutathione and cysteine, are also known to cause this conversion (Oshima 1995).

GTX 5 was partially degraded by ALUS253_18 (*Roseobater* clade), and after 48 h increases in unknown compounds were observed (data not shown) which remained stable over the 72 h time period examined (Fig. 4). No other bacterial isolates under these experimental conditions were capable of GTX 5 degradation and this bacterium was not capable of transforming GTX 1/4 or 2/3. However, a similar degradation route has been observed in a bacterial strain isolated from shellfish (Smith et al. 2001).

Almost no data is available on the enzymes involved in PST synthesis; the ability of bacteria to inter-convert these toxin analogues suggests more complicated pathways than have previously been envisaged with intermediates and end products not yet fully elucidated. The presence of bacteria living in close association with dinoflagellates with the ability to transform PST raises questions on the possible effects this may have on dinoflagellate toxicity.

CONCLUSIONS

A range of bacteria isolated from toxic *Alexandrium* spp. and non-toxic *Scrippsiella trochoidea* were capable of SCB activity, while bacteria isolated from a non-toxic *A. tamarense* strain did not demonstrate this trait. Continued molecular characterisation of SCB/toxigenic bacteria associated with a variety of *Alexandrium* spp. isolates originating from different locations will, however, be needed to better define any specificity between bacteria and dinoflagellate interactions.

Bacteria isolated from dinoflagellate cultures were also capable of utilising and transforming GTX toxins. However, under these experimental conditions there was little overlap between bacteria that demonstrated SCB activity and those that were able to modify PST, indicating that biosynthesis and catabolism of these compounds may not be related.

Further research into bacterial PST modifications offers the hope of unraveling how multiple PST derivatives are made, with perhaps a clearer picture emerging as to why the large range of numerous analogues with such marked differences in potency are synthesised.

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