Yessotoxin profiles of the marine dinoflagellates *Protoceratium reticulatum* and *Gonyaulax spinifera*

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Abstract

Yessotoxin (YTX) profiles were established for *Protoceratium reticulatum* isolated from the North Sea (Scottish east coast) and the Benguela Current (South Africa). The profiles were compared to those of plankton field samples from both regions. The presence of yessotoxins produced by *P. reticulatum* in South African waters is reported here for the first time. YTX was the predominant compound in isolates of *P. reticulatum* from both areas. Arabinosyl-YTX was found in both the South African isolate and field samples, but not in the material from the North Sea. An isolate of *Gonyaulax spinifera* from the North Sea was also analysed for yessotoxins, but none were found. The abundance of oxidized YTX-derivatives in the North Sea field samples containing *P. reticulatum* was unexpected and requires further investigation.

Introduction

Yessotoxins (YTXs) are a large group of ladder-frame disulphated polyethers, first shown to be produced by the marine dinoflagellate *P. reticulatum* in coastal waters of New Zealand (Yasumoto 1997). The three known primary sources of YTXs are all marine gonyaulacoid dinoflagellates, specifically *P. reticulatum*, *Lingulodinium polyedrum* (Paz 2004) and *G. spinifera* (Rhodes 2006). Yessotoxin and an array of derivatives can accumulate in suspension-feeding shellfish, leading to positive responses in the mouse bioassay for lipophilic marine biotoxins. Yessotoxins are globally distributed in coastal and shelf waters, having been reported from Japan, Norway, Chile, New Zealand, Italy and the North Sea. These compounds were originally classed as diarrhetic shellfish poisoning (DSP) toxins, but are now regarded as distinct, as they are not diarrheagenic.

We established the YTX composition of a *P. reticulatum* isolate from the Benguela Current off the west coast of South Africa and of isolates of *P. reticulatum* and *G. spinifera* from the North Sea along the east coast of Scotland. Analysis was performed by HPLC coupled with tandem mass spectrometry (LC-MS/MS). YTX profiles from the cultured isolates were compared to the profiles found in natural phytoplankton assemblages from both locations. These results are the first reports of YTXs in South African coastal waters.

Methods

Protoceratium reticulatum and *G. spinifera* were isolated from the Scottish east coast in June 2004. Clonal isolates were grown in f/2-enriched seawater growth medium at 16 °C on a 16:8 h L:D photocycle and harvested in late exponential phase. Samples of plankton assemblages, collected from the Scottish east coast at the same time as the clonal isolates, were harvested by centrifugation (15 min, 3220 x g) and the dry cell pellets were stored at -80 °C prior to extraction.

Protoceratium reticulatum was isolated from the west coast of South Africa in March 2000. The culture was maintained in K-medium at 17 °C on a 12:12 h L:D photocycle. Field samples were collected daily off Lambert's Bay on the west coast 21 March-6 April 2005. Samples of 200 ml were collected from 0 and 5 m depth and harvested by filtration through glass fibre filters. Cell pellets and filters were homogenized in a FastPrep instrument. Samples were centrifuged, supernatants were filtered and filtrates were analyzed by LC-MS/MS.

Mass spectral measurements were carried out on a triple quadrupole mass spectrometer (API 4000 QTrap, ABI-Sciex) with turbo spray ionization in negative ion mode. Mass spectrometric analyses for YTXs were performed on a Hypersil BDS C8 column (50 × 2 mm, 3 µm, 120 A) at a flow rate of 0.3 ml min^{-1} using an elution gradient with two eluents (A: water and B: 95 % acetonitrile/methanol (1:2 v/v) and 5 % water, both eluents containing 2.0 mM ammonium formate and 50 mM formic acid). Initial composition was 40% B with a linear gradient to 100 % B at 6 min, isocratic 100 % B until 15 min, then returning to initial conditions. Selected transitions (precursor ion → fragment ion) are given in Table 1. Yessotoxin was identified by comparing retention times and MS3 spectra of

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Table 1. Mass transitions used for LC-MS/MS sample analysis. a other YTX analogs with identical molecular mass are known.

Q1 Mass (amu)	Q3 Mass (amu)	Putative yessotoxin
991	911	#17 (Miles 2005)a
1047	967	41-ketoYTX a
1049	969	no proposed structure
1061	981	no proposed structure
1085	1005	#16 (Miles 2005)
1101	1021	TrinorYTX
1117	1037	no proposed structure
1131	1051	no proposed structure
1141	1061	YTX
1143	1063	no proposed structure
1155	1075	1-homoYTX a
1157	1077	45-hydroxyYTX
1159	1079	no proposed structure
1169	1089	9-Me-41a-homoYTX
1171	1091	45-hydroxy-1a-homoYTX
1173	1093	CarboxyYTX
1175	1095	44,55-dihydroxyYTX
1187	1107	no proposed structure
1189	1109	44,55-dihydroxy-41a-homoYTX a
1195	1115	no proposed structure
1203	1123	44,55-dihydroxy-9-Me-41a-homoYTX
1273	1193	32-O-arabinofuranosyl-YTX
1290	1210	#16 (Finch 2005)
1304	1224	#17 (Finch 2005)
1405	1325	32-O-[arabinofuranosyl-(5'->1'')]-arabinofuranosyl-YTX

samples and a reference standard (IMB-NRC, Halifax, Canada). Compounds with (M-H) masses of 1047, 1175 and 1273 were identified as YTX analogs by characteristic fragments (m/z 173, 855, 925) in MS3 experiments. Other compounds were not further characterised and have to be regarded as putative YTXs. Relative abundances are based on peak area comparisons, identical response factors for all transitions are assumed.

Results

The yessotoxin profiles of isolates from the Scottish east coast and the Benguela current consisted primarily of YTX, 99 % in the Scottish isolate and 90 % in the South African isolate. Cell quotas were 100 and 75 fg/cell for YTX, respectively. The South African isolate also contained 3 % arabinofuranosyl-YTX and 3 % putative 44,55-dihydroxyYTX. Other yessotoxins were present only in trace amounts. Arabinofuranosyl-YTX has previously been reported from Japanese (Konishi 2004) and European and New Zealand isolates (Miles 2006). This compound was detected in both the South African isolate of *P. reticulatum* and in field samples from the Benguela.

Whereas the profiles of YTXs of *P. reticulatum* batch cultures and field samples from South Africa showed similar patterns, large differences were seen between the Scottish cultures and field samples from three different North Sea sampling sites. For example, the toxin profile of the North Sea isolate of *P. reticulatum* consisted almost entirely of YTX, with all other YTX derivatives detectable only at trace levels. However, field samples from the same geographical area demonstrated rather different composition. In particular, compounds with the masses of oxidized YTX derivatives, such as carboxyYTX (previously detected only in shellfish), 45-hydroxydinorYTX and 44,55-dihydroxyYTX, were present in higher proportions than in the cultured isolate.

Owing to the recent report by Rhodes (2006) that *G. spinifera* isolates from New Zealand were a source of YTXs, we carefully analyzed for these compounds in an isolate of *G. spinifera* from the Scottish east coast. No YTXs were detected, which suggests that

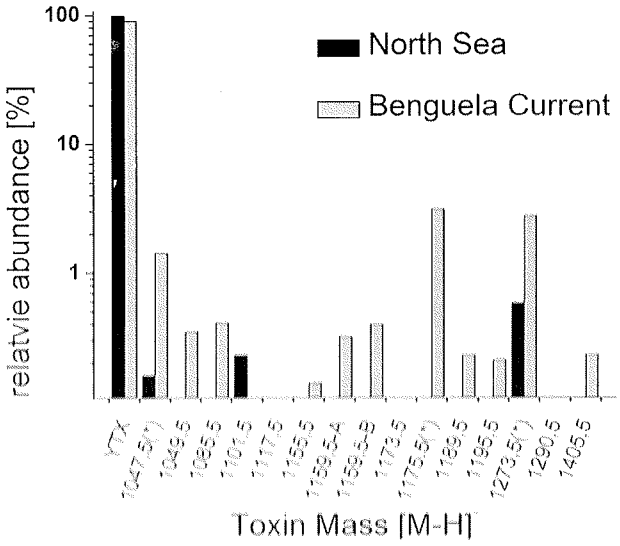

Figure 1. Yessotoxin profile of *P. reticulatum* isolates from the Scottish east coast and the Benguela Current. Compounds marked with (*) have been identified as YTXs by characteristic mass fragments (m/z 713, 855, 925) in MS3 experiments.

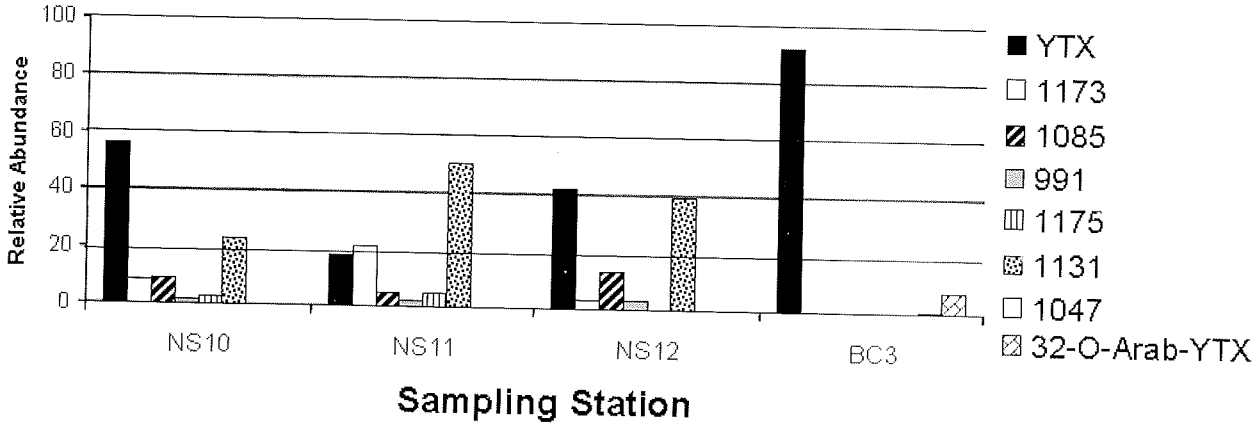

Figure 2. YTX profiles of field samples from the North Sea (NS) and the Benguela Current (BC).

G. spinifera did not contribute to the YTX profile in the mixed plankton assemblages collected from the North Sea.

Discussion

Yessotoxin profiles of the *P. reticulatum* isolate and phytoplankton field samples from the Benguela current were in good agreement, although minor differences were observed. For example a compound with the mass of 44, 55-dihydroxyYTX comprised up to 3 % of the toxin complement of the isolate, but could not be detected in the field samples. On the other hand arabinofuranosyl-YTX was more abundant in field samples than in the cultured isolate. These small discrepancies may be a consequence of the different environmental and harvest conditions to which the cultured material and field samples are subjected. In the North Sea, however, the yessotoxin profiles of cultures and field samples differed notably, and differences were also observed among field samples from different stations. This may reflect an underlying intra-specific genetic diversity in field populations. If this is the case, the toxin profile of an individual clonal isolate may not be strictly representative of the mixed toxin phenotypes present in natural assemblages. The presence of alternative sources of YTXs is an alternative explanation for this discrepancy, although *G. spinifera* is an unlikely source of YTXs along the Scottish coast because these compounds were not detected in the cultured isolated from this region. *L. polyedrum*, another potential source of YTXs, was not detected in the field samples. The field samples contained relatively high ratios of compounds with the masses of oxidized YTX-derivatives, such as 44, 55-dihydroxyYTX and carboxyYTX, the latter having previously been detected only in shellfish. This may be an indication of metabolic activity in the lower food web. In this scenario, the YTX metabolites may be present in fecal pellets and thus captured in mixed plankton samples. It is unknown whether zooplankton such as copepods metabolize YTXs.

The results presented here are derived from mass spectral data alone and only the identity of YTX has otherwise been confirmed. Identification of yessotoxins by mass spectrometry is based on SO3 loss after collision-induced fragmentation and for some compounds by MS3 experiments. At this point, we cannot exclude the (unlikely) possibility that sulphated compounds other than YTXs, but with the same range in molecular weight, have been misinterpreted. Further research is needed to confirm spectral identifications and to understand the fate of these marine biotoxins in the food web.

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