SEQUENCE COMPARISONS OF TOXIC AND NON-TOXIC ALEXANDRIUM TAMARENSE ISOLATES FROM UK WATERS

Wendy A. Higman, David M. Stone and Jane M. Lewis

Sequence analysis was used to determine the genetic relationship between toxic and non-toxic strains of *Alexandrium tamarense* in UK waters. Using sequence data from the D1/D2 region of the LSU rRNA gene two distinct classes were identified. In all cases, the strains shown to be non-toxic by ELISA were assigned to a Western European lineage whereas those producing the PSP toxin were assigned to the North American lineage. The data supports the hypothesis that native strains of *A. tamarense* are non-toxic and that it is the more recent introductions into the North Atlantic of *A. tamarense* that are proving to be problematic.

Introduction

Paralytic Shellfish Poisoning (PSP) is an increasing problem worldwide that is commonly associated algal cells of the genus *Alexandrium* (Dinophyceae). In the UK, PSP is linked to the *A. tamarense* species. However, both toxic and non-toxic strains of this species have been found in the UK and these strains cannot be readily differentiated morphologically. The ability of certain species of *Alexandrium* (including *A. tamarense*) to produce toxins appears to correlate with phylogenetic lineage[1]. North American and temperate Asian lineages consist exclusively of toxic strains and the western European line consists solely of non-toxic strains.

To date, all toxic strains of *Alexandrium* found in UK waters can be assigned to the North American lineage based on their LSU rRNA gene sequences, and it has been suggested that these strains are introductions, whilst the non-toxic strains are native to UK waters[2].

We have conducted studies to determine the genetic relationship between toxic and non-toxic strains of *Alexandrium tamarense* and to establish if the proposed correlation between toxicity and genetic lineage holds true for all strains isolated in UK waters.

Materials and methods

Alexandrium tamarense clonal cell cultures were established from water samples taken from Weymouth harbour during 1998 and single cells taken from sediment slurries of sediment sampled from two areas in Cork Harbour in 1999 (Table 1, Figure 1). Isolates were tested for toxicity by RIDASCREEN Saxitoxin ELISA using acetic acid extraction. Cultures were also provided from the University of Westminster culture collection (Table 1, Figure 1). With the exception of UW4 and 61 these cultures were all clonal. Toxicity of these isolates had previously been determined by HPLC[3].

DNA extractions were performed using a standard proteinase K/SDS digestion followed by a phenol/chloroform extraction and ethanol precipitation. The D1/D2 region of the LSU rRNA was amplified by polymerase chain reaction (PCR) in a 100µl reaction volume containing 1x PCR buffer (50mM KCl, 10mM Tris-HCl pH9.0, and 0.1% Triton X-100), 2.5mM $MgCl_2$, 0.25mM dNTPs, 500ng of both the forward (5'-TAAGTARGYGGTGGAAAWTRAACC-3') and reverse (5'-GTCAAGCAGAARCATNTTGCC-3') primers, 2.5 units of *Taq* polymerase and 100ng template DNA. Reaction mixes were overlaid with mineral oil and subjected to 30 amplification cycles of: 1 min at 95°C, 1 min at 55°C and 1 min at 72°C followed by a final extension step of 10 min at 72°C.

PCR products were purified using the GLEANCLEAN® (BIO 101 inc.) and ligated into the pGEM-T vector using a standard protocol. Both DNA strands were sequenced using the universal M13 sequencing primers and the ABI PRISM™ dye terminator cycle sequencing system (Perkin Elmer). Sequencing reactions were analysed on an ABI 310 genetic analyzer. Sequence alignments and the phenogram were generated using the CLUSTAL V algorithm within MEGALIGN (DNASTAR inc.).

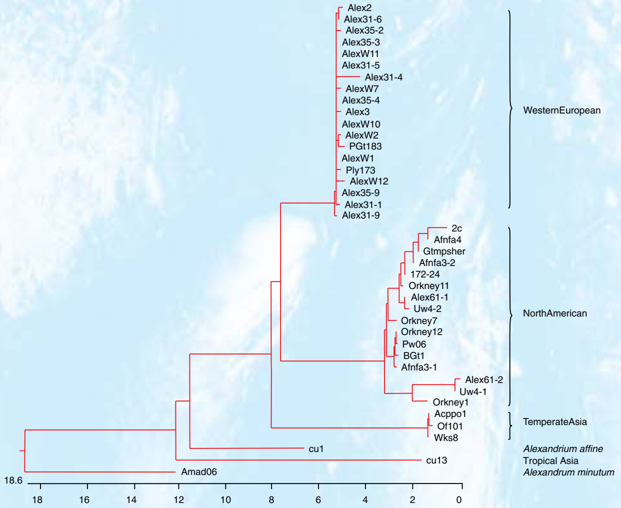

Figure 2: Phenogram showing the genetic relationship among Alexandrium isolates.
Analysis was performed using the Clustal analysis and tree drawing package within MEGALIGN (DNA star inc.).

***Table 1: Alexandrium tamarense* culture origins and toxicity**

Strain	Toxic	Species designation	Isolation locale
2c	Y	*A. tamarense*	Loch Ardtoe, Scotland
Alex UW42	N	*A. tamarense*	Belfast, Northern Ireland
Alex UW53	N	*A. tamarense*	Belfast, Northern Ireland
Alex 31-1	N	*A. tamarense*	Cork Harbour, Ireland
Alex 31-6	N	*A. tamarense*	Cork Harbour, Ireland
Alex 31-9	N	*A. tamarense*	Cork Harbour, Ireland
Alex 35-2	N	*A. tamarense*	Cork Harbour, Ireland
Alex 35-3	N	*A. tamarense*	Cork Harbour, Ireland
Alex 35-4	N	*A. tamarense*	Cork Harbour, Ireland
Alex 35-9	N	*A. tamarense*	Cork Harbour, Ireland
Alex 61	Y	*A. tamarense*	Firth of Forth, Scotland
Alex W1	N	*A. tamarense*	Weymouth, England
Alex W2	N	*A. tamarense*	Weymouth, England
Alex W10	N	*A. tamarense*	Weymouth, England
Alex W11	N	*A. tamarense*	Weymouth, England
Alex W12	N	*A. tamarense*	Weymouth, England
Ply 173	N	*A. tamarense*	Plymouth, England
UW4	Y	*A. tamarense*	Loch Ardtoe, Scotland

Figure 1: Site locations for culture isolation.

Results and Discussion

The LSU rRNA sequences obtained for the UK *A. tamarense* isolates were aligned with the published sequences for representatives of the *A. tamarense/ catenella/ fundyense* species complex, and the *A. affine* and *A. minutum* genetic lineage. Two distinct clusters were identified. Isolates derived from the Weymouth stock, and those collected from Belfast Harbour and the Tamar Estuary, Plymouth, clustered with the published sequence for the non-toxic strain, Pgt183. The cultures originating in Cork, an area prone to sporadic incidence of toxicity, were non-toxic by ELISA and were also assigned to the non-toxic Western European lineage. The isolates from Loch Ardtoe and the Firth of Forth, were assigned to the North American group together with several toxic isolates from Orkney[4]. These strains were also shown to be toxic by HPLC.

The sequence of the Western European group was highly conserved compared to the North American sequence with <1% nucleotide divergence. Sequences in the North American lineage were less well conserved and sequence heterogeneity was observed for both UW4 and Alex61 (UW4-1, UW4-2 Alex61-1 and Alex61-2). UW4-2 and Alex 61-1 were most similar to AFNFA3.2[1] and Orkney11[4] in that there was a nucleotide deletion at position 148, and the UUGUGGA motif at position 106-112 was substituted with UUUGUGG.

The UW4-1 and Alex 61-2 sequences showed greatest similarity to a toxic isolate from the Orkneys (Orkney 1), having a G at position 148, the UUGUGGA motif, the additional GT repeat at position 593-4 and a number of additional common substitutions when compared to AFNFA 3-1. However, Alex 61-2 and UW4-1 share some unique nucleotide substitutions not found in the other toxic strains and shared <97% nucleotide sequence identity with both Orkney1 and AFNFA 3-1.

Sequence heterogeneities were observed for some of the Cork isolates and were suggestive of a mixed population of both Western European and North American strains (results not shown). This could explain the sporadic occurrence of PSP in is this area and warrants further investigation.

It has been suggested by several groups that the toxic *Alexandrium* strains found in the Northern British Isles represent an ancestral population separated from the North American populations by dispersal events. Certainly, the sequence divergence observed in the D1/D2 region for UW4-1 and Alex61-2 would support the hypothesis that the populations were separated in recent evolutionary time. However, the close genetic relationship between UW4-2, Alex61-1, Orkney 11, Orkney 12 and the North American isolates suggests that they may be recent introductions from human activities such as ballast water transport.

References

1. Scholin, C.A., Herzog, M., Sogin, M. and Anderson, D.M. (1994). Identification of group and strain specific genetic markers for globally distributed *Alexandrium* (Dinophyceae). II. Sequence analysis of a fragment of the LSU rRNA Gene. J. Phycol. 30, 999-1011
2. Medlin, L.K., Lange, M., Wellbrock, U., Donner, G., Elbrachter, M., Hummert, C. and Luckas, B. (1998). Sequence comparisons link toxic European isolates of *A. tamarense* from the Orkney Islands to toxic North American stocks. Europ. J. Protistol. 34, 329-335
3. Higman, W.A. (1997). The distribution and toxicity of *Alexandrium* off the north east coast of Britain, Ph.D Thesis, University of Westminster, London.
4. Scholin, C.A., Villac, M.C., Buck, K.R., Krupp, J.M., Powers, D.A., Fryxell, G.A. and Chavez, F.P. (1994). Ribosomal DNA sequences discriminate among toxic and non-toxic *Pseudonitzschia* species. Natural Toxins 2,159-165

The Centre for Environment, Fisheries and Aquaculture Science (CEFAS), Barrack Road, Weymouth, Dorset, DT4 8UB, England http://www.cefas.co.uk