PRIMER NOTE

Six new microsatellite markers for the toxic marine dinoflagellate *Alexandrium tamarense*

TILMAN J. ALPERMANN,*  UW E. JOHN,*  LINDA K. MEDLIN,*  KEITH J. EDWARDS,†  PAUL K. HAYES†  and KATHARINE M. EVANS†

*Alfred Wegener Institute for Polar and Marine Research, Am Handelshafen 12, D-27570 Bremerhaven, Germany,
†School of Biological Sciences, University of Bristol, Woodland Road, Bristol BS8 1UG, UK

Abstract

We report the characterization of six new microsatellite loci for the toxic marine dinoflagellate *Alexandrium tamarense* (North American ribotype), using 56 isolates from a range of locations. The numbers of alleles per locus ranged from five to nine and gene diversities ranged from 0.041 to 0.722. We tested primers for these six loci on other *A. tamarense* ribotypes and on other *Alexandrium* species; the results suggest that the primers are specific to *A. tamarense* isolates belonging to the North American ribotype.

Keywords: *Alexandrium tamarense* ‘species complex’, dinoflagellate, harmful algal blooms, microsatellites

Received 14 February 2006; revision accepted 12 April 2006

*Alexandrium* is a marine, planktonic dinoflagellate genus, with haploid vegetative cells and diploid motile and nonmotile zygotes. Paralytic shellfish poisoning (PSP) neurotoxins are produced by nine of the 29 species so far described (Balech 1995). Three of these toxic *Alexandrium* species (*A. tamarense, A. fundyense* and *A. catenella*) form part of a taxonomically unresolved group, the *A. tamarense* ‘species complex’. Phylogenetic studies indicate that ribotypes cluster depending on geographical origin rather than morphological similarity (Scholin et al. 1994). So far, six different ribotypes have been identified (John et al. 2003). The North American (NA), temperate Asian and tropical Asian ribotypes are exclusively toxic, whereas the Western European, Tasmanian and Mediterranean ribotypes are exclusively nontoxic. The application of microsatellite markers in population studies will help to understand better the development and dynamics of *Alexandrium* blooms. Microsatellite markers have been developed for Japanese strains of *A. tamarense* (Nagai et al. 2004). Here we report the development of six new microsatellite markers for Scottish strains of *A. tamarense* (NA ribotype) and the results of cross-amplifiability tests in other ribotypes and species.

Genomic DNA was extracted from an exponentially growing culture of *A. tamarense* strain BAHME 182 using a modified cetyltrimethyl ammonium bromide (CTAB) method (Doyle & Doyle 1990). The DNA was enriched for CA repeats following Edwards et al. (1996). In brief, the DNA was digested with *Alu*I (New England Biolabs), ligated to a *Mlu*I adapter, amplified using one of the adapter oligonucleotides and hybridized to CA dinucleotide repeat oligonucleotides immobilized on Hybond N+ filter paper (Amersham Biosciences). The enriched microsatellites were re-amplified as above and cloned into pJV1 vector (Edwards et al. 1996). Cloned fragments were amplified using M13 forward and reverse primers (MWG Biotech) and sequenced using the DYEnamic ET Dye Terminator Cycle Sequencing Kit and a MegaBACE 1000 sequencer (Amersham Biosciences).

Ninety-five clones (47%) contained microsatellites. PRIMER 3 (Rozen & Skaletsky 2000) was used to design primer pairs flanking 13 cloned CA-repeats. Their ability to amplify the corresponding microsatellite loci was tested using a Mastercycler gradient thermal cycler (Eppendorf). The 20-µL reactions contained 10–20 ng of DNA, 10 mM Tris-HCl, 50 mM KCl, 1.5 mM MgCl₂, 0.2 mM dNTPs, 0.1 or 0.5 μM of each primer and 1 U of HotMaster™ Taq DNA polymerase (Eppendorf). After 5 min at 94 °C, 40 or 45 cycles were performed: 20 s at 94 °C, 10 s at 51.5–68.5 °C, and 30 s at 70 °C. The annealing temperature was either constant for 40 cycles, or was reduced by 0.3 °C per cycle for 25 cycles, followed by 20 cycles at a constant annealing temperature (Table 1).
Table 1. Attributes of *strain BAHME 182 (NA ribotype)* microsatellite loci: starting annealing temperature during touchdown PCR cycles (TD-\(T_a\)), annealing temperature during PCR cycles with constant annealing temperature (\(T_a\)), allele size range (bp), number of alleles (\(N_a\)) and number of non-amplifying samples (\(N_{\text{AO}}\)). The numbers of alleles exclusive to the North Sea and culture collection isolates are given as \(N_{\text{NS}}\) and \(N_{\text{CC}}\), respectively. All loci were tested on the total number of 56 isolates.

<table>
<thead>
<tr>
<th>Locus (GenBank Accession no.)</th>
<th>Primer sequence (5'-3')</th>
<th>Core sequence</th>
<th>Size range (bp)</th>
<th>TD-(T_a)</th>
<th>(T_a)</th>
<th>(N_a)</th>
<th>(N_{\text{NS}})</th>
<th>(N_{\text{CC}})</th>
<th>(N_{\text{AO}})</th>
</tr>
</thead>
<tbody>
<tr>
<td>ATB1 (DQ984621)</td>
<td>F: CAACACTGGAAGCGTGCTAA</td>
<td>(CTG-GTG)(_1)-(CA-GCT)(_2)-(CA-GCT)(_3)</td>
<td>163–197</td>
<td>61</td>
<td>54</td>
<td>9</td>
<td>3</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>ATB2 (DQ984622)</td>
<td>R: CCATGCGCTACCTCTTACA</td>
<td>(GT-GC)(_3)-(CA-GCT)(_3)-(CA-GCT)(_3)</td>
<td>258–315</td>
<td>61</td>
<td>54</td>
<td>9</td>
<td>3</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>ATB3 (DQ984623)</td>
<td>F: CATTTAGGTTGCGGTGCATA</td>
<td>(GT-GC)(_5)-(CA-GCT)(_3)-(CA-GCT)(_3)</td>
<td>263–278</td>
<td>61</td>
<td>54</td>
<td>9</td>
<td>3</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>ATB4 (DQ984624)</td>
<td>R: CCATGCGCTACCTCTTACA</td>
<td>(GT-GC)(_3)-(CA-GCT)(_3)-(CA-GCT)(_3)</td>
<td>258–315</td>
<td>61</td>
<td>54</td>
<td>9</td>
<td>3</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>ATB5 (DQ984625)</td>
<td>F: CAACACTGGAAGCGTGCTAA</td>
<td>(CTG-GTG)(_1)-(CA-GCT)(_2)-(CA-GCT)(_3)</td>
<td>163–197</td>
<td>61</td>
<td>54</td>
<td>9</td>
<td>3</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>ATB6 (DQ984626)</td>
<td>R: CCATGCGCTACCTCTTACA</td>
<td>(GT-GC)(_3)-(CA-GCT)(_3)-(CA-GCT)(_3)</td>
<td>258–315</td>
<td>61</td>
<td>54</td>
<td>9</td>
<td>3</td>
<td>2</td>
<td>2</td>
</tr>
</tbody>
</table>

Six microsatellite primer pairs (Table 1) generated amplification products of the expected size; 5' -fluorescently labelled forward primers (6-FAM, HEX or NED; Applied Biosystems) were used in repeat reactions using 74 isolates belonging to different *A. tamarense* 'species complex' ribotypes and *Alexandrium* species (*A. minutum, A. lusitanicum, A. insuetum, A. ostenfeldii* and *A. taylori*; list of strains is on request from authors). Fifty-six NA ribotype isolates were genotyped: 48 were collected in 2004 from one eastern Scottish North Sea water sample and eight came from culture collections. Amplification of glutamate synthase (GOGAT; Röttgers 2002) confirmed the amplifiability of each DNA template. One microlitre of the PCR generated amplicons in 15 \(\mu\)L of Hi-Di formamide (Applied Biosystems) and 0.5 \(\mu\)L of the size marker GENESCAN-500 [ROX] (Applied Biosystems) were sized using a 3130xl Genetic Analyser (Applied Biosystems). Microsatellite alleles were scored using GENEMAPPER version 3.5 software (Applied Biosystems). The six primer-pairs yielded 42 scorable microsatellite alleles with a minimum of five and a maximum of nine per locus (Table 1). ARLEQUIN version 2.000 (Schneider et al. 2000) was used to test pairs of loci for linkage disequilibrium (Slatkin 1994). Using the data derived from the 48 North Sea individuals, none of the pairs of loci was found to be in significant linkage disequilibrium \((P = 0.05)\).

Only NA ribotype isolates of the *A. tamarense* 'species complex' yielded amplification products, indicating that cross-amplification of the microsatellite loci does not occur between ribotypes and species. The numbers of alleles and gene diversities (Nei 1987) in the North Sea isolates suggest that the loci are sufficiently polymorphic for use in population studies. Eleven and 19 alleles were unique to the culture collection strains and North Sea isolates, respectively, suggesting a high degree of molecular diversity within the NA ribotype.

Acknowledgements

We thank Annette Richer and Urban Tillmann for their help with cultures of *A. tamarense* and the NERC (NER/A/S/2000/01252) and the HGF programme MARCOPOLI for financial support.

References


microsatellite markers in the toxic dinoflagellate *Alexandrium tamarense* (Dinophyceae). *Molecular Ecology Notes, 4*, 83–85.


