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Characterization of populations of *Alexandrium tamarense* by Genotypic and Phenotypic Markers

Abstract

Populations of the mixotrophic marine dinoflagellate *Alexandrium tamarense* were sampled along the Scottish North Sea Coast.
Clonal strains, derived from individually isolated cells, were genotyped by microsatellites and AFLP. The same strains were tested for the exhibition of phenotypic properties such as PSP toxin profiles and allelopathic effects on autotrophic and heterotrophic unicellular microplankton.
So far, the population markers tested do not correlate or seem to be interrelated.

Phylo-geography of *A. tamarense*

Populations of *Alexandrium* can be found with an almost globally distribution.
Three species or 'morpho-species' form a taxonomically unresolved group, the *A. tamarense* "species complex".
Phylogenetic studies indicate that ribotypes within this species complex cluster depending on geographic origin rather than morphological similarity.

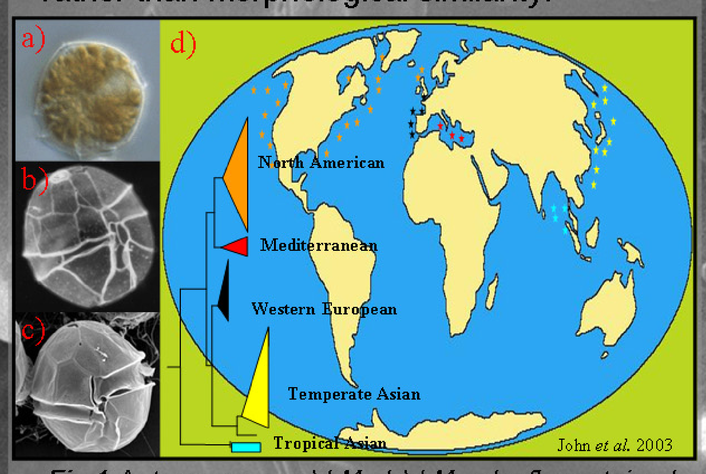

Fig.1 A. tamarense *a) LM, b) LM calcoflour-stained with epifluorescence, c) SEM and d) the present geographic distribution of 28S rDNA ribotypes.*

PSP in *A. tamarense ribotypes*

Three out of six ribotypes of *A. tamarense* produce *paralytic shellfish poisoning (PSP) toxins*. These potent neurotoxins are a group of about 20 derivatives of *saxitoxin*.
Blooms of toxic *Alexandrium* populations can lead to mortality of marine vertebrates and are a risk for human health.
Toxic *A. tamarense* exclusively belong to the North American, the Temperate Asian or the Tropical Asian ribotype.

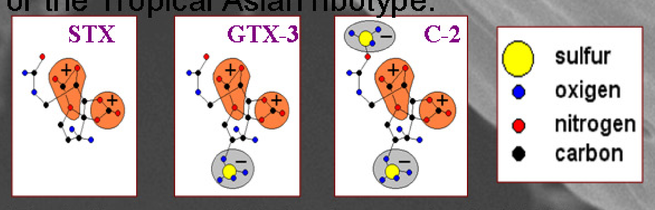

Fig.2 Structure of PSP toxins: saxitoxin (STX), gonyautoxin-3 (GTX-3) C-derivate 2 of STX(C-2) .

Sampling of *A. tamarense*

Populations of *A. tamarense* were sampled along the Scottish North Sea Coast.
For analysis of individuals, clonal cultures were established from vegetative dividing cells.

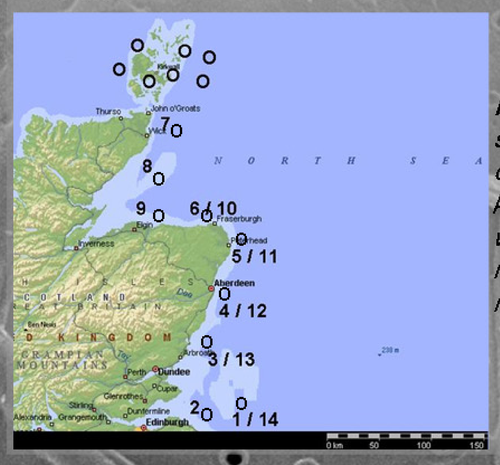

Fig.3 Sampling stations (open circles) where A. tamarense *was present are marked by a number.*

PSP-toxin profiles in *A. tamarense*

The PSP-toxin profile of *A. tamarense* isolates is stable under specific culture conditions. Since PSP-profiles vary considerably among clonal strains they are useful phenotypic markers.

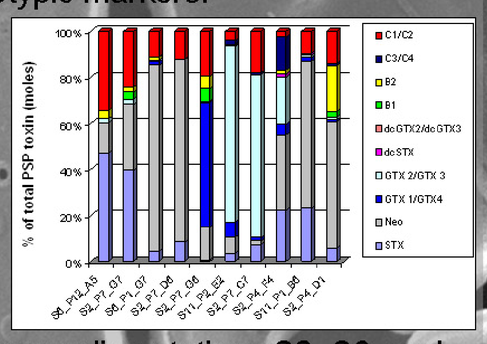

Fig.4 PSP-toxin profiles of 10 clonal isolates of A. tamarense *from stations S2, S6 and S11.*

Clones from sampling stations S2, S6, and S11 showed a broad variety of PSP-toxin profiles. However, a geographic separation by this marker could not be detected.

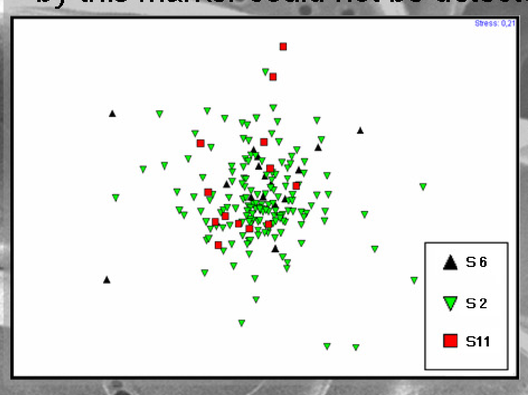

Fig.5 MDS plot of PSP-toxin profiles of clonal A. tamarense *isolates from stations S2, S6 and S11.*

Allelochemical properties

A. tamarense exerts lytic effects on other autotrophic and heterotrophic unicellular plankton organisms. These allelochemical properties may play an important role in the formation of blooms of *A. tamarense*, since autotrophic competitors might be inhibited and heterotrophic grazers deterred or eventually killed.
The allelochemical phenotype is constant for clonal isolates, but varies among clones, making it an interesting phenotypic marker.

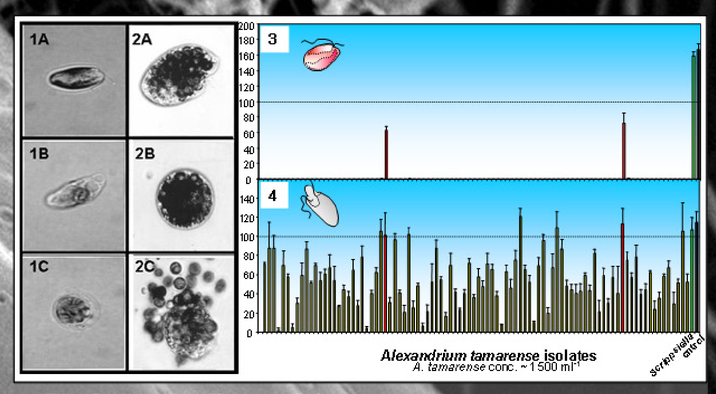

Fig.6 Lytic effects of A. tamarense against Rhodomonas sp. *(1) and* Oxyrrhis marina *(2): A) intact, B) affected, C) degraded cells, and survival of* Rhodomonas sp. *(3) and* Oxyrrhis marina *(4) after 24h culture with* A. tamarense *(cell conc. in percent of start concentration)*

AFLP and microsatellite markers

Six new microsatellite markers were developed for genotyping of clonal isolates of *A. tamarense*.

Fig.7 Electropherogram of two out of six new polymorphic microsatellite markers in three clonal isolates of A. tamarense.

Additionally, amplified fragment length polymorhisms (AFLP) was adopted for the genotyping of clonal isolates.
First results with microsatellites and AFLP indicate a high genetic diversity within the population from station S2. While both genotypic markers proofed suitable for the differentiation between individual clonal isolates, distance matrices created on basis of these two markers did not correlate, nor did they correlate with the expression of specific PSP-toxin profiles.
However, both genotypic markers will be used to study the genetic differentiation of natural populations of A. tamarense from the North Sea and the North Atlantic.

Conclusions and Perspectives

Populations of the mixotrophic dinoflagellate *A. tamarense* exhibit a broad spectrum of phenotypic and genotypic variability. AFLP and microsatellite markers proofed to be valuable tools for the genotyping of individual clonal strains.
These markers might be applied in further studies on the ecological role of allelo-chemial properties and PSP-toxin production. This provides a chance to better understand the development of *Alexandrium tamarense* blooms and the nature of its deleterious toxicity.

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