Bernd Krock[1], Haiyan Ma[1], Alexandr Muck[2,3], Natalie Wielsch[2], Aleš Svatoš[2], Allan D. Cembella[1]

Mass spectrometric characterization of allelochemicals produced by the marine dinoflagellate *Alexandrium tamarense*

Introduction

Allelopathic effects, including growth inhibition and cell lysis of target protistan species (Fig.1) have been investigated for decades among the toxic and harmful algal bloom (HAB) forming *Alexandrium* spp. [1,2], and are believed to relate to bloom formation of the producing organisms. However, neither the chemical character nor the mode of action of the compounds excreted into the surrounding medium by the producing organism have been elucidated.

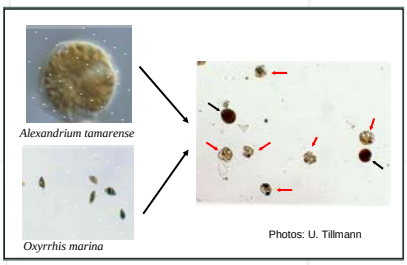

Fig. 1: Lytic effect of Alexandrium tamarense *on* Oxyrrhis marina *(small heterotrophic dinoflagellate). Black arrows:* Alexandrium *Red arrows:* Oxyrrhis *residues*

Experimental

Cell free culture medium of the lytic strain of *A. tamarense* (Alex2) was used for the purification of lytic compounds by bioassay (against the cryptophyte *Rhodomonas salina*) driven fractionation and a non-lytic strain (alex5) was used as a negative control. Lytic compounds were purified by reversed phase SPE and hydrophilic interaction liquid chromatography (HILIC) [3] and subsequently analyzed by several mass spectrometric techniques.

Results

QqQ and Orbitrap experiments

Two masses (*m/z* 1062 and 1292) were detected in the lytic strain Alex2, which were absent in the non-lytic strain Alex5. However, chromatography on different stationary phases showed that these masses only co-eluted with lytic activity on a C18 phase, but neither on C8 nor on HILIC (data not shown). Thus these masses were excluded as lytic compounds.

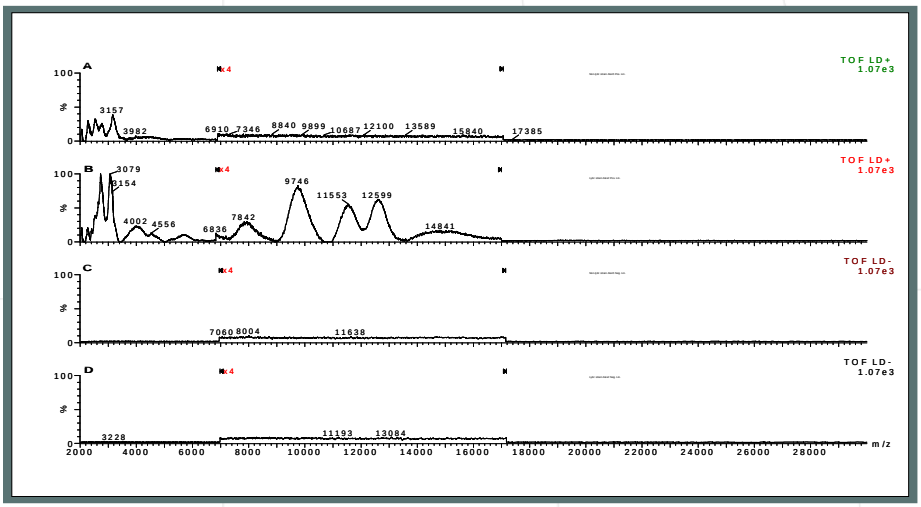

Fig. 2: MALDI-TOF mass spectra of HILIC fractions. A. Alex5, linear positive mode; B. Alex2, linear positive mode; C. Alex5, linear negative mode; D. Alex2, linear negative mode.

MALDI-TOF experiments

Since no unique masses of Alex2 could be detected in the low molecular range up to 2000 Da, ytic fractions were analyzed by MALDI-TOF in the linear mode. While negative ionization did not show any peaks (Fig. 2C/D), positive ionization revealed four mass clusters in the range between 7 and 15 kDa only in the lytic strain but not in the non-lytic one (Fig. 2A/B).

Trypsin digest and SEC

Biomolecules of large molecular weight usually are proteins. Therefore, the lytic fraction was incubated with trypsin and the digest chromatographed by size exclusion chromatography (SEC). However, lytic activity was unchanged compared to the untreated fraction in terms of lytic intensity and retention time (Fig 3.). Saccharides were also excluded by a photometric sugar assay (data not shown).

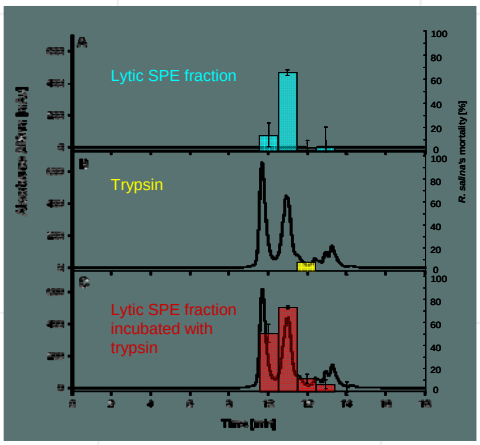

Fig. 3: SEC chromatograms of A: lytic SPE 80% methanol fraction; B: Trypsin; C: SPE + Trypsin. Left scale: absorbance at 280 nm, right scale: lytic activity of individual fractions

Conclusion

Alexandrium tamarense lytic compounds consist of a suite of 7 to 15 kDa molecular weight compounds, which are neither polysaccharides nor proteins. Further research is necessary to fully characterize their identiy.

References:

[1] Hansen, P.J., 1989. MEPS 53:105-116.
[2] Tillmann, U., 2002. MEPS 230, 47-58.
[3] Ma, H., 2009, Mar. Drugs, 7(4), 497-522.

Alfred-Wegener-Institut
für Polar- und Meeresforschung
in der Helmholtz-Gemeinschaft

www.awi.de

1) Alfred Wegener Institute
Am Handelshafen 12
27570 Bremerhaven, Germany

2) Max-Planck-Institut für Chemische Ökologie
Hans-Knöll-Str. 8
07745 Jena, Germany

3) current address: Waters Corp.
Helfmann-Park 10
65760 Eschborn, Germany