

Toxin profile of a *Dinophysis* sp. strain from the North Sea (Belgium) by ultra high performance liquid chromatography coupled to high resolution mass spectrometry

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Toxin producing marine phytoplankton are listed among the most important causative organisms of poisoning events for the seafood consumer. Two main groups of phytoplankton are responsible for poisoning incidents: dinoflagellates and diatoms. These unicellular organisms respond to favourable conditions in monospecific events, forming dense concentrations of cells, known as harmful algal blooms. Filter-feeding shellfish may ingest this toxic phytoplankton and act as a vector of toxins to humans, resulting in illness or death. Diarrhetic Shellfish Poisoning (DSP) is a gastrointestinal illness caused by the consumption of shellfish, which have been feeding on toxin producing dinoflagellates. DSP toxins may be classified into three groups: okadaic acid (OA)/dinophysistoxin (DTX) analogues, pectenotoxins (PTXs) and yessotoxins (YTXs). In the last decade, liquid chromatography (LC) coupled to tandem mass spectrometry (MS/MS) has proven to be a valuable analytical technique for the synchronized separation and detection of the 13 EU listed lipophilic marine toxins in terms of detection capability, sensitivity and specificity. However to date more than 200 different lipophilic marine toxins have been described. Current analytical developments such as high-resolution mass spectrometric devices may fill this gap by enabling the simultaneous detection of a wide variety of toxins. Therefore, this study intended to evaluate the marine toxin profile of one of the most important harmful algae in Europe, namely species from the genus *Dinophysis*. A first step was to isolate *Dinophysis* sp. cells from the Belgian coast. To this extent, samples were taken at different locations; nine offshore stations were sampled from the research vessel Simon Stevin during a vertical trajectory of the coastline. Inshore sampling was carried out at 15 locations (at the Ostend coast and the Spuikom) during the summer season of 2012. At each location sampling was carried out by filtering 100 liters of water using a plankton net with a mesh size of 0.20µm. The plankton was then collected in a bottle at the end of the net and stored in one liter of seawater at 4°C. Eventually, inshore samples obtained from the Spuikom, Ostend, showed a couple of *Dinophysis* sp. cells. Clonal cultures were established by feeding *Dinophysis* sp. with its prey *Mesodinium rubrum*, which on its turn was fed with the cryptophyte *Teleaulax amphioxeia*. Both prey species were kindly provided by Dr. Per Juel Hansen from the University of Copenhagen. Extracts of the cultures were analysed for the presence of toxins using a newly developed UHPLC-HR-Orbitrap-MS analysis method in negative ion mode. High resolution (50.000 FWHM) was used confirming the presence of two different marine toxins, namely okadaic acid and pectenotoxin-2. Further research will focus on profiling other relevant toxin metabolites using the database of Gerssen et al. (2011), and obtaining an in-depth knowledge on the ecological factors that influence marine toxin production in *Dinophysis* spp., both in terms of concentration and chemical profile.

References

Gerssen, A., P.P.J. Mulder and J. De Boer. 2011. Screening of lipophilic marine toxins in shellfish and algae: Development of a library using liquid chromatography coupled to orbitrap mass spectrometry. *Analytical Chimica Acta* 685:176-185.