Fishing for Toxins

ENVIRONMENTAL ANALYSIS

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Abstract

Researchers at the Alfred Wegener Institute (AWI) for Polar and Marine Research in Bremerhaven, Germany are applying advanced chemical and molecular biological technologies to answer ecological questions. They are analysing trace levels of natural bioactive substances in seawater and marine biotoxins produced by various phytoplankton (microalgae), bacteria and cyanobacteria ("blue-green algae"), trying to understand how these bioactive metabolites are produced and sequestered and what effects they have on the ecosystem.

Allan Cembella, Professor in the Faculty of Biology and Chemistry at the University of Bremen and Head of Biological Sciences at AWI, describes how the division is using sophisticated chemical analytical technology, including liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS), and limited genomics approaches to define the genetic regulation of marine biotoxin production and eventually determine the distribution and functional ecology of these toxins in marine food webs.

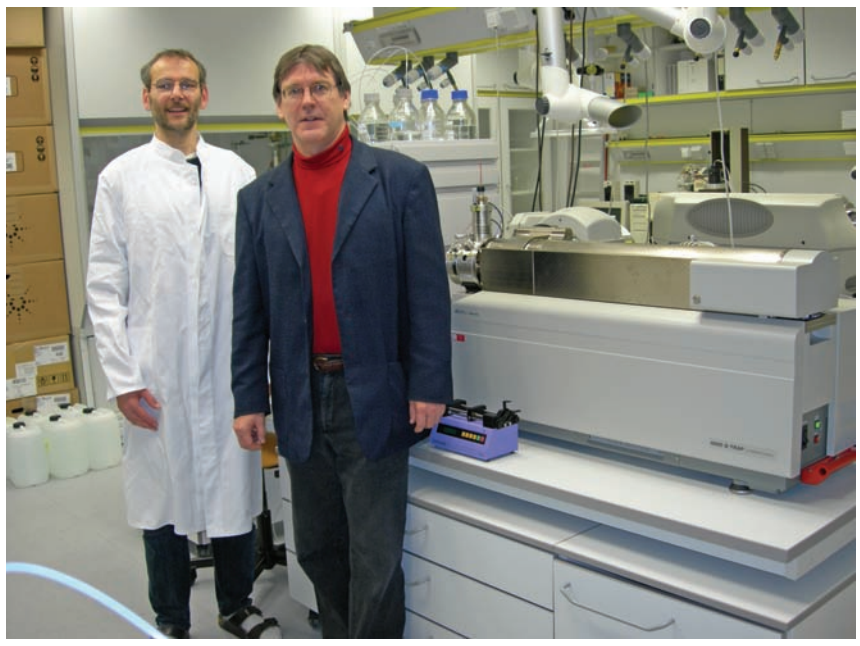
Introduction

The Alfred Wegener Institute in Bremerhaven, named after the famous polar explorer and geomorphologist responsible for the continental drift theory, was established in 1980 as a research institute focusing primarily on the polar regions. Since then its mandate has enlarged to embrace other disciplines in marine and coastal research in temperate waters and global climate change studies. The Division of Biological Sciences at the Institute is involved in research on projects covering organismal biology, physiology and genetics, functional ecology, molecular biology, marine and natural products chemistry and, now, ecological chemistry - chemical aspects of biological and ecological processes and mechanisms. The Institute established a new Ecological Chemistry section in order to unite trace organic analysis and natural products chemistry in an ecological context. Much of the work concentrates on finding out the functional significance of the production and sequestration of bioactive secondary metabolites by marine microorganisms (phytoplankton and bacteria), seaweeds, and invertebrate animals, such as sponges, sea slugs and jellyfish. Sophisticated bioanalytical chemistry is complemented by modern molecular biology and genomics tools.

Hidden reasons for toxicity

Many of the marine toxins of interest are produced by microalgal species that form Harmful Algal Blooms (HABs). These often dense proliferations of microalgae are a global problem occurring in many parts of the world, in both oceans and freshwater, wreaking havoc on ecosystems by causing damage to natural populations of fish, seagrass beds and marine mammals, causing injury to human health via drinking water contamination and seafood poisoning, and having a considerable negative economic impact on aquaculture and coastal tourism. While the biology and physiology of these causative organisms are rather well understood, little is known about the genetic and physiological regulation of their growth and toxin production. The major ecological questions here are what happens to the bloom when it dies; where do the toxins go; do the toxins remain dissolved in seawater; how persistent are they; are they transferred through the food web or do they sediment out with particles, such as bacteria? Answering these questions will undoubtedly help to map the toxin distribution and determine the function of these toxins that potentially affect a broad spectrum of organisms, from viruses to whales, in marine food webs.

A major focus of the AWI group working on the chemical ecology of these toxic microalgae is the effect of these toxins on the micrograzers in marine ecosystems, for example, the zooplankton - the small animals that graze on phytoplankton, the so-called 'grass of the sea'. Originally, ecologists assumed that since these compounds act as "toxins" (i.e. they are poisonous to mammals, especially to laboratory mice!) they must be produced as defensive compounds to ward off or kill their grazers. Indeed experiments done with copepods - little shrimp-like zooplankton that are the major grazer of phytoplankton - have shown some support for this hypothesis. Three different mechanisms have been proposed, all of which seem to be extremely species-specific in terms of predator and prey and are highly toxin specific. The first mechanism is pre-ingestive selection, where the predator senses danger through a chemosensory apparatus and swims away from the noxious algae - simple avoidance behaviour; the second is post-ingestive, where the predator eats a few algal cells and regurgitates the undigested material (whimsically called the 'jalapeno pepper hypothesis'); and finally ingestion of the toxic algal cells, followed by immobilisation, paralysis or physiological incapacitation of the predator.



Prof Dr Allan Cembella (right) with AWI analytical chemist Dr Bernd Krock (left)

Alas for such a straightforward explanation, the chemical defence hypothesis for the known phycotoxins is not always supported by the data. The team has found that in many cases, and somewhat surprisingly, these compounds, although they are very potent mammalian toxins, often do not seem to specifically inhibit grazing upon the producing species by copepods or even smaller microzooplankton. A general role for known phycotoxins in chemical defence is therefore unlikely. Further complications arise because of the production of other allelochemicals by phytoplankton that are lethal or noxious to microzooplankton grazers, but are not yet chemically described.

Many phycotoxins affect ion permeability across membranes and it has been suggested that their toxic effects on mammals are effectively 'collateral damage'. From an evolutionary perspective, it does not make sense for a phytoplankton cell to produce very potent toxins specifically to kill land mammals. This would suggest that the toxicity is only a by-product and in fact the toxins have an internal function in the cell that produces them. One alternative speculation is that the toxins may act as pheromones and so have a role in phytoplankton life cycles.

More theories abound and the team is systematically studying each one in depth to see which ones make the most sense, using the most sensitive technology possible and working towards methods that can identify the molecules and measure factors like toxicity in situ in field populations of natural blooms.

The chemical analytical technology

The development of atmospheric pressure ionisation mass spectroscopy literally revolutionised the study of marine biotoxins, making it possible for the first time to analyse all classes of these small molecules (the largest of which has a molecular weight of only 3200 daltons) from complicated cell matrices and seawater. The laboratory in Bremerhaven uses a 4000 Q TRAP® LC/MS/MS System, a hybrid triple quadrupole/linear ion trap mass spectrometer from Applied Biosystems, primarily because its sensitivity for detecting these compounds is typically 50 to 100 times greater than that of comparable instruments. It is now possible to measure these potent toxins in femtogram concentrations, both from extracted cells and when dissolved in seawater. And recently, a new separation method called hydrophilic interaction liquid ion chromatography (HILIC) has effectively brought a second wave of revolution. HILIC works extremely well with polar solutes, and the combination of the two techniques allows the separation of an extremely complex cocktail of over two dozen derivatives of five different classes of naturally occurring marine biotoxins. These methods are now used extensively, not only by primary research groups, but also by scientists involved in national programmes for seafood quality control, drastically reducing the number of mouse bioassays that were previously used for safety protocols.

Genetic regulation of marine biotoxin production

Another aspect of the AWI group's work focuses on the molecular biology behind the toxin production – hunting for the regulatory and biosynthetic genes that are involved in the expression of these toxins. Most of these marine biotoxins of algal origin are polyethers derived from polyketide metabolism, a common metabolic route for many antibiotics produced by bacteria and fungi. The first step has been to create a cDNA library, now completed for several groups of these toxic organisms. From there, the group has used gene sequencing to make expressed sequence tags (ESTs) and hunted through the annotated sequences to identify many hypothetical candidates for the polyketide synthase genes, which are those most likely to be responsible for the toxin biosynthesis.

Future directions

The current approach to monitoring potentially toxic phytoplankton blooms requires taking samples back to the laboratory and spending many hours tediously counting individual cells and trying to identify these without the certainty that the particular strain is a toxin-producing one. In the near future, the development of DNA microarray chips for putatively toxic species would enable rapid automated screening for expression of "toxin genes" (e.g., for specific polyketide synthases) in natural phytoplankton populations. This would save enormously on laborious field collection and microscopic counting of possible toxic organisms. Moreover, this technology would remove artefacts in the present monitoring schemes caused by high levels of inherent variability in toxicity of free-living phytoplankton populations. In situ monitoring based upon toxin- and taxon-specific probes is an important advancement in surveillance systems for early warning of impending toxicity levels in shellfish or finfish.