Re-Isolation of the azaspiracid producing dinoflagellate *Azadinium spinosum* from the Danish west coast

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Historic Background

Since 1995 new DSP-like poisoning events became public

MacMahon & Silke, 1996: Harmful Algae News, 14, 2

In 1998 Satake et al. identified azaspiracid-1 (AZA-1) as the causative compound in shellfish

J. Am. Chem. Soc., 120, 9967-9968

In 2003 James et al. suggested the heterotroph dinoflagellate Protoperidinium crassipes as the AZP causing organism

Toxicon, 41, 277-283

To date more than 30 structural azaspiracid variants are known

Moran et al. report that there was no correlation between the occurrence of *Protoperidinium* spp. in plankton and azaspiracids in shellfish in Irish waters over a four year period (2002 – 2006).

The authors exclude *Protoperidinium* as the source of azaspiracids.

Historic Background

First Isolation of the AZA-producing organism at Station 398 (57° 4’ N, 1° 30’ W)

Krock et al. (2009) Harmful Algae, 8, 254-263

and description as the new species *Azadinium spinosum*

July 2008: FK Uthörn Research Cruise Bremerhaven - Skagen
Second Isolation of *Azadinium spinosum* from Danish Waters

56° 15’ N, 7° 28’ E, (Station DK 21)
Second Isolation of *Azadinium spinosum* from Danish Waters
56° 15’ N, 7° 28’ E, (Station DK 21)

Two strains available:

Scottish strain 3D9 (2007)

Danish strain UTH E2 (2008)
Toxin Profiles of 3D9 and UTH E2

Ratio AZA-1/AZA-2

Ratio AZA-1/AZA-X

<table>
<thead>
<tr>
<th></th>
<th>3D9 2007 Scotland</th>
<th>UTH E2 2008 Denmark</th>
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<td>AZA-1/AZA-2</td>
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<td>AZA-1/AZA-X</td>
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Comparison of the Product Ion Mass Spectra of AZA-2 and AZA-X

AZA-2

AZA-X

m/z (ion mass)
Azadinium – Light Microscopy

Azadinium spinosum

Azadinium sp. 2
Scotland
2007

Azadinium sp. 3
Denmark
2008
Azadinium – Scanning Electron Microscopy

Azadinium sp. 3
Denmark
2008

Azadinium sp. 2
Scotland
2007

Azadinium spinosum

Azadinium – Scanning Electron Microscopy
### Azadinium – Species Comparison

<table>
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<tr>
<th>strain</th>
<th>species</th>
<th>isolation</th>
<th>azaspiracid</th>
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<tbody>
<tr>
<td>3D9</td>
<td><em>A. spinosum</em></td>
<td>Scotland 2007</td>
<td>+</td>
</tr>
<tr>
<td>2E10</td>
<td><em>Azadinium sp. 2</em></td>
<td>Scotland 2007</td>
<td>-</td>
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<tr>
<td>UTH D4</td>
<td><em>Azadinium sp. 3</em></td>
<td>Denmark 2008</td>
<td>-</td>
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<td>UTH C5</td>
<td><em>Azadinium sp. 3</em></td>
<td>Denmark 2008</td>
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<td>UTH C8</td>
<td><em>Azadinium sp. 3</em></td>
<td>Denmark 2008</td>
<td>-</td>
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<tr>
<td>UTH E2</td>
<td><em>A. spinosum</em></td>
<td>Denmark 2008</td>
<td>+</td>
</tr>
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</table>
April/May 2009: FS Heincke Research Cruise
North Sea - Irish Sea - Celtic Sea

AZA-1 detected

Station No.

AZA-1 [pg/l sea water]
April/May 2009: FS Heincke Research Cruise
North Sea - Irish Sea - Celtic Sea

Live Samples HE 302 (Station 48)

2 l sea water concentrated on 3 µm filters

Azadinium spinosum
Indication for AZAs in the South Pacific

A. spinosum culture

Plankton field sample from the Chilean coast (March 2009)
Conclusion

1. To date *A. spinosum* seems to be the only source for azaspiracids (AZAs)

2. *A. spinosum* produces only AZA-1, AZA-2 and an isomeric form AZA-X
   
   All other azaspiracids seem to be shellfish metabolites derived from these three compounds

3. Two other species of the genus *Azadinium* do not produce AZAs

4. *A. spinosum* is ubiquitous in the North East Atlantic

5. There is indication of the presence of *A. spinosum* in the South Pacific (Chile)
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Nicole Trefault, PUC Stgo. Chile
FK Uthörn Crew
FS Heincke Crew

…and for your attention