Azaspiracids and their producing organisms

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Summary
From 2007 until present a total of 11 species of the potentially toxigenic genera *Azadinium* and *Amphidoma*, a closely related, also toxigenic genus, have been discovered. However, among the eleven species azaspiracids (AZAs) have been found in only four of them. The first known AZA-producing species *A. spinosum* produces only AZA-1, -2 -33 and -34, but by now eight other AZAs have been discovered to be from dinoflagellate origin. Especially *A. poporum* have been shown to possess highly variable AZA profiles consisting of AZA-2, -36, -37, -40, -41 and -42 in various combinations including non-toxigenic strains. Among the AZAs of dinoflagellate origin, three structural types have been found: the 362-, 360- and 348-type, differing in the methylation and unsaturation of the H,I-ring system of the molecules. Until recently AZP appeared to be a merely European problem, but there is increasing evidence that *Azadinium* and *Amphidoma* and their respective AZAs have a worldwide distribution including the entire Pacific, the northern and south western Atlantic and thus may be a potential AZP risk elsewhere as well.

Introduction
Among the known marine shellfish poisoning syndromes azaspiracid shellfish poisoning (AZP) is the most recent one, which was observed for the first time in the Netherlands in 1995. Contaminated mussels cultivated in Ireland were consumed and intoxicated at least eight people. Three years later the implicated toxin was identified, isolated, structurally defined and named azaspiracid (now called azaspiracid-1 (AZA-1). As a result, the European Union has set a regulatory limit for maximum levels of AZA-1, -2 and -3 in shellfish (160 µg/ kg). Due to their structural characteristics AZAs were early suspected to be of dinoflagellate origin, however, the AZA-producing organism remained unknown until the isolation of *Azadinium spinosum* from the North Sea in 2007. In the following years 11 species of the genera *Azadinium* and *Amphidoma* were discovered. However, among the eleven species AZAs have been found in only four of them. While most of the to-date known AZAs are shellfish metabolites of AZA-1 and -2, recently a number of AZAs from dinoflagellate origin have been discovered.

Materials and Methods
Mass spectrometric experiments for the detection and characterization of AZAs were carried out on a LC-MS system consisting of an Agilent (Waldbronn, Germany) model 1100 LC coupled to an AB-SCIEX (Darmstadt, Germany) 4000 Q Trap triple quadrupole mass spectrometer. Single Reaction Monitoring (SRM) measurements were recorded for the detection of known AZAs, precursor scans for the detection of yet undescribed variants and Enhanced Product Ion scans for the characterization of AZAs as described in Krock et al. (2012).

Results and Discussion

To date eleven species of the genus *Azadinium* and *Amphidoma* have been described out of which four species have been reported to be AZA-producers: *Azadinium spinosum*, *A. poporum*, *A. dexteroporum* and *Amphidoma languida*. *A. spinosum*, the only producer of AZA-1 to date has only been reported from the north eastern Atlantic, however detection of AZA-1 has occurred in the Pacific area including Pacific USA, Chile and New Zealand, which suggests a more global distribution of this species. In addition to AZA-1, *A. spinosum* also produces AZA-2 and AZA-33, a variant without the A,B,C-ring moiety, and to a lesser extent AZA-34 and AZA-35. *A. pororum* does not only seem to have the widest geographic distribution throughout the Atlantic and Pacific of both hemispheres, but also shows the most diverse production of AZAs. To date seven different analogs are known from this species. In contrast to the other AZA-producing species which only have modifications of the carboxylic side chain and the terminal A,B,C-ring part of the molecules, the AZAs produced by *A. poporum* also differ in the nitrogen containing H-ring. In AZA-1 to -35 the I-ring has two methyl groups and no double bond. They are called the 362-type AZAs, according to the mass/ charge ratio (*m/z*) of the mass spectrometric H,I-ring fragments. AZA-36 to -40 only have one methyl group in the I-ring and thus belong to the 348-type AZAs. The third group of AZAs produced by *A. poporum* are the 360-type AZAs AZA-41 and AZA-42, which also have two methyl groups in the I-ring, but in contrast to the

362-type AZAs, a double bond. However, there seem to be some geographic peculiarities among the *A. poporum* AZA profiles. *A. poporum* strains available from the North Sea only produce the 348-type AZA-37, whereas strains isolated from the north western Pacific produce the 362-type AZAs AZA-2 and AZA-11, the 348-type AZAs AZA-36 and AZA-40, and the 360-type AZAs AZA-41 and AZA-42 in various combinations. Also non-toxic *A. poporum* isolates from this region have been reported. However, no AZA-37 has been detected in North West Pacific strains. In contrast, isolates of *A. poporum* from the South Western Atlantic (Argentina) and South Eastern Pacific (Chile) only produce the 362-type AZA-2.

Tab. 1: *Azadinium*/ *Amphidoma* species and their respective toxins

Species	AZAs	Literature
Azadinium spinosum	AZA-1, -2, -33, -34, -35	Tillmann et al. 2009, 2012a; Kilcoyne et al., subm.
Azadinium obesum	no AZAs	Tillmann et al. 2010
Azadinium poporum	AZA-2, -11, -36, -37, -40, -41, -42	Tillmann et al. 2011, Krock et al. 2014
Azadinium caudatum var. *margalefii*	no AZAs	Nézan et al. 2012
Azadinium caudatum var. *caudatum*	not tested	Nézan et al. 2012
Azadinium polongum	no AZAs	Tillmann et al. 2012a
Azadinium dexteroporum	tentatively AZA-3, -7	Percopo et al. 2013
Azadinium dalianense	no AZAs	Luo et al. 2013
Azadinium trinitatum	no AZAs	Tillmann et al. 2014
Azadinium cuneatum	no AZAs	Tillmann et al. 2014
Azadinium concinnum	no AZAs	Tillmann et al. 2014
Amphidoma languida	AZA-38, -39	Krock et al. 2012

The third AZA-producing species, *Amphidoma languida*, to date has only been reported from the North Atlantic. There are two isolates available: from the Irish West coast (Tillmann et al. 2012b) and the North West coast of Iceland (Tillmann et al, unpublished). Both isolates have the same AZA profile consisting of AZA-38 and AZA-39, both of the 348-type. Most recently a fourth toxigenic species, *A. dexteroporum* has been described from the Mediterranean. The AZAs produced by this strain have molecular masses identical of AZA-3 and AZA-7, but their identity has not yet been proven. It is noteworthy that an *A. dexteroporum* isolate from Iceland did not produce any AZAs (Tillmann et al. unpublished). To date the structures of AZA-1, -2, -11, and AZA-33 to AZA-37 have been fully elucidated. AZA-38 to AZA-42 have been identified by their collision induced dissociation spectra and full structural elucidation is still pending.

References

Krock, B., Tillmann, U., Voß, D., Koch, B. P., Salas, R., Witt, M., Potvin, É., Jeong, H. J. 2012. New azaspiracids in Amphidomataceae (Dinophyceae). Toxicon, 60: 830-839.

Krock, B., Tillmann, U., Witt, M., Gu, H., 2014. Azaspiracid variability of *Azadinium poporum* (Dinophyceae) from the China Sea. Harmful Algae, 36: 22-28.

Luo, Z., Gu, H., Krock, B., Tillmann, U. 2013. *Azadinium dalianense*, a new dinoflagellate species from the Yellow Sea, China. Phycologia, 52: 625-636.

Nézan, E., Tillmann, U., Bilien, G., Boulben, S., Chèze, K., Zentz, F., Salas, R., Chomerat, N. 2012. Taxonomic revision of the dinoflagellate *Amphidoma caudata*: transfer to the genus *Azadinium* (Dinophyceae) and proposal of two varieties, based on morphological and molecular phylogenetic analyses. Journal of Phycology, 48: 925-939.

Percopo, I.; Siano, R.; Rossi, R.; Soprano, V.; Sarno, D.; Zingone, A. 2013. A new potentially toxic *Azadinium* species (Dinophyceae) from the Mediterranean Sea, *A. dexteroporum* sp. nov. Journal of Phycology, 49: 950-966.

Tillmann, U., Elbrächter, M., Krock, B., John, U., Cembella, A. D. 2009. *Azadinium spinosum* gen. et sp. nov. (Dinophyceae) identified as a primary producer of azaspiracid toxins. European Journal of Phycology, 44: 63-79.

Tillmann, U., Elbrächter, M., John, U., Krock, B., Cembella, A. D. 2010. *Azadinium obesum* (Dinophyceae), a new nontoxic species in the genus that can produce azaspiracid toxins. Phycologia, 49: 169-182. Tillmann, U., Elbrächter, M.; John, U., Krock, B. 2011. A new non-toxic species in the dinoflagellate genus *Azadinium*: *A. poporum* sp. nov. European Journal of Phycology, 46: 74 - 87.

Tillmann, U., Soehner, S., Nézan, E., Krock, B. 2012a. First record of the genus *Azadinium* (Dinophyceae) from the Shetland Islands, including the description of *Azadinium polongum* sp. nov. Harmful Algae, 20: 142-155.

Tillmann, U., Salas, R., Gottschling, M., Krock, B., O'Driscoll, D., Elbrächter, M. 2012b. *Amphidoma languida* sp. nov. (Dinophyceae) reveals a close relationship between *Amphidoma* and *Azadinium*. Protist, 163: 701-719.

Tillmann, U., Gottschling, M., Nézan, E., Krock, B., Bilien, G. 2014. Morphological and molecular characterization of three new *Azadinium* species (Amphidomataceae, Dinophyceae) from the Irminger Sea. Protist, 165: 417-444.