Interim Report on laboratory culture of marine microalgae of the Great Barrier Reef toxic dinoflagellate cultures established at the North Queensland Algal Identification/Culturing Facility (NQAIF)

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Background

Many toxic dinoflagellates are benthic, living on macroalgae and in the top layer of marine sediments. The dinoflagellate flora of the Great Barrier Reef (GBR) is largely unknown. This lack of knowledge applies not only to the identity of toxin-producing species that might be present but also to their seasonality, distribution and abundance patterns. Ciguatera (tropical reef fish poisoning) is a tropical illness brought about by the consumption of large predatory finfish that are contaminated with dinoflagellate toxins. Queensland in particular is a globally known ciguatera hot spot. The main dinoflagellate implicated in ciguatera poisoning is *Gambierdiscus*. Eleven species of *Gambierdiscus* species are currently recognised but these species are almost indistinguishable using morphology (see below). Other dinoflagellates implicated in ciguatera poisoning events include *Amphidinium* spp. (e.g. *A. carterae*), *Coolia* spp. and *Ostreopsis* spp. Other toxic dinoflagellates lead to diarhetic shellfish poisoning (*Prorocentrum* spp. and *Dinophysis* spp.) and paralytic shellfish poisoning (*Alexandrium* spp. and, for example, the tropical *Pyrodinium bahamense* var. *compressa*).

A typical characteristic of dinoflagellate morphology is the presence or absence of armour (theca). All dinoflagellates have a single layer of vesicles underneath the plasma membrane, making the plasma membrane the outermost layer of the cell, which is in contact with the seawater environment. The vesicle system underneath the plasma membrane is known as amphiesma, a morphological trait shared with ciliates and apicomplexans (e.g. malaria parasites). Some dinoflagellates are heavily armoured (the amphiesma vesicles contain thick cellulose plates), while other dinoflagellates are either protected by a very thin and delicate theca (amphiesma vesicles contain thin cellulose plates) or are unarmoured (amphiesma vesicles are empty). Thus dependent on amphiesma content, dinoflagellates are divided into armoured or thecate species and unarmoured or athecate species. Those that are athecate are not easily preserved in fixative and are therefore largely unidentifiable in preserved samples.

The identification of dinoflagellates to species level almost always requires scanning electron microscopy and more often than not the development of species-specific molecular probes (see Blair *et al.* 2009). The use of scanning electron microscopy alone has several disadvantages. Without critical-point drying, the technique will only allow the identification of heavily armoured dinoflagellates, as athecate species are destroyed in the preparation and dehydration process. Furthermore, scanning electron microscopy is not suitable for enumerating dinoflagellate abundance in large sample sets due to the time consuming nature of image acquisition. Molecular techniques on the other hand can be used for species identification and for quantification of toxic dinoflagellate abundance; however its development relies initially on established and identified cultured material for probe validation.

To produce material for morphological identification and to aid molecular probe development specific for toxic dinoflagellate species in the GBR, we have started to isolate dinoflagellates from the GBR and neighbouring tropical locations to establish monoclonal cultures.

Methods

Macroalgal samples, unless stated otherwise, were collected from Nelly Bay, Magnetic Island between May and September 2008. Macroalgal material was washed in filtered seawater to flush epiphytic dinoflagellates off their surfaces. The washwater was concentrated via sequential filtration through a 53 and 20 µm nylon mesh filter. Organisms collected on the mesh filters were taken up in filtered seawater and examined in a Petri dish for the presence of dinoflagellates under 10x magnification on an inverted Olympus microscope (CKX-41).

Culture isolation and establishment was carried out by the curator of NQAIF, Stanley Hudson. Individual dinoflagellate cells were isolated using the micro-capillary single-cell capturing technique (Anderson and Kawachi, 2005). Isolated cells were washed at least five times *via* micro-capillary re-capture and transfer into filtered and sterile seawater. Isolated cells were viewed daily for a period of up to three weeks to ensure that they were contaminant-free. Contaminant-free isolates that were dividing and/or motile were transferred into sterile 20 mL L1 medium (Anderson *et al.* 2005) four weeks after initial isolation. Cultures were maintained in L1 medium at a 12:12 h diurnal photoperiod cycle and a light intensity of 43 µmol photons m^{-2} s^{-1} at 24°C. Cultures are being maintained monthly by inoculating 20 mL of freshly autoclaved L1 medium.

Results and Planned Activities

A total of nineteen culture attempts survived and were established; two cultures were donated by S. Murray (Table 1). Most established cultures are potential toxin-producers (Table 1) and are currently being sub-cultured to establish larger volume batch cultures.

Table 1: Established dinoflagellate cultures at the North Queensland Algal Identification/Culturing Facility (NQAIF).

NQAIF culture number	Cultured organism	Theca*	Potential toxicity	Isolator
NQAIF033	*Amphidinium* sp.	3	yes	S. Hudson
NQAIF035	*Protoperidnium* sp.	1-2	unknown	S. Hudson
NQAIF042	*Prorocentrum* sp. 1	2	yes	S. Hudson
NQAIF043	*Prorocentrum* sp. 2	2	yes	S. Hudson
NQAIF056	*Prorocentrum* sp. 3	2	yes	S. Hudson
NQAIF060	*Coolia monotis* (Fiji)	2	yes	S. Murray
NQAIF070	*Amphidinium* sp.	3	yes	S. Hudson
NQAIF071	*Amphidinium* sp.	3	yes	S. Hudson
NQAIF090	*Coolia monotis*	1-2	yes	S. Hudson
NQAIF103	*Protoperidinium* sp.	1-2	yes	S. Hudson
NQAIF116	*Gambierdiscus australis*	2	yes	S. Hudson
NQAIF203	*Prorocentrum* sp. 5	2	yes	S. Hudson
NQAIF210	unknown	unknown	unknown	S. Hudson
NQAIF211	*Gyrodinium* sp.	1	unknown	S. Hudson
NQAIF252	*Gymnodinium* sp.	3	unknown	S. Hudson
NQAIF260	*Gymnodinium* sp.	3	unknown	S. Hudson
NQAIF262	unknown	unknown	unknown	S. Hudson
NQAIF263	*Prorocentrum micans*	2	yes	S. Hudson
NQAIF270	*Gymnodinium* sp.	3	unknown	S. Garrard
NQAIF276	*Ostreopsis* sp.	1	yes	S. Hudson
NQAIF280	*Gymnodinium sanguineum*	3	yes	S. Hudson

* 1 = Thick thecal plates; 2 = Thin thecal plates; 3 = Athecate.

This material will serve to design suitable molecular probes for species identification (see Blair *et al.* 2009) and for scanning electron microscopy. In the absence of a critical point dryer, we are currently working on a suitable method for the unaltered preservation of naked athecate species for scanning electron microscopy.

We will continue to expand the current dinoflagellate culture collection with live material to be sampled from Orpheus Island and surrounding mid-reefs during the next field trip (March 2009) and the third field trip in August (dependent on funding).

References

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