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Evaluation of California isolates of *Lingulodinium polyedrum* for the production of yessotoxin

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Yessotoxin (YTX) is a newly discovered type of phycotoxin that is commonly produced by two dinoflagellates, *Protoceratium reticulatum* and *Lingulodinium polyedrum*. *P. reticulatum* has been confirmed to produce YTX and other analogues from isolates in New Zealand, Norway, Spain, Italy, Canada, the United Kingdom and the United States. *L. polyedrum* has been determined to produce YTX in isolates from Italy, the United Kingdom, Ireland and, most recently, Spain. *L. polyedrum* is present most years off Baja, California, and bloom events of this species in southern California coastal waters have been recorded as far back as 1901. Three cultures of *L. polyedrum* isolated from southern California coastal waters were tested for the presence of YTX using Biosense Laboratory ELISA kits. Yessotoxin-like activity was detected in the particulate phase of two out of three cultures. Toxin was also detected in the dissolved phase. However, this is probably the result of salt matrix effects rather than measurable toxin. This is the first study to confirm the potential for YTX production in California isolates of *L. polyedrum.*

Keywords: harmful algal blooms, *Lingulodinium polyedrum*, yessotoxin

Introduction

Yessotoxin (YTX) is a newly discovered type of phycotoxin that has been shown to produce cardiotoxic effects (Terao *et al*. 1990, Aune *et al*. 2002). It was first isolated by Murata *et al*. (1987) from scallops *Patinopecten yessoensis* in Mutsu Bay, Japan. The absolute configuration of yessotoxin and two of its analogues (45-hydroxYTX and 45,46,47-trinorYTX) was determined by Satake *et al.* (1996) using the same species of scallop collected from Mutsu Bay. The presence of YTX has been confirmed in shellfish from Chile (Yasumoto and Takizawa 1997), New Zealand (Yasumoto and Takizawa 1997, MacKenzie *et al*. 2002), Norway (Ramstad *et al*. 2001), Italy (Ciminiello *et al*. 1997, Draisci *et al*. 1999) and Ireland (J Silke, Marine Institute, Ireland, pers. comm.).

There are two known biological origins of YTX. The dinoflagellate *Protoceratium reticulatum* was the first confirmed producer of both YTX and the analogue 45,46,47-trinorYTX identified in Japan (Satake *et al*. 1997, 1999). This species has also been identified and confirmed to produce YTX and other analogues from isolates in New Zealand (Satake *et al*. 1997, 1999), Norway (Ramstad *et al*. 2001, Samdal *et al*. 2004), Spain (P Riobo, Instituto Español de Oceanografía, Vigo, Spain, unpublished data), Italy (L Boni, Scienze Ambientali, Università di Bologna, Italy, unpublished data), Canada (LA Stobo, Fisheries Research Services, Aberdeen, UK, unpublished data), the United Kingdom (LA Stobo, unpublished data) and the United States (Paz *et al*. 2004). Another dinoflagellate, *Lingulodinium polyedrum,* (formerly called *Gonyaulax polyedra*, reclassified by Dodge (1989)) was determined to produce YTX, as well as the analogue, homoYTX in isolates from the Adriatic Sea, Italy (Tubaro *et al*. 1998, Draisci *et al*. 1999). YTX was also detected in a *L. polyedrum* isolate from the United Kingdom (LA Stobo, unpublished data), two isolates from Spain (Paz *et al*. 2004) and multiple isolates from Ireland (J Silke, pers. comm.). Table 1 summarises all of the studies that have tested *L. polyedrum* isolates for YTX production in different geographic locations.

Interestingly, the report from the Adriatic Sea was not the first report of toxicity of *L. polyedrum*. Prior to this, a bloom of *L. polyedrum* in the fall of 1988 from the same geographical region was analysed by mouse bioassay and high-performance liquid chromatography with fluorescence detection (HPLC-FLD) (Bruno *et al*. 1990). The mice displayed symptoms of a neurotoxin, concluded to be saxitoxin. Another report of saxitoxin was from California isolates of *L. polyedrum,* determined by Schradie and Bliss (1962). They purified an acid extract from *L. polyedrum* cells, isolated from southern California, and used paper chromatography and mouse bioassay to reveal a toxin similar to that produced by the dinoflagellate *Gonyaulax catenella* (renamed *Alexandrium catenella* Balech 1985), with an activity of 195 mouse units from 35 x 10^6 cells. Their work has been criticised owing to the large amount of saxitoxin used as the standard, possible contamination of the results, and no confirmation of toxicity from other blooms

Table 1: Summary of studies confirming yessotoxin (YTX) production in *L. polyedrum* isolates from different geographic locations

Location of isolate(s)	YTX (pg cell^{-1})	Reference
United Kingdom	0.02	LA Stobo, unpublished data
Italy	Present	Draisci *et al*. (1999)
Norway	0	Ramstad *et al*. (2001)
Spain	0.0009	Paz *et al*. (2004)
Spain	0	P Riobo, unpublished data
Ireland	0.3	J Silke, pers. comm.
California	0.002–0.02	This study

tested along the coast of southern California (Bates *et al*. 1978) using standard methods for detecting saxitoxins.

California isolates of *L. polyedrum* have not been tested for toxin since the early work of Schradie and Bliss (1962). The present study confirms that *L. polyedrum* isolated from California coastal waters produces YTX-like activity in culture.

Material and Methods

Three cultures of *L. polyedrum* — CCMP 1931, CCMP 1936 and 104A (received from P Franks, Scripps Institute of Oceanography, not part of a commercial culture collection) — isolated from Scripps Pier in La Jolla, California, were grown up in batch culture in f/2 medium and phosphate-limited L1 medium (phosphate concentration 27µmol l^{-1} in the f/2 medium and 15µmol l^{-1} in the L1 phosphate-limited medium). All cultures were grown at 21°C, under 87µmol photons m^{-2} s^{-1} using Sylvania 'grow-lite' spectrally corrected light sources on a 14:10h light:dark cycle.

Indirect competitive enzyme-linked immunosorbent assay (ELISA) kits (Briggs *et al*. 2004) were used to test all samples for toxin. These preliminary kits produced by Biosense Laboratories are not yet commercially available, but have been tested against reliable methods such as liquid chromatography mass spectrometry (LCMS). This competitive binding assay uses a 96-well plate coated with a YTX-conjugated protein that competes with free YTX (in the sample) to bind with specific anti-YTX antibodies labelled with horseradish peroxidase (HRP). The concentration of YTX in a sample is inversely proportional to the amount of anti-YTX-HRP conjugate bound in the wells. The ELISA kits include a purified YTX standard. The ELISA kits have been used successfully to analyse YTX in *P. reticulatum* in both field and culture samples (Samdal *et al*. 2004). The general procedures outlined in Samdal *et al*. (2004), which involve separate determination of YTX in particulate and dissolved phases of the samples, were followed in this study.

Results

The CCMP 1931 culture grown in f/2 medium was in the stationary growth phase at the time of harvest, with 4 700 cells ml^{-1}, a growth rate of 0.13 day^{-1}and a phosphate concentration of 17µmol l^{-1} (initially 27µmol l^{-1}). The CCMP 1931 culture grown in phosphate-limited L1 medium did not grow and was not assayed for toxin.

The 104A isolate was also in stationary growth when harvested for toxin, with the culture grown in f/2 medium at 5 700 cells ml^{-1}, a growth rate of 0.25 day^{-1} and a phosphate concentration of 13µmol l^{-1}. The 104A culture grown in phosphate-limited L1 medium was at 5 200 cells ml^{-1}, with a growth rate of 0.25 day^{-1} and a phosphate concentration of 6µmol l^{-1} (initially 13µmol l^{-1}), and was in exponential growth at the time of harvest. The f/2 medium cultures displayed YTX-like activity in the particulate phase of 0.002pg cell^{-1} (9.85pg ml^{-1}) for the CCMP 1931 culture and 0.002pg cell^{-1} (12.8pg ml^{-1}) for the 104A culture. Yessotoxin in the particulate phase of the 104A culture grown in phosphate-limited L1 medium was also detected at 0.001pg cell^{-1} (8.06pg ml^{-1}). It is important to note that culture 104A grown in phosphate-limited L1 medium was not actually limited by phosphate at the time these toxin samples were taken.

The CCMP 1936 culture was grown in f/2 medium only and no YTX was detected in the particulate phase of these samples. There were also positive YTX results for the dissolved phase from these samples.

The dissolved extraction method is currently being optimised for this dinoflagellate species and these results are most likely due to matrix effects from salt and do not reflect actual toxin (I Samdal, National Veterinary Institute, Oslo, Norway, pers. comm.). The ELISA protocol requires diluting dissolved samples to <100 cells ml^{-1} to avoid the matrix affect. However, these protocols were designed to detect YTX production in *P. reticulatum,* which produces significantly higher toxin in culture than *L. polyedrum*. The dissolved samples of *L. polyedrum* cultures tested in this study were run both undiluted and diluted. The undiluted samples resulted in a positive measurement of toxin by the ELISA analysis and the diluted (by 10- to 100-fold) samples had no YTX detected by this method. These results are therefore preliminary and have not yet been validated. However, several negative controls were used (such as a non-toxic culture, a f/2 medium free of culture cells, and filtered seawater). Based on these negative controls, we are confident that there is measurable YTX in the particulate phase from two of the three cultures tested.

Discussion

The results, albeit preliminary, indicate the presence of YTX-like activity in the particulate phase in two southern California isolates of *L. polyedrum*. The detection of YTX in samples in the dissolved phase is most likely due to

matrix effects. However, the possibility of toxin existing in the dissolved phase is highly likely given that it has been detected in the particulate phase of these cultures and in the dissolved phase of other cultures (Paz *et al*. 2004) of *L. polyedrum*.

The results of the dissolved samples analysed in this experiment are difficult to interpret, because the amount of toxin produced by *L. polyedrum* in culture is significantly lower than that produced by *P. reticulatum.* The ELISA method used here was developed for *P. reticulatum*, so the dilution of the dissolved samples could have reduced the toxin to an undetectable level using this method of analysis.

Additional isolates of *L. polyedrum* from California will be tested for yessotoxin production using HPLC-FLD, as described by Yasumoto and Takizawa (1997), and these cultures will be sent to the National Research Council, Canada, for validation of toxin production using LCMS. Multiple batch culture experiments will be conducted to establish cellular toxicity of California isolates compared to European strains, and the toxicity under different conditions of macronutrient concentration, temperature and light regime.

In summary, both dinoflagellate species, *P. reticulatum* and *L. polyedrum*, isolated from California coastal waters produce YTX-like activity in culture. Given that *L. polyedrum* is geographically dominant from central California southwards, whereas *P. reticulatum* is more prevalent from central California northwards, YTX is potentially present along much of the US west coast.

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References

Aune T, Sorby R, Yasumoto T, Ramstad H, Landsverk T (2002) Comparison of oral and intraperitoneal toxicity of yessotoxin towards mice. *Toxicon* **40**: 77–82

Balech E (1985) The genus *Alexandrium* or *Gonyaulax* of the *tamarensis* group. In: Anderson DM, White AW, Baden DG (eds) *Toxic Dinoflagellates.* Elsevier, New York, pp 33–38

Bates HA, Kostriken R, Rapoport A (1978) The occurrence of saxitoxin and other toxins in various dinoflagellates. *Toxicon* **16**: 595–601

Briggs LR, Miles CO, Fitzgerald JM, Ross KM, Garthwaite I, Towers NR (2004) An ELISA for the detection of yessotoxin and its analogs. *Journal of Agricultural and Food Chemistry* **52**: 5836–5842

Bruno M, Margherita P, Gucci B, Pierdominici E, Ioppolo A, Volterra L (1990) Presence of saxitoxin in toxic extracts from *Gonyaulax polyedra. Toxicon* **28**: 1113–1116

Ciminiello P, Fattorusso E, Forino M, Magno S, Poletti R, Satake M, Viviani R, Yasumoto T (1997) Yessotoxin in mussels of the Northern Adriatic Sea. *Toxicon* **35**: 177–183

Dodge JD (1989) Some revisions of the Family Gonyaulacaceae (Dinophyceae) based on a scanning electron microscope study. *Botanica Marina* **32**: 275–298

Draisci R, Ferretti E, Palleschi L, Marchiafava C, Poletti R, Milandri A, Ceredi A, Pompei M (1999) High levels of yessotoxin in mussels and presence of yessotoxin and homoyessotoxin in dinoflagellates of the Adriatic Sea. *Toxicon* **37**: 1187–1193

MacKenzie L, Holland P, McNabb P, Beuzenberg V, Selwood A, Suzuki T (2002) Complex toxin profiles in phytoplankton and greenshell mussels (*Perna canaliculus*), revealed by LC-MS/MS analysis. *Toxicon* **40**: 1321–1330

Murata M, Kumagai M, Lee JS, Yasumoto T (1987) Isolation and structure of yessotoxin, a novel polyether compound implicated in diarrhetic shellfish poisoning. *Tetrahedron Letters* **28**: 5869–5872

Paz B, Riobo P, Fernandez ML, Fraga S, Franco JM (2004) Production and release of yessotoxins by the dinoflagellates *Protoceratium reticulatum* and *Lingulodinium polyedrum* in culture. *Toxicon* **44**: 251–258

Ramstad H, Hovgaard P, Yasumoto T, Larsen S, Aune T (2001) Monthly variations in diarrhetic toxins and yessotoxin in shellfish from coast to the inner part of the Sognefjord, Norway. *Toxicon* **39**: 1035–1043

Samdal IA, Naustvoll LJ, Olseng CD, Briggs LR, Miles CO (2004) Use of ELISA to identify *Protoceratium reticulatum* as a source of yessotoxin in Norway. *Toxicon* **44**: 75–82

Satake M, Ichimura T, Sekiguchi K, Yoshimatsu S, Oshima Y (1999) Confirmation of yessotoxin and 45, 46, 47-trinoryessotoxin production by *Protoceratium reticulatum* collected in Japan. *Natural Toxins* **7**: 147–150

Satake M, MacKenzie L, Yasumoto T (1997) Identification of *Protoceratium reticulatum* as the biogenic origin of yessotoxin. *Natural Toxins* **5**: 164–167

Schradie J, Bliss CA (1962) The cultivation and toxicity of *Gonyaulax polyedra*. *Lloydia* **25**: 214–221

Satake M, Terawawa K, Kadowaki Y, Yasumoto T (1996) Relative configuration of yessotoxin and isolation of two new analogs from toxic scallops. *Tetrahedron Letters* **37**: 5955–5958

Terao K, Ito E, Oarada M, Murata M, Yasumoto T (1990) Histo-pathological studies on experimental marine toxin poisoning – 5. The effects in mice of yessotoxin isolated from *Patinopecten Yessoensis* and of a desulfated derivative. *Toxicon* **28**: 1095–1104

Tubaro A, Sidari L, Della Loggia R, Yasumoto T (1998) Occurrence of yessotoxin-like toxins in phytoplankton and mussels from Northern Adriatic Sea. In: Reguera B, Blanco J, Fernández ML, Wyatt T (eds) *Harmful Algae.* Xunta de Galicia and Intergovernmental Oceanographic Commission of UNESCO, Santiago de Compostela, pp 470–472

Yasumoto T, Takizawa A (1997) Fluorometric measurement of yessotoxins in shellfish by high-pressure liquid chromatography. *Bioscience, Biotechnology, Biochemistry* **61**: 1775–1777

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