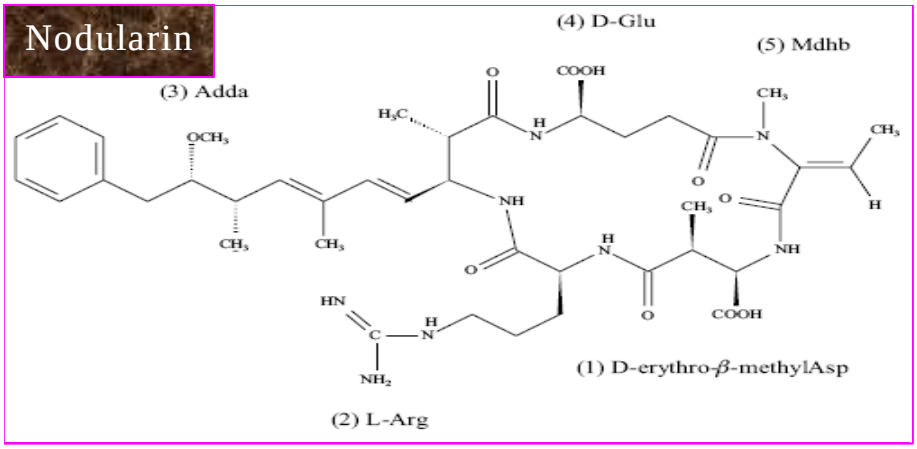
Evaluation of Cyanotoxins in the Farmington Bay, Great Salt Lake, Utah.



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ABSTRACT

The presence of toxic cyanobacteria in several marine environments world wide is well documented. However, the presence of cyanobacteria in the Farmington Bay of the Great Salt Lake, Utah, is largely unexplored. Recently, a great concern is being raised over severe eutrophication in the Farmington Bay due to the presence and the inflow of nutrients in the bay from various sources. Excess growth of algae, especially during summer months, has been observed in the Farmington Bay. In brackish waters, such as in the Great Salt Lake, the cyanobaceria belonging to the genus *Nodularia* generally occurs and produces the toxin nodularin. This study quantified nodularin in representative and homogeneous water samples collected from six different locations in the Farmington Bay of the Great Salt Lake at four different occasions during the summer of 2008. A significant portion of the research was also devoted to analytical method development and quality control tests in our laboratory. An ultra performance liquid chromatography-based analytical method was developed for nodularin found in brackish waters. When deionized water was spiked with nodularin to give a final concentration of 1 μg/L, more than 99 % toxins could be recovered from deionized water, thus demonstrating the ability of the newly developed method to detect and quantify low concentrations of nodularin. The Farmington bay water matrix interfered with nodularin extraction and reduced the extraction recovery to 78 % when the filtered water from the Farmington Bay was spiked with 1 μg/L of nodularin. Sites 4 and 6 in July and sites 4 and 5 in September had nodularin concentrations of 0.207 and 0.384 μg/L and 0.331 and 0.250 μg/L, respectively. Molecular identification based on 16S rDNA cloning and sequencing

provided very few partial DNA sequences that matched with cyanobacteria with none of the sequences related to any species of *Nodularia*. The majority of the sequences fell within the classes of β- *proteobacteia*, γ-*proteobacteia*, *Bacteroidetes* and *Sphingobacteria*.

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INTRODUCTION

The Great Salt Lake, which is located on a shallow playa, is a remnant of the prehistoric Lake Bonneville. The lake is located in the northern part of Utah, is the largest salt lake in the western United States, and is the fourth largest terminal lake in the world (U.S.G.S. 2001). The salinity of the lake is typically 3 to 5 times higher than that in the ocean (U.S.G.S. 2001) which gave the name "Great Salt Lake" to the lake. The lake contributes to the state's commerce by its mineral salts and brines production which is worth over $200 million annually, brine shrimp-cyst harvesting which is worth more than $100 million annually and recreational activities such as sailing. The marshes and wetlands surrounding the lake constitute ecological aspects which play an important role in nutrients and metals dynamics. Brine shrimp and brine flies serve as food to many birds and attract shorebirds which is important to the ecosystem of the lake. At least 22 species of shorebirds utilize the lake during migration and another eight more species nest in habitats associated with the lake (Cavitt et al. 2006). Consequently, the Great Salt Lake is recognized as a site of hemispheric importance within the Western Hemisphere Shorebird Reserve Network (Andres et al. 2006) which makes the ecosystem of the lake very interesting and has very high environmental values (Gwynn 2008).

The lake is divided into three major bays: the Gilbert Bay, the Gunnison Bay, and the Farmington Bay (figure 1). A solid-fill railroad causeway divides the lake into two parts: the north part, which represents the Gunnison Bay, which receives little freshwater inflow, and the south part, which represents the Gilbert Bay, receiving almost all the inflow from tributaries (the Bear River, the Weber River, and the

Jordan Rivers) (U.S.G.S.2001). Gunnison Bay is extremely saline with 28% salinity. The salinity in Gilbert Bay is approximately 11%, and the salinity in the Farmington Bay varies generally between 3 to 5% due to water dynamics (U.S.G.S.2001).

Because of its importance to North American, the Great Salt Lake has been studied extensively. One study investigating the fate and origin of hydrogen sulfide (H_2S) in the Farmington Bay found that hydrogen sulfide gas was produced in the deeper layers of sediments in Farmington and Gilbert Bays in the Great Salt Lake. Both Farmington and Gilbert bays were shown to release H_2S into the air shed, thus contributing to odor problems. In a separate study aimed at measuring phosphorus at several locations in the main channel of the Farmington Bay (Schulle 2008), it was noticed that sites near the shore had the highest phosphorus concentrations and were mostly comprised of ortho-phosphorus in the range of 0.9 to 1.0 mg P/L. Common heavy metals such as selenium and mercury are also found at different locations in the Great Salt Lake. Studies in the past have also evaluated the possible reasons of hyper eutrophication in the Farmington Bay, and one such study by Wurtsbaugh et al. (2006) attributed the dynamics of cyanobacteria to nutrients in the lake and variations in the lake salinity. Cyanobacteria, commonly known as blue-green algae or cyanophytes, are photosynthetic prokaryotes found in marine and freshwater aquatic environments throughout the world, including Antarctica. Cyanotoxins are compounds produced by cyanobacteria and are either intracellular (inside the cell) or extracellular (outside the cell as dissolved compounds).

Wurtsbaugh et al. (2006) showed *Nodularia spumigena* as the dominant cyanobacteria in the Farmington Bay of the Great Salt Lake and was shown to

produce the toxin nodularin in the lake at levels much greater than the maximum concentration recommended by the World Health Organization (WHO 1999).

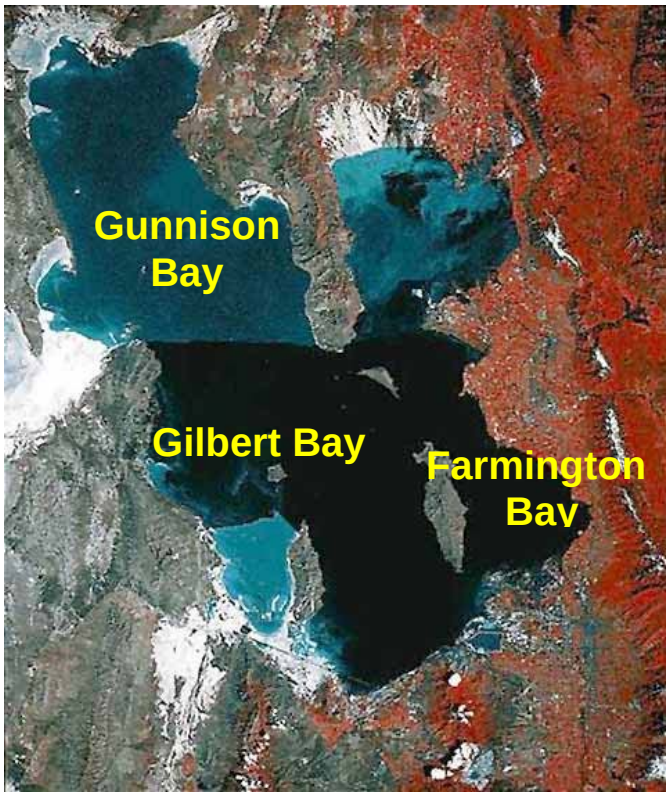

Figure 1. Great Salt Lake map (Utah Education Network)

Nodularin is recognized as one of the most potent natural toxins capable of risking human health, poisoning animals, causing fisheries poisonings, and impeding recreational use of waters (Edler et al. 1985, GuBmann et al. 1985, Main et al. 2005, Van Hankleren et al. 1995, Karjalainen et al. 2005, Bartram et al. 1999).

Problem Statement and Objectives

Because of the natural resources in the lake, unique species such as brine shrimp, and being a stop over for thousands of migratory birds, much attention is being given to various environmental issues related to lake ecology and water quality in recent times. Eutrophication in the lake is a well-accepted fact, and the presence of

heavy metals such as selenium and mercury needs no further proof. The major contaminants which affect the use of water by aquatic life are suspended sediments, nutrients and metals. (Baskin, et al. 2002). As a whole, the Great Salt Lake can be termed as a complex ecosystem surrounded by numerous environmental challenges. Among several environmental concerns, eutrophication in the lake has emerged as one of the major environmental challenges. In particular, Farmington Bay receives the majority of treated municipal and industrial wastewater from the Salt Lake City metropolitan area, as well as runoffs from non-point sources (Marcarelli et al., 2003).

Excess algal bloom was observed and reported in the bay (Wurtsbaugh et al. 2006). Since the presence of excess nutrients in any water body can promote eutrophication, it was hypothesized that discharges into the Farmington Bay directly or by means of wetlands, promote the bloom of algae. Previous studies (Wurtsbaugh et al., 2006) have shown the presence of *Nodularia spumigena* as the dominant cyanobacteria in the Farmington Bay of the Great Salt Lake, contributing to nodularin in the lake. Although the study by Wurtsbaugh et al., 2006 was important and demonstrated the presence of *Nodularia spumigena,* this study seems inconclusive in some sense due to the following reasons (based on the report by Wurtsbaugh et al. 2006 posted on the Central Davis Sewer District web page).

1. It was stated that *Nodularia spumigena* was the dominant cyanobacteria contributing toxin into the bulk water. However, no methodology either based on microscopic examination or molecular approach targeting DNA was presented in the materials and methods section of the report.
2. Information was missing on how the representative water samples were collected. It was not clear whether the algal biomass floating on the water

surface was skimmed off or a homogeneous sample from the water column was collected.

3. It was reported that in certain samples, nodularin could not be quantified because the concentration was too high to be quantified by the instrument. Generally, the samples anticipated to have high concentrations of contaminants are diluted to bring the concentrations within instrument range.
4. It is not clear whether nodularin was measured in the bulk liquid or in the cell mass. When cyanobacteria decays, the intercellular toxins are released into the bulk liquid.

The specific objectives of the research in this study are outlined below.

- Establish an "in house" analytical method for cyanotoxins, mainly nodularin.
- Quantify variations of concentrations of nodularin in the Farmington Bay in summer and establish genetic identification of bacteria in the water column at sampled sites.

MATERIALS AND METHODS

Water sampling*:* Water samples were taken in June, July, September and November 2008. The six locations (shown in figure 2) were either receiving treated discharges from wastewater treatment plants or from storm and agricultural runoffs. The latitude and longitude coordinates of these six locations are given in table 1. Unlike previous research efforts by others, we employed a modified sampling strategy where the focus was to collect representative and homogeneous water samples over the water column at any sampling location. A PVC tube of approximately 20 cm diameter and 76 cm length was gently placed on the lake sediment to create a water column.

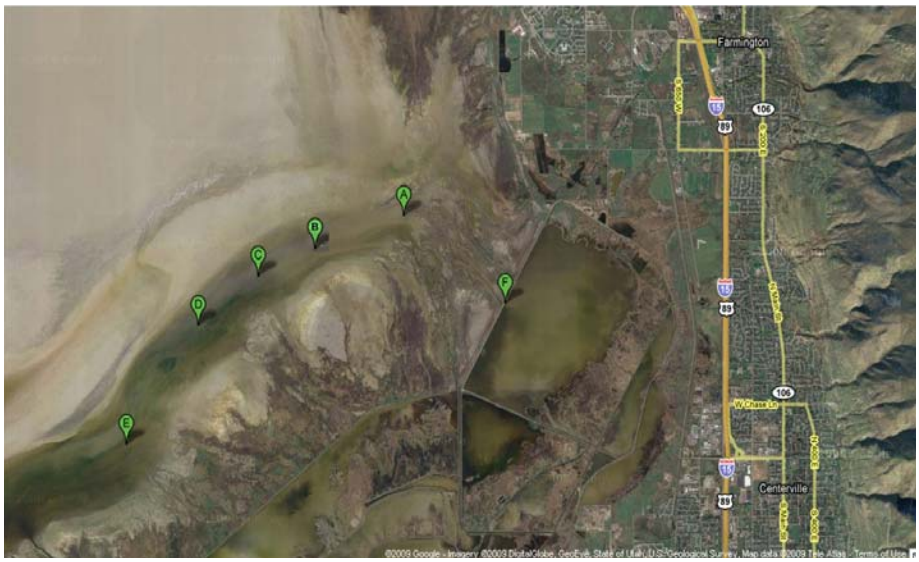

Figure 2. Sampling locations in the Farmington Bay

The water column was then allowed to stand still in order to avoid mixing of the underlying sediments. Samples were collected from the top, middle and bottom portion of the column and were mixed together in a 1-liter glass bottle to provide a composite sample at that location. Care was taken as not to disturb the sediments during the sampling. The bottles were stored on ice in a Coleman cooler. Figure 3 shows the column and a graduate student mixing the water column inside the PVC column before collecting the samples.

Figure 3. Sample collection apparatus and use

Table 1. Sampling sites with GPS coordinates

Sampling site	Coordinates
1	40.96N 111.96W
2	40.95N 111.98W
3	40.95N 111.99W
4	40.94N 112.00W
5	40.92N 112.02W
6	40.94N 111.94W

Cyanotoxin Extraction from Samples

We employed a freeze and thaw method, as recommended by World Health Organization, followed by sonication to release the intracellular toxins into the bulk liquid. Freeze and thaw was accomplished by dipping 100 mL of a composite sample contained in a flask into liquid nitrogen followed by placing the sample in hot water bath at 75^0C. This was repeated for 6 times. The sample was then subjected to sonication using an ultrasonic processor (Ace glass incorporated, Vineland, NJ). After these procedures, the water sample was filtered to remove cell debris and other suspended particles. Toxins from the filtrate were recovered using solid phase extraction (SPE). SPE is widely used for three main purposes, (1) sample concentration enrichment, (2) eliminate interfering matrix elements, and (3) eluting the attached and concentrated sample in proper solvent for subsequent analysis.

The SPE cartridge was first conditioned using 5 mL methanol (Sigma Aldrich, Saint Louis, MO) at a flow rate of 5 mL/min to remove any impurities from the cartridge. The cartridge was then equilibrated by using 5 mL ultra-pure water (Millipore water system) at the same flow rate. After drying for 30 minutes, the sample filtrate containing toxins was passed through the SPE cartridge at a flow rate

of 2.5 mL/min. The impurities were then washed off with 5 mL of 5% methanol in water at a flow rate of 5 mL/min. After this clean-up step, the analyte was eluted twice with 5 mL methanol. All SPE cartridges were placed on a VisiPrep Solid Phase Extraction Vacuum Manifold (Sigma Aldrich, Saint Louis, MO) through a vacuum pump. The set-up (Figure 4) is a screw-type solvent-resistant vacuum with bleed gauge which provides better sealing. For the vacuum control, the valve system of the apparatus allows rotating individual valves to control flow through each SPE tube. The disposable liner in the vacuum manifold eliminates cross-contamination during sample processing.

Figure 4. Visiprep Solid Phase Extraction Vacuum Manifold

The eluted sample was concentrated by first drying under nitrogen in a 40ºC water bath (TurboVap LV evaporator, figure 5, Caliper Life Sciences, Inc., Hopkinton, MA). The dried sample was reconstituted to 1 mL which was then transferred to a 2.5 mL vial for chromatographic analysis.

Figure 5. TurboVap LV evaporator

Analytical method for toxins

An ultra performance liquid chromatogram–mass spectrometer (UPLC-MS) (Waters, New Castle, DE) was used in this study for sample chromatographic analysis. This UPLC-MS system used sub-2 μm particle columns which offered the most promise for LC to meet disparate challenges without sacrificing performance. The use of sub-2 μm particle columns produced higher backpressures than conventional 5 and 3 μm columns (Waters 2008). Therefore, this application provided sharper peaks, increased peak heights, better signal-to-noise performance and led to a greater MS sensitivity.

The samples were analyzed using a 250×4.6 mm Luna C18 column with a 4-μm particle size (Phenomenex, Torrance, CA). Chromatographic solvents used in this analysis were A: water containing 0.1% TFA (Trifluoroethanoic acid), B: HPLC-grade acetonitrile. The UPLC gradient conditions were as follows: 95% solvent A and 0.5% solvent B were started at the flow rate of 0.6 mL/min for 3.5 minutes. The mobile phase was changed to 15% solvent A and 85% solvent B between 3.5 and 4 minutes. The mobile phase was then changed to 100% solvent B from 4 minutes to

4.25 minutes and from 4.25 minute until the end, the gradient was changed back to 95% solvent A and 5% solvent B.

The MS analysis was performed using a Micromass Quattro II - Triple Quadrupole Mass Spectrometer with electospray ionization in positive mode. The target mass (in terms of m/z value) for nodularin was 825.

Instrument performance and calibration curve

High performance liquid chromatography followed by mass spectrometry (HPLC/MS) is the commonly used method for analyzing cyantoxins in environmental samples. However, the UPLC method developed for this study gave even better sensitivity. Pure nodularin was used to test UPLC/MS instrument performance and optimize the response. A stock solution (1000 μg/L) was prepared with pure nodularin (≥99 % pure) bought from Sigma Aldrich. Serial dilutions using methanol were done to obtain calibration standards of 5 μg/L, 10 μg/L, 25 μg/L, 50 μg/L, and 100 μg/L. For reproducibility and quality control, triplicate injections were made for each concentration standard. Peak heights and total integrated responses from each standard were compared, and the average responses were taken to obtain the calibration curve.

Quality Control Tests

Comparison of SPE columns: Two types of cartridges, C18 and HLB (Hydrophilic-Lipophilic-Balance), with reversed phase packing were compared in this study. Both cartridges were purchased from Waters (Waters, New Castle, DE).

Reversed phase SPE involves the separation of a polar or moderately polar sample matrix and a nonpolar stationary phase through which the sample passes. The reversed phase of C18 cartridge packing contains alkyl- or alryl-bonded silicas, where the hydrophilic silanol groups at the surface of the raw silica packing are chemically modified. Retention of the organic analytes from polar solutions (i.e., water) onto the SPE materials results primarily from the attractive forces between the carbon-hydrogen bonds in the analyte and the functional groups on the silica surface. The Oasis HLB sorbent is a macroporous copolymer made from a balanced ratio of two monomers, the hydropoilic N-vinylpyrrolidone and the lipophilic divinlylbenzene.

To compare both columns, 200 mL deionized water was spiked with a known concentration of nodularin to give a final concentration of 1 μg nodularin/L, and the volume of DI water was divided into two 100-mL aliquots. Each 100 mL was passed through C18 and HLB columns respectively, and the toxin attached to the matrix was eluted with 10-mL methanol. Methanol was concentrated to 1 mL using the TurboVap system. Nodularin was analyzed using UPLC/MS.

Matrix, freeze/thaw and sonication effects: The composition of Great Salt Lake water is complex and includes many salts and other unwanted constituents. Therefore, an attempt was made to evaluate the effect of this complex matrix on nodularin extraction. A water sample (100 mL) from Farmington Bay was filtered through 0.45 μm filter paper to remove cells and other suspended debris. The filtered sample was spiked with nodularin to give a final concentration of 1 μg nodularin /L. One more 100 mL sample was also taken from Farmington Bay and was filtered through 0.45 μm filter paper. This second sample was not spiked with nodularin and

was treated as the control blank. Both samples were subjected to the toxin extraction protocol described above, and extracted samples were analyzed using UPLC/MS.

Genetic identification of bacteria in water samples

We employed 16S rDNA-based molecular techniques to identify the bacterial population in water samples. Cells in the water samples were first pelleted by centrifuging 90-mL samples. After centrifugation, the supernatant was discarded, and the cell pellet was re-suspended 1 mL autoclaved DI water. The resulting cell solution was used for DNA extraction. DNA was extracted from each water sample using the UltraClean Soil DNA kit (MoBio Laboratories, Inc., CA) as per manufacturer's instructions. The presence of genomic DNA was confirmed using gel electrophoresis on 1 % agarose.

Gel Electrophoresis

The gel was prepared by dissolving 1 g of agarose powder in 100 mL of TBE buffer (Tris/Borate/EDTA) by heating the solution in the microwave for one minute. The mixture was then allowed to cool to approximately 60ºC, and a few drops of ethydium bromide were added to it. This was poured into the electrophoresis plate containing a comb to make wells in the gel, and allowed to solidify. The gel plate was placed into the electrophoresis box (Fotodyne, WI) containing TBE buffer that covered the plate. Approximately 10 µL of the DNA sample was loaded on to the wells, and the gel was run under 85 volts for 30 minutes. After 30 minutes, the plate

was removed and the gel was analyzed under a UV gel imaging lamp to view the DNA.

16S rRNA Polymerase Chain Reaction (PCR)

Eubacteria-specific primers 8f (5' GAGGCCTCAATTGCAGGCAGGC 3') and 1492r (5' GGTTACCTTGTTACGACTT 3') were used to identify bacteria, including cyanobacteria, present in the water column. The temperature cycle was maintained at the following conditions: initial denaturation at 94°C for 3 minutes, 30 cycles consisting of 1 min denaturation at 94°C, 35 seconds annealing at 55°C, and 2 min extension at 72°C. An additional final extension at 72°C was carried out for 2 minutes. The PCR products were then separated by electrophoresis on an agarose gel. The resulting PCR product was verified by 1% agarose gel electrophoresis and purified using a QIAquick gel extraction kit (Qiagen, Valencia, CA) according to manufacturer's instructions. The purified DNA was used for cloning and sequencing.

Cloning and Sequencing

The TOPO cloning kit (Invitrogen Corp., Carlsbad, CA) was used to clone the 16S rDNA fragment into a vector. The vector was supplied with kanamycin resistance to select *E. coli* which contained the plasmid. After ligation and transformation into competent *E. coli*, ligated and transformed cells were transferred to LB plates containing 50 μg/mL kanamycin. Since the plasmid of interest codes for kanamycin resistance, *E. coli* cells that had no plasmids in them could not grow to produce colonies. Distinct clones from the growth medium were selectively picked

and were subjected to further screening by growing them on the same growth medium to confirm that the clones making up the colonies contained inserts of interest. Plasmid DNA was then extracted using the Wizard Plus Miniprep DNA purification system (Promega, Madison, WI). To verify the presence of inserts in the purified plasmids, a rapid screening was conducted using agarose gel electrophoresis. The plasmid inserts were amplified with the Big Dye sequencing reaction (Applied Biosystems, Foster City, CA) using 8F as the forward primer, and the products were purified using Cleanseq (Agencourt Biosciences, Beverly, MA) before being analyzed on an automated DNA sequencer (ABI model 3730 96-capillary sequencer, Applied Biosystems, Foster City, CA). The retrieved sequences were compared with available 16S rDNA sequences from Ribosomal Database Project (RDP)-Release 10, and they were aligned using ClustalX (Thompson et al. 1997) software. A phylogenetic tree was constructed using TreeView software, and a phylogenetic table was created by using RDP Basic Local Alignment Search Tool (BLAST).

RESULTS AND DISCUSSION

Comparison of SPE columns: The recovery of toxin using a C18 column was 77.3 %, whereas the recovery was 99 % using an HLB column. Therefore, the HLB cartridge was selected as the SPE cartridge for this study. Figure 6 shows chromatographs obtained after running recovered samples using HLB and C18 columns. Better recovery (~99%) is evident in the chromatograph by the response value of 32307 as compared to the response value of 24215 using C18 column.

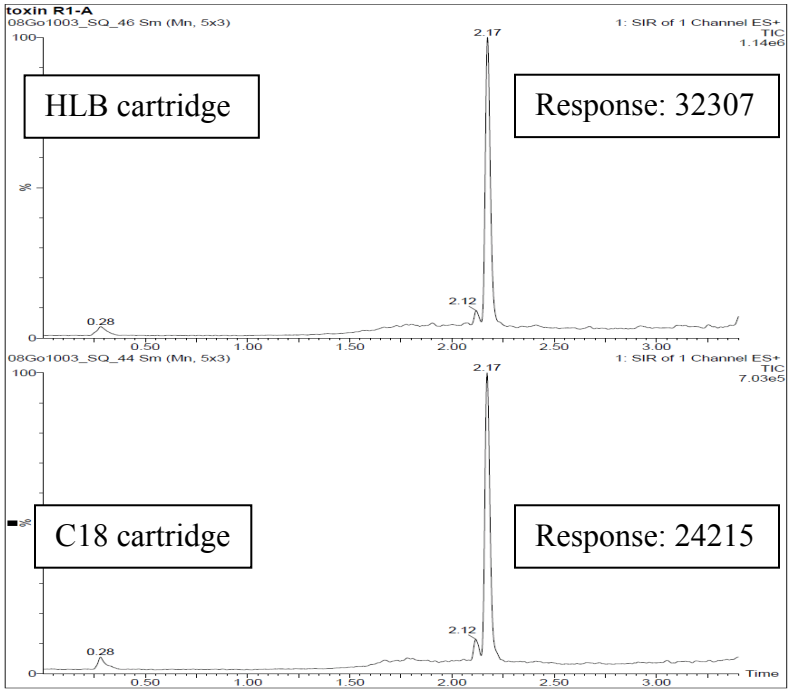

Figure 6. Response chromatographs of nodularin using HLB and C18 catridges

Recovery and Matrix Tests: No nodularin was detected in the control sample. Duplicate samples from the spiked Farmington Bay water were subjected to UPLC/MS analysis. The duplicate chromatographs for the spiked water sample are shown in fig 7. Since the 100 mL filtered water sample containing 1 µg/L was subjected to freeze, thaw, sonication and was passed through the HLB cartridge to recover the nodularin and finally to concentrate it to 1 mL, the expected nodularin concentration in 1 mL concentrated sample was 100 µg/L. Chromatograph analysis showed that the final concentration of nodularin in the concentrated samples was 78.1 µg/L, resulting in 78.1% recovery. Hence, the recovery factor of 0.78 was used in rest of the analysis. This recovery factor is comparable to other published values.

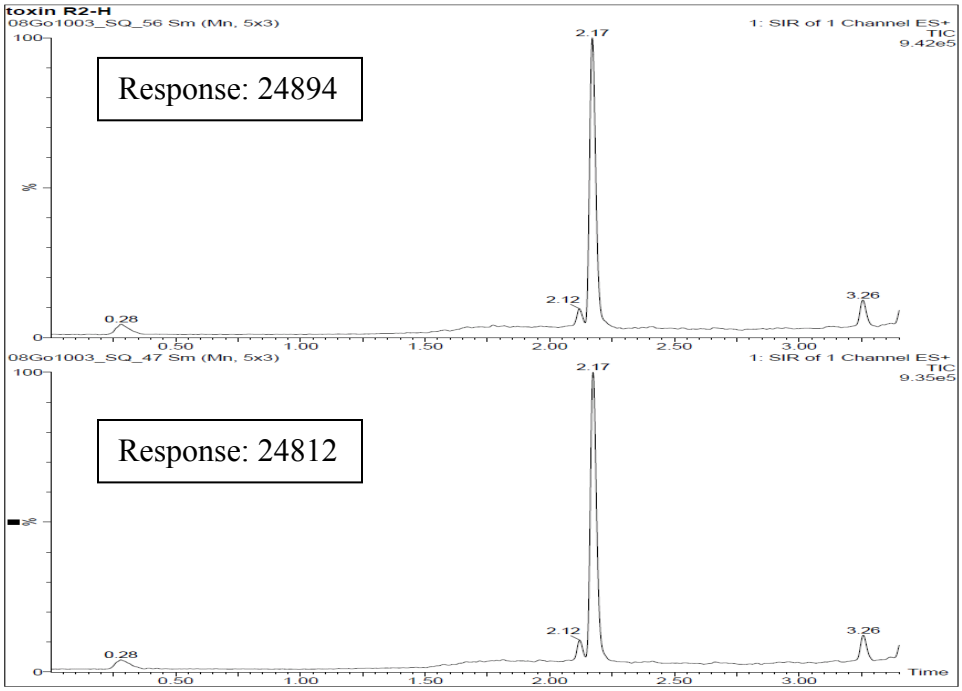

Figure 7. Matrix effect on recovery of nodularin in water sample from the Farmington Bay

Instrument performance and calibration curve: The calibration curve was obtained with nodularin standards ranging from 5 µg/L to 100 µg/L. Figure 8 shows the optimized instrument chromatograph at different concentrations of nodularin standards ranging from 5 µg/L to 50 µg/L (100 µg/L is not shown). The instrument gave good reproducibility as evidenced by the triplicate analysis results. The average response for each concentration was recorded and was plotted on x-axis and standard concentrations at y-axis. The resulting calibration curve is shown in figure 9. A R^2 value of 0.9989 indicates a linear calibration curve. The instrument quantitation limit was 5 µg/L. In each analysis, the sample was concentrated by a factor of 100. Considering this concentration factor and the instrument quantitation limit, the overall method quantitation limit was 0.05 µg/L for nodularin.

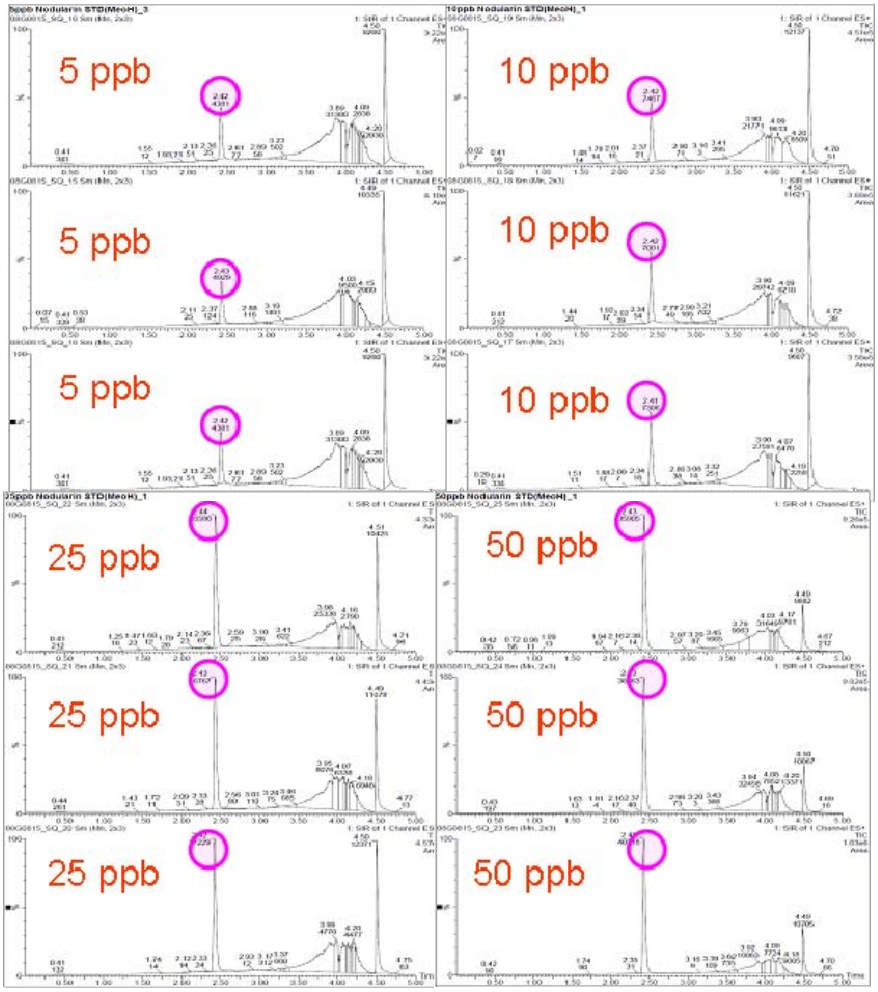

Figure 8. Chromatographs showing instrument response in triplicates at different concentrations of nodularin

Nodularin concentrations in monthly samples: Samples in the months of July, August, September and October were collected from six locations in Farmington Bay. In each month, all six samples were subjected to toxin extraction simultaneously, and the calibration curve was verified each time. Quality control tests were also performed in monthly analyses by spiking the samples with a known concentration of nodularin apart from standard recovery and matrix effect control tests described above. Table 2 summarizes analysis results for nodularin for all samples collected over four months.

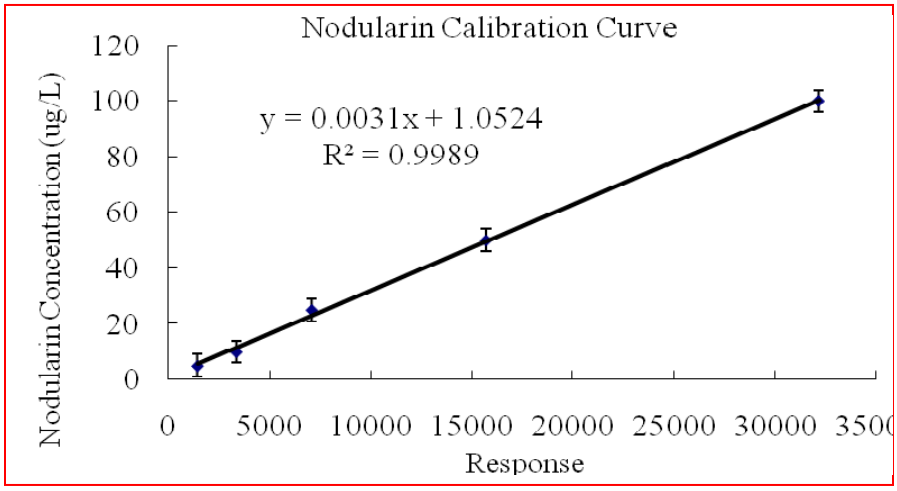

Figure 9. Calibration curve obtained using pure compound of nodularin

Two out of six samples each in July and September gave positive results for nodularin. Samples from sites 4 and 6 in July gave nodularin concentrations of 0.207 and 0.384 µg/L, respectively. Similarly, samples from sites 4 and 5 in September gave nodularin concentrations of 0.331 and 0.250 µg/L, respectively. The concentrations reported in table 2 were corrected for matrix interferences and were obtained after applying the 0.78 recovery factor. These values are well below nodularin concentrations in Farmington bay reported by others previously. No other samples showed the presence of nodularin.

Based on four sampling events, UPLC/MS analysis, quality control and recovery tests, it can be concluded that nodularin was either completely absent or present in concentrations in Farmington Bay well below 0.05 µg/L. The algal bloom occasionally observed in the Farmington bay may not necessarily always consists of blue green algae, a form of cyanobacteria responsible for toxin production.

Table 2. Samples with positive Nodularin concentration

Date	**Site**	**Nodularin concentration** (µg/L)
July 2008	4	0.207
	6	0.384
September 2008	4	0.331
	5	0.250

Molecular identification of bacteria in water column: Universal primers were used to track the bacterial community. Genomic DNA samples from the water samplescontaining nodularin were mixed together, and the template needed for polymerase chain reaction (PCR) was taken from this mixed DNA sample. This approach was taken to avoid the cost of multiple cloning and sequencing experiments. Furthermore, eubacteria-specific universal primers were used to track all species of *Nodularia* ,if present, and other bacterial communities. Figure 10 shows the phylogenetic distribution of 96 partial DNA sequences that were recovered as a result of cloning and sequencing. The tree also includes sequences from publicly available databases such as RDP and GenBank for comparison purposes. The results are also summarized in table 3. Table 3 presents all recovered DNA sequences at phylum, class, order, family and genus levels. The majority of the sequences fell within the classes of *β- proteobacteia, γ-proteobacteia, Bacteroidetes and Sphingobacteria*.

Molecular identification based on 16S rDNA cloning and sequencing provide very few partial DNA sequences matching with cyanobacteria with none of the sequences were related to *Nodularia*. Two out of the 96 sequences matched the genus

Bacillariophyta, and unidentified *GpI*. *Bacillariophyta* are the diatoms. *Bacillariophyta* are extremely abundant both in the plankton and in sediments in marine and freshwater ecosystems, and are photosynthetic.

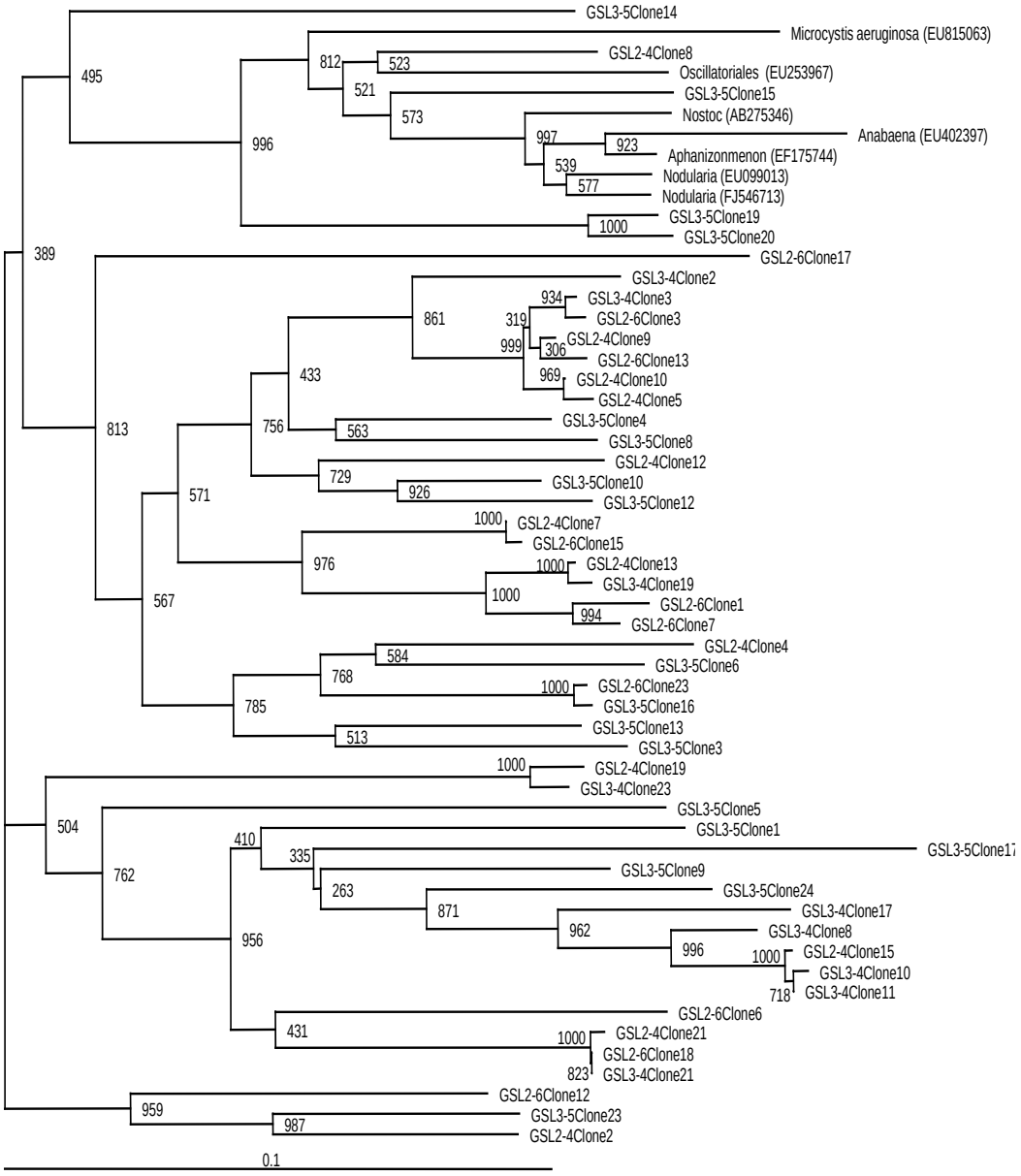

Figure 10 Phylogenetic tree showing the distribution of 96 sequences

Pseudomonas represented 14 % of the total sequences. *Pseudomonas* is the member of the *γ-proteobacteria* class of bacteria. Members of the genus *Pseudomonas* are well known to plant microbiologists because they are one of the

few groups of bacteria that are true pathogens of plants. The next abundant genus in the sequenced clones was *Haliscomenobacter*. *Haliscomenobacter* are filamentous bacteria which generally cause bulking in activated sludge systems. The interesting observation was the presence of hydrogen oxidizing bacteria belonging to the genus *Hydrogenophaga* and theromophilic bacteria *Thermodesulfobacterium*. *Hydrogenophaga* is a new genus of hydrogen-oxidizing bacteria. *Thermodesulfobacterium* is a thermophilic, sulfate-reducing bacterium. *Thermodesulfobacterium* is a strict anaerobe, cannot utilize acetate as an electron donor in its energy metabolism and instead uses compounds like lactate, pyruvate, and ethanol to reduce SO_4^{2-} to H_2S.

The absence of *Nodularia*-specific cyanobacteria, despite the presence of nodularin in the water column samples, is interesting but not surprising. There could be two possible reasons for this apparent inconsistency. The first reason could be the selective amplification of other bacteria during PCR because PCR has its own biases. The second reason could be the absence of *odularia*, and the nodularin that was detected in the July and September water samples was a result of residual concentrations in the water column due to earlier presence of *Nodularia*, albeit in very low quantity. However, a more detailed study will be needed specifically targeting the dynamics of *Nodularia* in Farmington bay starting from late spring (early summer) until late summer. Based on these cloning and sequencing results, it can be concluded that the possibility of the presence *Nodularia* in the Farmington Bay cannot be ruled out, but the quantity of bloom did not exceed a level as to produce nodularin beyond acceptable limits proposed by WHO.

Table 3. Phylogenetic group table of bacteria in Farmington Bay of Great Salt Lake

Class	Order	Family	Genus	No. of Clones
Gammaproteobacteria	Pseudomonadales	Pseudomonadaceae	Pseudomonas	13
Betaproteobacteria	Burkholderiales	Oxalobacteraceae	Janthinobacterium	4
Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	Rhodobacter	3
Bacteroidetes	Bacteroidales	Porphyromonadaceae	Paludibacter	9
Gammaproteobacteria	Aeromonadales	Aeromonadaceae	Aeromonas	5
Betaproteobacteria	Burkholderiales	Comamonadaceae	Acidovorax	2
Epsilonproteobacteria	Campylobacterales	Campylobacteraceae	Arcobacter	7
Sphingobacteria	Sphingobacteriales	Saprospiraceae	Haliscomenobacter	12
Thermo desulfobacteria	Thermo desulfobacteriales	Thermo desulfobacteriaceae	Thermo desulfobacterium	3
Betaproteobacteria	Burkholderiales	Comamonadaceae	Hydrogenophaga	2
"Clostridia"	Clostridiales	Clostridiaceae	Caldithrix	2
Fusobacteria	Fusobacteriales	Fusobacteriaceae	Fusobacterium	3
Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	Porphyrobacter	2
Gammaproteobacteria	Chromatiales	Ectothiorhodospiraceae	Thioalkalivibrio	2
Cyanobacteria		Chloroplast	Bacillariophyta	2
Gammaproteobacteria	Alteromonadales	Shewanellaceae	Shewanella	1
Cyanobacteria		Family I	GpI	1
Bacteroidetes	Bacteroidales	Porphyromonadaceae	Parabacteroides	1
Alphaproteobacteria	Rhodospirillales	Acetobacteraceae	Acidiphilium	1
Betaproteobacteria	Burkholderiales	unclassified_Burkholderiales		1
Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	Roseovarius	1

LIMITATIONS AND FUTURE STUDY

For the consecutive second year, we have demonstrated that Nodularin is either absent in Farmington Bay at the sampled locations or it is present in very low concentrations. Central Davis Sewer District funded research study in the summer of 2007 by Ms Mary Ellen in which the objective was to conduct synoptic sampling at selected locations in the main channel of Farmington Bay for different water quality parameters including dissolved and total phosphorus. In our study, we sampled 6

locations in the main channel whereas study by Mary Ellen included more sampling locations further downstream in the main channel. Hence, we firmly believe that; (1) more sampling locations should be included in the future study, and (2) the additional sampling locations should match with those used by Mary Ellen in her study. This strategy will enable us to formulate a holistic mechanism of Nodularin in the main channel. Furthermore, future studies should also include nutrient flux from sediments, phosphorus speciation, in situ tests to evaluate whether the bay of nitrogen limited or phosphorus limited.

CONCLUSIONS

The following conclusions are formulated based on this research.

- A newly developed UPLC/MS method was successful and was able to quantify nodularin concentration as low as 5 µg/L. Based on this instrument quantitation limit and a sample concentration factor of 100, the overall method limit of quantitation for nodularin was 50 ng/L.
- The analysis was reproducible which was evident from triplicate analysis of nodularin standards ranging from 5 to 100 µg/L.
- The HLB soild phase extraction cartridge provided more than 99 % recovery of nodularin from water samples, making it more suitable than the C18 cartridge for this analysis.
- A recovery factor of 0.78 based on matrix interferences was in close proximity of previously reported values.

- Only two samples in the month of July and two samples in the month of September gave positive results for nodularin. The nodularin concentration in these samples was well below 0.5 μg/L.
- Molecular identification of bacteria revealed a diverse community of bacteria with no sequences matching with the genus *Nodularia*. This means that *Nodularia* was present in very low quantities even if heavy algal bloom was observed in the summer.
- A more detailed study aimed at identifying and quantifying *Nodularia* in Farmington Bay will be needed using *Nodularia*-specific gene markers to establish the presence and ecology of this cyanobacteria.

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