Harmful Algae 16 (2012) 98–107

Contents lists available at SciVerse ScienceDirect

Harmful Algae

journal homepage: www.elsevier.com/locate/hal

Characterization of paralytic shellfish toxins from *Lyngbya wollei* dominated mats collected from two Florida springs

Amanda J. Foss [a,b,*], Edward J. Phlips [a], Mete Yilmaz [a], Andrew Chapman [b]

[a] The Fisheries and Aquatic Sciences Program in the School of Forest Resources and Conservation, Institute of Food and Agricultural Sciences (IFAS), University of Florida, 7922 NW 71st Street, Gainesville, FL 32653, USA
[b] GreenWater Laboratories/CyanoLab, 205 Zeagler Drive, Palatka, FL 32177, USA

ARTICLE INFO

Article history:
Received 2 August 2011
Received in revised form 8 February 2012
Accepted 8 February 2012
Available online 17 February 2012

Keywords:
Cyanobacteria
Lyngbya wollei
Saxitoxin
Paralytic shellfish toxin
Florida springs
16S rRNA
sxtA
sxtG
Mass spectrometry

ABSTRACT

Lyngbya wollei, a commonly observed cyanobacterium in Florida's spring fed systems, is considered a nuisance organism due to its formation of large benthic and floating mats. Standing crops and mats of *Lyngbya* from two Florida springs, Silver Glen Springs (Ocala National Forest) and Blue Hole Spring (Ichetucknee Springs State Park), were sampled and characterized via microscopy. A near full-length 16S rRNA gene sequence recovered from genomic DNA preparation of a filament collected from Silver Glen Natural Well was 99% identical to another *L. wollei* sequence. Paralytic shellfish toxin (PST) biosynthesis genes *sxtA* and *sxtG* were also detected in the filament DNA and were 97% and 98% identical in sequence, respectively, to those of *L. wollei*. PSTs were characterized utilizing High Performance Liquid Chromatography (HPLC) coupled with Mass Spectrometry (MS). Analysis of extracted algal material with LC/MS/MS verified that PSTs decarbamoylgonyautoxin 2&3 (dcGTX2&3) and decarbamoylsaxitoxin (dcSTX) were present in *L. wollei* mats in Florida springs and provided evidence supporting the presence of all *L. wollei* toxins (LWT 1-6). Levels of quantifiable toxins (dcGTX2&3 & dcSTX) ranged from 19 to 73 μg STX-eq (g dry weight)$^{-1}$. Although *L. wollei* toxins 1–6 could not be quantified due to a lack of available standards, their presence indicates samples may be higher in toxicity. This is the first detailed study confirming PST presence in *L. wollei* dominated mats in Florida spring systems.

1. Introduction

Cyanobacteria produce many active metabolites, some of which induce toxic responses, including harmful effects on human and animal populations (Sivonen and Jones, 1999). In Florida's spring fed rivers, one of the dominant cyanobacteria is *Lyngbya wollei* (Farlow ex Gomont) Speziale, which forms large benthic and floating mats (Cowell and Botts, 1994). A survey submitted to the Florida Department of Environmental Protection (FDEP) evaluating algal growth and nutrients in 21 different springs, found that *L. wollei* is one of the most common "macroalgae" present and has become a management concern (Stevenson et al., 2007). *L. wollei* has also been shown to produce toxins (Seifert et al., 2007; Berry et al., 2004; Teneva et al., 2003; Carmichael et al., 1997). Toxins of concern include dermatoxins (toxins that affect the skin), hepatotoxins (toxins that affect the liver and other internal organs), and neurotoxins (toxins that affect nerve cells) (Landsberg, 2002). Although anecdotal reports of adverse skin reactions (i.e. rashes, hives, and blisters), gastrointestinal disorders, respiratory illness, and even temporary loss of consciousness following potential exposure to cyanobacteria in Florida waterways have been reported to the Florida Department of Health (FDOH) (personal communication), detailed studies relating cyanobacterial toxins to health effects in Florida are limited. The FDOH initiated an evaluation of freshwater *Lyngbya* and its toxins in Florida springs in 2004 (PBS&J [Post, Buckley, Schuh, and Jernigan] 2007). The study reported the presence of "saxitoxin-like" compounds, which were designated unknown paralytic shellfish toxins (PSTs), since methods used at the time (i.e. ELISA) were not sufficiently selective to specify variants.

Paralytic shellfish toxins, commonly referred to as saxitoxins or paralytic shellfish poisons, are a group of neurotoxic alkaloids produced primarily by marine dinoflagellates and freshwater cyanobacteria, with over 57 structural variants reported to date (Wiese et al., 2010). Because PSTs mainly act by blocking voltage-gated sodium and calcium channels (Kao and Levinson, 1986; Su et al., 2004), exposure to these toxins may result in illness, paralysis, and even death (Kao, 1993). PSTs are potentially harmful to aquatic and terrestrial animals (Landsberg, 2002) in addition to humans. Analysis of *L. wollei* samples from Lake Guntersville in Alabama (USA) have yielded the PSTs decarbamoylgonyautoxins

* Corresponding author at: GreenWater Laboratories, 205 Zeagler Drive Suite 302, Palatka, FL 32177, USA. Tel.: +1 386 328 0882.
E-mail address: amandafoss@greenwaterlab.com (A.J. Foss).

1568-9883/$ – see front matter
doi:10.1016/j.hal.2012.02.004

2&3, decarbamoylsaxitoxin, and six novel PSTs, (*L. wollei* toxins 1-6) (Carmichael et al., 1997; Yin et al., 1997, Onodera et al., 1997). No other detailed reports of *L. wollei* toxins have since come out in the peer-reviewed literature. The goal of this study was to conduct detailed analyses of the PSTs associated with *L. wollei* dominated mats in Florida. Since the morphologic and molecular identification of *L. wollei* is under some scrutiny (Joyner et al., 2008) and toxin production cannot be determined by simply determining the presence of *Lyngbya*, the characterization of PST toxins in Florida's *L. wollei* mats was an important goal.

2. Materials and methods

2.1. Sampling site description

Three sites were selected for *Lyngbya* collection, one in Blue Hole Spring (Ichetucknee State Park) and two in the Silver Glen Springs Recreation Area (Ocala National forest). These springs were chosen because they contain extensive mats dominated by the cyanobacterium *L. wollei*. Silver Glen Springs is located in the Ocala National Forest in Marion County Florida and is utilized as a recreation area maintained by the United States Department of Agriculture (USDA) Forest Service. The spring run has a large combined pool emanating from two vents (east and southwest vents). The main pool is sectioned off with ropes into three regions, two of which contain large *Lyngbya* dominated mats (Fig. 1). The eastern spring vent is a 1st magnitude spring (flow rate > 100 cubic feet per second, [cfs]) and is frequented by swimmers. Very little *Lyngbya* is present in the latter region. The southwest vent (also known as the "Natural Well") is a 2nd magnitude spring (flow rates 10–100 cfs) and contains extensive *Lyngbya*-dominated mats. Both regions with mats were sampled over the study period. An underwater view representing the expansive nature of the *L. wollei* mat in Silver Glen Springs is shown in Fig. 2(A).

Blue Hole Spring (aka Jug Spring) is a 1st magnitude spring (flow rate >100 cfs) in the Ichetucknee Springs State Park located in Columbia County, FL. Blue Hole Spring is one of many springs that feed into the Ichetucknee River system, which empties into the Santa Fe River. Although recreational activities such as tubing and canoeing are allowed in the main spring run, the activities in Blue Hole are limited to swimming, cave diving and snorkeling. *Lyngbya*-dominated mats in this spring are located along the bottom as well as vertically distributed along the northern face of the spring hole, attached to the roots of bald cypress trees (*Taxodium distichum*). Mat samples were collected from the vertically distributed mat (Fig. 2(B)).

Fig. 1. Silver Glen Springs Recreational Park photo; white lines represent ropes restricting general access; Natural Well and Main Stem were sampled in this study. Photo Courtesy of St. Johns River Water Management District.

A B

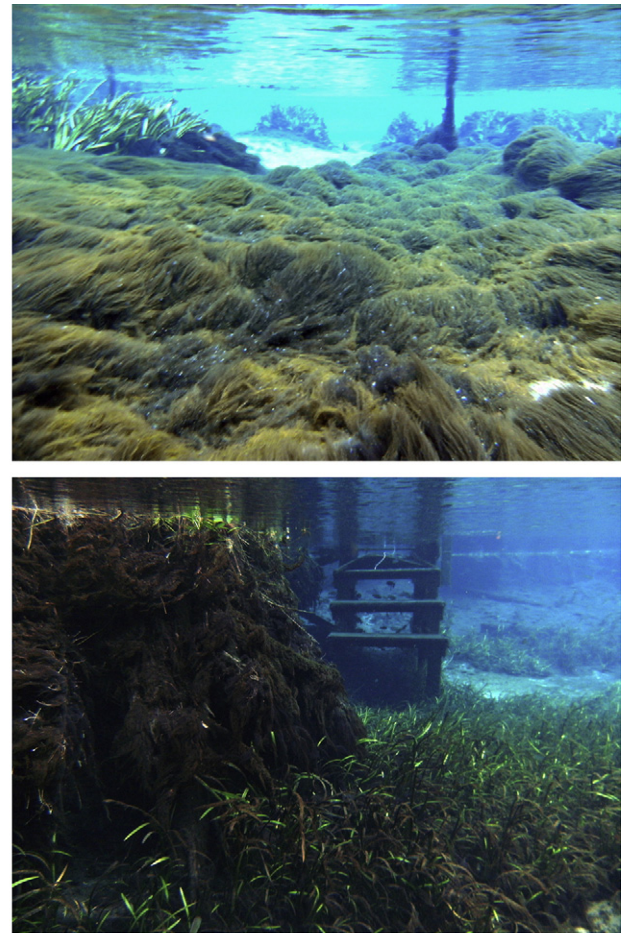

Fig. 2. Underwater images of *L. wollei* mats at the study sites (A): Silver Glen Natural Well (southeastern vent) standing mat (August 2009), (B) Blue Hole (Jug) Spring vertically distributed mat loosely attached to tree roots (left). Mat is in close proximity to stairs utilized recreationally to access to spring head.

2.2. Collection

A single line transect was used to collect *Lyngbya*-dominated mat samples. A 37 m transect was used in the main stem area of Silver Glen Spring. A surveyor tape was run across the length of the benthic mat from east to west. The Natural Well transect was 9 m in length, running along the bottom across the mat from north to south. Because the mat at Blue Hole Spring was not horizontally distributed, a 5 m transect was set up at a depth of 1 m (half the maximum depth) along the root line of bald cypress trees.

Algal mat samples were collected as grab samples at five randomly selected locations along each transect. Each grab sample consisted of approximately 20 g wet weight, which were deposited in 10 in. × 10 in. plastic bags for transport and analysis (approximately 100 g wet weight total). Large invertebrates and debris were lightly rinsed from the composited mat samples using spring water. There were six collection events during which all three sites were visited. One composited collection per site was taken during a sampling event, with a field replicate collected during each event. A total of 24 samples were collected for this study (Table 1). The samples were maintained below 10 °C after collection and for transport. All sample preparation for lyophilization, DNA isolation, and algal analysis was conducted within 4 h of collection.

Additional data collected included tree canopy coverage (densiometer, Wildlife Supply Company, Yulee, FL), site depth,