Using DNA Barcoding Assays to Identify Diatoms in New York City Waterways for Drowning Investigations

Amani Fawaz[1], Chepeara Curry[2] Mentor: Richard Li Ph.D.[3]

[1]Brooklyn Technical High School, [2]Kappa International High School, [3]John Jay College of Criminal Justice

Abstract

Diatoms are single-celled eukaryotes that are found in different bodies of water. Diatoms have been used in forensic investigation to help determine if someone died from drowning. Our aim was to generate a method to confirm when an individual drowned by identifying the diatom species present in the victim's bloodstream and the crime scene. To address this issue, we carried out a pilot study to find out if diatom species at a particular site vary at different times (intra-day variations) and days (inter-day variations). Our results were obtained through Sanger sequencing and the data was analyzed using DNA Subway software. The analysis showed the presence of the most abundant diatoms in our samples at one location. The results show that *Thallassiosira* , a predominant genus of marine diatoms, is constantly present in most of the samples we collected. The results of this study have important implications. This shows that the determination of diatoms present in New York City waterways can be potentially helpful in drowning investigations by law-enforcement agencies.

Introduction

Diatoms are single-celled eukaryotes that can be found in adequately lit lakes, rivers, oceans, marshes, and bogs [6, 4]. Diatoms are ranged from 2 to 500 micrometers in size (Figure 1). Diatoms have been used in forensic investigations to assist in determining whether an individual has drowned. The presence of diatoms found in a person's blood stream indicate that they drowned and the absence may indicate that the person may have expired prior to being found in a body of water [5]. Additionally, diatoms have been used in forensic science to help determine whether a person drowned in freshwater lakes, rivers or oceans.

We are developing a method, based on the comparisons of diatom species present in an individual's bloodstream and a drowning site, to confirm where a person drowned. However, because the currents of New York City waterways may change which diatoms are present in the sample depending on the time of investigation, we carried out a pilot study to find out if diatom species at a particular site vary at different times (*intra-day variations*) and days (*inter-day variations*). This information can be helpful to law enforcement agencies, providing proper time frames of sample collection for investigation.

Materials & Methods

A small trial of experiments was designed to collect water samples from one location (Figure 2) of New York City waterways. Water samples were collected at different times for intra-day (high tide and low tide; Figure 3) and different days for inter-day variation studies. Collected water samples were extracted using the QIAamp DNA Micro Kit (QIAGEN). The diatom barcoding region is described in Figure 4. DNA amplification was carried out using the AmpliTaq Gold 360 PCR Master Mix (Thermo Fisher Scientific) consisting of primers D512 [3, 7]. Primers were held at 94°C (90s) to denature template followed by 34 cycles: 94°C (40s), 51°C (40s), and 72°C (60s) and the final extension at 72°C (5 min.) [3]. PCR succession was analyzed on 1.5% agarose gel. The amplified products were sequenced using Sanger sequencing [2]. The DNA sequence data was analyzed using DNA Subway software and compared using BLAST (Basic Local Alignment Search Tool) to identify the species of diatoms.

Results

Intra-day variation studies consist of two types of samples: samples collected at high tide and low tide (Figure 3). For inter-day variation studies, five pairs of water samples at different days were collected. Some of these samples produced DNA sequences of multiple species, which cannot be resolved using Sanger sequencing thus they were not analyzed further. The analyzed sequences were compared and the most abundant diatom species are described in Table 1. The electropherograms of representative inter-day variation samples are shown (Figure 5).

Sample Collection	Description	Most abundant specie(s)	Alignment Length (bp)	E-Value
February 9th, 2016	High Tide	*Bacterosira sp., Thalassiosira sp.*	382	1.00E-160
	Low Tide	*Bacterosira sp., Thalassiosira sp.*	383	1.00E-163
February 23rd, 2016	High Tide	*Bacterosira sp.*	397	0
	Low Tide	*Thalassiosira gessneri*	397	1.00E-134
March 8th, 2016	High Tide	*Bacterosira sp., Thalassiosira sp.*	399	0.0, 0.0
	Low Tide	*Thalassiosira gessneri*	289	1.00E-103

Table 1. The sequence analysis for diatom species identification from the water samples collected.

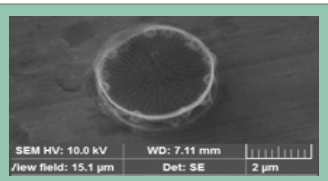

Figure 1. Scanning electron micrograph of marine *diatom Thalassiosira pseudonana* (Williams and Li, unpublished).

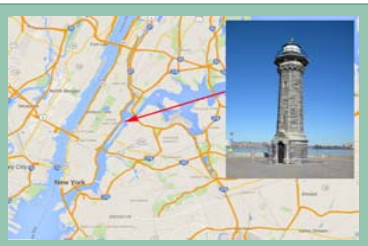

Figure 2. *Geographical location* at which the water samples were collected.

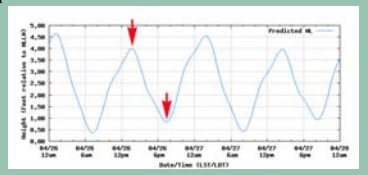

Figure 3. A representative example of time points of sample collection for intra-day studies (low tide and high tide).

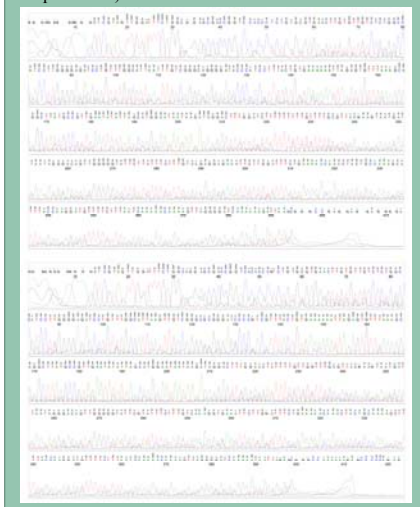

Figure 5. A representative example of identification of diatom species. An electropherogram of sequencing of a high-tide diatom sample (top) and an electropherogram of sequencing of a low-tide diatom sample (bottom).

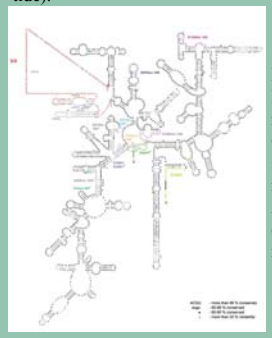

Figure 4. Barcoding Region of diatom 18S rRNA gene DNA. Consensus secondary structure of the 18S rRNA is shown. Amplified region corresponds with the region V4 in red [7].

Discussion

Our results indicated that the *Bacterosira* and/or *Thalassiosira* genus are the most abundant diatoms present in all the samples collected (Table 1). It is known that *Thalassiosira* is the predominant genus of marine diatoms [1]. In this pilot study, it was observed that *Thalassiosira* was constantly present in most of our samples collected. Additionally, the same sequences were matched to *Bacterosira* in many of the samples (Table 1). The DNA sequences of the barcoding region used in this study are identical between *Bacterosira spp.* and *Thalassiosira spp. (Li et al., unpublished).* Recently, studies have revealed that *Bacterosira* was closely related to *Thalassiosira* [1]. Based on these findings, a phylogenetic re-definition of the genus *Bacterosira*, to belong to *Thalassiosira,* is proposed [1]. The results of this pilot study imply that the predominant genus of diatom is constantly present in the intra-day and inter-day samples. This finding indicates that identification of diatom species can potentially be useful for drowning investigations in New York City waterways. In future studies, detailed profiles of diatom species at additional locations of New York City waters will be studied.

References

1. Alverson, A. J., Lee, J.H., Park, J. S.. 2016. A Phylogenetic Re-definition of the Diatom Henus Bacterosira (Thalassiosirales, Bacillariophyta), with the Transfer of Thalassiosira Constricta Based on Morphological and Molecular Characters. Phytotaxa. 245(1). 1.
2. Campbelll, M. Capillary Electrophoresis [Internet]. Davidson, NC: Davidson College; 2002. Available from: http://www.bio.davidson.edu/courses/genomics/method/capillary.html
3. Chen, X. G., Huang, Y., Hou, Y. P., Zhang, J.. 2013. Diatom Taxa Identification Based on Single-cell Isolation and rDNA Sequencing. Forensic Science International: Genetics Supplement Series. 4(1). e308-e309
4. Maddison, D. R, K.-S. Schulz (eds.); Diatoms [Internet]. 2007. Available from: http://tolweb.org/Diatoms/21810
5. MK G., Vichar M., Vinyak V.. 2013. Diatom Fingerprinting to Ascertain Death in Drowning Cases. 4(5). 1.
6. Spaulding, S.A., Lubinski, D.J., Potapova, M. Diatoms of the United States [Internet]. 2010. Boulder, CO: Colorado Univ.; n.d; Available from: http://westerndiatoms.colorado.edu/
7. Zimmermann, J., Jahn, R., Gemeinholzer, B.. 2011. Barcoding diatoms: evaluation of the V4 subregion on the 18S rRNA gene, including new primers and protocols. Org. Divers. Evol. 11(3). 173.

Acknowledgements

This project was supported by the Urban Barcode Research Program. We thank Dr. Christina Marizzi, (DNA Learning Center, Cold Spring Harbor Laboratory), Maria Faure-Betancourt and Desiree Williams (John Jay College of Criminal Justice) for their helpful assistance.