Analysis of microorganisms in concentrated and non-concentrated samples from the Susquehanna River
Laura Robusto
Mentored by Dr. Vicky Bevilacqua, Dr. Samir Deshpande, Dr. Rabih Jabbour and Patrick McCubbin

Introduction

With average temperatures increasing throughout the year around the Susquehanna River, the organism profile in the river might be expected to change over time. Pollutants from shipping companies and commercial businesses can also be a threat to the river and its ecological equilibrium. A baseline of organisms present should be established and followed over time to avoid irreversible damage to the ecosystem. Edgewood Chemical Biological Center (ECBC) has developed an approach using liquid chromatography-tandem mass spectrometry (LC-MS-MS) based proteomics with software known as Agents of Biological Origin Identifier (ABOiId; Deshpande et al., 2011) for the identification of microbes in environmental samples (patent # 8,224,581). In addition, the Applied Biosensors Laboratory at the University of South Florida (USF) is in the process of patenting a device known as the Portable Multi-use Automated Concentration System (PMACS) (Leskinen et al., 2012) that concentrates water samples from 1000 L down to ~400 mL.

This project included three main objectives. The first objective was to determine whether microbes in the samples concentrated with the PMACS could be detected and identified using the ABOiId-MS method. The next objective was to determine whether concentration on-site would result in the identification of additional microorganisms compared to a grab sample (no concentration). The final objective was to determine whether secondary concentration using 30 mL resulted in better detection relative to secondary concentration of a 10 mL sample.

Materials and Methods

With the approval of Steve Young, the City Yacht Basin Manager of Havre de Grace Marina, the PMACS was used to collect and concentrate Susquehanna River samples from the pier near Concord Point Lighthouse (39°32'25.28" N, 76°05'02.41" W). Collection of one concentrate Susquehanna River samples from the pier near Concord Havre de Grace Marina, the PMACS was used to collect and one grab sample were obtained on the following dates for comparison: 10/11/2012, 10/17/2012, 10/24/2012, 11/01/2012, 11/08/2012, 11/1/2012 and 11/14/2012.

LC-MS-MS and analysis were performed according to the procedures previously reported (Jabbour et al., 2010; Deshpande et al., 2011). The LC-MS-MS analysis was split between 02/05/13 and 02/21/13. On 02/05/13, 8 aliquot samples from 10/11 were run – 4 PMACS samples (10 and 30 mL) and 4 grab samples (10 and 30 mL). On 02/21/13, six 30 mL undiluted aliquots from all six PMACS sample dates and one 10 mL undiluted aliquot from 10/11 were run.

The mass spectrometry data was used as input for ABOiId. The software searches for related files to provide the probabilities of the peptide sequence assignments and uses spectrum-to-sequence matches to generate a sequence-to-organism assignment. The identified organism names were input into the NCBI genome browser to obtain their descriptions, functions and categories.

Results

Graph 1: Number of organisms identified using LC-MS-MS and ABOiId for various concentration levels from the collection on 10/11/2013.

Graph 2: Number of organisms identified for 02/05/13, 02/21/13 (both blue), and a Buffer Blank (red) A - Aquatic, B - Aquatic Sediment, C - Host Associated, D - Multiple, E - Terrestrial, and F - Unknown

Environmental conditions during water collection

Keratin from human hair is a common contaminant seen during MS proteomics sample preparation. Pantoaea at 9h and Rhodospirillum photometricum were observed in more than one sample. Also, the storm resulted in a high level of silt that made sample preparation difficult.

Conclusions

There was not appreciable reproducibility between samples to verify the presence of the majority of microbes identified. Even though reproducibility was not significant, some microbes identified were types that are expected to be present in river water (such as aquatic and aquatic sediment microbes or terrestrial microbes). These results indicate that the observed microbes were present in the Susquehanna River on the dates of collection. The lack of reproducibility, even at a high level of concentration, in different samples from different collection days could result from several possible factors. These include weekly variation of the river microbe profile, microbes that may not have been concentrated enough during for detection, and variation in the environment around the collection site with the occurrence of Superstorm Sandy. Not only was there variation within the samples after the storm, but fewer organisms were identified in samples collected after the storm than from the first three collection dates. Further concentration would be required prior to analysis to obtain a more accurate microbe profile for the Susquehanna River by the ABOiId-MS method.

Acknowledgements

I would like to thank Mr. Gareth Davis as my faculty advisor and Dr. Vicky Bevilacqua as mentor and collaborator, as well as Dr. Michael Stanford for transportation and other employees of ECBC. Also, thanks to Drs. Daniel Lim, Stephanie Leskinen, Elizabeth Kearns, and Sonia Magaña of the USF Applied Biosensors Laboratory for the loan of the PMACS, training on its use, and helpful discussion on data analysis.