Real time PCR for the detection of *Aeromonas hydrophila*

Joshua Ensor
Mentored by Brett Pearce and Elenyah Klein

Introduction

Determining the presence of *Aeromonas hydrophila* in local water sources was proposed as an action that helps ensure human safety. *Aeromonas* is a genus of bacteria that is found in samples of food and water. The different strains of *Aeromonas* cause human health concerns such as gastroenteritis, septicemia, and various wound infections (Gadonna-Widehem, Maaroufi, & Trakhna, 2013), as well as being lethal to many species of fish and amphibians. The purpose of this research was to use real time PCR (qPCR) to determine the presence of *Aeromonas hydrophila* in local bodies of water. Samples were taken from various locations on the shoreline of the Loch Raven Reservoir, Susquehanna River, Bush River, and Gunpowder River. Usage of contaminated water for drinking or for recreational activity can result in exposure to the bacteria in the surrounding population. In this research the BD Max™, an automated polymerase chain reaction machine, qualified the presence of bacteria and determined whether the presence of *Aeromonas* within each water source differs under the hypothesis that positive proportions are equal between samples (H_0: $\lambda = \lambda_0$).

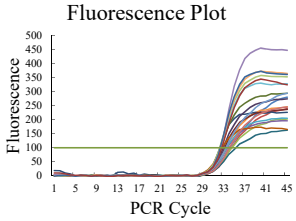
Materials and Methods

Water samples were collected from various local water sources (Figure 1) in 50 ml containers. The samples were vortexed and inverted before a 750 µL portion was inserted into each sample buffer tube. A sample buffer tube, along with a DNA extraction tube, master mix tube, and conical tube were then placed into a Exk-DNA-3 kit DNA strip. The BD MAX™ automatically extracts and concentrates DNA from each sample. Cell lysis was performed on each sample, and the DNA within was attracted by a magnetic bead. The bound DNA was then released from the magnetic beads and mixed with a dry master mix, which contains the necessary dNTPs (deoxynucleotide triphosphates), which are nucleotides used by DNA polymerase to extend the DNA on an annealed primer. These are necessary for efficient DNA amplification. Finally PCR inhibitors are removed using a series of buffers. During the annealing phase of qPCR a section of nucleotides, known as primers, was bound to a section of the split DNA which was then extended by the DNA polymerase. A probe is a fluorescently labelled section of DNA that detects the presence of a complimentary nucleotide target sequence. The probe has a fluorophore molecule attached to its 5' end, and a quencher molecule attached to its 3' end (Figure 2). When a complementary sequence was created the probe became bound to it during the annealing step. Then the quencher and the fluorophore then to separated (Sigma Aldrich, n.d). As the two molecules separated the quencher molecule eventually fails to dissipate released fluorescence. This results in increased detection of fluorescence by the BD MAX™.

Materials and Methods (cont.)

The level of fluorescence was then reported to a fluorescence curve (Sigma Aldrich, n.d) (Graph 1). If the fluorescence reached the cycle threshold (100), the level at which fluorescence was significantly above the background interference, before the 40th cycle, then the sample was considered a positive value. Following the test, the positive values were then compared to a standard curve, and the sample concentration was calculated by plotting predetermined concentrations of *Aeromonas* vs. cycle time. A two-sample Poisson test was then used to compare pairs of the water sources. The alpha level for this test was 0.05.

Figure 1: (Right) This picture shows an offshoot of the Gunpowder River that was used to collect samples. Multiple locations were selected for each source, and several samples were taken from each location. These samples were collected from locations along the shoreline using a hand filling method.



Graph 1: (Left) Fluorescence plot from Gunpowder River sampling. Each color represents a different sample from the testing. If fluorescence goes above 100 RFUs (represented by the horizontal reference line at 100) by the 40th cycle, the sample is positive for *Aeromonas*.

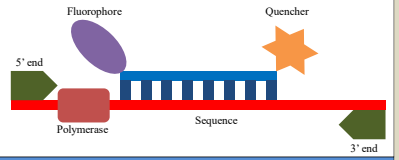
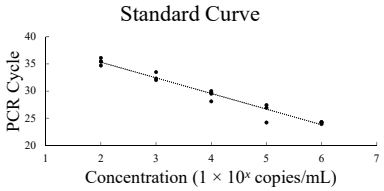


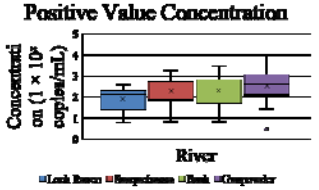
Figure 2: (Right) Diagram of annealing step of qPCR. The distancing of the fluorophore and quencher creates fluorescence.

Results



Graph 2: (Above) Standard curve used to determine the concentration of *Aeromonas hydrophila* at each location.

Results (cont.)



Graph 3: (Left) Location contamination represented in a box plot. These are the sample concentrations calculated using the standard curve. The sources are listed in ascending order by mean contamination per sample. Total sample count (N) = 111.

Through a 2-sample Poisson test comparing each of the sources (α = 0.05), it was determined that the p-value for each test was well below the alpha level. Therefore the null hypothesis of H_0: $\lambda = \lambda_0$ can be rejected. It can be concluded that there is a statistically significant difference in the amount of positives at each testing location.

Conclusions

This research was conducted in order to determine whether or not there was contamination of our local water bodies with *Aeromonas hydrophila*. This helps to prevent the suffering of people who could become exposed to this bacteria. The results show that there is contamination noted in many parts of each location, and that this contamination varies in its concentration, with locations exhibiting larger proportions of positives also exhibiting larger concentrations of *Aeromonas* (copies/mL) in the water. It was noted that the level of positive values within the Loch Raven Reservoir was much lower than the other tested bodies. This is an important sign of good health in the reservoir as this location is used as a source of drinking water for the inhabitants of Baltimore City. The Gunpowder river showed the highest level of both presence and bacterial concentration. Recreational activity should be limited in this area to avoid exposure. Future testing in this area could include a larger range of testing at each location. This research was limited to land based testing which affected our ability to sample from more remote regions of the water bodies. This type of research could allow future scientists to determine locational density of the bacteria which would allow them to identify environmental contributors to the growth of the *Aeromonas* bacterium.

References

Gadonna-Widehem, P., Maaroufi, A., & Trakhna, F. (2013). Using a real-time quantitative polymerase chain reaction (PCR) method for reliable enumeration of *Aeromonas hydrophila* in water samples. *African Journal of Microbiology Research*, *7*(19), 2119-2126.

Sigma Aldrich. (n.d). *qPCR Technical Guide*. Available from http://www.sigmaaldrich.com/life-science/molecular-biology/pcr/pcr-technologies-guide-pdf-download.html