Temperature and Oxygen Abuse of *Crassostrea virginica* and its Effect on *Vibrio* Bacteria

Lawrence Lam, Advisor Donna L. Rhoads
University of New Haven Summer Undergraduate Research Fellowship (SURF)

Introduction

Vibrio parahaemolyticus (V.p.) and *Vibrio vulnificus (V.v.)* are bacterium under the same family as *Vibrio cholerae*. Under the genus "*Vibrio*", indicating crescent like cell structure, *parahaemolyticus* and *vulnificus* are gram-negative, facultative aerobic, halophiles[6,7]. Upon entering the body through open wounds or consumption, usually associated with raw bivalves, a *Vibrio* infection may occur, resulting in seafood-borne gastroenteritis. According to the Center for Disease Control and Prevention, there is an estimated 4500 cases of *V. parahaemolyticus* infections each year in the United States[6].

According to research done by the CDC, from 2008 to 2012, there was a noted 43% increase in *Vibrio* populations occupying U.S. coastal waters[3]. Largely attributed to rising water temperatures, there is a significant increase of *Vibrio* populations in northern waters. Furthermore, a transportation period of up to 10 hours prior to treatment in the process of harvesting *Crassostrea virginica* (eastern oysters) is concerning.

The purpose of this research project was to examine the behavioral patterns of *V.p.* and *V.v.* under different experimental environments. By doing so, standards may be arrived at in which it is most optimal to store harvested oysters to control levels of *Vibrio* bacteria.

Materials and Methods

Eastern oysters were collected throughout the research period. Upon collection, the samples were divided up into equal experimental groups and placed under different temperatures and levels of oxygen availability. In intervals of 1, 2, 4, and 7 days, samples stored under each experimental condition were shucked, homogenized, and plated on CHROMagar-vibrio plates, a specialized media that is selective for *Vibrio* bacteria and differential among the species through chromogenic indicators targeting specified enzymatic pathways. All experimental procedures were performed while practicing aseptic technique. Upon an incubation period of 24 hours at 37°C, the bacterial growth on each plate was counted in colony-forming units (CFU) and recorded. For the experimental group of oysters stored at 37°C, CFU counts were done through the use of a quadrant count as approximation, due to extremely high confluence .

Polymerase chain reaction (PCR) and electrophoresis were performed as confirmatory tests to ensure that the observed bacterial colonies were in fact *V.p.* and *V.v.*

Images

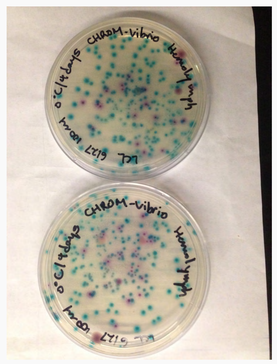
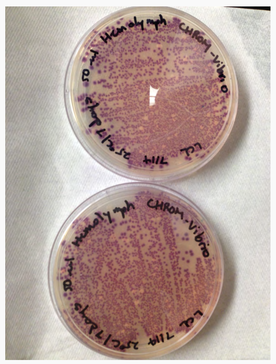


Figure 1: CHROMagar-*Vibrio* plates demonstrating an abundance of *V.v.*



Firgure 2: CHROMagar*Vibrio* plates demonstrating an abundancce of *V.p.*

Results

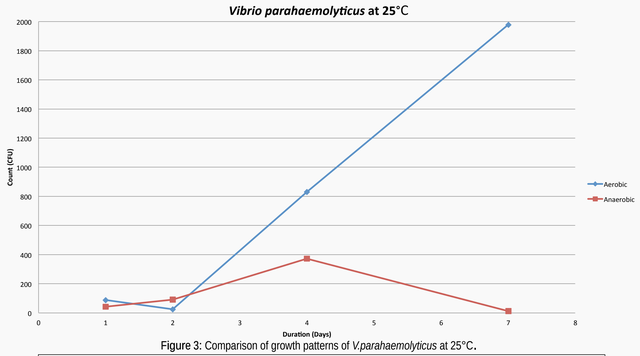


Figure 3: Comparison of growth patterns of *V.parahaemolyticus* at 25°C.

Figure 3 provides a comparison of the growth of *V.p.* under an aerobic and an anaerobic environment at 25°C. As visibly evident, the stress on the bacteria caused by the absence of oxygen resulted in a noticeable decline in population after an experimental period of 4 days.

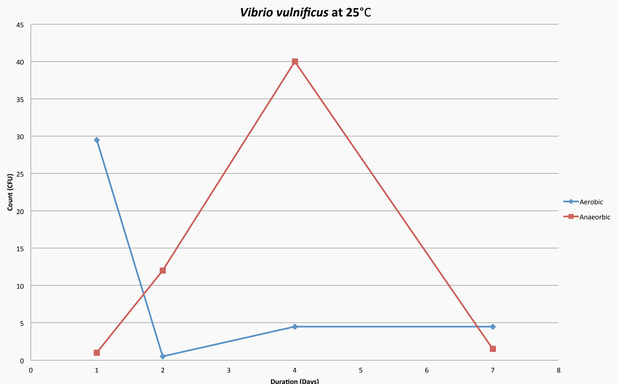


Figure 4: Comparison of growth patterns of *V.vulnificus* at 25°C.

Figure 4 portrays a relatively inconsistent correlation between the growth patterns of *V.v.* under aerobic and anaerobic environments. The lack of a noticeable pattern suggests the absence of influence of oxygen availability on the viability of *V.v.* for up to 7 days.

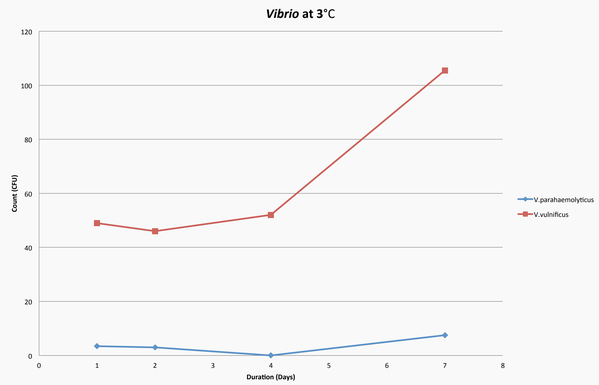


Figure 5: Comparison of the growth between *V.parahaemolyticus* and *V.vulnificus* at 3°C. As observed, *V.v* grew significantly better than *V.p.* at lower temperatures.

Figure 5 portrays an interesting observation. Although both species of *Vibrio* produced significantly lowered counts, *V.v.* grew noticeably more abundant than *V.p.*.

Results

Table 1: Additional trials under different temperatures. Due to the use of different aliquots during the sampling process, all calculated results were standardized to colony-forming units (CFU) per 100 grams of homogenized oyster tissue.

Temperature (Celsius)	Incubation Period	Average CFU (50 ul) (V.v/V.p.)	CFU/100g V.v.	CFU/100g V.p.	Average CFU (100 ul) (V.v./V.p.)	CFU/100g V.v.	CFU/100g V.p.
-20	1 days	0/1.5	0	12682.22	0/2.5	0	10568.51
-20	2 days	0/1.5	0	13112.28	0/1	0	4370.76
-20	4 days	0/0.5	0	5173.19	0/0.5	0	2586.59
-20	7 days	0/0.5	0	3556.19	0/0	0	0
0	1 day	14/0	259259.26	0	101.5/3	939814.81	26455.03
0	2 days	18.5/2	237179.49	25641.03	43/3.5	275641.03	22435.89
0	4 days	45/4.5	495927.17	49592.72	156/24.5	859607.09	135002.39
0	7 days	2.5/0.5	43346.01	8669.2	0/0.5	0	4334.6

Temperature (Celsius)	Incubation Period	CFU/100g V.v.	CFU/100g V.p.
37	1 day	4366.81	543668.12
37	2 days	936739.66	7591240.88
37	4 days	283018.87	14182389.94
37	7 days	0	96296296.3

Table 2: Additional trials performed at 37°C. The execution of these trials were done using different aliquots and proportions during the process in determining the best procedure. All values were standardized to CFU per 100 grams.

Additional trials demonstrated the same general behavioral patterns. At lower experimental temperatures, both species showed a decrease in total *Vibrio* population. However, *V.v.* was consistently more abundant than *V.p.* at lower temperatures. Inversely, at higher temperatures, *V.p.* grew exponentially while *V.v.* produced negligible counts.

Conclusion Discussion

The purpose of this research project was to attempt to determine the temperatures and levels of oxygen availability at which would prove most stressful for *Vibrio parahaemolyticus* and *vulnificus*. By doing so, it would be possible to arrive at an optimal procedure to limit the replication of *Vibrio* bacteria in eastern oysters during postharvest transportation and treatment. Although this goal was not arrived at, through the observation and analysis of the collected data, a preliminary pattern for the behavior of *Vibrio* bacteria under environmental stress was discovered.

Being facultative aerobes, *V.v.* showed little to no alterations in patterns of growth under anaerobic conditions. *V.p.* ,on the other hand, showed significantly lowered rates of growth after four days of incubation at 25°C. At -20°C, as expected, neither species produced representable counts. *V.v.* was completely undetected, while *V.p.* provided insignificant counts, varying between one and two CFU. Under standard refrigeration temperatures of 0°C and 3°C, neither species of *Vibrio* grew optimally. After an experimental period of 24 hours under these conditions, *V.p.* was noted to be nonviable. However, interestingly, *V.v.* was still able to grow under these conditions, although providing drastically lower counts. On the other hand, at the highest experimental temperature of 37°C, it was observed that *V.v.* was not present after extended periods of incubation. In comparison, *V.p.* replicated at an exponential rate. Noting that the incubation temperature of 37°C was intended to simulate the conditions inside the human body, this result was unexpected, but not inconsistent as the same observation was noted for both trials at 25°C and 37°C. The reasoning behind this is unknown. However, one possibility could be that *V.v.* is incapable of competing with *V.p.* for resources as at higher experimental temperatures, the CHROMagar plates were highly confluent, resulting in increased competition for space and nutrients.

Conclusion and Discussion

In addition to this, the results of PCR and electrophoresis did confirm that the counts recorded were, in fact, for *V.p.* and *V.v.* and not *V. cholerae.* Reflecting on the data produced, many minor inconsistencies may be noted. However, the scope in which this project was performed must be taken into account. Due to constraints in time and resources, multiple trials under each experimental conditions were not performed, resulting in less than desirable statistical variation.

Acknowledgements

- Special thanks to Rebecca Gratz and the Sound School Regional Vocational Aquaculture Center for providing school space used during research.
- Special thanks to Gabriel Neil Geist for providing lab space and guidance in the handling and treatment of *Crassostrea virginica.*
- Special thanks to the Carruba Family for their funding and support for the SURF program.
- Special thanks to the University of New Haven and the Summer Undergraduate Research Fellowship program for providing the opportunity and resources to make this project possible.

.

References

1. "Bivalve Depuration: Fundamental and Practical Aspects." *Bivalve Depuration: Fundamental and Practical Aspects*. N.p., n.d. Web. 05 Mar. 2014. <http://www.fao.org/docrep/011/i0201e/i0201e00.htm>.
2. "Chromogenic Technology." *Chromogenic Agar Technology*. N.p., n.d. Web. 06 Mar. 2014. <http://www.chromagar.com/p-Chromogenic_agar_Technology.html?PHPSESSID=f312d95cdb1b7a6d96a498a6c554f83a#.Uxhr1F5w-tc>.
3. "Food Safety News." *Food Safety News*. N.p., n.d. Web. 06 Mar. 2014. <http://www.foodsafetynews.com/2013/04/campylobacter-and-vibrio-rates-rose-in-2012-cdc-progress-report/#.UxflS15w-tc>.
4. K, Melody, Senevirathne R, Supan J, Janes M, and Jaykus LA. "Effectiveness of Icing as a Postharvest Treatment for Control of Vibrio Vulnificus and Vibrio Parahaemolyticus in the Eastern Oyster." *National Center for Biotechnology Information*. U.S. National Library of Medicine, n.d. Web. 05 Mar. 2014. <http://www.ncbi.nlm.nih.gov/pubmed/18680950>.
5. " Vibrio." *East Coast Shellfish Grower's Association*. N.p., n.d. Web. 11 Mar. 2014. <http://www.ecsga.org/Pages/Issues/Human_Health/vibrioPortal.htm>.
6. "Vibrio Parahaemolyticus." *Centers for Disease Control and Prevention*. Centers for Disease Control and Prevention, 21 Oct. 2013. Web. 10 Mar. 2014. <http://www.cdc.gov/vibrio/vibriop.html>.
7. "Vibrio Vulnificus." *Centers for Disease Control and Prevention*. Centers for Disease Control and Prevention, 21 Oct. 2013. Web. 10 Mar. 2014. <http://www.cdc.gov/vibrio/vibriov.html>.