Epigenetic Effects of Salinity on eastern oyster Crassostrea virginica

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Introduction.

Decreasing salinity worldwide due to anthropogenic climate change has major effects on oysters, such as increased mortality, decreased energy storage, and decreased disease resistance. Across the southeast part of the U.S., the frequency and intensity of precipitation events has been increasing (1), with effects of decreasing salinity particularly evident in Louisiana estuaries. Oyster reefs provide habitat for entire estuarine ecosystems, they filter water to decrease turbidity and enhance primary production, and they serve as breakers during hurricanes and flooding events. The Gulf of Mexico alone contributes 80-90% of the United States' 30 million pounds of oysters to the industry. Therefore, it is vital for oyster populations to be able to adapt to the changing environment because of their economic and environmental importance. Studies indicate that DNA methylation may provide a means for individual organisms to survive these changes and even pass on adaptations to the next generation (2). Methylation, a mechanism for encoding epigenetic information, has the potential to alter the DNA structure and gene expression in response to environmental stressors, such as salinity (4). In this project, the methylation-sensitive amplification fragment length polymorphism (MS-AFLP) technique (3) will be developed further to analyze the methylation patterns of DNA in the eastern oyster, *Crassostrea virginica*, to determine the effects of salinity on the occurrence of epigenetic events.

Methods.

Tissue samples were taken from oyster populations at sites with varying salinities (Table 1, already collected as a part of the LASG-funded masters project of Scott Riley). Using the MS-AFLP technique to analyze the methylated DNA, I will be able to determine DNA fragment length, allowing me to identify where methylation occurred in each population as well as the frequency of epigenetic events in response to the salinity stressor (3). The initial protocol on which the project depended required extensive optimization, as the methodology had never been used on oyster tissues until now. Restriction enzymes EcoRI, HpaII, which is methylation-sensitive, and MspI, which is

not methylation-sensitive and will cut more frequently in heavily methylated genomes than HpaII, were used (Taylor et al 2010). Fluorescent primers were included to aid in analysis through PeakScanner software. The optimized MS-AFLP (Appendix 1) can be used to analyze oyster tissues and, theoretically, any other test organism.

Table 1. Locations and mean salinities (USGS) of proposed sampling locations to test effects of natural salinity variation on genomic methylation in eastern oysters. These locations were chosen based on mean salinity and standard deviation of the mean. Site 1 provides samples from a salinity that is mostly moderate with the occurrence of low and high salinity events. Site 2 provides samples low salinity. Site 3 provides samples from moderate salinity. Site 4 provides samples from an extreme low salinity.

Location	Mean Salinity (ppt)	Standard Deviation
1. Grand Isle	19.96935	+-4.219634
2. Caillou Lake (Sister Lake)	11.72006	+-2.894487
3. Lake Calcasieu near Cameron	19.40974	+-2.573968
4. Vermillion Bay Cypremort Point	3.956184	+-1.94717

Results.

The success of the protocol was indicated on the gel image below, indicating a difference between methylation sensitive and normal restriction enzymes. This gel was run in September and was repeatable.

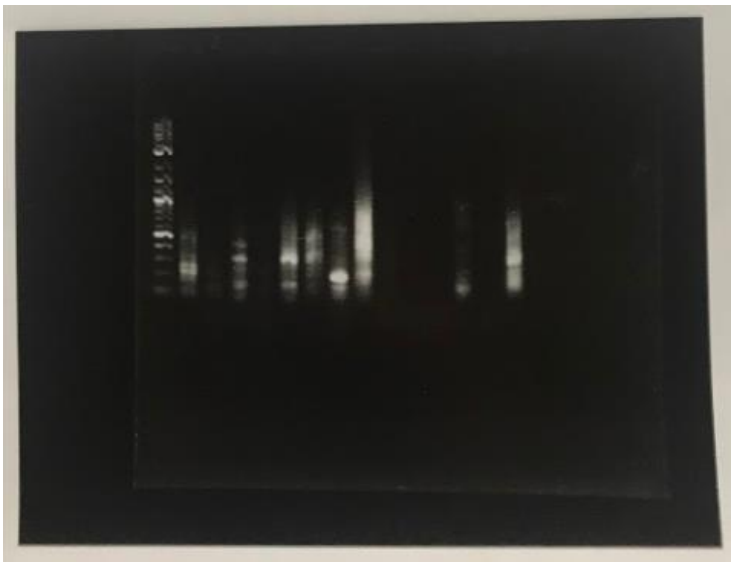


Image 1. BioRad image of gel electrophoresis run in September of 2017 indicated the first successful trial of the protocol. It was observed that the first seven samples, which were digested by Msp1, presented more bands than those by Hpa11.

The functionality of MS-AFLP was then confirmed after the addition of fluorescent primers and peak analysis. In the figures below, the Hpa11 digested sample presents significantly few peaks than the Msp1 digested sample. The optimized protocol can now be used to compare oyster tissues taken from locations of varying salinities.

Figure 1.

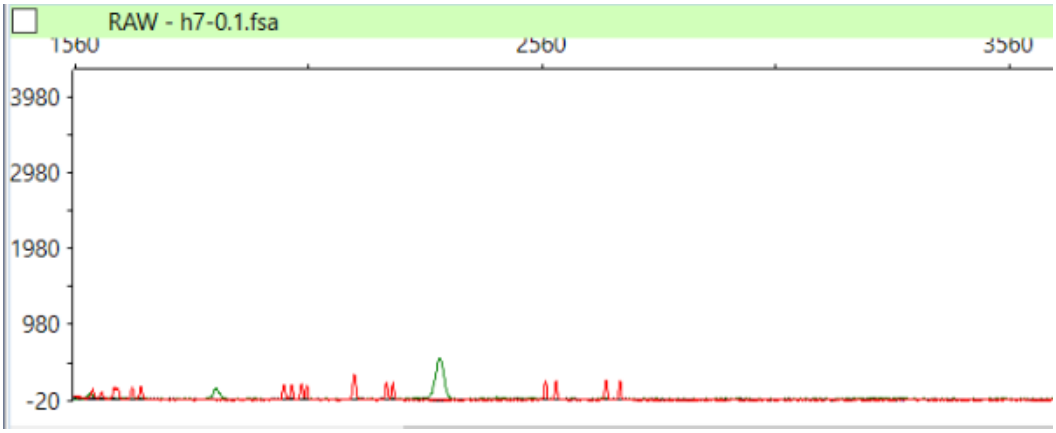
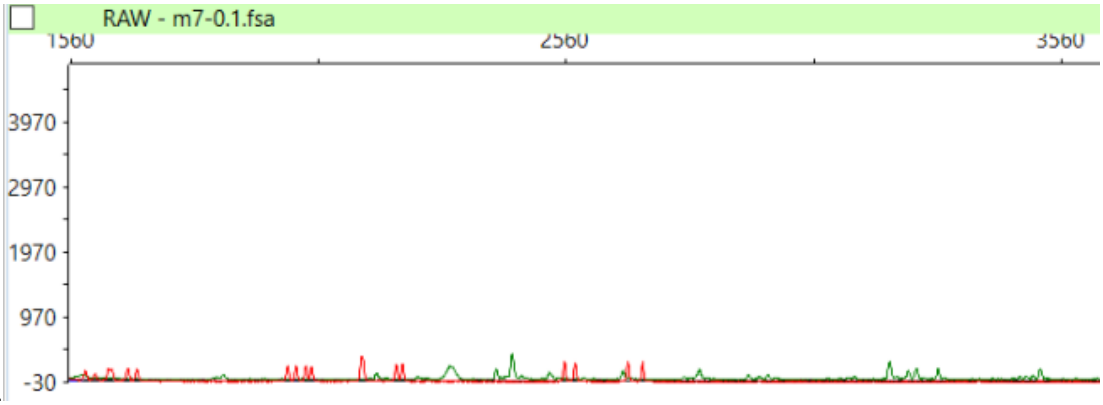


Figure 2.



Figures 1 and 2. Figure 1 is the raw peak scan of a sample with restriction enzyme Hpa11 (methylation sensitive) while Figure 2 is the raw peak scan of a sample with restriction enzyme Msp1. The red peaks indicate the ladder used, and the green peaks are the fragments of DNA that resulted from each digestion. The number of peaks is greater in Figure 2.

Conclusion.

Serving as a mediator of environmentally-induced plasticity, epigenetic research is cutting-edge, and we are still evolving our understanding of the mechanisms that

cause it as well as how the epigenome is passed to offspring (4). This research will provide a tool necessary to achieve that understanding and will aid efforts in monitoring and conservation of oysters for economic and ecological purposes. With an optimized protocol and method for analysis, next steps include the analysis of possible differences between oyster samples from varying salinities. Future work may involve exploring if and how the epigenetic changes are passed to offspring, how those heritable traits impact offspring survival, and epigenetic responses can extend to other environmental stressors, including temperature and pH.

Literature cited

(1) Powell, E.J. & Keim, B.D. (2015) Trends in Daily Temperature and Precipitation Extremes for the Southeastern United States: 1948–2012. *Journal of Climate*, 28, 1592–1612.

(2) Riviere, G. (2014) Epigenetic features in the oyster *Crassostrea gigas* suggestive of functionally relevant promoter DNA methylation in invertebrates, Frontiers in physiology, 5, 129, 10.3389/fphys.2014.00129, 2014.

(3) Taylor, E., Blouin, M.S., Thuillier, V., Cooper, B., Amarasinghe, V., Cluzel, L., Araki, H. & Grunau, C. (2010) No evidence for large differences in genomic methylation between wild and hatchery steelhead (Oncorhynchus mykiss). *Canadian Journal of Fisheries and Aquatic Sciences*, 67, 217–224.

(4) Hofmann, Gretchen E. (2017) Ecological Epigenetics in Marine Metazoans. Frontiers in Marine Science, 4. 10.3389/fmars.2017.00004

Appendix 1.

MSAP Procedure

Digestion with restriction enzymes:

MspI

DNA sample	18 ul
CutSmart Buffer	2 ul
EcoRI	1 ul
MspI	1 ul

Total Volume = 22 ul

HpaII

DNA sample	18 ul
CutSmart Buffer	2 ul
EcoRI	1 ul
HpaII	1 ul

Total Volume = 22 ul

Incubate in thermocycler at 37 C for 1 hour

Ligation of adapters:

Digested DNA	10 ul
Ligase Buffer	2.5 ul
HpaII/MspI adapter	1.3 ul
EcoRI adapter	1.3 ul
Ligase	1.3 ul

Total Volume = ~16.5

Run in thermocycler at 22 C for 2 hours, then 65 C for 10 minutes

Dilute 4-fold by adding 45 ul of nuclease free water

Preselective Amplification:

Water (nuclease free)	4 ul
Ligated DNA product	8 ul
Taq Buffer	3 ul
EcoRI preselect primer	0.75 ul
HpaII/MspI preselect primer	0.75 ul
dNTPs	1 ul
Taq	0.5 ul

Total Volume = 15 ul

Preselective Thermocycler Conditions (Protocol PRESELEC)

72 C for 2 minutes
20 cycles of:

1. 94 C for 1 second
2. 56 C for 30 seconds
3. 72 C for 2 minutes

60 C for 30 minutes
4 C infinite hold

Dilute 4-fold by adding 55 ul of nuclease free water

Selective Amplification:

Water (nuclease free)	10 ul
Preselect DNA product	9 ul
Taq Buffer	3 ul
EcoRI primer X*	0.75 ul
HpaII/MspI primer X*	0.75 ul
dNTPs	1 ul
SelectiveTaq	0.5 ul

Total Volume = 25 ul

*7 primer pairs are used to perform 7 parallel amplifications

Selective Thermocycler Conditions (Protocol SELECT)

94 C for 2 minutes
9 cycles of

1. 94 C for 1 second
2. 65 C (-1 degree for each cycle) for 30 seconds
3. 72 C for 2 minutes

23 cycles of

1. 94 C for 1 second
2. 56 C for 30 seconds
3. 72 C for 2 minutes
4. 60 C for 30 minutes
5. 4 C infinite hold