Quantifying the expression of oil-degrading functional genes in Louisiana saltmarsh sediments

By: Tyler Harris

Introduction:

The Macondo 252 oil spill, also known as the BP or Deepwater Horizon spill, is the largest oil contamination event that has occurred in U.S. history. It is estimated that the spill released approximately 4.2 million barrels of oil into the Gulf, affecting hundreds of miles of shoreline including the salt marshes that border Louisiana (Cleveland, 2013). Nearly five years after the spill, there are still sites that contain high levels of oil. It is well established that bacteria play a large role in the natural response to oil contamination. Certain bacteria such as *Alphaproteobacteria* contain genes that code for proteins which facilitate the breaking of long chain hydrocarbons into smaller molecules such as carbon dioxide and water (Kostka et al., 2011). This explains why oil-degrading bacteria are the subject of many researchers in recent years.

Research Activities:

Three sites bordering Barataria Bay, off the coast of Southeast Louisiana, were previously distinguished and labeled as heavily oiled (HV) moderately oiled (MD) and unaffected by oil (RF) based on the observable amount of oil present at each site. Seven soil samples from random locations within each site were extracted and frozen with dry ice. The samples were then taken back to the lab for analysis. The water content of each sample was

determined by weighing a portion of the samples before and after desiccation then dividing the difference by the weight before desiccation.

MoBio Powersoil® DNA Isolation kits were used in order to extract the DNA in each sample while MoBio Powersoil Total™ RNA Isolation kits were used to extract the RNA. Additionally the RNA was purified using RNase free DNase from Qiagen®. Upon extraction and purification the DNA and RNA quality and concentration was analyzed via spectrophotometric analysis using the Thermo Scientific Nanodrop 2000C. A260/A280 ratios were compared with DNA/RNA of known purity before proceeding. RNA was converted to cDNA using SuperScript® cDNA preparation kits by Invitrogen®. The purity and concentration of the cDNA was ensured via spectrophotometric analysis.

The cDNA and DNA were prepared for qPCR utilizing Bio-Rad SsoAdvanced™ SYBR® Green Supermix. Supermix contains the necessary stabilizers, enhancers, dNTPS, SYBR® Green dye, buffer, $MgCl_2$ and hot start polymerase. Each PCR reaction mixture contained 5.00 µl of Supermix, 0.05 µl each of forward and reverse primers, 0.90 µl nuclease free water, and 4.00 µl sample. The abundance and expression of three oil degrading genes, alkB, GN PAH-RHDα and GP PAHα were quantified as well as one gene common to nearly all bacteria, 16Sr RNA. The standard curves were created using serial dilutions of standardized DNA fragments acquired from the Genbank database. The accession numbers are as follows: M83949 (GN PAH-RHDα, *Pseudomonas putida*), KC207085 (16Sr RNA, *Pseudomonas putida*), DQ846881 (GP PAH-RHDα, *Rhodococus opacus*), and AJ233397 (alkB, *Pseudomonas putida*). Nuclease free water combined with the SYBR® Green Supermix solution and primers were used as controls. The qRT-PCR was carried out using the Bio-RAD CFX Connect™ Real-time system as triplicates.

The thermal cycling conditions were programmed for each gene as follows:

- 16Sr RNA, 95ºC for 5 minutes followed by forty cycles of: 30 seconds at 95ºC; 30 seconds at 56ºC, 40 seconds at 72ºC, and seven minutes at 72ºC. (Cébron et al., 2008)
- AlkB, 94ºC for 15 minutes followed by forty cycles of: 20 seconds at 84ºC, 30 seconds at 50ºC, 45 seconds at 72ºC; and one minute at 45ºC. (Powell et al., 2006)
- GP PAH-RHDα, 95ºC for 5 minutes followed by forty cycles of: 30 seconds at 95ºC, 30 seconds at 56ºC, 40 seconds at 72ºC, and seven minutes at 72ºC. (Cébron et al., 2008)
- For the GN PAH-RHDα, 95ºC for 5 minutes followed by forty cycles of: 30 seconds at 95ºC, 30 seconds at 57ºC, 40 seconds at 72ºC, and seven minutes at 72ºC. (Cébron et al., 2008)

Results:

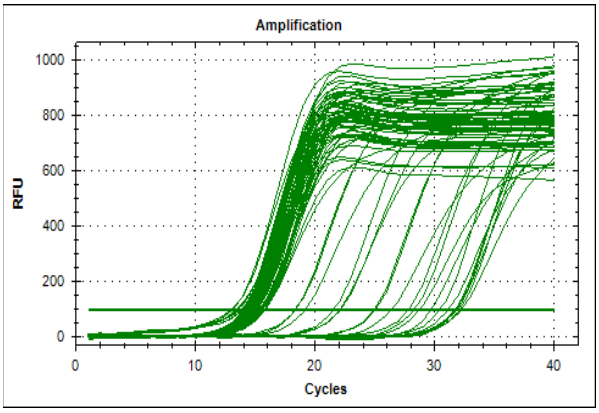

Figure 1. Graphic depiction of the amplification and quantification of the 16Sr RNA DNA.

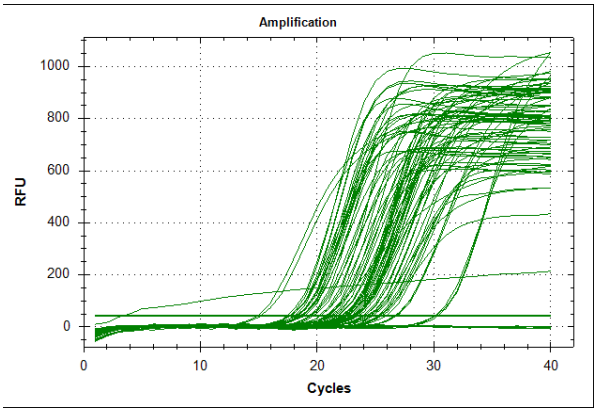

Figure 2. Graphic depiction of the amplification and quantification of the16Sr RNA cDNA.

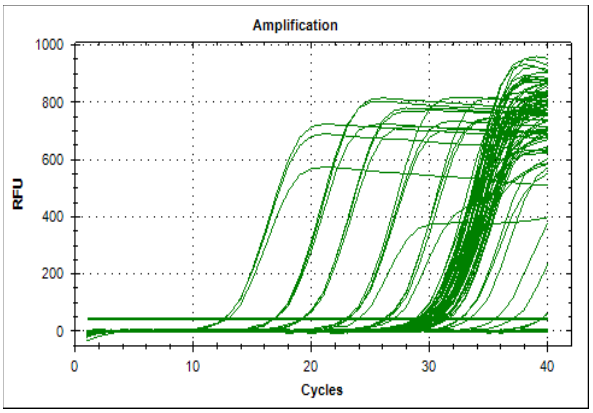

Figure 3. Graphic depiction of the amplification and quantification of the AlkB DNA.

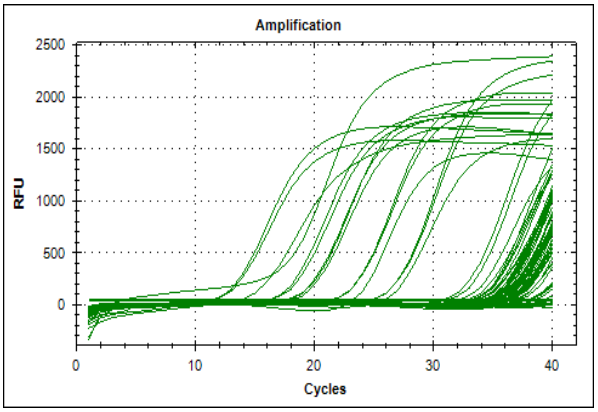

Figure 4. Graphic depiction of the amplification and quantification of the AlkB cDNA.

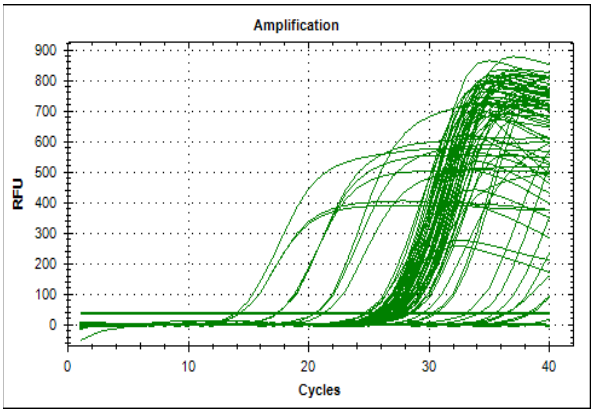

Figure 5. Graphic depiction of the amplification and quantification of the GN PAH-RHDα DNA.

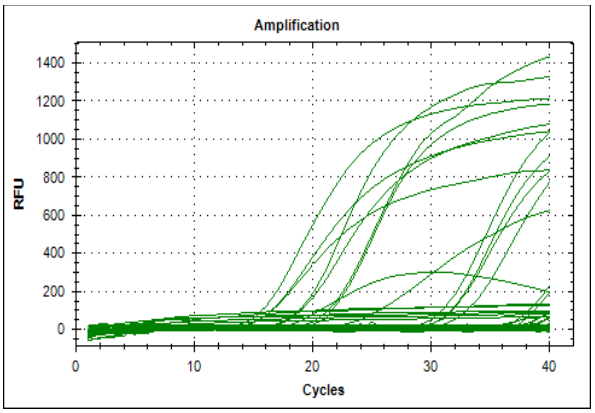

Figure 6. Graphic depiction of the amplification and quantification of the GN PAH-RHDα cDNA.

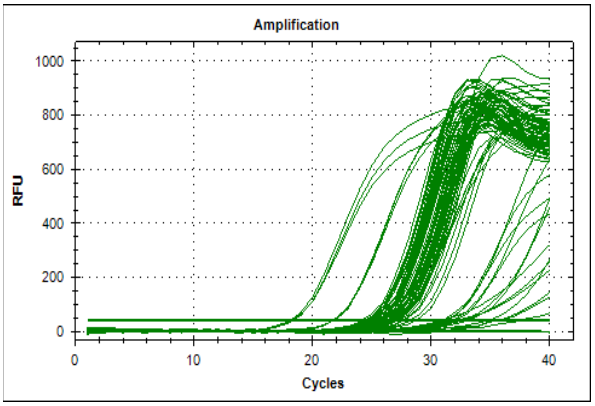

Figure 7. Graphic depiction of the amplification and quantification of the GP PAH-RHDα DNA.

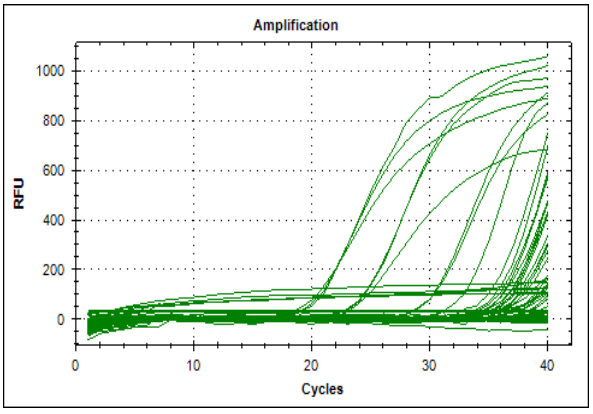

Figure 8. Graphic depiction of the amplification and quantification of the GP PAH-RHDα cDNA.

Conclusions and Significance:

The results of this experiment give a snapshot view into the abundance and expression of oil-degrading bacterial populations in Louisiana salt marshes approximately four years post-oil contamination. When combined with the results of earlier sampling events and analyses it becomes apparent that the oil-degrading genes have been increasing in abundance and expression relative to the 16Sr RNA gene indicating the oil degrading populations are increasing in proportion to the total number of bacteria living in the moderately oiled and heavily oiled salt marsh sediments. This can be attributed to the ability of the bacteria carrying the alkB, GN PAH-RHDα and GP PAHα genes having the ability to utilize oil as a carbon source. It was also noted that the abundance and expression of the alkB gene was statistically, significantly lower than the abundance and expression of both PAH-RHDα genes. This is believed to be the result of the depletion of n-alkanes by alkB carrying bacteria. With the abundance and expression trends of these genes combined with soil characteristics such as DOC, TPH, SUVA254, as well as respiration will give further insight into the roles gram negative and positive bacteria play in PAH degradation. This study was unable to analyze the genes involved in degradation of specific hydrocarbons which would give further insight into the dynamics of how each component of crude oil degrades, what genes are involved, and the timeframe in which degradation takes place. This information is critical to determining the type, quantity, and ratios of bacteria necessary to the remediation of oil-contaminated soils.

Acknowledgements:

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References:

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