Culture Dependent vs. Culture Independent 16S Sequencing for Bacterial Communities during Decomposition of Shrimp

Marlee Hayes-Mims[1], Kristin Bjornsdottir-Butler [1] , and Ronald A. Benner, Jr.[1]

[1]U.S. Food and Drug Administration, Center for Food Safety and Applied Nutrition, Gulf Coast Seafood Laboratory, Dauphin Island, AL 36528, USA

Abstract

Understanding bacterial community changes of seafood during storage is critical in developing methods for bacterial biomarkers of decomposition. However, culture dependent and independent methods may yield different results when evaluating bacterial community compositions. The study objective was to compare culture dependent and independent 16S sequencing methods to evaluate bacterial community composition during storage of shrimp at different temperatures. Expired beheaded shrimp were incubated at 0, 12, 24, and 36ºC for 20 days, 72, 24, and 12 hours, respectively. At each sampling point, triplicate samples were collected and metagenomic analysis using the16S rRNA gene amplicons was conducted (culture independent). Samples were homogenized (1:10), spread plated on TSA, and incubated under the same conditions as indicated above. DNA from 48 colonies (from countable plates) at each sampling point was purified for 16S rRNA gene sequencing via Sanger Sequencing (culture dependent). At 0ºC, culture dependent results showed the initial bacterial community consisted primarily of *Psychrobacter*, *Arcobacter*, and *Planococcus* spp. By day 20, it consisted predominantly of *Shewanella* spp. The culture independent method detected *Shewanella* spp. initially and tracked its increase throughout storage. The presence/increase of *Shewanella* spp. was observed on different days based on the two methods. At 36ºC, based on both methods, the initial bacterial community was highly diverse. However, the culture dependent method showed the dominant bacteria were *Vibrio* and *Photobacterium* spp. by hour 12, whereas the culture independent method demonstrated a much less pronounced decrease in diversity. Generally, the culture independent method identified a more diverse community composition compared to culture dependent method. Understanding the difference in composition and changes of bacterial communities during decomposition at various temperatures and assessing the most effective and reliable methods for evaluation will aid in the detection of seafood decomposition and may ultimately help identify bacterial biomarkers thereof.

Plain Language Synopsis:
Seafood bacterial communities are constantly changing during storage. Understanding those communities and how they transform relative to conditions of storage is important to ensure that safe, high-quality seafood makes it to the consumer. In this study, we compare two methods of assessing bacterial communities in shrimp throughout storage.

Introduction

Seafood decomposition can be assessed by microbiological, molecular, sensory, and chemical means. Understanding each of these types of indicators along with their relationship to each other may aid in recognizing potential biomarkers for application as evidence of seafood decomposition.

Bacterial communities associated with seafood products change under various time/temperature storage conditions. Evaluating how the microflora changes during storage is critical in discovering biomarkers of decomposition. These biomarkers, when applied as evidence of seafood decomposition, will be paramount for minimizing distribution of adulterated seafood in interstate commerce and mitigating human health risks associated with consumption of seafood products. The objective of this study was to compare results from 16S Sanger sequencing (culture dependent) and metagenomic 16S sequencing (culture independent) at various storage temperatures during decomposition of shrimp.

Results and Discussion

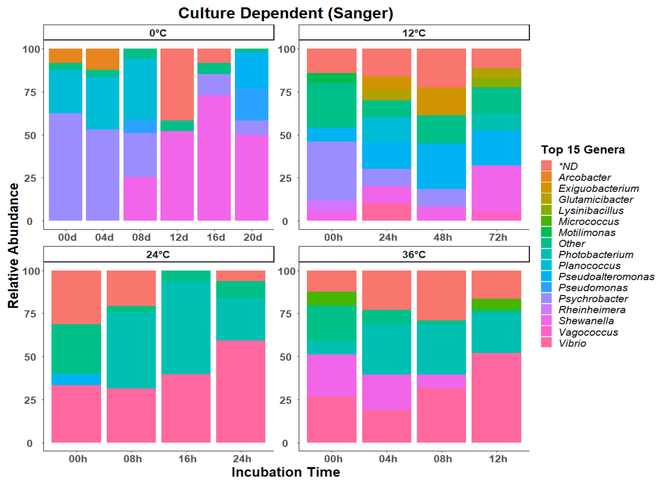

Figure 1. Shrimp bacterial community composition via 16S Sanger sequencing shown by genus abundance. *ND-not determined (isolates which could not be regrown or were unable to be distinguished through 16S Sequencing); **Other** includes genera not in the top 15.

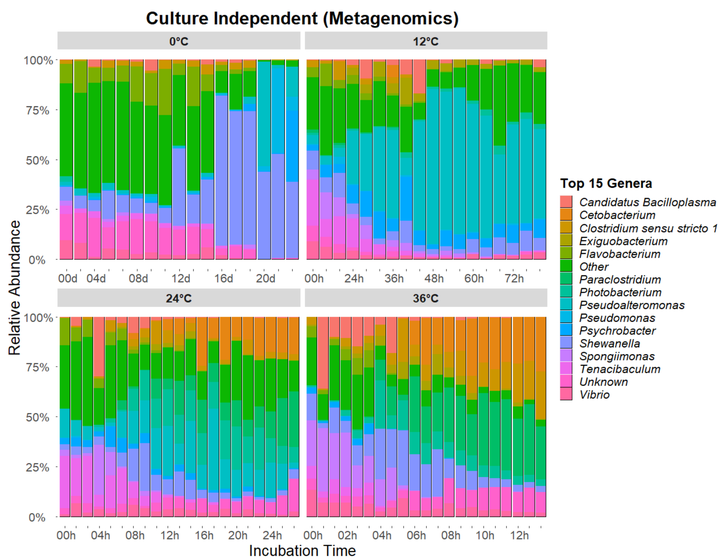

Figure 2. Shrimp bacterial community composition via 16S Metagenomic sequencing shown by genus abundance. *ND-not determined; **Other** includes genera not in the top 15.

Culture Dependent (Sanger Sequencing)

0ºC - the initial bacterial community consisted primarily of *Psychrobacter, Planococcus,* and *Arcobacter* spp. By day 20, it consisted predominantly of *Shewanella, Pseudomonas,* and *Pseudoalteromonas* spp.
12ºC - the initial bacterial community was diverse, including *Shewanella, Vibrio, Photobacterium,* and *Psychrobacter* spp. and this diversity continued into hour 72, with increases in *Shewanella* and *Photobacterium* spp.
24ºC - the initial bacterial community consisted of *Vibrio* and **Other** or not determined spp. and by hour 24 was mainly *Vibrio* and *Photobacterium* spp.
36ºC - the initial bacterial community was highly diverse, including *Shewanella, Vibrio, Photobacterium,* and *Psychrobacter* spp. By hour 12, the dominant bacteria were *Vibrio* and *Photobacterium* spp.

Culture Independent (Metagenomics)

0ºC - the initial bacterial community consisted primarily of **Other/Unknown** spp. By day 20, it consisted predominantly of *Shewanella, Pseudomonas, Psychrobacter,* and *Pseudoalteromonas* spp.
12ºC - the initial bacterial community was diverse, during hours 24-72 an increase in *Pseudoalteromonas* spp. became pronounced.
24ºC - the initial bacterial community was diverse and by hour 24 some slight changes were seen in the composition, such as, increases in *Vibrio* and *Photobacterium* spp.
36ºC - the initial bacterial community was highly diverse, including *Shewanella, Vibrio,* and *Psychrobacter* spp. By hour 12, the dominant bacterial flora were *Paraclostridium, Clostridium,* and *Cetobacterium* spp.

Materials and Methods

Live shrimp were acquired from a local fisherman in the Gulf of Mexico and expired in an ice-water bath immediately. Shrimp were then beheaded, rinsed, and weighed into plastic sample bags.
Sample bags, 3 replicates per time point, were incubated at 0, 12, 24, and 36ºC for 20 days, 72 hours, 24 hours, and 12 hours, respectively. At each sampling point, every 4 days, 12 hours, 8 hours, and 4 hours at 0, 12, 24, and 36ºC respectively, 10 g were blended 1:10 with sterile saline and serial dilutions were plated onto TSA and incubated as described above.

For Sanger Sequencing, single colonies were picked from countable plates at each time point and streaked twice on TSA for purity. The pure isolates were inoculated in TSB at their representative incubation temperature/ times and stored in TSB + 20% glycerol until further analysis.
A 2-ml subsample of the broth culture was centrifuged at 13,000 x *g* for 2 min, the supernatant poured off, and DNA was extracted from bacterial pellets by QiaCube (DNeasy Blood and Tissue Kit). Isolates were identified by the 16S rDNA sequence, using Eubac27F and 1492R primers, and via sequencing on the AB3730 DNA Analyzer. Sequences were analyzed using the CLC Workbench and data using R studio.
For metagenomic sequencing (samples collected after incubation), a low spin (310 g for 3 min) was performed to remove shrimp tissue homogenate and bacterial DNA was extracted using ZymoBIOMICS MagBead DNA Kit. The 16S gene was amplified and barcoded using 26F1 and 534R1 primers and sequenced via the Illumina MiSeq V3 platform. Sequences were analyzed using the QIIME2 pipeline. The resulting data was analyzed in R studio.

Conclusion

In general, the culture independent method resulted in a greater number of genera identified. Culture dependent methods may have limitations in this respect as it is limited to culturable bacteria. However culture dependent methods may yield more clarity at the species level (data not shown). For both methods, bacterial community diversity either decreased or remained the same over time. Decreases in diversity were more pronounced at lower temperatures.

At each temperature, an increase in genus abundance of the community over time may suggest a good candidate(s) as decomposition indicators. Some examples include *Shewanella* or *Photobacterium* spp. at 0ºC or *Vibrio* spp. at 24ºC. In these examples, both methods reflect similar results. At 12ºC, the culture independent method points to *Pseudoalteromonas* spp. as a potential indicator of decomposition but the culture dependent method does not illustrate the same possibility. At 36ºC the culture dependent method suggests *Vibrio* and *Photobacterium* spp. as possible biomarkers, whereas the culture independent method leaned more toward *Paraclostridium*, *Clostridium*, and *Cetobacterium* spp.

The comparison of these two methods helps to illustrate the complexity of identifying and applying specific biomarkers of decomposition in shrimp. Further understanding bacterial communities responsible for decomposition and their relationship to molecular, sensory, and chemical indicators will help advance reliable methods for detection of decomposition in shrimp, as well as other seafood commodities.