Staphylococcus and Psychrobacter are the most abundant bacteria genera identified in deep-sea viperfish stomach and intestine

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Summary

- The stomach and intestinal DNA of a viperfish were isolated and sequenced to study the gut microbial community of the deep-sea fish.
- The most abundant genus is Psychrobacter, and the most abundant species is *Psychrobacter halodurans*.

Abstract

Viperfish are found in the mesopelagic zone at depths of 400 m to 1000 m in the ocean. This deep-sea environment is characterized by harsh conditions such as high pressure, low light, and low oxygen levels. We analyzed the gut microbiome of viperfish to understand how they survive in this extreme environment. The intestine and stomach were dissected from four individual viperfish, and total DNA were extracted and purified from each sample via DNeasy Blood & Tissue kit. The samples were then processed with 16S Barcoding Kit 24 V14 library prep kit sequenced on a Nanopore MinION sequencer. 16S rRNA sequences was used to identify the species and determine relative abundance of bacteria within our collected samples. The most relative abundant marine bacteria were *Psychrobacter halodurans*, *Planococcus Maritimus*, and *Psychrobacter nivimaris*.

Introduction

Extreme environment of the deep-sea results in unique traits

- At depths 200 meters and below, ocean organisms must adapt to
  - crushing water pressure
  - lack of light
  - low temperature
  - low oxygen levels
- Such adaptations manifest in a variety of ways
  - bioluminescence or large, photosensitive eyes (Monterey Bay Aquarium, 2022)
  - anti-freeze proteins (Johnston, 1990)
  - low metabolic rate and low energy expenditure (Seibel and Drazen, 2007)

Office of Ocean Exploration and Research, National Oceanic and Atmospheric Administration (NOAA). https://oceanexplorer.noaa.gov/facts/deep-ocean.html

- Information on the gut microbiota can help us better understand the mechanisms of these adaptations, especially metabolism
  - We used the 16S rRNA, a gene conserved across microbes, to identify bacterial species

Methodology

Sample Collection

- Measured the size of the fish: about 5.5 cm
- Made a slight incision at the viperfish abdomen and pulled open to reveal the organs
- Isolated stomach and intestine and stored them at -80°C until DNA isolation

DNA Isolation

- Total DNA extracted using DNeasy Blood and Tissue kit, following manufacturer's protocol
- Quantity and quality checked with Nanodrop

Library Preparation

- Used 15 ng of each DNA sample for library prep via Nanopore 16S Barcoding Kit 24 V14

Nanopore Sequencing and Data Analysis

- Sequencing data were processed through EPI2ME with the default Fastq 16S workflow

Results

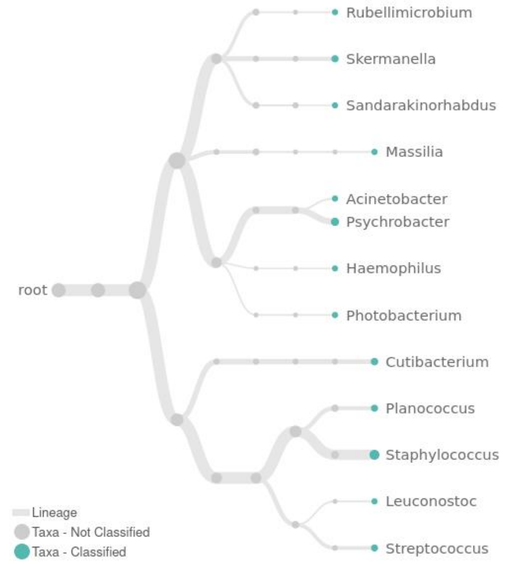

Figure 1. Thirteen bacteria genera have read numbers over 1% of total reads. Staphylococcus and Psychrobacter are the most abundant genera.

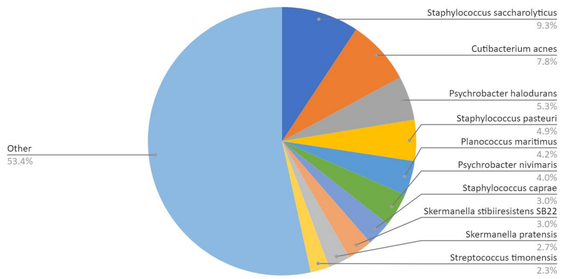

Figure 2. Ten most abundant bacteria species. Out of these, three are marine bacteria. These three are *Psychrobacter halodurans*, *Planococcus maritimus*, and *Psychrobacter nivimaris*. The "Other"category represents 110 species with a relative abundance of 2.1% or less.

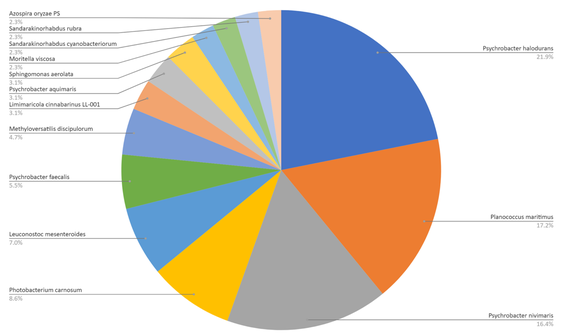

Figure 3. Top fourteen abundant marine bacteria species. The top three abundant bacteria (*Psychrobacter halodurans*, *Planococcus maritimus*, and *Psychrobacter nivimaris*) are represented in Figure 2. Other bacteria of marine origin have relative abundance of less than 2.3%.

Discussion

120 species of bacteria were identified from 526 reads following the amplification of the 16S rRNA gene using total DNA extracted from fish stomach and intestine samples.

- Deep-sea fish possess bacteria unique to the marine environment.
- The most abundant genus, Staphylococcus (Figure 1), has a wide range of habitat from the skin of mammals to marine sponge.

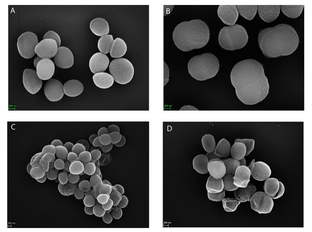

Figure 4. Scanning electron micrograph (SEM) of *Staphylococcus saccharolyticus* (Bruggeman et al., 2019). This is the most common bacteria overall, identified in 9.3% of reads. It is found in human blood and on the skin.

- The second most abundant genus is Psychrobacter (Figure 1), which mostly inhabit cold environments such as the deep sea.
- Out of the top 15 marine bacteria in terms of relative abundance by the number of reads, *Psychrobacter halodurans*, *Planococcus maritimus*, and *Psychrobacter nivimaris* account for 55.5% of total abundance, with the remaining bacteria reads being under 10% in terms of relative abundance (Figure 2-3).
  - *Psychrobacter halodurans* has been isolated from marine sediment in Weihai, China. *Psychrobacter nivimaris* and *Psychrobacter halodurans* are known for radiation resistance (Shang et al., 2022; Yang, 2014)
  - *Planococcus maritimus* is known for biosurfactant production, starting oil scavenging in areas polluted by oil (Waghmode et al., 2019)

Our data contributes to the knowledge of the bacteria in the viperfish. The various types of marine bacteria identified are the first steps to understanding their potential functions in their symbiotic relationship with the viperfish, as well as their roles in the deep-sea ecosystem.

Study Limitations & Future Directions

- We only isolated bacterial DNA from the stomach and intestine samples. Bacteria in other organs were not addressed.
  - The viperfish has photophores that help them illuminate the surrounding area. Identifying bacteria species from the photophores may help us better understand the viperfish adaptation to the low light environment.
- The sample size of was limited to four viperfish. This research presents a unique preliminary data, but more samples should be used to verify the results.

References

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