Two Bacterial Species Capable of Degrading Toxic Compounds are Among the Most Abundant Bacteria in the Gut of Deep-Sea Dragonfish, Owlfish, and Lanternfish

Callos, R.[1], Zuniga, J.[1], Feng, Z.[1,2], Najera, K.[2], Bozinovic G.[1,2]

Portland State University[1], Boz Life Science Research and Teaching Insititute[2]

Abstract

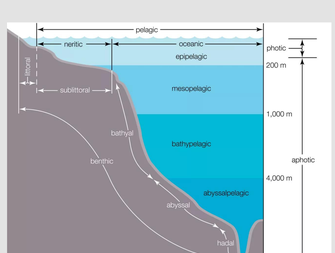
- Deep sea is a challenging environment for inhabitants.
- DNA from gut microbiomes of four deep-sea fish from three families was isolated
- Nanopore 16S sequencing was performed to identify bacterial species
- Three bacterial species, *Sphingomonas leidyi, Nocardioides pyridinolyticus*, and *Caulobacter vibrioides*, are the most abundant ones and consistently present across all fish families.
- *Nocardioides pyridinolyticus* can degrade pyridine, a highly soluble chemical often released from industrial activities

Hypothesis

There are bacteria in deep-sea fish gut microbiome that can metabolize harmful organic compounds in the ocean.

Introduction

- Mesopelagic and bathypelagic fish live in harsh environments with high pressure and temperatures between 5ºC and 20ºC
- Microbiota have been shown to be associated with[1]:
  - Defense mechanisms
  - Carbohydrate transport
  - Signal transduction.
  - Metabolism
- Fish microbiome composition depends on[2]:
  - Immunity
  - Host selection
  - Metabolism
  - Diet
  - Environmental factors
- Common bacterial families found in the guts of deep-sea fish, from the same families as dragonfish and lanternfish[3]:
  - Lachnospiraceae
  - Muribaculaceae
  - Vibrionaceae
  - Moraxellaceae
  - Eimeriidae.
  - Pseudoalteromonadacea
- Diversity and abundance of bacterial communities vary with environmental changes, such as pressure levels[4]
- Mesopelagic and Bathypelagic fish mostly live in cold and low nutrient areas, and their microbiota should be capable of utilizing a wide range of organic compounds as energy sources, including chemicals that are toxic to the fish

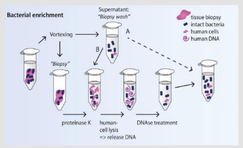


Deep Sea Zones

Methodology and Materials

Fish Collection

- Five fish were collected near the Oregon coast with a trawling net at 600-800 m below the surface
- Stomachs and intestines were dissected and stored at -20ºC

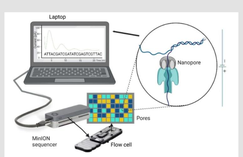


Host DNA Depletion

- Tissue samples were cut to ~2 mm in diameter
- Followed host-depletion protocol[5] to minimize fish DNA

Bacterial DNA Isolation

- MO BIO Powersoil DNA isolation Kit and QIAGEN Blood & Tissue kit were used for bacterial DNA purification to compare extraction efficacy



Library Preparation & Nanopore 16S Sequencing

- DNA was processed with Oxford Nanopore 16S Barcoding Kit 24 V14 following manufacturer's protocol
- Used MinION for sequencing

Data Analysis

- EPI2ME 16S workflow for bacterial identification
- Data were formatted in R version 4.4.2, and figures were generated using the packages ggplot2 version 3.4.0, and ggVenndiagram

References

Results

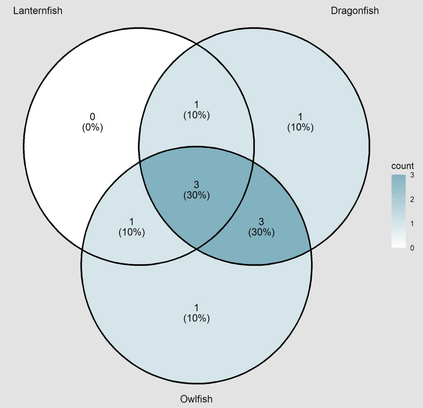


Figure 1. Shared and unique bacteria species across deep-sea fish families. Samples from the same family were combined: there are four dragonfish samples, two lanternfish samples, and six owlfish samples. Bacteria with reads below five, and probable human skin bacterial contaminants were excluded.

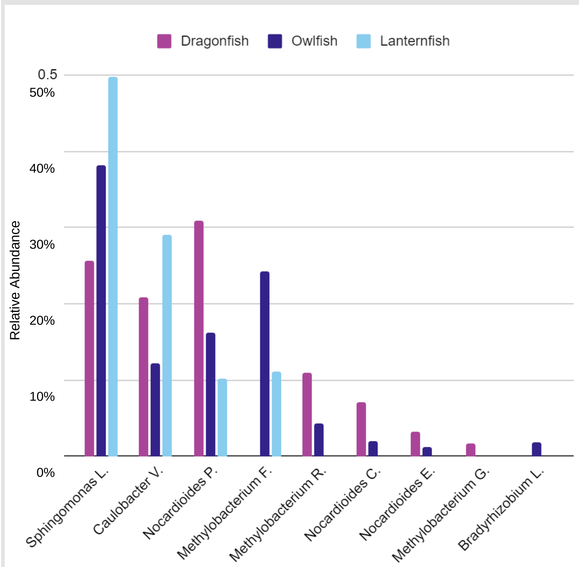


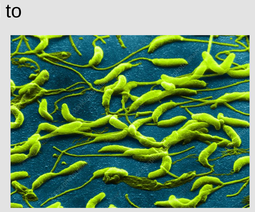
Figure 2. Most common bacteria found throughout three fish families. Samples from the same family were combined, and the relative abundance of each bacteria from a fish family was calculated as the percentage of its reads relative to the total reads of the fish family. There are four dragonfish samples, two lanternfish samples, and six owlfish samples. Bacteria with reads below five, and probable human skin bacterial contaminants were excluded.

Discussion

- Three most abundant bacteria found were present in the gut of all three fish families (Figure 1).
- *Sphingomonas leidyi* bacteria could be involved in detoxification. Bacteria in the *Sphingomonas* genus have been discovered in the gut of healthy Atlantic salmon (*Salmo salar*), and were involved in both nutrient metabolism and the degradation of toxic compounds and pollutants in white shrimp (*Lutopenaeus vannamei*)[6,7].
- The presence of *Caulobacter* in marine and freshwater environments is usually distinct[8]. *Caulobacter vibriodes* (*crescentus*) is generally considered as a freshwater bacteria species, but is surprisingly one of the most abundant bacterial species in deep-sea fish.
- *Nocardioides pyridinolyticus*, another one of the three most prevalent bacteria identified (Figure 2), can degrade pyridine. Pyridine is a compound harmful to aquatic life, and *N. pyridinolyticus* may help lower the toxicity potential for the deep-sea fish if they are exposed to pyridine[9].
- Bacteria in the genus *Nocardioides* generally can survive well in low-nutrient conditions, and can break down pollutants to extract carbon and nitrogen[10].
- Our identified bacteria are not what we expected from commonly reported deep sea fish gut microbiome composition[3]. This suggests that microbiota may be highly diverse among deep-sea fish across different regions.

Limitations and Future Direction

- **Contamination.** Two samples contain human-associated bacteria, which were considered contaminants and excluded from the analysis. Improving proper aseptic technique should mitigate this issue in future studies.
- **Low sample size.** We have a small sample size which was further compromised by contaminated samples. Increasing the number of fish per family in future studies should help confirm our results.
- **Limited families.** Only three fish families were examined. Including additional families could provide more comprehensive understanding of bacterial diversity in different fish guts.
- **Further investigation into *Sphingomonas leidyi*/*Caulobacter leidyi*.** *Caulobacter leidyi* was reclassified to *Sphingomonas leidyi* in 2012 [11]. Considering their detection along with *Caulobacter vibriodes*, further research into their activities in the fish gut should help better understand and characterize these closely related species.



***Caulobacter* bacteria.**