Dragonfish and medaka fish share similar bacteria that may benefit their digestive and immune systems

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Abstract

The dragonfish lives in the challenging deep-sea environment, characterized by low temperature, high pressure, and low oxygen levels. Research has shown that gut microbiota promotes tolerance against harsh environmental factors. We hypothesized that microbiota in the dragonfish allow the dragonfish to survive in these extreme conditions. We also compared the dragonfish microbiota to the medaka fish microbiota as a reference point. Organs and tissues were dissected from the fish, and bacterial DNA was extracted from each sample. We performed Nanopore 16S rRNA gene sequencing and identified bacteria in each sample. Several genera, *Staphylococcus, Cutibacterium,* and *Serratia*, are abundant in both fish. While we did not detect bacteria that may directly protect dragonfish against the extreme environmental conditions, certain bacterial species provide digestive or immune benefits that can support their overall health and survival.

Introduction

Hypothesis

- Dragonfish microbiota help them survive conditions of low oxygen, temperature, light, and high pressure in the deep-sea environment.

Deep sea

- Low oxygen: for example, the oxygen concentration in the Pacific Basin is below 0.2 ml/L at the depth of 1,500 m or deeper (Kamykowski and Zentara, 1990).
- Low temperature: on average, the ocean water below 200 meters is around 4°C (NOAA, 2018).
- High pressure: at 1,000 m below the surface, water pressures reach about 40 to 110 times of the Earth's atmosphere (NOAA, 2020).

Deep Sea Dragonfish vs. Medaka fish

- The dragonfish (*Stomiidae*) (Aguilar et al. 2018), is found circumglobally in the mesopelagic and bathypelagic zones (Villarins et al. 2022),
  - Diel vertical migrators moving at 400 – 1000 m below the sea surface (Eduardo et al., 2020).
  - Have been recorded in temperatures from 4 to 14.5°C, but are and undergo daily temperature changes of up to 7°C (Bergman et al. 2023).
- The Medaka fish, *Oryzias latipes,* is found in East Asia, typically living in shallow fresh or brackish water close to the shoreline (Hilgers and Schwarzer, 2019).
  - Inhabiting the temperate zone, they endure temperatures from 4°C to 40°C (Hilgers and Schwarzer, 2019).

Gut Microbiome

- Microbiomes in the gut promotes immunity and tissue repair.
  - The bacteria *Carnobacterium spp.* in Atlantic salmon display antimicrobial effects on seven bacterial pathogens (Robertson et al., 2000).
  - Gut microbiota in Zebrafish stimulates epithelial proliferation (Rawls et al. 2004).
- Microbiomes promotes tolerance to extreme temperatures.
  - Germ-free mice transplanted with microbiota from the fecal matter of mice exposed to –5°C showed less decrease in rectal temperature after exposure to -5°C (Wang et al., 2024).

Methodology

Sample Collection

- Dragonfish were collected via net trawling off the coast of Oregon around 650 meters below the surface at
  - Latitude DD: 44.652
  - Longitude DD: -126.05
- Medaka fish were collected from the stock maintained at the Boz Institute

Tissue Dissection and DNA Isolation

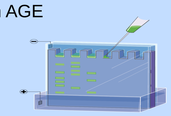
- Organs and tissues were dissected from Dragonfish and medaka and stored at –20°C
- QIAGEN DNEasy PowerLyzer PowerSoil kit was used for isolating bacterial DNA from each sample.
  - A modified protocol was used (Bruggeling et al., 2021).

Polymerase Chain Reaction (PCR) and Agarose Gel Electrophoresis (AGE)

- The V4 region of the 16S gene was amplified with PromegaGoTaq Green master mix and the following primers:

Forward 5'-GACGCTCTTCCGATCTTATGGTAATTGTGTGCCAGCMGCCGCGGTAA-3'
Reverse 5'-TGTGCTCTTCCGATCTAGTCAGTCAGCCGGACTACHVGGGTWTCTAAT-3'

- PCR success was confirmed with AGE



Nanopore sequencing and Data Analysis

- 16S Barcoding Kit 24 V14 was used for DNA library prep following manufacturer's protocol.
- Prepared library was sequenced using Oxford Nanopore's MinION sequencer.
- Sequencing data was processed with EPI2ME for species identification

Results

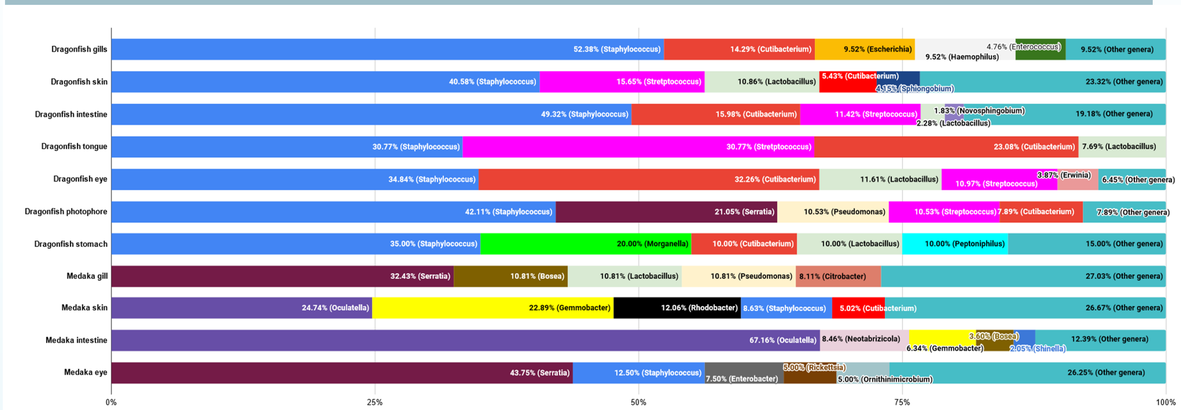


Figure 1. Relative abundance of bacteria genera by sample type. *Staphylococcus* is the most abundant genera in all dragonfish samples. The relative abundance values were calculated by dividing the number of reads from a specific genus by the total number of reads in the sample.

Discussion

The dragonfish and medaka have drastically different microbiome composition, but certain bacteria were detected in both fish.

- The *Streptococcus* genus is abundant in all dragonfish samples except for gill and stomach (Figure 1).
  - One of the most prevalent species, *S. thermophilus*, is associated with the production of antioxidant compounds, which have anti-inflammatory effects and antimutagenic effects (Martinovic et al., 2020).
- The *Staphylococcus* genus is abundant in all samples except for medaka gill and intestine, and is especially abundant in Dragonfish samples (Figure 1).
  - *Staphylococci* can produce biosurfactants, which reduces surface tension between two immiscible liquids and allows greater metabolism efficiency through increased reuptake (De Jesús Cortés Sánchez, A. et al., 2017).
  - *S. hominis* can produce lipase and contains genes that encode resistance to various antibiotics. (Kapusta et al., 2021).
- The *Cutibacterium* genus is also found in both fish (Figure 1).
  - *C. acnes* has a linoleic acid isomerase that produces conjugated linoleic acid, a chemical associated with enhanced immune defenses (Bruggemann et al., 2021).
  - Nevertheless, *C. acnes* and some other detected species likely originated from the human skin, and were probably contaminations introduced into the samples.
- While we did not detect bacteria that may directly help the dragonfish survive the extreme environment in the deep-sea, several identified species should provide benefit for their overall health and survival.

Study Limitations and Future Directions

- Due to the unexpectedly low reads of many samples, the bacteria detected may not be fully representative of the true microbiome.
  - Methodology should be further optimized to maximize the yield during library preparation.
- Some detected bacterial species are commonly found on human skin, and were likely introduced during dissection or DNA extraction.
  - Better sterile technique should be practiced in the future to minimize contamination.
- Only one dragonfish was tested, and multiple technical replicates were used from the same intestine sample.
  - More biological replicates should be tested to verify the patterns observed in our data

References

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