Streptococcus thermophilus, a probiotic bacteria, was detected in the intestine of deep-sea dragonfish

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Abstract

In many animals, gut microbiota play a critical role in digestion, homeostasis, and protection against pathogens. Deep sea is a unique environment where sources of nutrient is extremely rare; we thus expect the intestine of deep-sea fish to host bacteria that can produce specialized digestive enzymes with high energy-extraction efficiency. We dissected a deep-sea dragonfish and six fresh/brackish water medaka and isolated the bacterial DNA from their intestine samples. We performed Nanopore 16S rRNA gene sequencing and identified bacteria in the intestines of medaka and dragonfish. The composition of gut microbiome in the dragonfish is distinct from that in the medaka. One of the most abundant bacteria, *Streptococcus thermophilus*, is a probiotic bacteria commonly found in yogurt. This discovery leads us to believe that there are undiscovered bacteria within deep-sea organisms with characteristics potentially equal or greater in significance of contributing to host survival.

Introduction

Hypothesis:

- Deep-sea dragonfish contain unique microbiota capable of producing digestive enzymes with higher nutrient-extraction efficiency than medaka

Background:

Deep-sea environment (Jorgensen and Boetius, 2007; He et al., 2017)

- low oxygen
- high pressure
- low temperature
- low light
- limited nutrient availability

Gut microbiota are crucial to digestive functions of deep-sea fish (Ley et al., 2008; Nicholson et al., 2012)

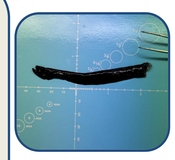
- Deep-sea fish should favor the growth of microorganisms providing support for their survival
  - For example, those producing enzymes that support metabolism or fluoresce (Collins, Fergus W.J. et al., 2021)

Dragonfish lives in the deep sea, while the medaka fish naturally inhabit small rivers and rice fields, which provide more sources of nutrient (Inoue and Takei, 2002)

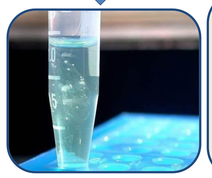
Methodology

Sample Collection and Fish Dissection

- One dragonfish was collected from ~650 m depth off Oregon coast
- Six medaka fish were collected from lab stock
- Isolated intestine, brain, stomach, aorta, kidney, skin, photophore, eyes, and tongue were stored at -20°C



Bacterial DNA Isolation



- Following bacterial DNA isolation protocol (Bruggeling et al.), the presence of fish DNA was minimized, and bacterial DNA was extracted

Polymerase Chain Reaction and Agarose Gel Electrophoresis

- Amplified 16S V4 region with Promega GoTaq® Green Master Mix and the following primers:

Forward 5'-GACGCTCTTCCGATCTTATGGTAATTGTGTGCCAGCMGCCGCGGTAA-3'

Reverse 5'-TGTGCTCTTCCGATCTAGTCAGTCAGCCGGACTACHVGGGTWTCTAAT-3'

- Checked PCR success with agarose gel electrophoresis

Nanopore 16S Library Preparation

- Followed library prep protocol (Oxford Nanopore Technologies)
- Annealed barcode sequences to sample DNA
- Ran PCR to amplify entire 16S gene

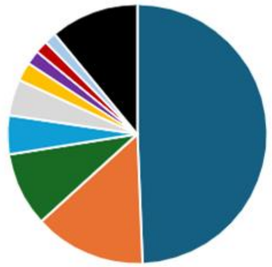
Nanopore DNA Sequencing and Data Analysis

- Prepared library was sequenced with Nanopore MinION, and sequencing data were processed with EPI2ME for species identification

Results

(A)

Bacteria abundance in medaka intestine sample 1



(B)

Bacteria abundance in medaka intestine sample 2

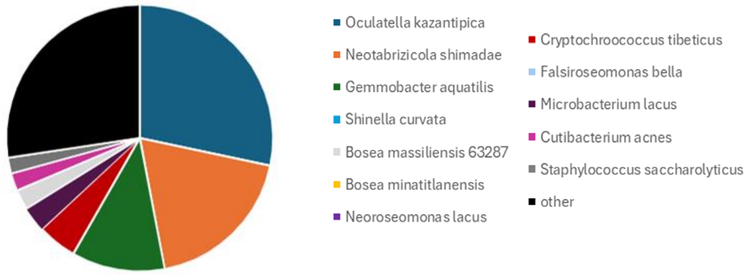
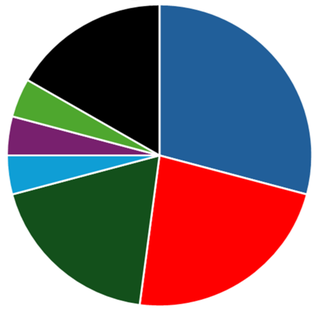


Figure 1: Abundance of bacteria in medaka intestine samples 1 (A) and 2 (B) based on number of 16S gene reads. *Oculatella kazantipica*, *Neotabrizicola shimadae*, and *Gemmobacter aquatilis* are the most abundant ones in both samples, making up over 50% of all bacteria identified.

(A)

Bacteria abundance in dragonfish intestine sample 1



(B)

Bacteria abundance in dragonfish intestine sample 3

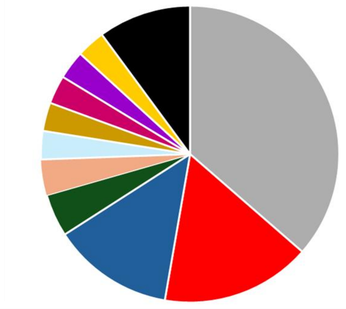


Figure 2: Abundance of bacteria in dragonfish intestine samples 1 (A) and 3 (B) based on number of 16S gene reads. dragonfish intestine samples 2 and 4 yielded too few reads and were not included. *Cutibacterium acnes*, *Staphylococcus saccharolyticus*, and *Streptococcus thermophilus* are among the most abundant species in both samples. Other relatively abundant bacteria include *Corynebacterium bouchesdurhonense* (4%) in sample 1 and *Staphylococcus hominis* (35%) in sample 3.

Discussion

We did not detect bacteria that produce digestive enzymes with high nutrient-extraction efficiency in the deep-sea dragonfish.

Our hypothesis is however partially correct, as the medaka and dragonfish do have drastically different intestine microbiome composition.

<u>*Bacteria unique to medaka intestine*</u>

- *Neotabrizicola shimadae*
  - Commonly found in the waters of Japanese hot springs
  - Produces multiple enzymes critical for digestion, including β-galactosidase, a lactose digestive enzyme; chymotrypsin, a protein and peptide digestive enzyme; and trypsin, a cysteine protease capable of breaking down aromatic amino acids (Muramatsu et al., 2022).

<u>*Bacteria unique to dragonfish intestine*</u>

- *Streptococcus thermophilus*
  - A probiotic bacteria key for reducing uremia, synthesizing metabolic end products, and reducing pathogenic bacteria infections (Martinović et al., 2020, Uriot et al., 2017).
  - Reduces indoxyl sulphate, a waste product contributor of uremia and secretes antagonistic compounds that attack potentially pathogenic bacteria harmful to the gut (Vitteta et al., 2019).

- Many of the abundant bacteria identified in the dragonfish intestine samples, such as *S. saccharolyticus* and *S. hominis*, likely originated from human skin or respiratory tract.
  - Sterile technique should be better practiced during future dissections and sample processing to minimize potential contamination.

Oculatella kazantipica and *Cutibacterium acnes* are the only two bacterial species shared between the two fish.

<u>*Bacteria in common*</u>

- *O. kazantipica* , a species of cyanobacteria, habitat soil, freshwater, and marine environments (Vinodagrova et al., 2017, Panou and Gkelis, 2022).
- *C. acnes* is commonly found on human skin, and is likely contamination introduced during the experiments.

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