Bacteria from the *Sphingomonas* Genus was Discovered in Deep Sea Fish, which Could Help Them Degrade Toxicants

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Abstract

Symbiosis is a type of mutualistic relationship in which two or more species benefit from each other. For example, the relationship between humans and the bacteria living in our digestive systems benefits both parties. These bacteria outnumber the human body cells by several magnitudes, and they support many of our vital functions. Similarly, deep-sea fish microbiota should also have a mutualistic relationship with their hosts. We extracted and purified bacterial DNA from deep-sea fish and sequenced their 16S rRNA gene to profile their gut microbiota. *Sphingomonas leidyi*, a bacteria capable of degrading a variety of toxic pollutants, is the most abundant bacteria in dragonfish and lanternfish. Using this data, we hope to peer through the mesopelagic veil, directly into the various tissues of deep-sea fish to find concrete evidence of symbiosis helping them survive their extreme environment.

Introduction

The deep Sea
- Harsh environment
- Lacks food (Collins et al. 2021)

Deep-sea Fish
- Thrive despite food scarcity
- Intestines nourish microbes (Collins et al. 2021)

Gut Microbiome
- Helps land vertebrates digest efficiently (Clarke et al. 2014)
- Present and may have similar functions in deep-sea fish

Hypothesis
- We will discover microbes that have biological functions that may help the deep-sea fish survive the harsh environment

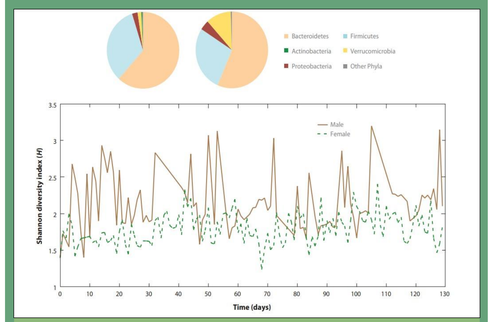
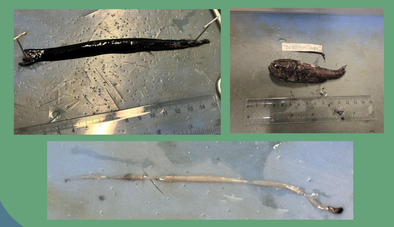
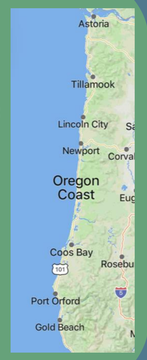


Figure 1: Intraindividual variation over time. Depending on the temporal scale, an individual's gastrointestinal microbiota can vary as measured using the overall community diversity measure, Shannon diversity (H). From "The human microbiome: from symbiosis to pathogenesis," by E.A. Eloe-Fadrosh & D.A. Rasko, (2013), *Annual review of medicine*, 64(1), DOI: 10.1146/annurev-med-010312-133513.

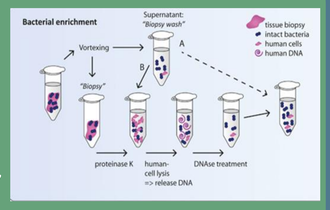
Methodology

Collection and Dissection
- Fish collected from 600-800 m deep water off Oregon coast
- Fish as identified based on morphology: Dragonfish (Stomiidae); lanternfish (Myctophidae); owlfish (*Pseudobathylagus milleri*)
- Fish dissected; Organs sorted and collected





Host depletion and bacterial DNA extraction
- Followed protocol outlined by Bruggeling et al.
- Fish tissue vortexted; digested with proteinase K, and fish DNA digested with DNase
- Bacteria treated with mutanolysin; heat-shocked, and lysed via bead beating



- Experimented with two kits for DNA purification: MO BIO Powersoil DNA Isolation kit and QIAGEN DNeasy Blood & Tissue kit

Library construction and Nanopore 16S sequencing
- Amplified 16S gene DNA were attached with barcode and adapter protein, then loaded onto MinION sequencer

Data analysis
- Sequences matched to species using EPI2ME
- Figures generated with R version 4.4.2 using the package ggplot2 version 3.4.0

PCR amplification & gel electrophoresis
- Tissue samples combined with DreamTaq Green master mix in PCR tubes; placed in thermal cycler following recommended cycles
- 16S rRNA gene samples amplified using primers provided by CosmosID
- CosmosID primers produced no results (primer dimers); different primers used in library prep
- Quality control step to check the presence of bacterial DNA

Results

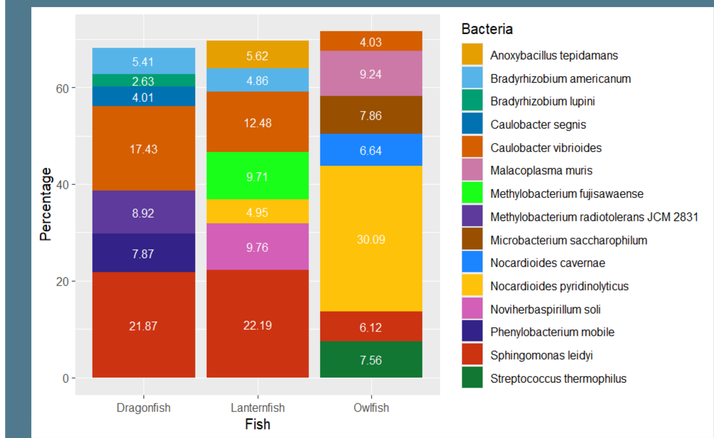


Figure 2: *Sphingomonas leidyi* is the most abundant bacteria in both dragonfish and lanternfish. Fish families were compiled from skin, intestine, and stomach samples from several individual fish using morphological features to determine identity. Figure shows top 7 most abundant bacteria with sample identity on the x-axis. Bar height represents percentage of total reads from each family, which is also labelled on each segment. *S. lediyi* and *C. vibroides* were the only bacteria present in all 3 families.

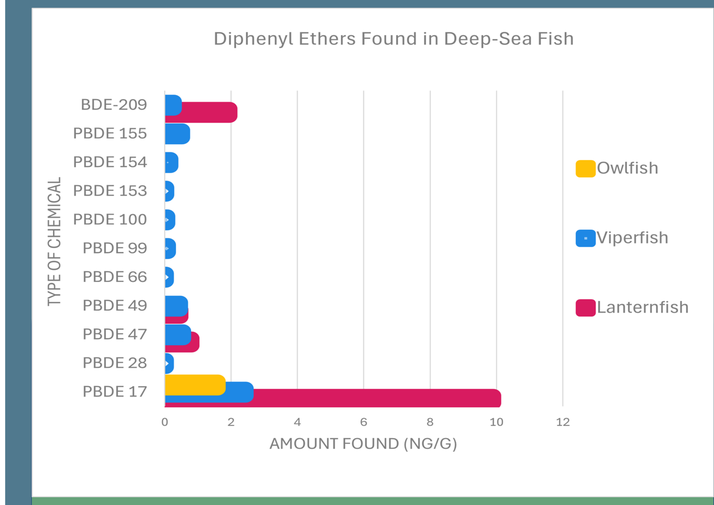


Figure 3: Diphenyl ethers were found in livers of deep-sea fish families. Bar graph shows the amount of chemical detected per gram of liver in fish groups determined by morphological features. Chemicals listed on y-axis are brominated flame retardants (BFRs) that were detected in any samples. N=4 for lanternfish, N=2 for owlfish and viperfish. Viperfish contained the most types of BFRs, while their total concentrations in lanternfish are the highest.

Discussion

- Our samples contain high levels of *Sphingomonas leidyi*
  - Bacterial species from the Sphingomonas genus can break down polybrominated diphenyl ethers (Schmidt et al. 1992a, b, c).
  - Diphenyl ethers were detected in liver samples from other fish from the same family.
  - *S. leidyi* may help break down pollutants in the fish's body.
  - Polybrominated diphenyl ethers belong to a group of environmental toxicants known as brominated flame retardants(BFRs)
- Several bacteria in the *Sphingomonas* genus also have the capacity to break down other harmful chemicals
  - Pesticides, like 2'4 DE, (Ka et al, 1994) Pentachlorophenol (PCP) (Karlson et al, 1995) Carbofuran (Ogram et al, 2000) which can cause environmental toxicity and reproductive dysfunction
  - Polycyclic Aromatic Hydrocarbons, (PAHs) (Dagher, 1997) some of which are linked to cancer and can end up in sediment
  - PAH metabolite gentisate (Huang et al, 2015)
- BFRs, PAHs, and pesticides can end up in deep sea environments through stormwater runoff and cause health issues in exposed organisms
  - If bacteria species in the fish can metabolize the harmful toxicants, their toxic potential in the fish should be lowered

Future Directions

- Investigate if *S. leidyi* can be used for bioremediation to break down persistent environmental toxicants, and examine their potential for cleaning up pollution in the deep sea.
- Confirm if *S. leidyi* and other Sphingomonas bacteria actively break down toxic chemicals in the deep-sea fish. A comprehensive correlation analysis between Sphingomonas abundance and toxic chemical abundance should be helpful.

Bibliography