Bacteria degrading toxic aromatic and sulfur compounds identified in Dragonfish (*Stomiidae*) near hydrothermal vents

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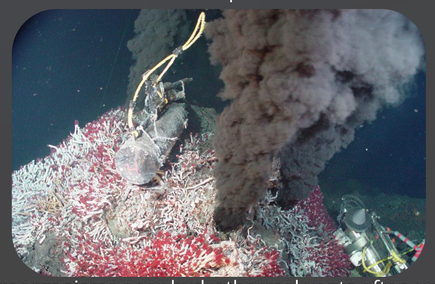
Abstract

Hydrothermal vents release toxic chemicals that can be harmful to the fish inhabiting the deep-sea, while many bacteria in the same area can metabolize these chemical for energy. The deep-sea fish may thus form a symbiotic relationship with bacteria that can degrade toxic chemicals and reduce the exposure levels in the host. We dissected a dragonfish living near a hydrothermal field and isolated bacterial DNA. We performed 16S rRNA gene sequencing and identified bacteria that can metabolize toxic chemicals, such as aromatic and sulfur compounds.

Introduction

Hypothesis - Dragonfish (*Stromiidae*) living near hydrothermal vents have unique microbiome composition that help them survive in the harsh deep-sea environment.

- Deep-sea environment is extreme and harsh compared to surface waters.[1]
  - -low temperatures
  - -low oxygen supply
  - -low light
  - -high water pressure
  - -toxic chemicals from hydrothermal vents
    - When hot, metal-infused fluids are released from the vents , toxic compounds can form from sulfur, nitrogen, iron, calcium, and diffuse into the deep-sea water[2]

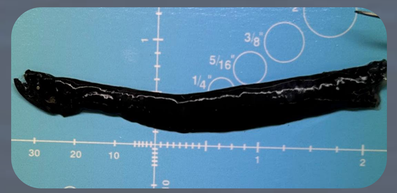


- Microorganisms near hydrothermal vents often use the released mixture as a source of energy through chemosynthesis.
  - Many of these microbes cannot thrive in the cold deep-sea waters on their own, and they often rely on a host for support.[3]

Methodology

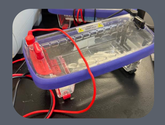
Fish collection - An unknown species of *Stomiidae* was obtained via net trawling off the coast of Portland, Oregon at 44.652, -126.05 at about 650 meters below sea level.

Dissection - The dragonfish was dissected to extract tissues and organs, which were stored at -20°C until DNA extraction .



DNA extraction - Bacterial DNA was extracted from isolated tissues using the protocol provided described in Bruggeling et al. to remove most of the fish cells and DNA. [4] Isolated bacterial DNA were stored at -80°C.

Polymerase chain reaction (PCR) and agarose gel electrophoresis (AGE) - Promega GoTaq Green Master Mix, [5] and the following primers were used to amplify the 16S V4 region of the extracted DNA
Forward 5'-GACGCTCTTCCGAT CTTATGGTAATTGTGTGCCAGC MGCCGCGGTAA-3'
Reverse 5'-TGTGCTCTTCCGAT CTAGTCAGTCAGCCGGACTAC HVGGGTWTCTAAT-3'



Agarose gel electrophoresis was performed to verify PCR results

Nanopore 16S sequencing – 16S 24 V14 kit was used for library prep [6]; sequencing was performed on MinION, and sequencing data were processed with EPI2ME

Results

Figure 1. Locations of hydrothermal vents and dragonfish collection site off the coast of Oregon.
The blue pin indicates the location of where the dragonfish was collected (44.652, -126.05). White pins indicate the locations of all other hydrothermal vents in the area. The hydrothermal vent field closest to the sampling location is at the East Blanco Depression (44.217, -129.63).

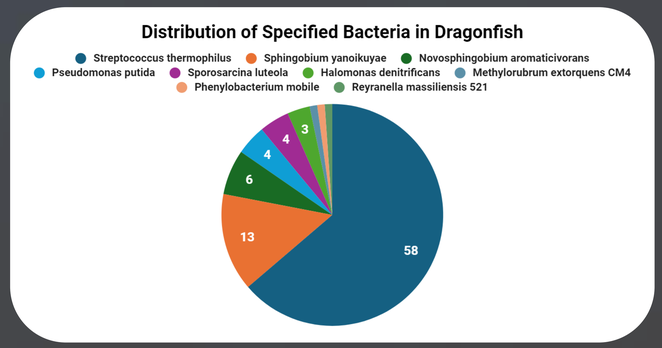


Figure 2. Bacterial species that can protect the dragonfish from harmful substances produced by hydrothermal vents.
Each slice represents a bacterial species, with slice sizes proportional to their relative abundance within the samples. The number indicate the total read of each bacteria species.

References

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Discussion/Conclusion

- Many hydrothermal vents are located near the coast of the Pacific Northwest.
  - The East Blanco Depression hydrothermal field [6] is very close to the location where the dragonfish was collected (Figure 1).
- Multiple dragonfish species are known to travel from deeper (bathypelagic) to more shallow waters (mesopelagic) when hunting for prey.[7, 8]
  - Water in the deep sea contain chemicals (such as hydrogen sulfide and methane) that could harm the dragonfish.[3, 9]
- **We detected bacteria from the dragonfish that may protect the fish from these agents**

<u>*Metabolism of toxic sulfur compounds*</u>

- *Phenylobacterium mobile:* utilizes the chloridazon, antipyrin, and linear alkylbenzene sulphonate (LAS) as sole carbon sources.[10]
- *Reyranella massiliensis:* contains genes involved in encoding for sulfur metabolism and metabolism of aromatic compounds.[11]
- *Streptococcus thermophilus:* capable of breaking down and neutralizing volatile sulfur compounds.[12]
- *Sphingobium yanoikuyae:* uses biohazardous saturated hydrocarbons and aromatic compounds as sole carbon sources.[13]
- *Pseudomonas putida:* utilizes sulfhydration for synthesis of cysteine and homocysteine.[14]

<u>*Metabolism of toxic aromatic compounds*</u>

- *Methylorubrum extorquens:* able to degrade aromatic compounds; utilizes methanol, acetate, and succinate as a carbon source.[15]
- *Novosphingobium aromaticivorans:* metabolizes a wide range of aromatic compounds.[16]

<u>*Metabolism of other toxic chemicals*</u>

- *Sporosarcina luteola:* capable of sequestering toxic metals and reducing oxidation of residual sulfides.[17]
- *Halomonas denitrificans:* found near deep-sea hydrothermal vent chimneys. Known to reduce dinitrogen gas.[18, 19]

- The characterized dragonfish microbiome could protect the dragonfish by degrading toxic substances in the deep sea. Other deep-sea fish near hydrothermal vents could be examined to confirm if this is a common symbiotic relationship.