Pollutant-degrading bacteria identified from viperfish gut via 16S rRNA sequencing

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Abstract

Polycyclic aromatic hydrocarbons (PAHs) are a class of toxic chemicals that occur naturally in coal, crude oil, and gasoline, and become released upon combustion. PAHs have been found to bind to sediments suspended in the water. To determine if there are symbiotic bacteria that may protect deep-sea fish from toxic effects of PAHs, we dissected viperfish stomach and intestine, and isolated total DNA for species identification. We amplified the 16SrRNA, a commonly used barcoding gene for bacteria identification, and discovered bacteria that are known to break down PAHs, among other pollutants. These bacteria could be potentially used for bioremediation in polluted deep-sea environment.

Introduction

Hypothesis:
- Pollutant degrading bacteria symbiotically reside in viperfish gut microbiome.

Polycyclic Aromatic Hydrocarbons (PAHs)
- PAHs are toxic chemicals often released to the environment when burning organic material, as gas or attached to particles. [1]
- Can cause cancer in humans. [2]
- Can cause hepatic lesions and reproductive impairment in fish. [3]
- PAHs have been detected in the deep ocean.

The Gut Microbiome
- The gut microbiome has been found to reinforce digestive and immune systems. [4]
- Bioluminescent symbiont bacteria can emit light in the photophores.

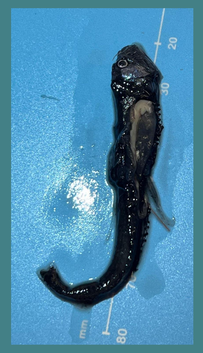
Viperfish
- Viperfish are any species of fish in the genus *Chauliodus*. They are deep-sea fish that reside 250-5000 meters under the sea. [5]

The 16S Gene
- The 16S ribosomal RNA is the RNA component of the 30S subunit of a prokaryotic ribosome (SSU rRNA).
- Often used in reconstructing phylogenies via barcoding, due to slow evolution rates of the sequence.

Methodology

Sample Collection and Processing

- Samples were collected off the coast of Oregon (Latitude DD: 44.652, Longitude DD: -126.05)
- Dissected viperfish and removed intestines and stomach
- Stored at -80°C until DNA extraction



DNA Extraction

- QIAGEN DNeasy Blood & Tissue Kit
- Followed manufacturer's procedure

Nanopore Sequencing Library Preparation

- 16S gene amplified during library prep
- Samples sequenced on MinION Mk1B

Sequencing Data Analysis

- EPI2ME data analysis software
- Fastq 16S and Fastq WIMP workflows

EPI2ME

Results

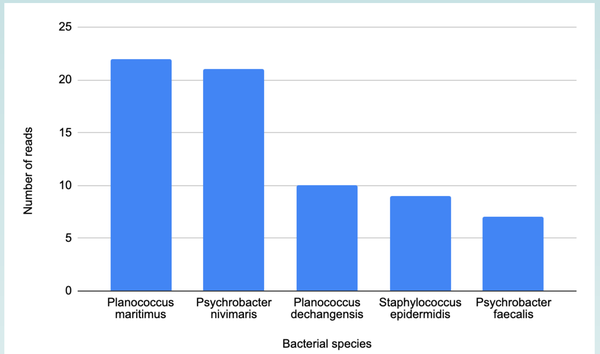


Figure 1. Five most abundant PAH-degrading bacteria detected in viperfish stomach and intestine samples.

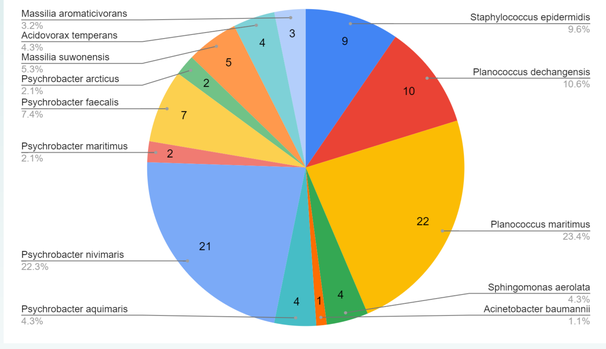


Figure 2. A total of thirteen PAH-degrading bacteria species were identified.

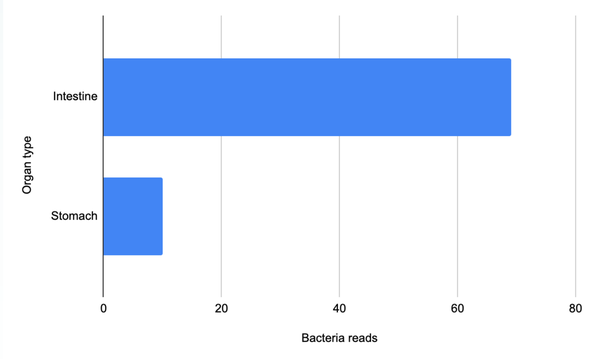


Figure 3. The PAH-degrading bacteria are more abundant in intestine than in stomach.

Discussion & Conclusion

The identification of pollutant-degrading bacteria in our samples suggest that bacteria may help protect viperfish from the negative effects of PAHs in the environment.

- The most abundant PAH-degrading bacteria species are *Planococcus maritimus* with 22 reads and *Psychrobacter aquimaris,* with 21 reads (Figure 1).
- Psychrobacter are often found in cold environments such as the deep sea.
- Many of the detected Psychrobacter species are known to break down PAHs, including *P. arcticus*, *P. faecalis, P. maritimus*, *P. nivimaris*, and *P. aquimaris* (Figure 2).
- *P. nivimaris* has hydrolytic capabilities and have been found to reduce perchlorate, another environmental pollutant, in marine sediments.
- The majority of the PAH degrading bacteria were found in the intestine (Fig. 3). Their high abundance in the gut microbiota may protect viperfish from the toxic effects of PAH exposure.

Considering pollutants such as PAHs are contaminating the deep-sea environment, the bacteria species identified in this study are also candidates for bioremediation.

Limitations & Future Directions

- Only four viperfish were used. More samples should be used to verify the results and may provide additional gut microbiome information.
- The dataset can be further explored to identify bacteria capable of degrading other pollutants in the deep sea.
- During DNA extraction, all bacteria were lysed. Future experiments can focus on isolating and culturing identified pollutant-degrading bacteria species and test their efficiency/potential for bioremediation.

Citations