Identification of Two Bacterial Species Based on Their Ability to Bind and Metabolize Motor Oil

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Introduction

- Since the second industrial revolution, the petroleum industry has heavily influenced the world in both ways: good and bad.
- Contamination from petroleum products from processing crude oil can be physically and chemically damaging to the ecosystem (Chaudhary & Kim, 2019)
- Cleaning oil-contaminated sites is a difficult and complicated procedure due to many factors. Especially because the composition of oil includes a variety of molecular weights of hydrocarbons (Leahy et al., 1990).
- Bioremediation is an alternative way that is cheap, environmentally friendly, efficient and highly specific for restitution of oil-contaminated sites (Atlas, 1995; Margesin & Schinner, 2001).
- Some microorganisms can thrive in this oil-contaminated environment by producing surfactants (Margesin & Schinner, 2001). Biosurfactants can emulsify and reduce surface tension for bacteria to bind and metabolize the hydrocarbons (Uzoigwe, 2025).
- The goal of this project is to identify bacterial species based on their ability to bind and metabolize motor oil.

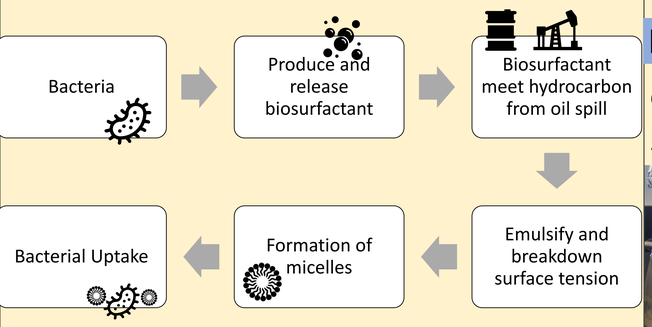

Figure 1: How bacteria metabolize oil

Purpose

To identify bacterial species that have ability to bind and metabolize oil.

Material and methods

- The Bacteria taken from the bacterial collection of Derkson et al. (2021) were chosen for their ability to bind and metabolize motor oil.
- Cultures were grown in Bushnell Haas Brotyh (BHB) with motor oil.
- Genomic DNA was isolated using a Sigma GeneElute™ Bacterial Genomic DNA Kit.
- 5 µL of genomic DNA was loaded into 0.8% (w/v) agarose gel. Lambda *Hin*d III digested DNA was used as a standard.
- PCR of 16S rRNA was performed. The primers used in PCR were also from Derkson et al. (2021).
- 5 µL of PCR products were loaded into 1.5% (w/v) agarose gel. Quick load® 100bp DNA ladder was used as a standard.
- Serial dilutions were performed to investigate the growth rate of the bacteria in BHB.

General workflow

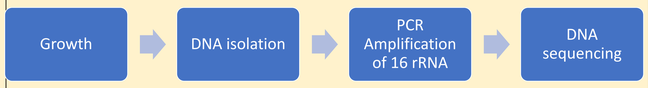

Figure 2: General workflow of the project

Results

Growth of bacterial culture in BHB with motor oil

A B

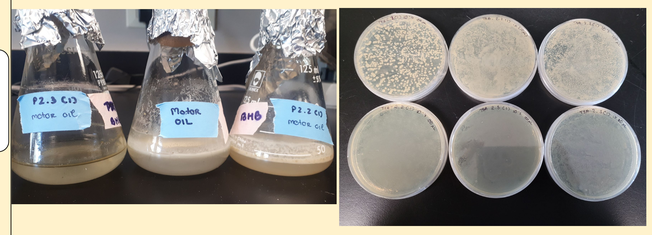

Figure 3:

A. Growth of bacterial species in 25 mL BHB with 1.0mL of motor oil
B. TSA plates showing results of a serial dilution of the bacterial cultures in BHB with 1.0mL of motor oil

Genomic DNA isolation

1 2 3 4 5

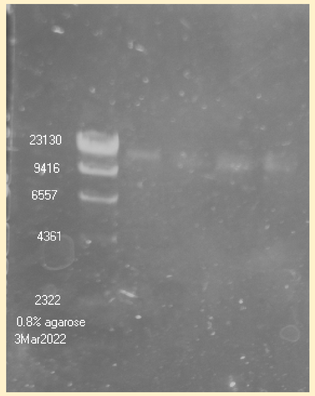

Figure 4:
Lane 1: Lambda DNA/ HindIII standard
Lane 2: 2.2(1) old
Lane 3: 2.2(1) new
Lane 4: 2.3(1) old
Lane 5: 2.3(1) new

The isolated genomic DNA was of high quality and was used for PCR.

PCR amplification of the first 500 bp of 16s rRNA

1 2 3 4 5

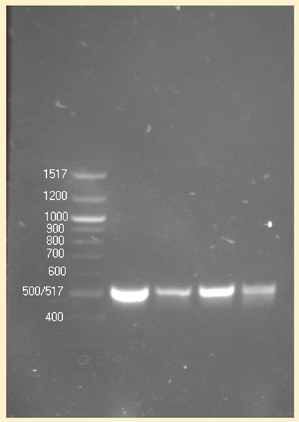

Figure 5:
Lane 1: Quick-load 100bp DNA ladder
Lane 2: 2.2(1) old
Lane 3: 2.2(1) new
Lane 4: 2.3(1) old
Lane 5: 2.3(1) new

Analysis of the PCR products showed them to be of the correct molecular weight.

The PCR samples have been submitted for DNA sequencing and identification of the isolates

Discussion

- The bacterial species P2.2(1) and 2.3(1) grow well in the BHB with motor oil as the sole source of carbon.
- The cultures displayed different grow rates:
  - In BHB with motor oil, P2.3(1) had high grow rate in week 1, 2.2(1) had high grow rate in week 2. (Derksen et al. 2021).
  - In TSA and TSB, P2.3(1) grew faster than P2.2(1) in TSB, but P2.2(1) grew faster than P2.3(1) in TSA.
- Genomic DNA isolation resulted in a high-quality DNA samples and PCR products have been sent for sequencing in order to identify the bacterial species.
- Overall, we successfully isolated genomic DNA and amplified the first of 500bp of the 16S rRNA gene of P2.2(1) and P2.3(1).

Future Research

The data shows some potential for future research:

- The observation showed two bacterial species potentially have different mechanism to survive in motor oil (Figure 3). Previous data showed that P2.2(1) has the ability to produce biosurfactant (Makkar et al., 202(1). This suggests a study on whether P2.3(1) produces biosurfactant, and if not. what mechanism P2.3(1) uses to grow well and metabolize motor oil.
- The DNA sequence and identification of the isolates may suggest further research on these bacteria, including the determination of what genes are responsible for the metabolism of motor oil

Acknowledgement

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