

Methylotrophic Bacteria

Research Question

Methylotrophic bacteria are a special type of bacteria that can live off simple carbon chemicals like methanol or methane, things most other organisms can't use for energy. They're found almost everywhere, including soil, lakes, oceans, and even on plant leaves, which is what we used for this project. In this project we will be focusing **if we can isolate Methylotrophic bacteria from live leaves.**

Project Design

The objective of this project was to take pink bacteria from the surface of leaves and follow them through a full, step-by-step process to grow and study them. Through a full series of lab techniques. We used methods such as leaf printing, culturing, DNA extraction, PCR, and gel electrophoresis to move from a natural sample to a clear picture of the bacteria's DNA.

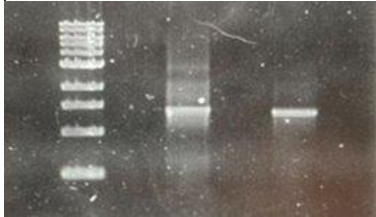
Data Analysis & Results

After running the gDNA in a gel, we were successfully able to see results from 2 samples. We then sequenced these samples using a nucleotide blast program and were able to identify one of our results as a Methylotrophic bacteria.

Conclusions

We were able to successfully isolate methylotrophic bacteria from one of our leaf samples, which was a big accomplishment for our experiment. Even though we started with six different leaves each, only one of them ended up growing the bacteria we were looking for. This isn't surprising, because growing microbes in the lab can be very difficult, most microorganisms don't grow well outside their natural environment.

1kb B1 B2 B3 Z1

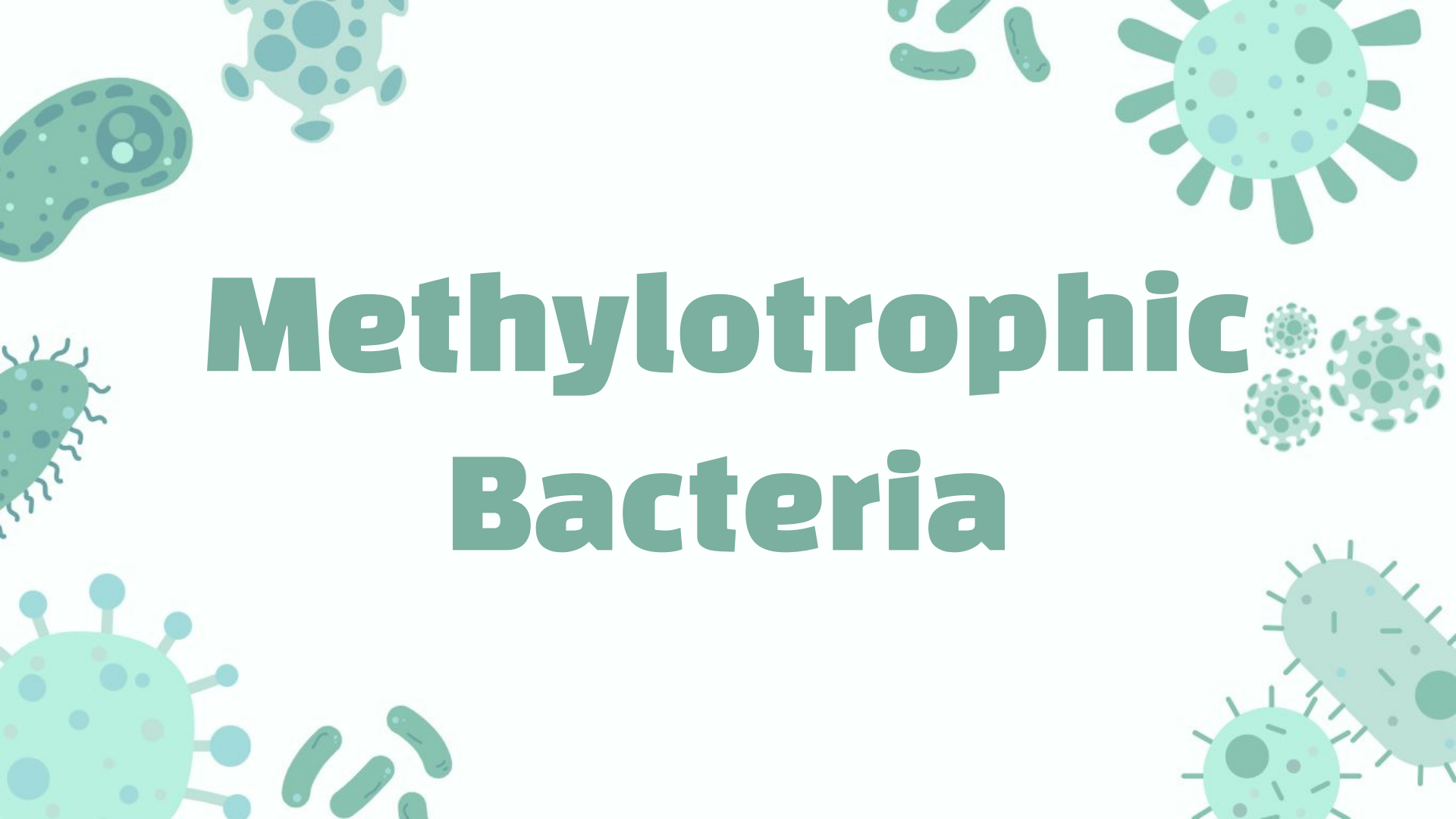


Sequences producing significant alignments

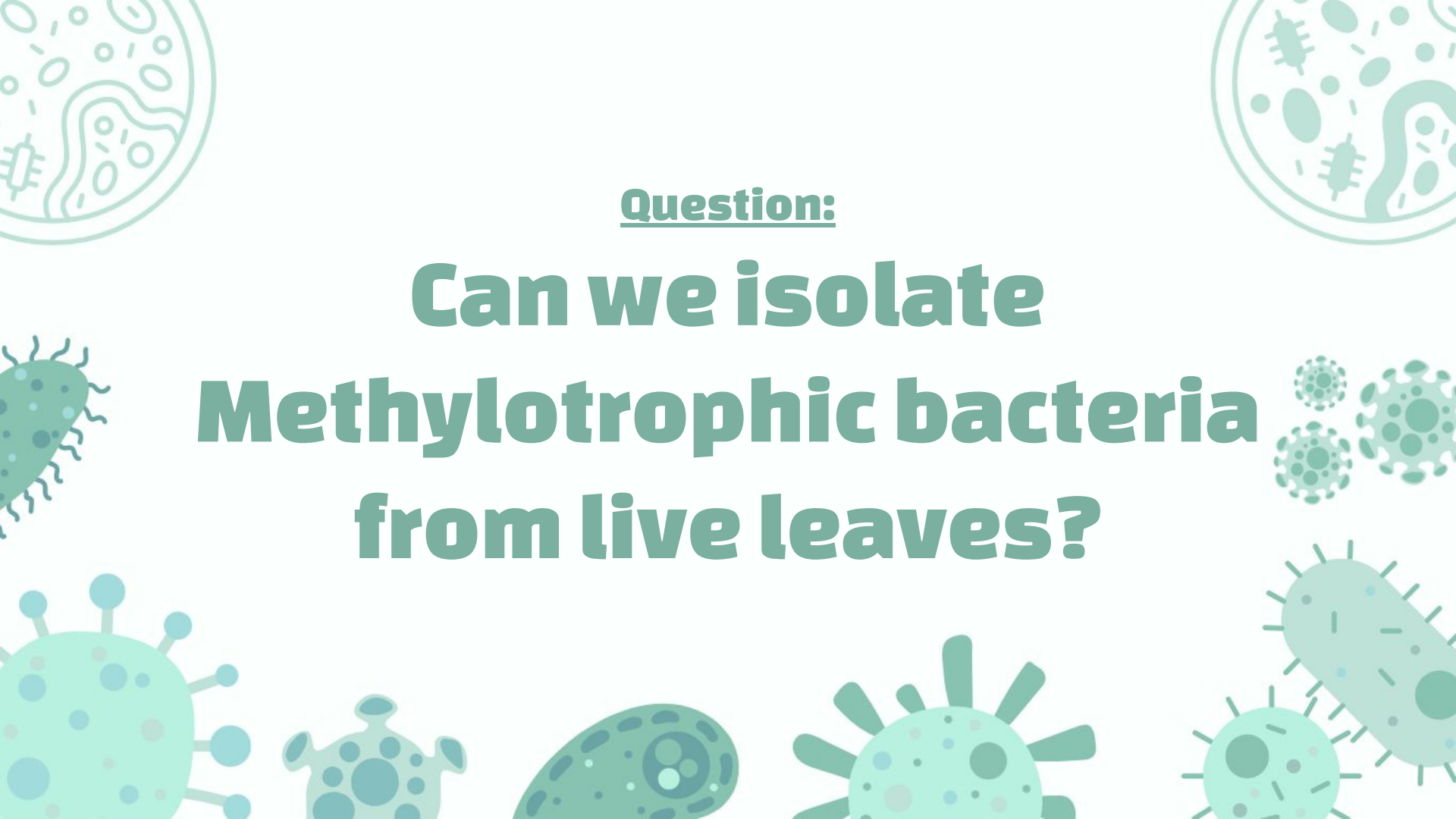
Description	Score	Expect	Query	%	Pos	Acc
Uncultured bacterium clone G1452212.1 (JGI) (Methylobacterium sp. strain J1452212.1)	153	100	100%	100%	100	100%
Uncultured bacterium clone G1452212.1 (JGI) (Methylobacterium sp. strain J1452212.1)	153	100	100%	100%	100	100%
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Methylobacterium sp. strain J1452212.1 (JGI) (Methylobacterium sp. strain J1452212.1)	153	100	100%	100%	100	100%
Methylobacterium sp. strain J1452212.1 (JGI) (Methylobacterium sp. strain J1452212.1)	153	100	100%	100%	100	100%

The background of the slide is white and decorated with various stylized green microorganisms. These include several rod-shaped bacteria, some with flagella, and several spherical viruses with spikes or surface proteins. The organisms are scattered around the central text, with some appearing larger and more detailed than others.

Methylotrophic Bacteria

The background of the slide is decorated with various stylized illustrations of microorganisms. In the top left and top right corners, there are petri dishes containing a mixture of different bacterial shapes, including rods and cocci. Along the bottom edge, there are several individual bacterial forms: a large, spiky, spherical bacterium on the left; a smaller, multi-lobed bacterium; a smooth, oval-shaped bacterium; a bacterium with long, radiating flagella; and a large, elongated rod-shaped bacterium with many short flagella on the right.

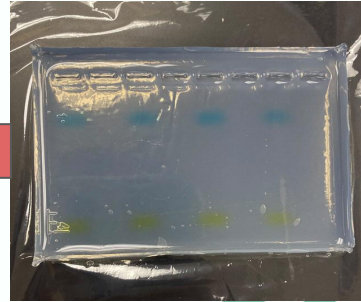
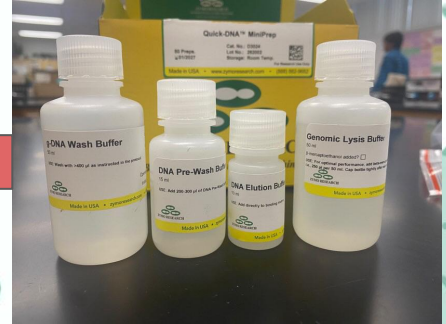
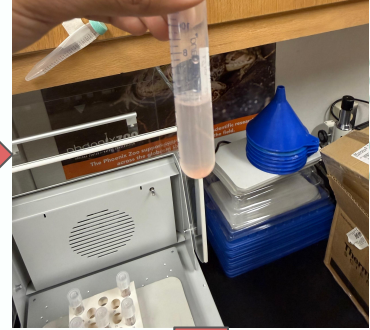
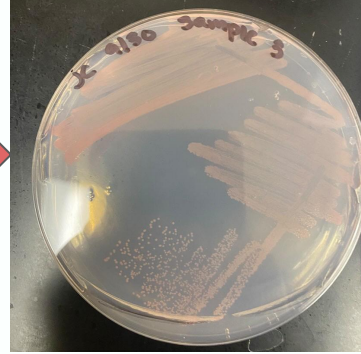
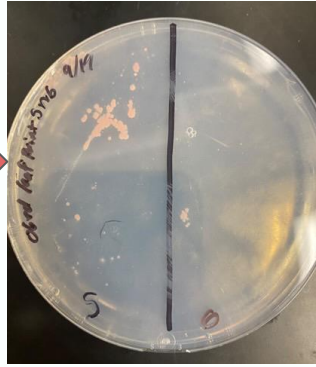
Question:

**Can we isolate
Methylophilic bacteria
from live leaves?**

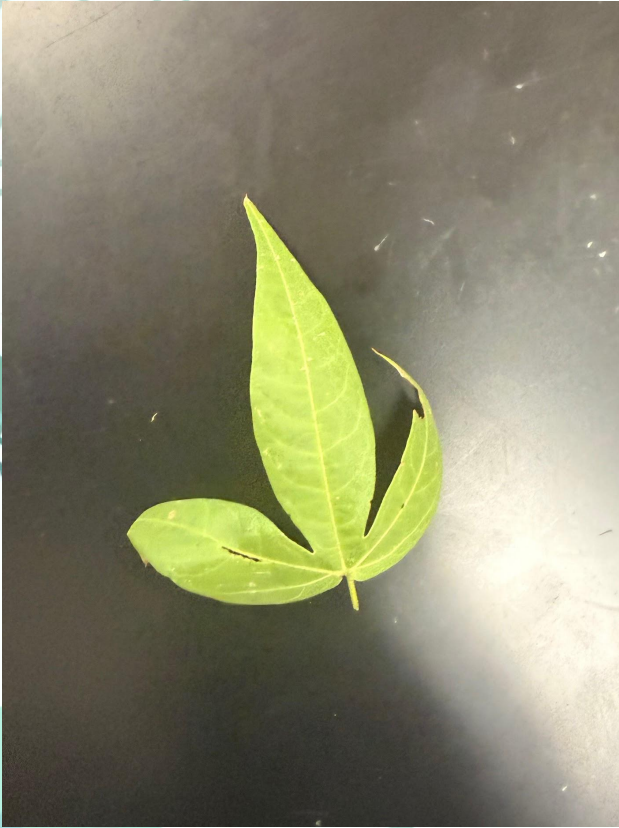
What are Methylophilic bacteria?

Methylophilic bacteria are a special type of bacteria that can live off simple carbon chemicals like methanol or methane, things most other organisms can't use for energy. They're found almost everywhere, including soil, lakes, oceans, and even on plant leaves, which is what we used for this project. What makes them stand out is that they have enzymes that let them break down these gases and chemicals, which helps clean up the environment and reduce things like methane, a strong greenhouse gas. Because they can live in many places and use unusual energy sources, scientists also study them for things like pollution cleanup and eco-friendly technology.

Project Pipeline

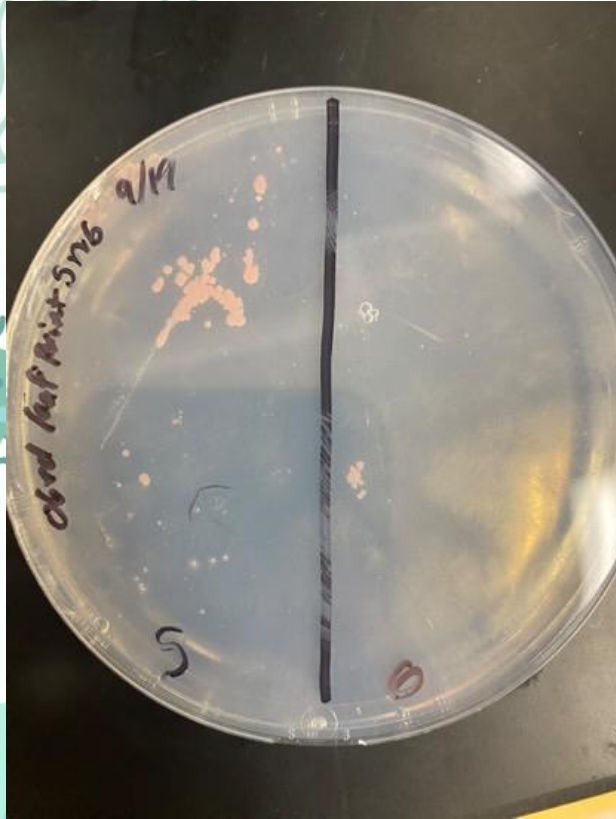


Leaf Print Pt.1



- Leaf printing is the first step of our project
- We leaf print to see which bacteria can grow on leaf surfaces
- After leaf printing them on the selective media plates, it took around 2-3 weeks until we saw some pink colonies

Leaf print Pt.2



- We noticed that after leaf printing some plates had more pink colonies than others
- Some plates had no colonies at all
- We leaf print so we can isolate specific bacteria from the leaf surface and culture it on selective agar plates
- We are looking for individual pink colonies! (No fungal growth)

Subculture

- After leaf printing and identifying individual colonies, we do a process called subculturing
- Why do this? To isolate only the pink colonies and pick a random lucky lonely pink colony to use for our research.



Liquid Culture



- We did liquid cultures - which was selecting one pink colony and putting it into selective liquid media
- We let the liquid culture grow so we could extract genomic DNA (gDNA) later on
- Liquid cultures are another way to grow up a bacterial strain - they grow in a solution rather than on an agar plate

gDNA extraction



- To do gDNA Extraction, we started with a little cell pellet and followed the Zymo Quick-DNA kit
- Genomic Lysis Buffer was used to open the cell of the pink bacteria and it helps break down the cell membrane
- A plastic column tube with a cotton disc was used to help separate all the cellular components - like a sieve!
- gDNA Wash Buffer was used to get rid of the salts and unwanted cellular debris so that only gDNA remained
- DNA Elution Buffer was used to release the gDNA from the cotton disc and suspend it in solution

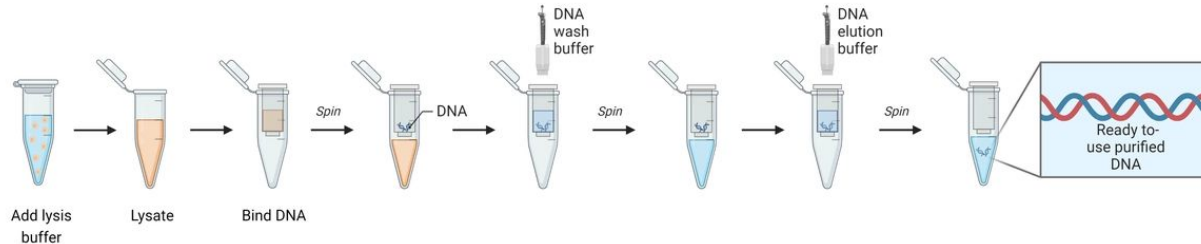
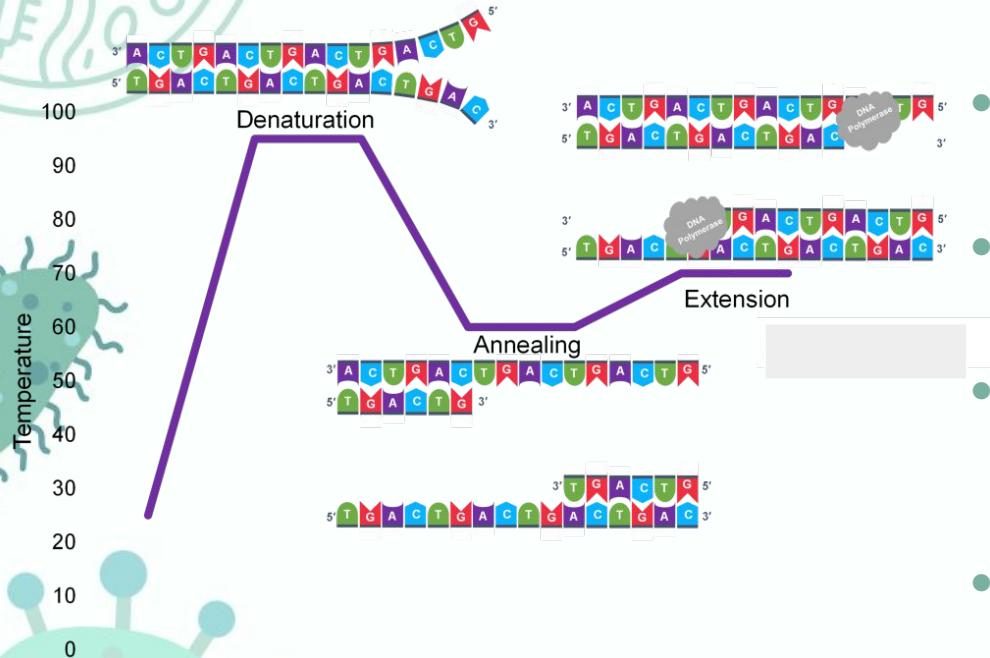


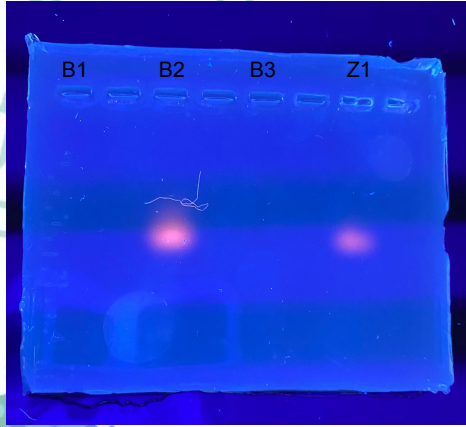
Image citation: [Overview of DNA Extraction Methods | AAT Bioquest](#)

PCR

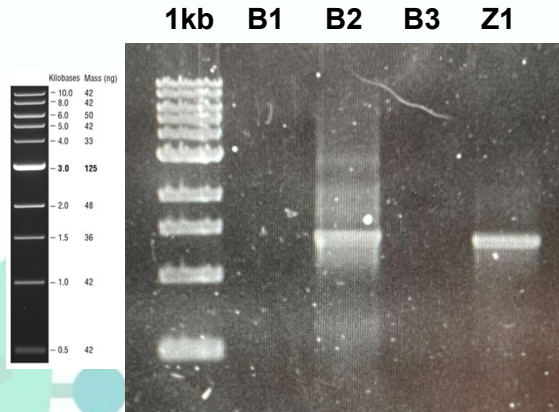


- PCR was used to amplify the 16S rRNA gene so we could get enough DNA to work with and analyze.
- The purpose of PCR was to make many copies of this gene so we could sequence it and determine what bacteria we had.
- PCR allowed us to check if our colony was a methylotroph by giving us enough DNA to run BLAST and match it to known bacterial species in databases.
- Without PCR, the bacterial DNA would be too small in quantity to analyze properly, so PCR made the identification step possible.

Gel Electrophoresis



- We plugged in the samples into a 1.2% gel at 100V for 25 mins - this allowed us to check the +/- and size of each sample
- Only B2 and Z1 showed up in the gel because they had 16S rRNA gene (the DNA)
- B1 and B3 did not have any DNA present so they didn't show up on the gel



Z1

Sequences producing significant alignments

Download Select columns Show 100

☒ select all 100 sequences selected[GenBank](#) [Graphics](#) [Distance tree of results](#) [MSA Viewer](#)

Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
☒ Methylobacterium sp. strain IIRRSV22-5 16S ribosomal RNA gene, partial sequence	Methylobacterium sp.	1267	1267	100%	0.0	100.00%	1309	QP748943.1
☒ Methylobacterium sp. strain BA 16S ribosomal RNA gene, partial sequence	Methylobacterium sp.	1267	1267	100%	0.0	100.00%	989	OR208701.1
☒ Methylobacterium oryzae strain LH2N24 16S ribosomal RNA gene, partial sequence	Methylobacterium oryzae	1267	1267	100%	0.0	100.00%	1377	OP080820.1
☒ Methylobacterium sp. strain 391_Methyba2 16S ribosomal RNA gene, partial sequence	Methylobacterium sp.	1267	1267	100%	0.0	100.00%	1371	QI798007.1
☒ Methylobacterium radiotolerans strain HP17.1 16S ribosomal RNA gene, partial sequence	Methylobacterium radiotolerans	1267	1267	100%	0.0	100.00%	1362	MK968397.1

B2

Sequences producing significant alignments

Download Select columns Show 100

☒ select all 100 sequences selected[GenBank](#) [Graphics](#) [Distance tree of results](#) [MSA Viewer](#)

Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
☒ Uncultured bacterium clone C04_MSD120 16S ribosomal RNA gene, partial sequence	uncultured bact...	355	355	63%	7e-93	78.99%	850	JQ357778.1
☒ Parafilimonas rhizosphaerae strain T16E-198 16S ribosomal RNA, partial sequence	Parafilimonas r...	353	353	54%	2e-92	80.71%	1466	NR_157628.1
☒ Uncultured bacterium clone MRD120_C08 16S ribosomal RNA gene, partial sequence	uncultured bact...	350	350	54%	3e-91	80.81%	851	JQ357442.1
☒ Uncultured bacterium clone MSD18_D08 16S ribosomal RNA gene, partial sequence	uncultured bact...	350	350	54%	3e-91	80.81%	858	JQ357622.1
☒ Chitinophaga sp. MJM 38 16S ribosomal RNA gene, partial sequence	Chitinophaga s...	350	350	54%	3e-91	80.81%	1515	KF534707.1

Sequencing

- For Z1, very sequence is labeled *Methylobacterium* sp., *Methylobacterium oryzae*, *Methylobacterium radiotolerans*, *Methylobacterium tardum*, etc, which are classic pink-pigmented methylotrophic bacteria
- The BLASTn also shows that we have a 100% Query Cover, 100% Percent Identity, and an E-value = 0.0, which means our 16S rRNA sequence is essentially a perfect match to *Methylobacterium* species.
- Our B2 sample did not match *Methylotrophs* well, showing hits like *Chitinophaga*, *Uncultured bacterium* clone, and *Flexibacter* which aren't methylotrophic bacteria, it comes to show that this particular sample wasn't a methylotroph colony.

Data and observations

- Only 2 of 4 samples had positive results on the gel
- Both samples were from the same field place (the Ag center)
- We believe the reason 2 of our samples didn't appear on our gel was because the lysis buffer wasn't able to properly break the cell membrane and get to the gDNA

Conclusion

We were able to successfully isolate methylotrophic bacteria from one of our leaf samples. Even though we started with six different leaves each, only one of leaves was able to be cultured, growing microbes in a lab setting can be extremely challenging. Only about 2% of all microbes on Earth can actually be cultured in a laboratory. Thankfully, by going through each step of the process, culturing the bacteria, extracting their gDNA, running PCR, and finally sequencing the 16S rRNA gene, we were able to confirm that this sample matched known *Methylobacterium* species. This shows us that our methods worked and that leaf surfaces can vary a lot in the types of bacteria they carry.