

The isolation and characterization of a novel species, *Bradyrhizobium cervisia*, from a shelf-stable beer.

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INTRODUCTION

Within the brewing industry, microbes are the lifeline of any brewing process with the most known microbe being *Saccharomyces cerevisiae*. However, there are other microbes that are often found within the brewing process. Many brewers have been examining the role of bacterial species in the brewing process, and their effect on their products. Bacterial presence can be both a positive and a negative within beers, as some may produce desirable flavors while some can create off-flavor compounds that will compromise their products.

Within this research, we aim to understand the microbial makeup of different shelf stable beers; however, through our work we were able to potentially discover a novel species of bacteria in the genus *Bradyrhizobium*. We can determine this through multiple phenotypic tests, 16s rRNA analysis and Whole Genome Sequencing.

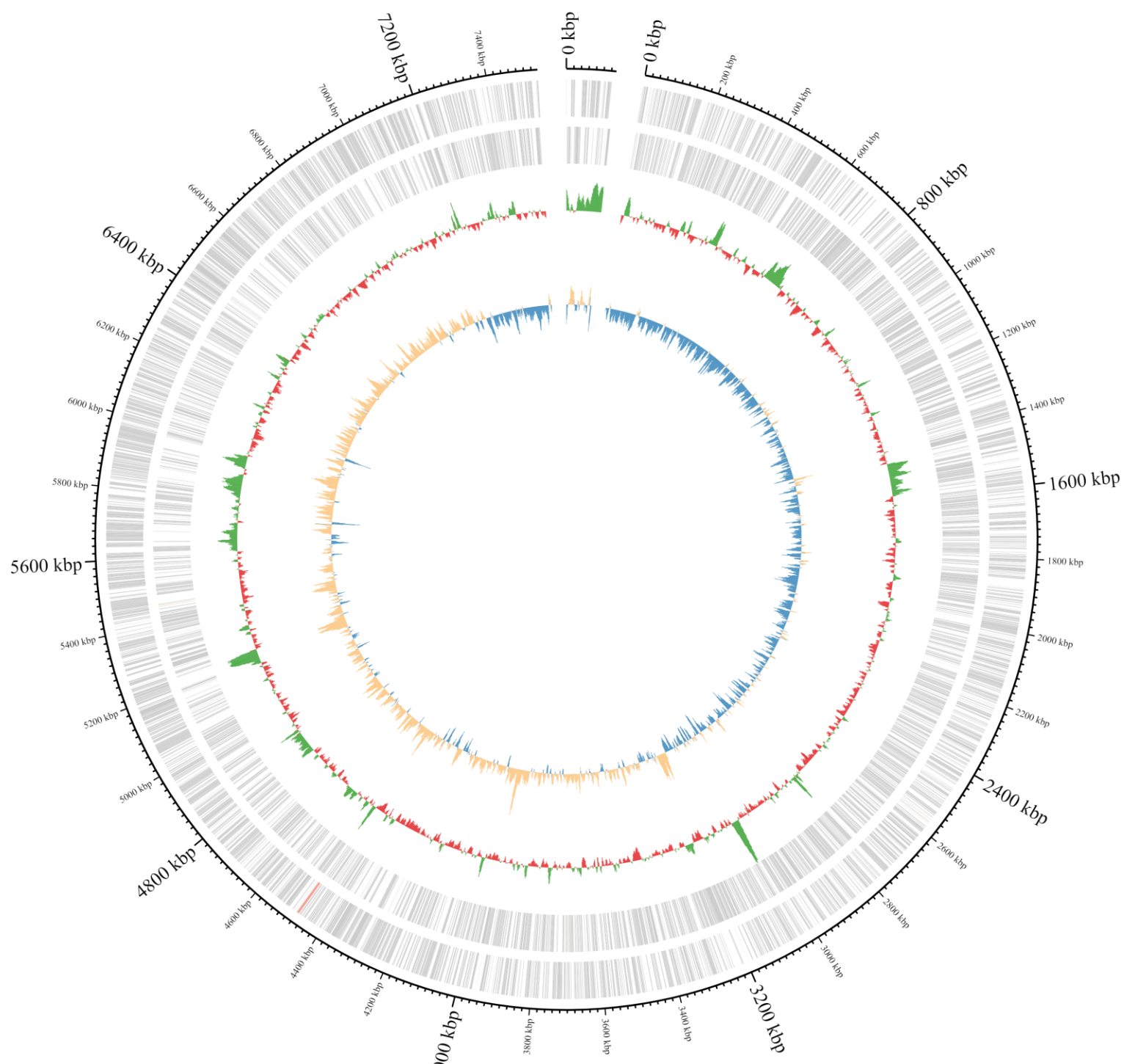
Bradyrhizobium as a genus is commonly found within soil environments and is a common nitrogen fixer within the environment.¹ It is commonly found around legumes but can also be seen living in soil with wheat growing in it.² This provides an insight into why this species may be found in a beer.



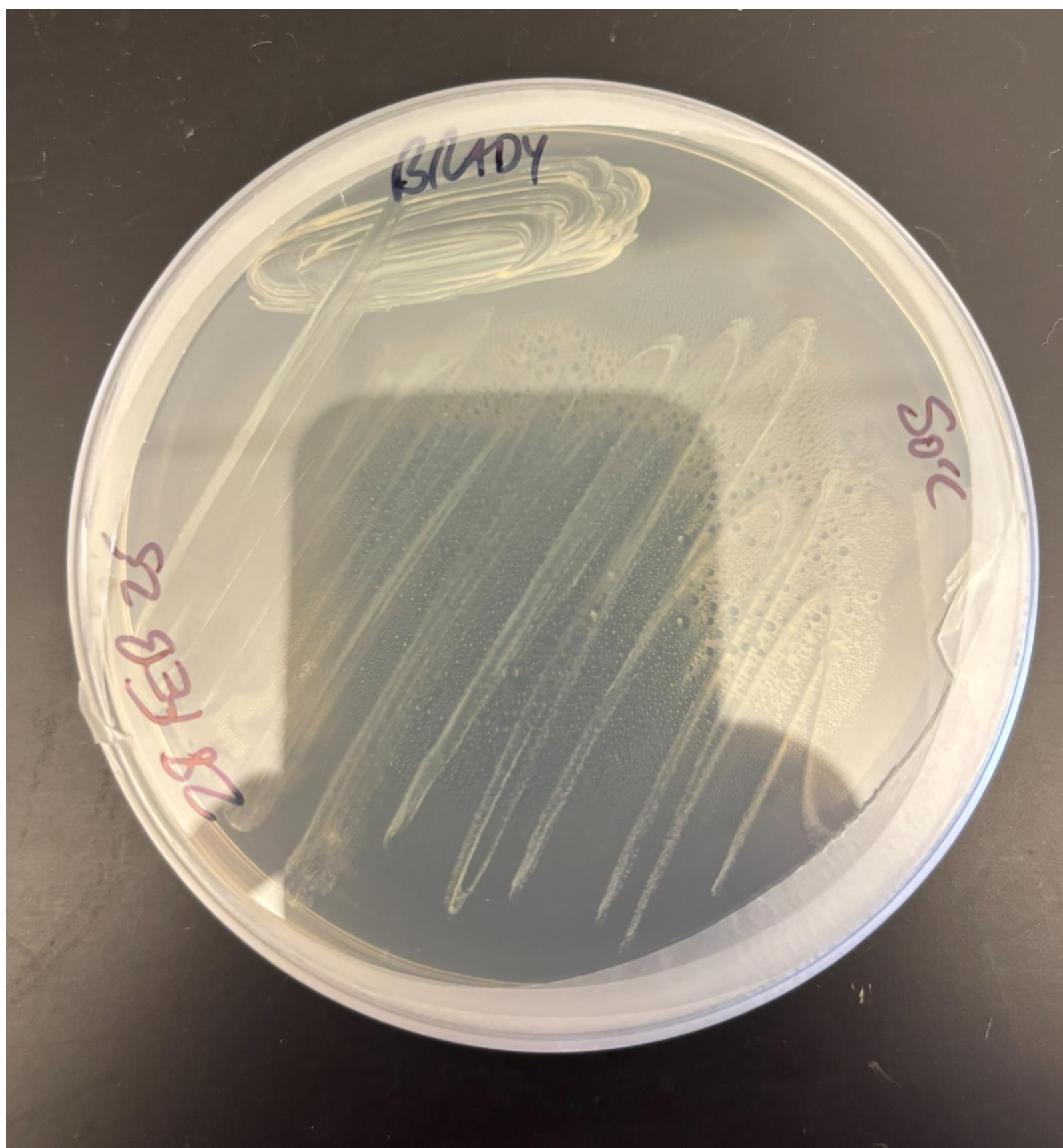
the isolate of interest.

METHODS

- Performed dilution sampling from ten self-stable beers at a 10⁻¹ dilution, isolate cultures of interest.
- Ran phenotypic tests included measuring growth at different temperatures, pH levels, NaCl levels, as well as determining growth on specialized media.
- Performed gram staining and negative staining to understand the shape and the structure of the isolate.
- Conducted 16s rRNA sequencing and compare the results to known species.
- Performed Whole Genome Sequencing and determine species and genome characteristics.



Whole Genome Sequencing of unknown *Bradyrhizobium* isolate.

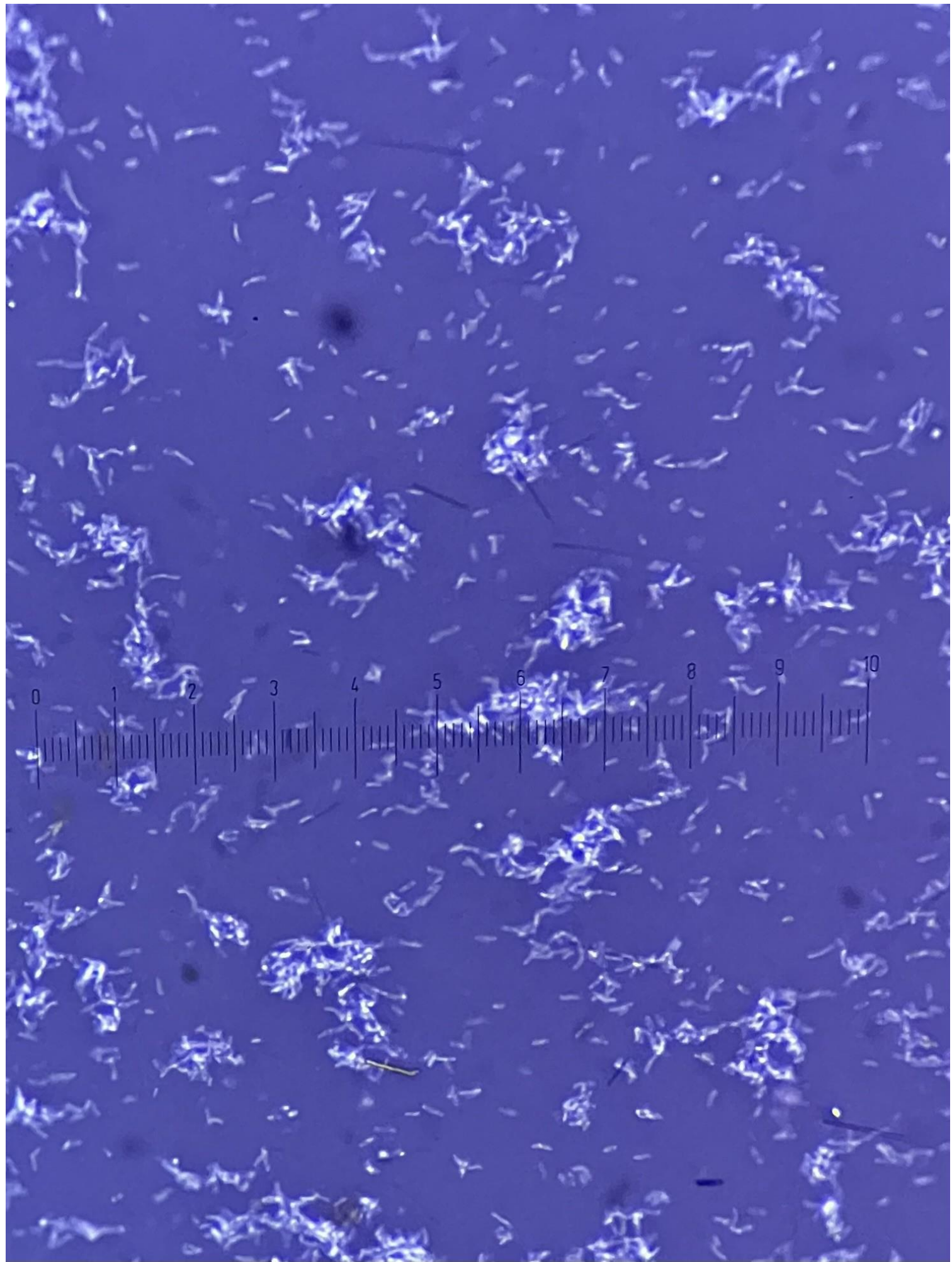


Streak plate of *Bradyrhizobium* isolate

RESULTS

Phenotypic Results

- Temperature: Growth observed between 4° - 37° C
- pH Levels: Growth observed between 3 – 9 pH.
- Microscopy: Rod Shaped, Gram Negative



Negative Staining results of Isolate

16S rRNA Sequencing Results

Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
Bradyrhizobium embrapense	2497	2497	100%	0.0	100.00%	1450	NR_145861.1
Bradyrhizobium viridifuturi	2497	2497	100%	0.0	100.00%	1468	NR_145860.1
Bradyrhizobium septentrionale	2492	2492	100%	0.0	100.00%	1421	NR_178802.1
Bradyrhizobium quebecense	2492	2492	100%	0.0	100.00%	1421	NR_178800.1
Bradyrhizobium ripae	2475	2475	100%	0.0	99.70%	1484	NR_179732.1
Bradyrhizobium elkanii	2475	2475	100%	0.0	99.70%	1414	NR_112927.1
Bradyrhizobium pachyrhizi	2475	2475	100%	0.0	99.70%	1483	NR_043037.1

Whole Genome Sequencing Results

- The closest match was *Bradyrhizobium viridifuturii* (93.1%)
- When uploaded to TYGS, genome did not match any published genome in the database, indicating a potential new species.

CONCLUSIONS

We can determine that the species that was isolated is a novel species belonging in the genus *Bradyrhizobium*. There are key differences that support our hypothesis of the novel species, such as growth at lower temperatures and a wide range of pH levels.

Whole genome sequencing allowed for us to determine and map the genome, as well as compare it to known genomes within the genus. The genome provides essential insight into multiple processes that this species performs, such as the use of maltose for energy, and when comparing to known genomes within *Bradyrhizobium*, these processes differentiate the isolate from known species.

This novel species is one of interest, as *Bradyrhizobium* is commonly found in soil. It is possible that this species was present in the soil that was used to grow the ingredients and was able to survive the brewing process and grow and live within the finished product.

With the isolate being considered a novel species, the hypothesized name of this species is *Bradyrhizobium cervisia*, as *cervisia* is the Latin translation for beer.

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References

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