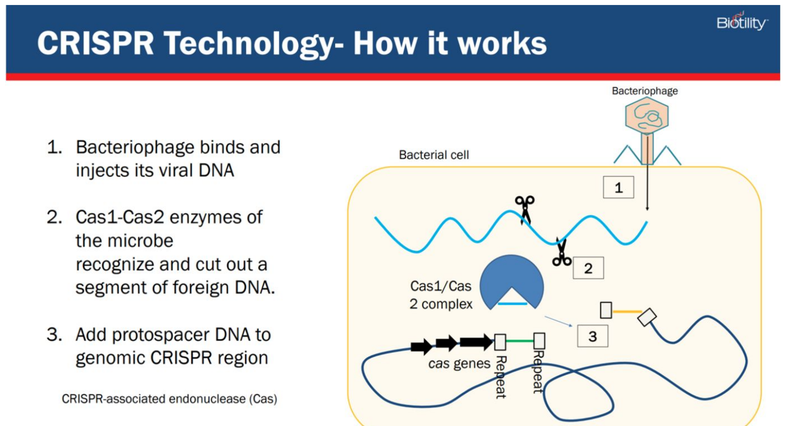
# There's CRISPRs in my Yogurt?!

Project by: Reegan West, Bianca Lungu, Melyna Nguyen
Mentor: Kimberly McClure
Program: Biology

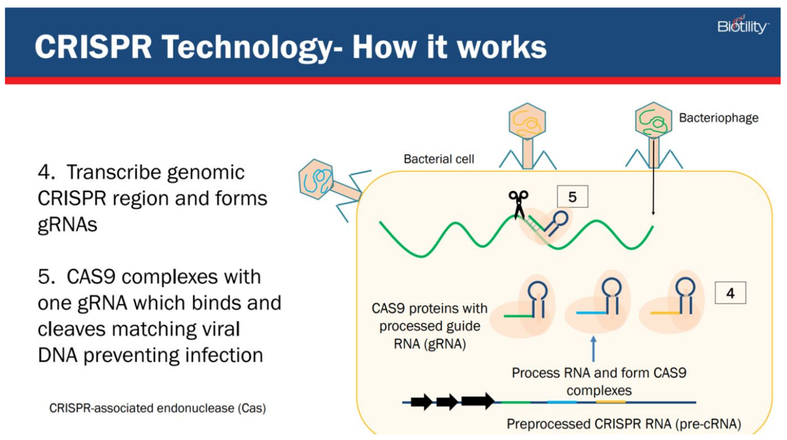
## Background

Many dairy products, such as yogurt and cheese, use live bacteria in their

The bacteria used in yogurt have an adaptive immune system called the Clustered Regularly Interspaced Short Palindromic Repeat (CRISPR)-Cas System. The CRISPR System protects bacteria from repeat viral infections due to the cells' ability to store fragments of viral DNA, where it can then degrade any identical DNA.




*Source: Biotility: Applied Biotech Training, University of Florida*




*Source: Biotility: Applied Biotech Training, University of Florida*

## Methods

- Heirloom yogurt was made from an undefined mixture of microbes
- Commercial yogurt used was MountainHigh™ Plain yogurt

*Figure 1: MountainHigh™ and inoculated heirloom yogurt*

- Colonies of heirloom and commercial yogurt were isolated on MRS agar plates

*Figure 2: Isolation of heirloom and commercial microbes on MRS agar plates*

- Heirloom and commercial bacteria colonies were gram stained

*Figure 3: Gram Stain of commercial yogurt bacteria (1000x oil immersion)*

*Figure 4: Gram stain of heirloom yogurt bacteria (1000x oil immersion)*

- A polymerase chain reaction (PCR) was used to amplify the CRISPR region
- Gel electrophoresis used to analyze PCR products

## Results

### Gel Electrophoresis of CRISPR Samples

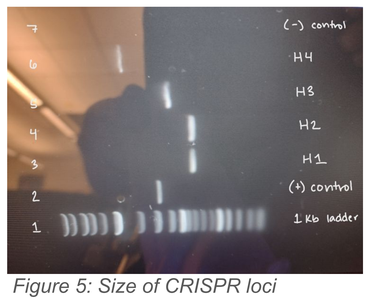



*Figure 5: Size of CRISPR loci (amplified by PCR) from heirloom yogurt bacteria (S. Thermophilus)*

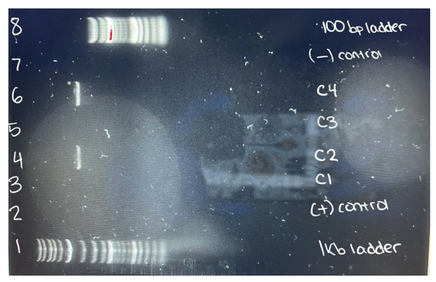



*Figure 6: Commercial PCR gel results labeled with positive control*

### CRISPR Repeat sequences

| Sample | Amplicon Size | Repeat sequence | # of spacers |
|---|---|---|---|
| Heirloom 2 | 850 bp | GTTTTTGTACTCTCAAGATTTAAGTAACTGTACAAC | 6 |
| Commercial 2 | 2400 bp | GTTTTTGTACTCTCAAGATTTAAGTAACTGTACAAC | 14 |

*Table 1: Heirloom 2 bacteria from **Fig. 5-4** and Commercial 2 bacteria from **Fig. 6-4**. Repeat sequence, number of spacers, and estimate amplicon size in CRISPR locus is shown.*

### Commercial Yogurt Bacteria CRISPR Loci sequence

| Spacer Sequences | Identity of Virus (Phage) | E-value |
|---|---|---|
| AAAATGGATTATCATATATTCATAAAGTGA | MAG: Bacteriophage sp. isolate 1807_32025, partial genome | 2.70E+01 |
| AACGCTTCCTAGGTTTAAGCCGTCTTTTCC | Streptococcus phage CHPC1041, complete genome | 4.00E-04 |
| TTACACAAAATTGGTTAACGTCAACGACAT | Streptococcus phage CHPC1041, complete genome | 2.00E-06 |
| AAATTGTCGTTCATTGCCGTTCAACACCTC | Streptococcus phage SW23, complete genome | 2.00E-06 |
| ACAAAGGTTGTTATTACAGTTGGTTTATA | MAG TPA_asm: Caudoviricetes sp. isolate cthvO2, partial genome | 2.40E+01 |
| ATTTAAAGAGTTGATTAAGTGCGTTACTGT | MAG TPA_asm: Caudoviricetes sp. isolate ctJ298, partial genome | 6.70E+00 |
| AAATGGGTTATGCTGTTCAATATGCGTCCC | Streptococcus phage SW12, complete genome | 2.80E-02 |
| AAACTGAAAACAACACAGACAATTCAACAA | No significant match | |
| GCCCAAAATGCTAGACGTTTGAATGACGGC | Streptococcus phage CHPC663, complete genome | 2.00E-06 |
| ATGAAGAACGTGATTCACCTACGGTATGCT | MAG TPA_asm: Caudoviricetes sp. isolate ctFZS48, partial genome | 2.00E-06 |
| TTATGTACATTTCAAAAGGTGTCATCCATA | Streptococcus phage SW12, complete genome | 2.00E-06 |
| AATACGATTCATTGCAAGAGTATGTTAATG | Streptococcus phage SW12, complete genome | 2.00E-06 |
| AATATGAAATCACAAGTAATGAGCCTAGCA | Streptococcus phage CHPC663, complete genome | 7.00E-06 |
| AAAACATTACAAGAGACTACTTTTACAAT | Streptococcus phage Javan536, complete genome | 6.00E-06 |

*Table 2: BLAST analysis of the spacers in Commercial 2 (See **Table 1**) for bacteriophage identity, and its E-value.*

### Heirloom Yogurt Bacteria CRISPR Loci sequence

| Spacer Sequences | Identity of Virus (Phage) | E-Value |
|---|---|---|
| AGATACTCTTGTCGCCTCTGAACAACCAG | Streptococcus phage pST | 4.00E-04 |
| TTTGATGGCTCTTGGTAGGGAACTGGATAT | Streptococcus phage pST | 7.00E-06 |
| AAAGACAAGCCCAAGGGATTGAACTAGCAA | Bacteriophage sp. | 1.05E+02 |
| CTACATTATTGATCATGTTTTTTCTCCTGT | Streptococcus phage CHPC925 | 2.80E-02 |
| TAGAAGGCTCTGGAAATACAAAGCAATTCT | Caudoviricetes sp. | 6.7 |
| CGAACAGTTGGCGAGAAATCCGTCTGGCGT | Streptococcus phage CHPC929 | 2.0E-03 |

*Table 3: BLAST analysis of the spacers in Heirloom 2 (See **Table 1**) for bacteriophage identity, and its E-value.*

## Conclusion

Our research compared the CRISPR immune systems of *S. thermophilus* in commercial and heirloom yogurts. We hypothesized that commercial yogurts would have superior immunity because companies want to prevent infection by viruses. However, we obtained less data from commercial samples due to less successful PCR electrophoresis, limiting our comparison.

On average, commercial samples had more spacer sequences (13.5) than heirloom samples (8.4), suggesting potentially better viral resistance. This is significant for human health as we prefer more resistant bacteria in our diet.

Our study was limited by the insufficient cultures and genetic information from commercial yogurt. Future studies could benefit from equal PCR reactions and cultures for both types.

Potential improvements could include exploring Lactobacillus CRISPR loci differences and comparing various yogurt types, such as lactose-free yogurts, or Greek yogurt. This could provide a broader understanding of the microbial differences and similarities in our food.

## References

Barrangou, Rodolphe, and Philippe Horvath. "CRISPR: New Horizons in phage resistance and strain identification." Annual Review of Food Science and Technology, vol. 3, no. 1, 10 Apr. 2012, pp. 143–162, https://doi.org/10.1146/annurev-food-022811-101134.

Zhang H, You C. 16S ribosomal RNA-depletion PCR and its application in cause analysis of yogurt package shrinkage. J Dairy Sci. 2022 Sep;105(9):7288-7297. doi: 10.3168/jds.2021-21575. Epub 2022 Aug 2. PMID: 35931476.

Andrey N. Shkoporov, Colin Hill, Bacteriophages of the Human Gut: The "Known Unknown" of the Microbiome, Cell Host & Microbe, Volume 25, Issue 2, 2019, Pages 195-209, ISSN 1931-3128, https://doi.org/10.1016/j.chom.2019.01.017.

*CRISPR Technology- How it works.* Biotility: Applied Biotech Training. University of Florida

Lake Washington Institute of Technology