

Developing Methods for Testing Inhibition of *E. coli* Strains by Urinary *Lactobacilli*

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Overview

The urinary tract is known to host a urinary microbiome that plays a role in urinary health and disease, but there is a need to better understand the extent. Earlier work was successful in isolating 29 strains of *Lactobacilli* from urine. In this project, 2 of these isolates, *Lactobacillus gasseri* and *Lactobacillus delbrueckii*, were tested for inhibition of 4 *Escherichia coli* strains. Initial results demonstrated the need for increased standardization of the well-diffusion assays employed.

Experimental Approach

MRS media was initially observed to “caramelize” with increased sterilization time. It was hypothesized that shorter sterilization time would produce more consistent, measurable results due to more readily available carbon-sources for organisms to use.

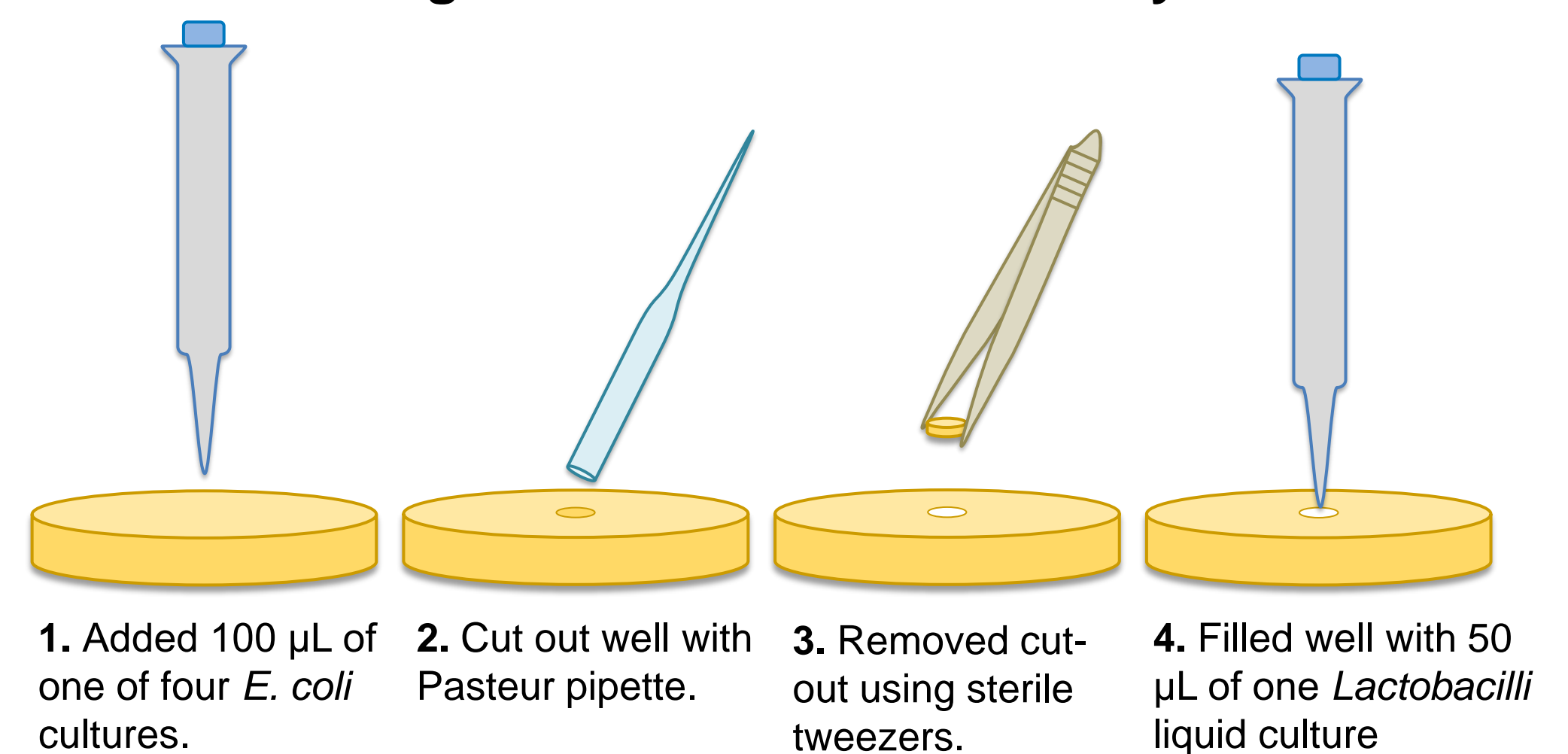
Preparation of Lactobacilli Media

- Two sterilization times for MRS media were compared:
 - 20 minutes at 121 °C = **“Light MRS”**
 - 35 minutes at 121 °C = **“Dark MRS”**
- Similar media were used for *Lactobacilli* liquid cultures
- 25 mL of MRS media were poured into each plate and allowed to dry for 1 hour, uncovered, in laminar-flow hood

E. coli Liquid Cultures

- 4 strains were grown in separate LB broth cultures:
 - Background MG1655
 - SVS1144-Wild Type¹
 - Uropathogen CFT073
 - SVS1144-K90D (Mutant)¹

Figure 1. Well-Diffusion Assay



Results & Discussion

Figure 2. Inhibition by *L. gasseri*

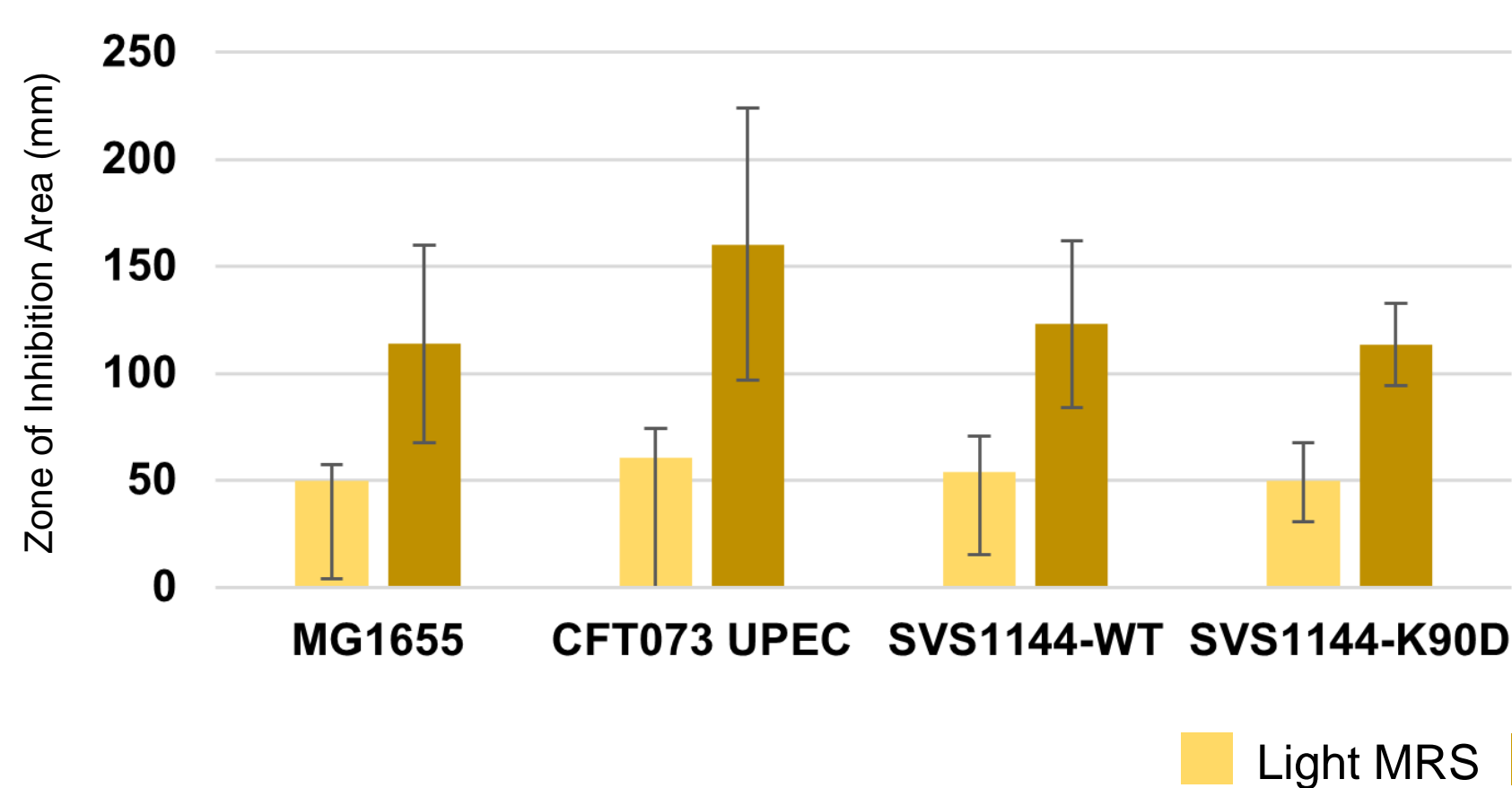


Figure 3. Inhibition by *L. delbrueckii*

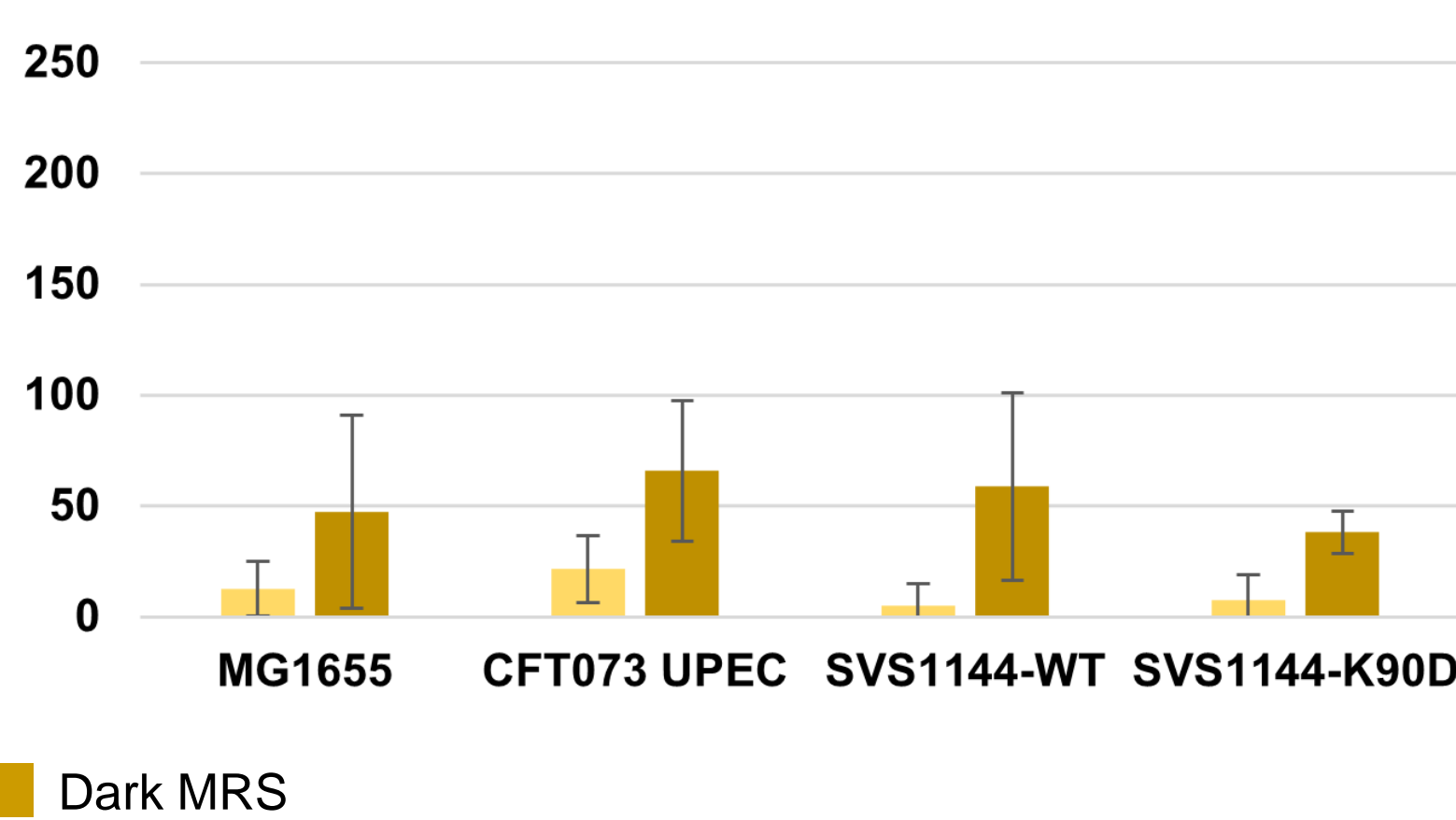
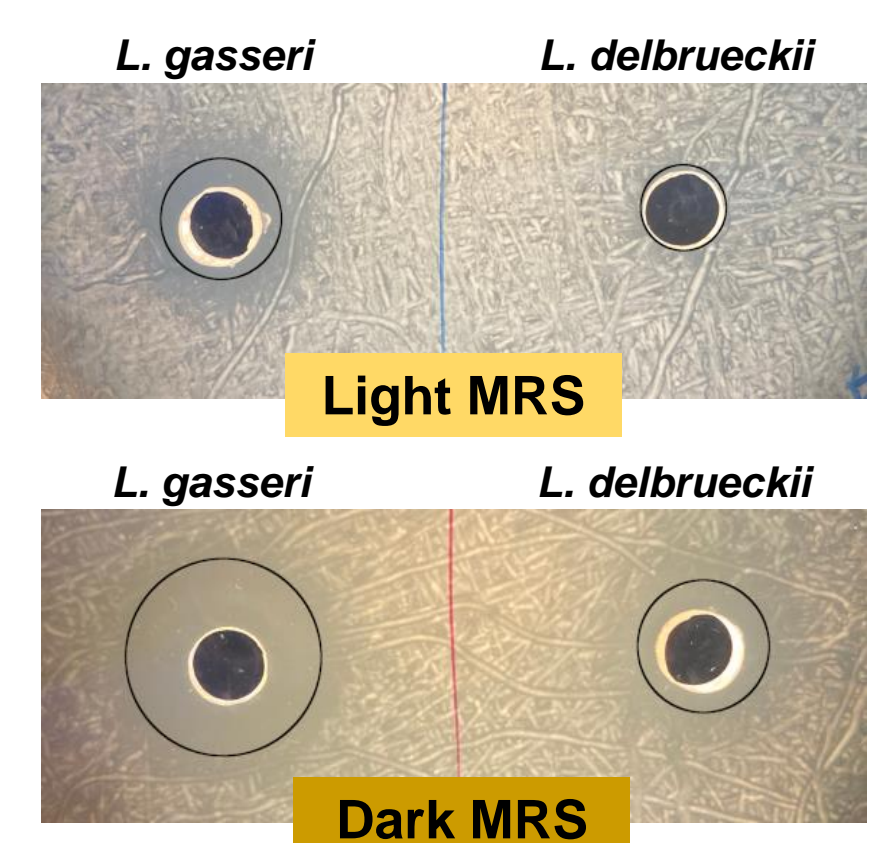


Figure 4. GIMP Zones for CFT073 UPEC



- Plates were imaged and zones of inhibition were quantified in GIMP; measurements were analyzed in MS Excel (Fig. 2-3).
- Measured only clear zones = where observed streaks/haze of lawn growth distinctly ended (i.e., end of gradient. Fig. 4).
- All zones used this definition and were measured independently of one another to minimize visual biases.
- Combining qualitative + quantitative measurements was often inefficient and highly prone to very wide deviations.
- Light MRS produced clearer, more defined zones that potentially minimize these issues.

Conclusions & Future Work

- Urinary *Lactobacilli* may inhibit various strains of *E. coli*.
- Light MRS proved to be more efficient and consistent for measurements than dark MRS.
- However, dark MRS was more efficient in the inhibition process.
- More work is needed to refine these methods and to fully understand what caused the observed inhibition.
- Tests will also include running assays in samples of urine to better simulate the urinary tract conditions.

References

- Franklin, E. A., Worthan, S. B., Pham, C., Yap, M.-N. F., & Cruz-Vera, L. R. (2021). A ribosomal protein variant that confers macrolide resistance differentially regulates acid resistance, catabolism, and biofilm formation related genes in *Escherichia coli*. <https://doi.org/10.1101/2021.04.10.439278>

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