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Investigating the Effects of Kidney Stones on our Microbiome: Lactobacilli Growth in the Presence of Oxalate

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Overview

Lactobacillus is a genus of bacteria that might play an important role in the prevention of urinary tract infections via uropathogen inhibition in humans. Oxalate is a compound that causes kidney stones via formation of insoluble calcium oxalate forms in the urinary tract. Research into the effects of oxalate on the growth of urinary lactobacilli could provide vital insights regarding the relationship between kidney stones formation and UTIs as well as possible probiotic treatments to kidney stones. We aimed to determine the effect of oxalate on the growth of two strains of lactobacilli isolated from human urine *(L. gasseri and L. delbrueckii)*.

Methods

Growth media:
- Light MRS (LMRS) and Dark MRS (DMRS) broth prepared and autoclaved for 20 min and 35 min respectively (Fig. 1)

Starter cultures:
- Liquid cultures of *L. gasseri* and *L. delbrueckii* grown in either broth for 48 hours under static conditions

Recorded growth in 96-well-plates in plate reader:
- Inoculated lactobacilli cells at 1:10 and 1:100 dilutions (Fig. 2)
- 24 hours at 37° C with readings taken every 15 min
- Plate shaken in a double orbital pattern prior to each read
- Sodium oxalate to the final concentration of 10 mM or water added to the growth media (Fig.3-4)

Fig. 1 DMRS (left), LMRS (right)

Fig. 2 Process of Creating a 1:100 Diluted Well

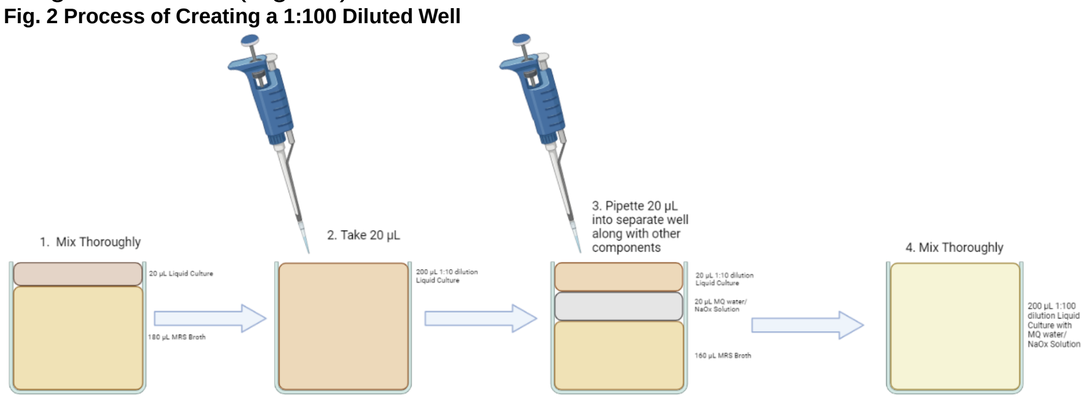
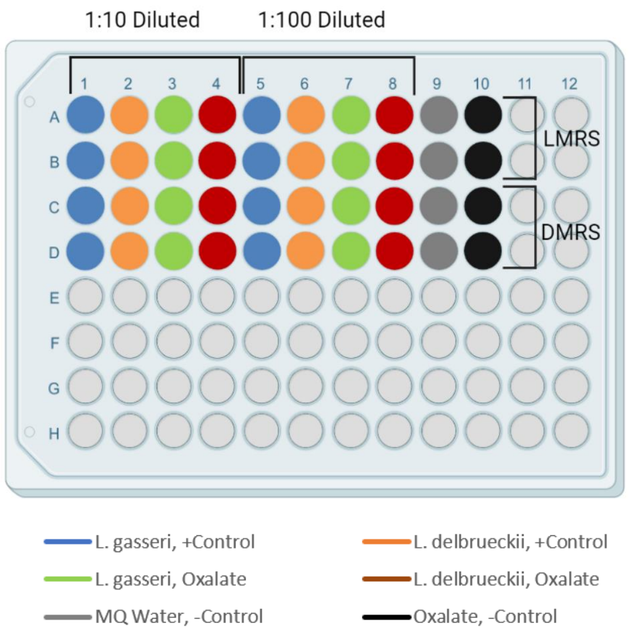


Fig. 3 Components of Each Well Type

+Control	Oxalate	-Control
• 20 µL Liquid Culture • 20 µL MQ Water • 160 µL MRS Broth	• 20 µL Liquid Culture • 20 µL 100 mM NaOx Solution • 160 µL MRS Broth	• 20 µL MQ Water/100 mM NaOx Solution • 180 µL MRS Broth

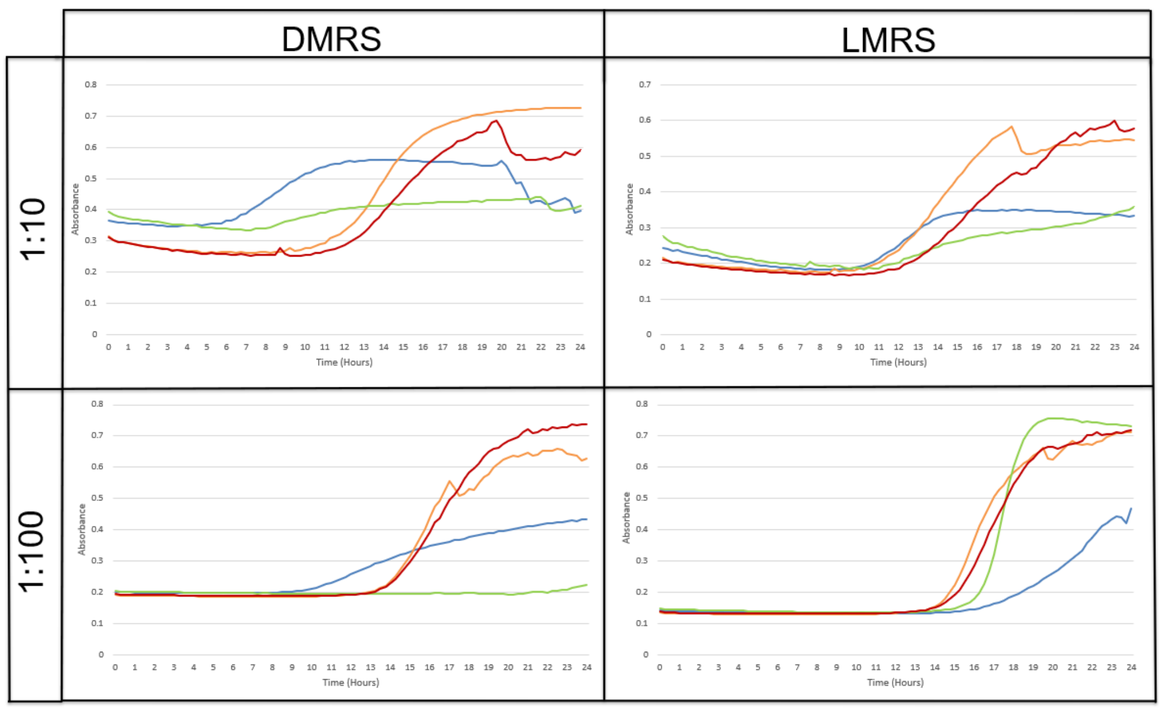
Fig. 4 96-Well-Plate Layout



Results

- There are differences in urinary lactobacilli growth between LMRS and DMRS as well as with and without oxalate (Fig. 5)

Fig. 5 Growth Curve Results



- Continuous measurement of urinary lactobacilli growth is complicated by cell-cell aggregation at different growth stages (Fig. 6) that affects measurements of turbidity

Fig. 6 Bottom-View of 96-Well-Plate after 24 Hours of Growth

Conclusions

We saw some inhibition of growth in the presence of oxalate for both strains of lactobacilli. However, there were exceptions to this trend in wells that were prepared with 1:100 dilutions of starter culture. This could suggest that the initial density of lactobacilli in a urinary microbiome determines the effect oxalate will have on lactobacilli growth. Future experiments could observe the growth over longer times to ensure the entire growth curve is being accounted for. The unusually long lag phase for many of the samples could also addressed in the future.

References

1. **McCullars R. (2022). Initial phenotypic characterization of bacterial isolates from human urinary microbiome**
2. **Images created with BioRender.com**

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