

# INTERACTIONS OF COMMENSAL URINARY LACTOBACILLI WITH URINARY PATHOGENS

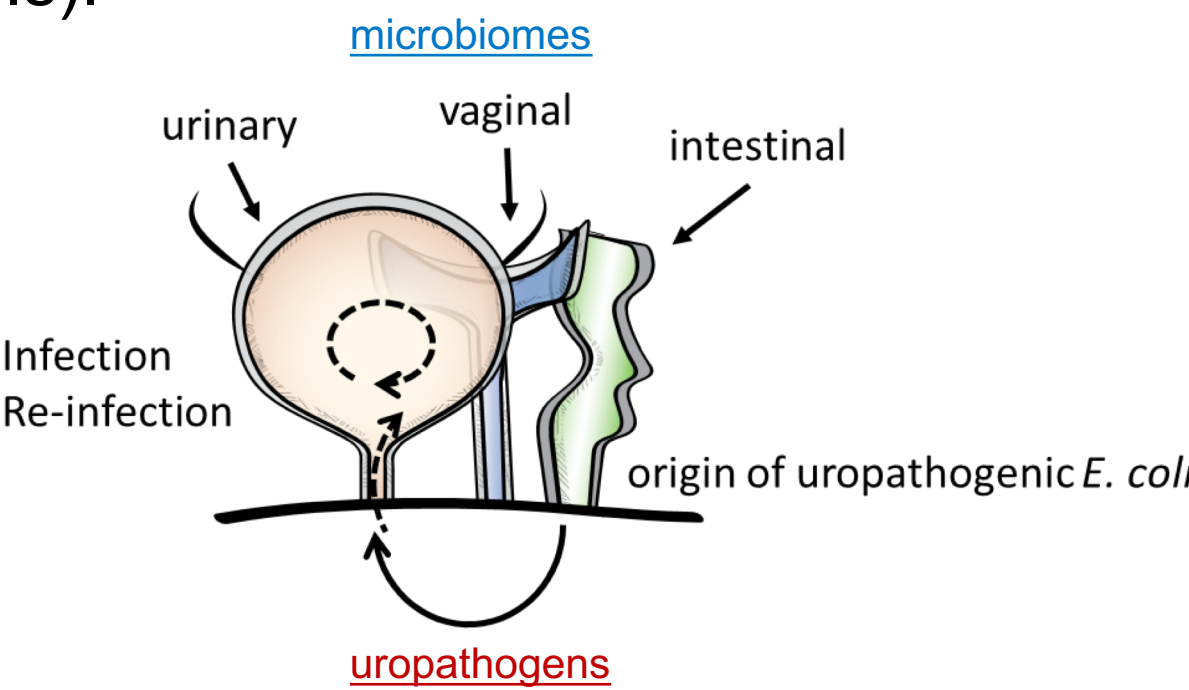
Lydia F. Delaney<sup>1</sup>, Rebecca M. Sloan<sup>1</sup>, James A. Johnson<sup>1</sup>, Monique Vaughan<sup>2</sup>, Nazema Y. Siddiqui<sup>2</sup>, Tatyana A. Sysoeva<sup>1</sup>

<sup>1</sup> Department of Biological Sciences, The University of Alabama in Huntsville, Huntsville, AL

<sup>2</sup> Department of Obstetrics & Gynecology, Division of Urogynecology and Reconstructive Pelvic Surgery, Duke University Medical Center, Durham, NC

## INTRODUCTION

- Over 170 million cases of the urinary tract infections are registered annually around the world. Majority of the UTIs are caused by enteric bacteria (*Escherichia coli*, *Klebsiella pneumoniae*, *Enterococcus faecalis*). The standard treatment for UTIs is a course of antibiotics but the urinary pathogens are becoming increasingly resistant to antibiotics.
- It has become evident that diverse commensal bacteria inhabit the lower urinary tract. The composition of the urinary microbiome correlates with several urological conditions, including urinary tract infections (UTIs).



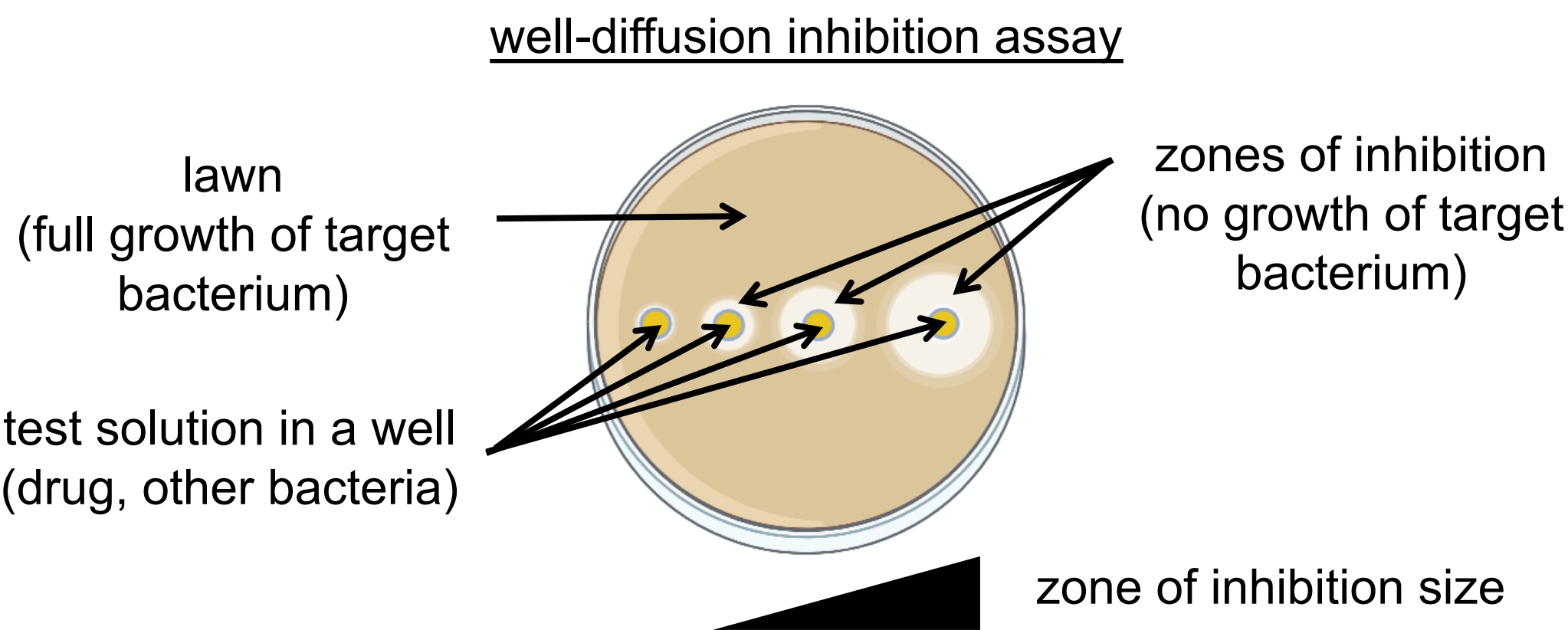
Can urinary microbiome interfere with urinary tract infections?

- Often, urinary microbiome is found to contain lactic acid forming bacteria – lactobacilli. Lactobacilli are long known to humankind as beneficial bacteria that are extensively used in food preparation and preservation due to their ability to inhibit growth of other bacteria. Lactobacilli are also found in human oral, intestinal and vaginal microbiomes and are often applied as probiotic for various health conditions.
- Many lactobacilli secrete compounds that inhibit the growth of other microbes, including pathogenic bacteria.

Can urinary lactobacilli inhibit the growth of uropathogens?

## METHODS

- Growth optimization of urinary commensals in aerobic and anaerobic conditions
- Whole genome sequencing using Illumina platform followed by *de novo* assembly by Unicycler program
- Well-diffusion inhibition assay to test interaction of lactobacilli with other bacteria



## RESULTS

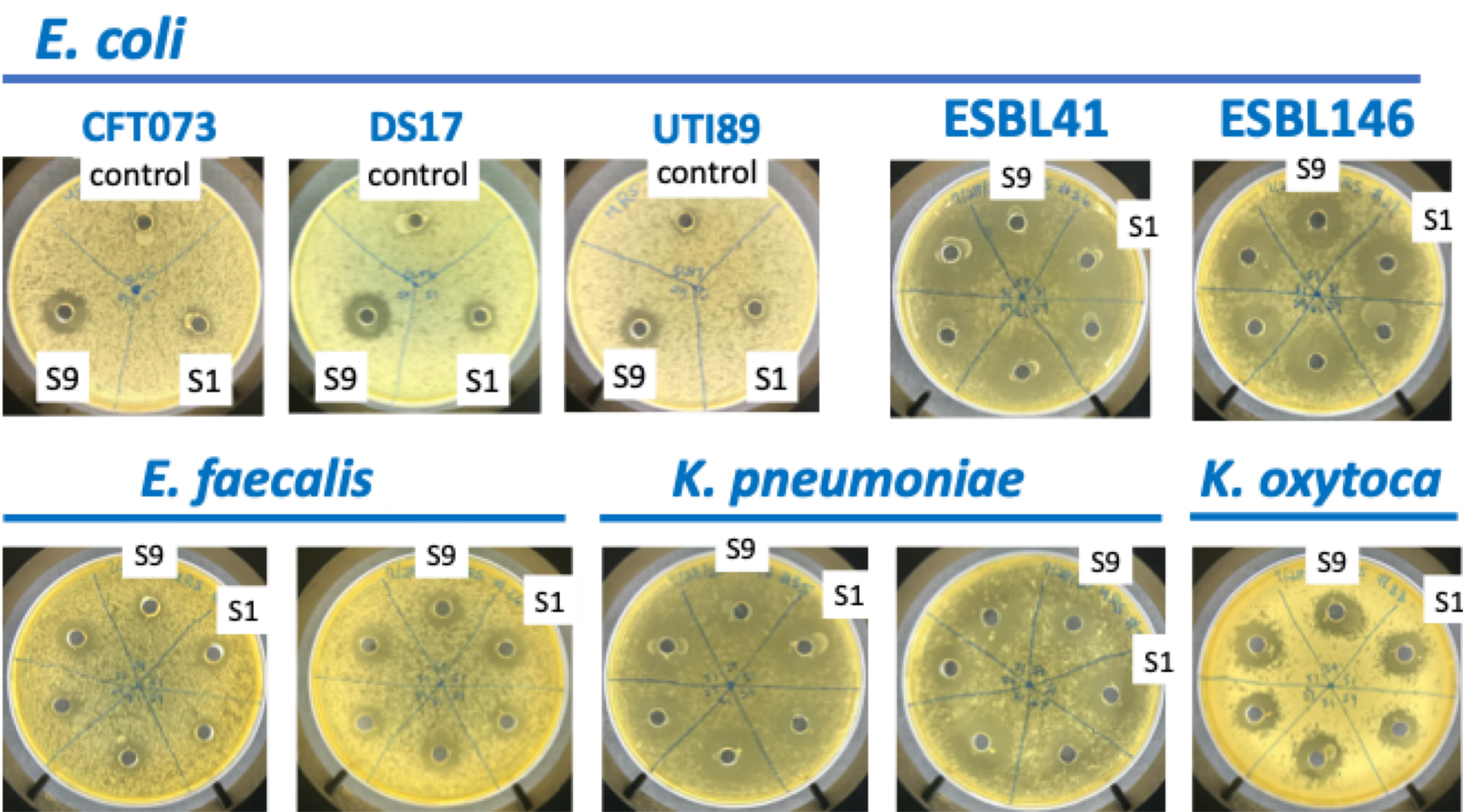
### Collecting and characterizing a small repository of urinary commensal bacteria

- 64 isolates of urinary commensal microbes were collected from asymptomatic postmenopausal women using Enhanced Quantitative Urine Culture technique
- There are 27 lactobacilli strains that belong to seven different species: *L. gasseri*, *L. delbrueckii*, *L. rhamnosus*, *L. acidophilus*, *L. jensenii*, *L. iners*, *L. crispatus*
- There are also 14 strains of classic urinary pathobionts such as *E. coli*, *K. pneumoniae*, *K. oxytoca*, and *E. faecalis*
- Draft genomes of *L. gasseri* S1, S2, S3, *K. pneumoniae* S8, *L. delbrueckii* S9 were established. Lactobacilli genomes range from 1.7 to 1.8 Mb and found to be closely related to vaginally isolated strains. *K. pneumoniae* S8 strains contains several antibiotic resistance genes.

### Inhibition tests with model uropathogens and resistant clinical isolates

- We tested first two urinary lactobacilli strains from two different species in well-diffusion inhibition assays against model uropathogenic strains of *E. coli*, *K. pneumoniae* and *E. faecalis*.
- Growth of the model uropathogenic *E. coli* (UPEC) strains CFT073, DS17, UTI89 and *K. pneumoniae* TOP52 is inhibited by urinary lactobacilli *L. gasseri* S1 and *L. delbrueckii* S9 while *E. faecalis* strains (OG1RF, JH2-2, V583 and MH594) appear to be more resistant to these urinary lactobacilli.
- Well-diffusion tests showed that multidrug resistant clinical isolates of *E. coli* are being inhibited by the two tested lactobacilli strains as well.

**Figure 1. *L. gasseri* S1 and *L. delbrueckii* S9 inhibit the growth of major uropathogens.** Images of well-diffusion inhibition assay on the MRS agar is shown with the target bacteria indicated above the plates. Clearings around the wells with S1 and S9 lactobacilli indicate a different level of inhibition. CFT073, DS17, UTI89 – model uropathogenic *E. coli* strains, ESBL41 and ESBL146 – examples of multidrug-resistant *E. coli* tested. Six-sector plates contain triplicates of the measurements with S1 and S9.



### Screening a broader range of urinary lactobacilli

- Another 18 strains of urinary lactobacilli were tested for their ability to inhibit the growth of UPEC CFT073 strain and most were shown to inhibit the uropathogen to varying degree
- Preliminary quantification of the inhibition does not show strong indication of any particular lactobacilli species and thus careful genetic and phenotypic analysis of individual strains is warranted

**Figure 2. The screen of urinary lactobacilli for inhibition of uropathogenic *E. coli* CFT073.** Well-diffusion inhibition assay with strains from *L. gasseri*, *L. rhamnosus*, *L. jensenii*, *L. crispatus*, *L. acidophilus*, *L. delbrueckii*, untyped *Lactobacillus* species were quantified.

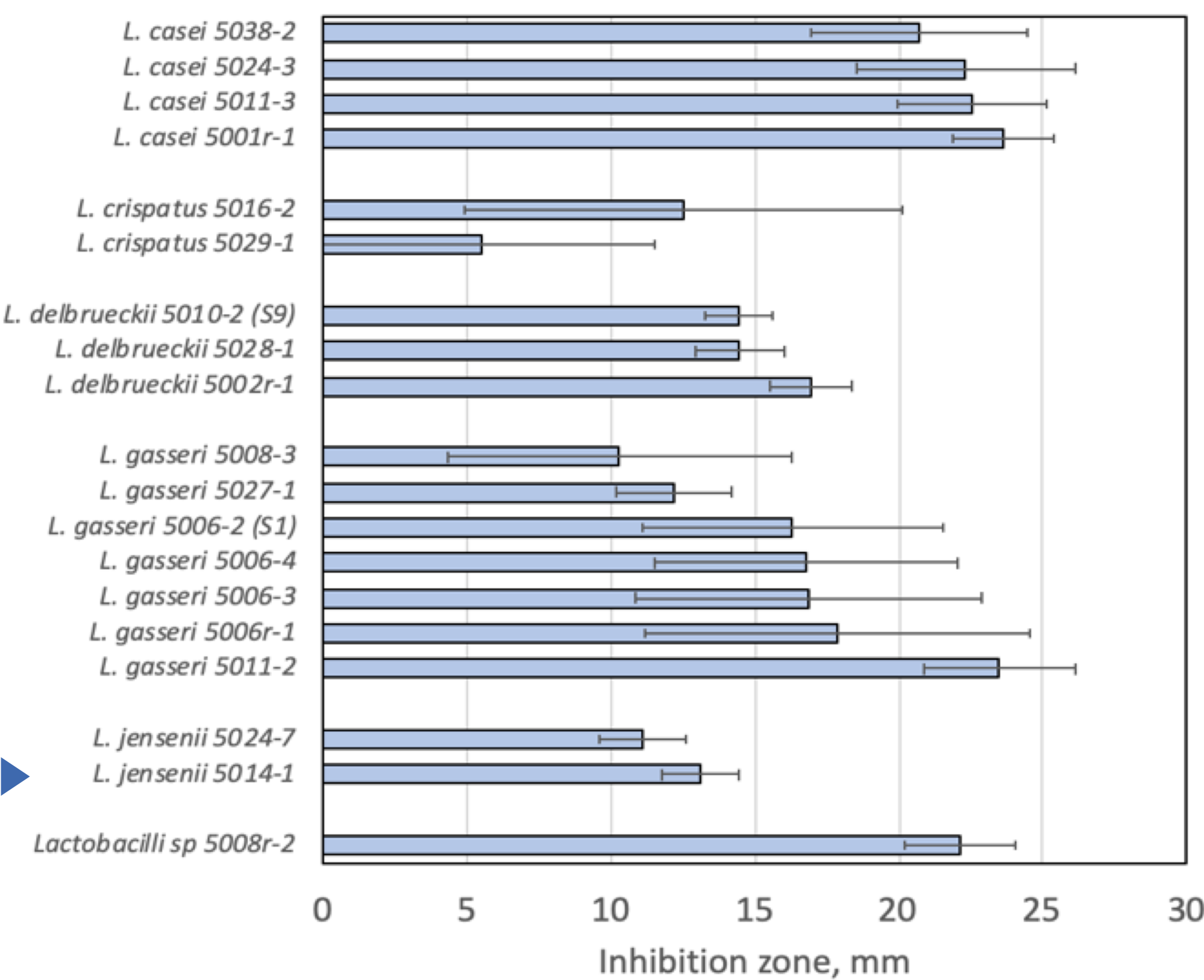


**Table 1. Collection of urinary commensals**

| Urinary Isolates Repository*                                     | # |
|------------------------------------------------------------------|---|
| <b>Lactobacilli (27)</b>                                         |   |
| <i>Lactobacillus acidophilus</i> gasseri                         | 8 |
| <i>Lactobacillus casei</i> rhamnosus/paracasei                   | 5 |
| <i>Lactobacillus crispatus</i>                                   | 5 |
| <i>Lactobacillus delbrueckii</i>                                 | 4 |
| <i>Lactobacillus iners</i>                                       | 1 |
| <i>Lactobacillus jensenii</i>                                    | 2 |
| <i>Lactobacillus spp</i>                                         | 2 |
| <b>Strains from well-established uropathogen species</b>         |   |
| <i>Escherichia coli</i>                                          | 5 |
| <i>Klebsiella pneumoniae</i>                                     | 3 |
| <i>Klebsiella oxytoca</i>                                        | 1 |
| <i>Proteus mirabilis</i>                                         | 1 |
| <i>Enterococcus faecalis</i>                                     | 4 |
| <b>Strains from commensal and opportunistic pathogen species</b> |   |
| <i>Corynebacterium coyleae</i>                                   | 1 |
| <i>Staphylococcus epidermidis</i>                                | 4 |
| <i>Staphylococcus hominis</i>                                    | 1 |
| <i>Staphylococcus lugdunensis</i>                                | 1 |
| <i>Streptococcus anginosus</i>                                   | 6 |
| <i>Streptococcus bovis</i> group                                 | 1 |
| <i>Streptococcus sanguinis</i> alpha                             | 1 |
| <i>Streptococcus sp</i>                                          | 1 |
| <i>Aerococcus urinae</i>                                         | 3 |
| <i>Leuconostoc sp</i>                                            | 1 |
| <i>Raoultella ornithinolytica</i>                                | 1 |
| <i>Diphtheroids</i>                                              | 3 |
| <b>Others (fungi)</b>                                            |   |
| <i>Candida albicans</i>                                          | 2 |
| <i>Candida glabrata</i>                                          | 1 |

\* species are typed by MALDI-TOF

## FUTURE DIRECTIONS

### Establishing the mechanism of growth inhibition

- Genome analysis of S1 and S9 identifies several genes that encode protein toxins – bacteriocins
- More functional and genetic studies are needed to show whether bacteriocin production is responsible for the observed inhibition of uropathogens

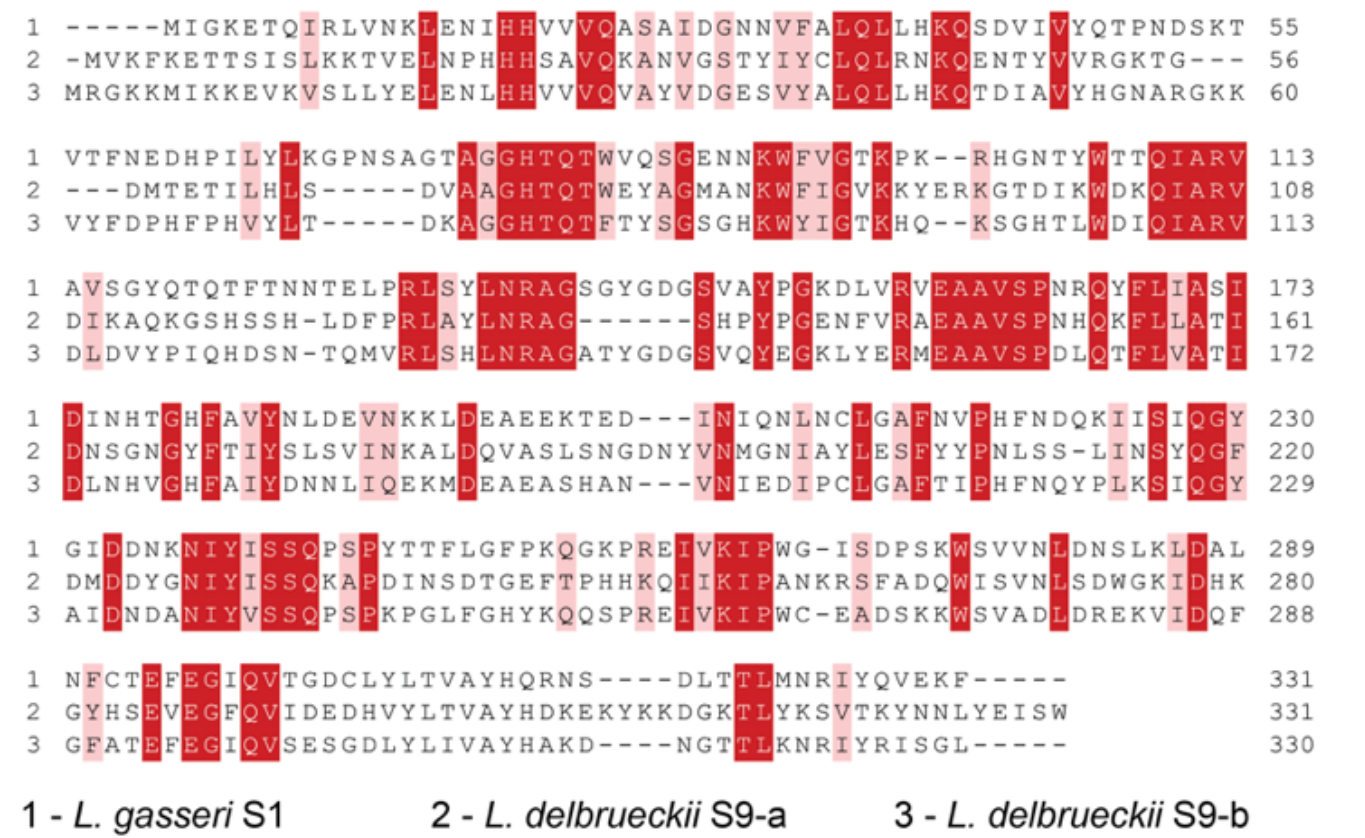


**Figure 3. Comparison of predicted helveticin J homologues identified in genomes of urinary lactobacilli.** 1 – homologue from *L. gasseri* S1, 2 and 3 – two homologues from *L. delbrueckii* S9 genome.

## SUMMARY

We collected a repository of commensal urinary bacteria containing 27 strains of urinary lactobacilli, and established draft genome sequences of several first isolates

Urinary lactobacilli *L. gasseri* S1 and *L. delbrueckii* S9 inhibit growth of major uropathogens including multidrug resistant *E. coli*

Other urinary lactobacilli are capable of inhibiting uropathogenic *E. coli* growth to different extents

## ACKNOWLEDGMENTS

UAH start up fund awarded to TAS. Sequencing data collection was sponsored by the NIH K12 DK100024 award to TAS. All assemblies were performed using services provided by the Alabama Supercomputer Authority.

## REFERENCES

- Flores-Mireles, A. L., Walker, J. N., Caparon, M., & Hultgren, S. J. Urinary tract infections: Epidemiology, mechanisms of infection and treatment options. *Nature Reviews Microbiology* 13, 269–284 (2015).
- Wolfe, A. J. et al. Evidence of uncultivated bacteria in the adult female bladder. *J. Clin. Microbiol.* 50, 1376–1383 (2012).
- Hilt, E. E. et al. Urine is not sterile: Use of enhanced urine culture techniques to detect resident bacterial flora in the adult female bladder. *J. Clin. Microbiol.* 52, 871–876 (2014).
- Neugent, M. L., Hulyalkar, N. V., Nguyen, V. H., Zimmern, P. E., & De Nisco, N. J. Advances in understanding the human urinary microbiome and its potential role in urinary tract infection. *MBio* 11, (2020).
- Fouts, D. E. et al. Integrated next-generation sequencing of 16S rDNA and metaproteomics differentiate the healthy urine microbiome from asymptomatic bacteriuria in neuropathic bladder associated with spinal cord injury. *J. Transl. Med.* 10, (2012). Wick, R. R., Judd, L. M., Gorrie, C. L. & Holt, K. E. Unicycler: Resolving bacterial genome assemblies from short and long sequencing reads. *PLoS Comput. Biol.* (2017).
- Zacharof, M. P. & Lovitt, R. W. Bacteriocins Produced by Lactic Acid Bacteria a Review Article. *APCBE Procedia* 2, 50–56 (2012).