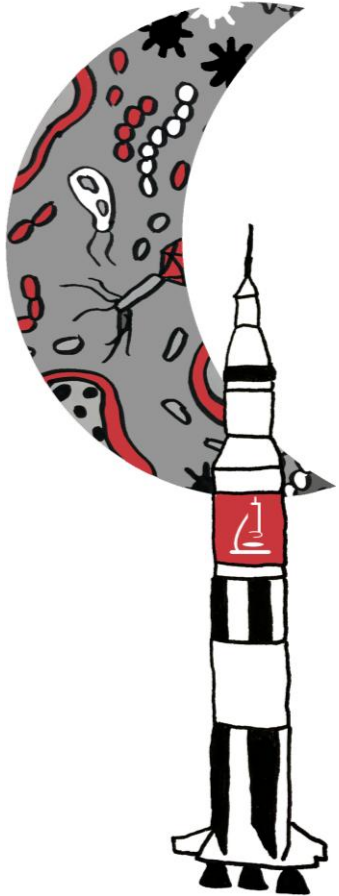


Characterization of microbial interactions in urobiome

Tanya Sysoeva

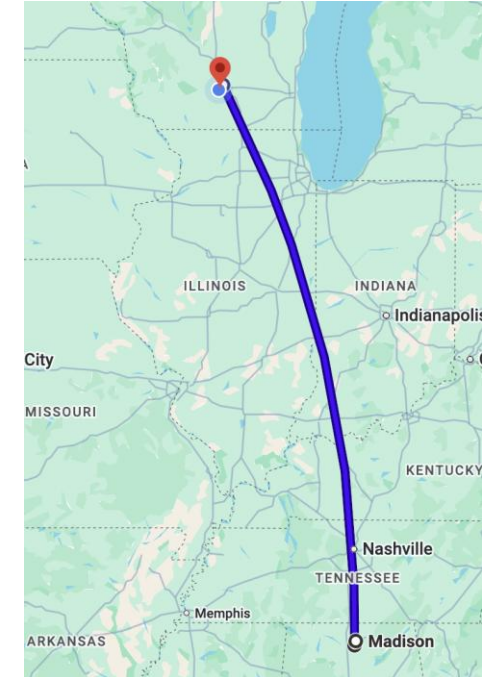
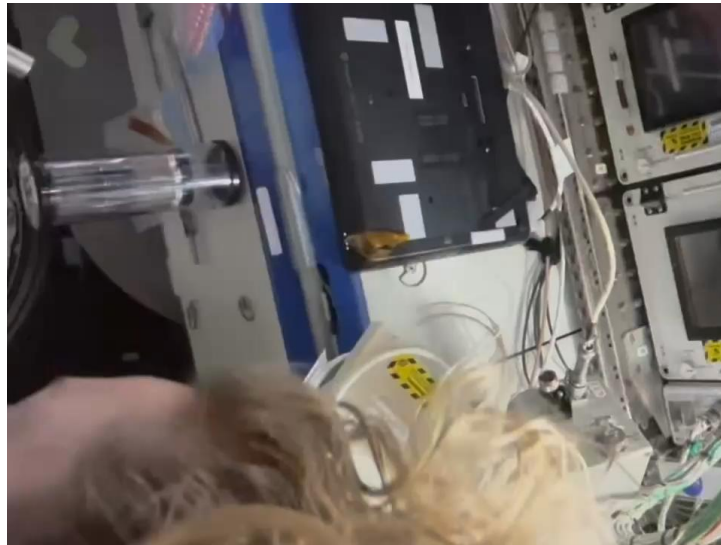
University of Alabama in Huntsville



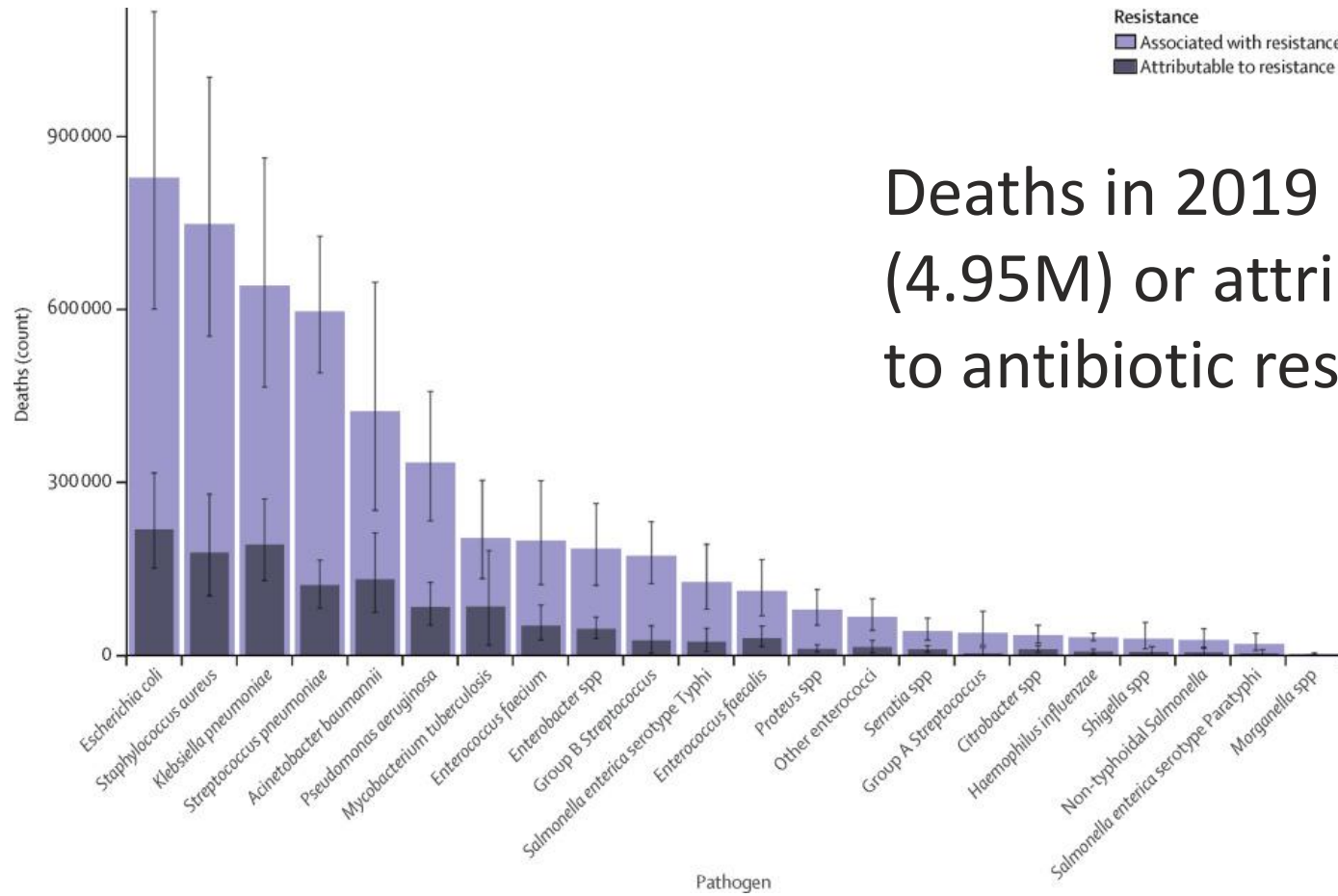


Madison, AL to Madison, WI

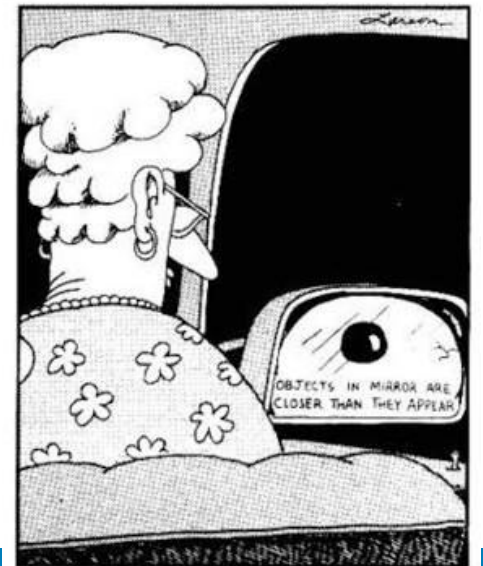
Huntsville – Rocket city



Antibiotic resistance is a major threat to public health



Deaths in 2019 associated
(4.95M) or attributed (1.27M)
to antibiotic resistant bacteria

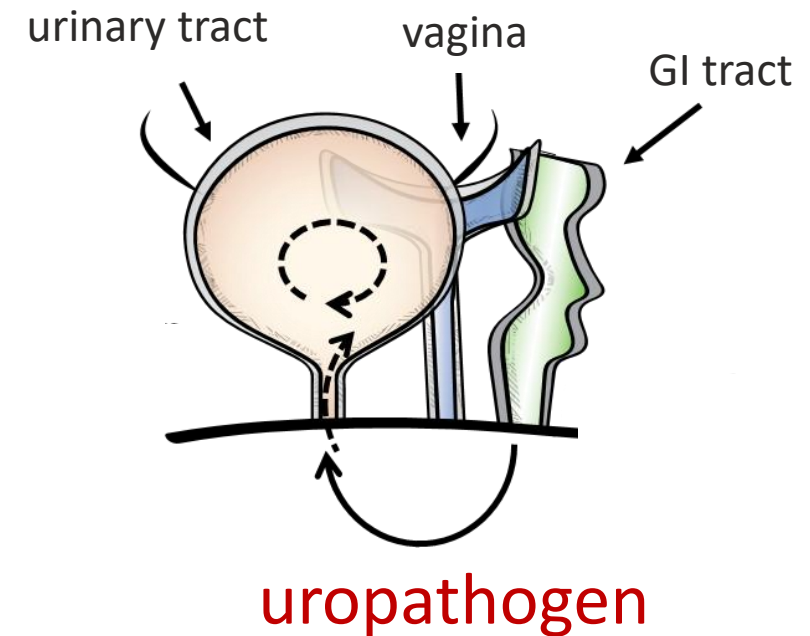


Objects in mirror are closer than they appear

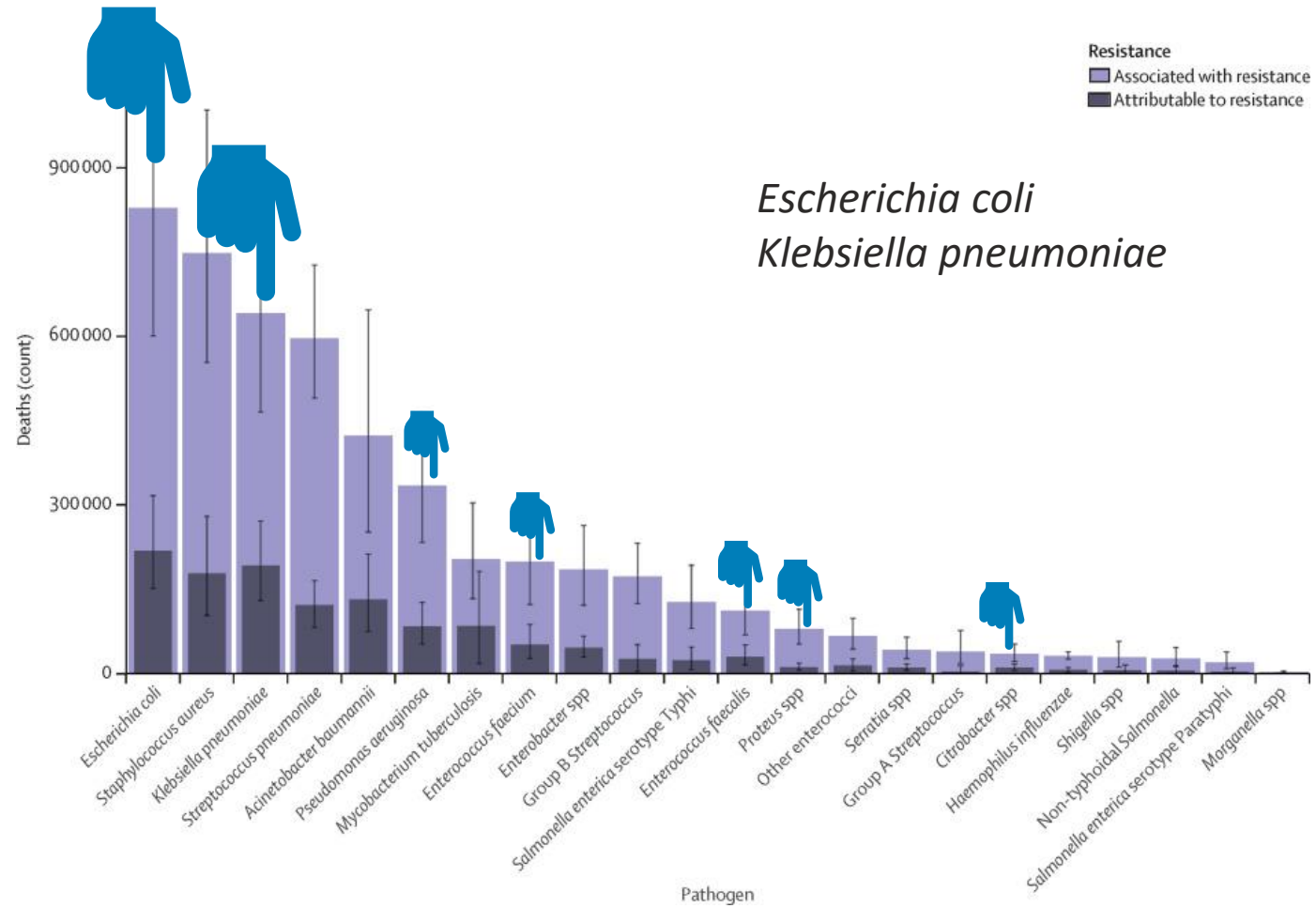
Global burden of bacterial antimicrobial resistance in 2019: a systematic analysis
Murray et al 2022 *The Lancet* doi.org/10.1016/S0140-6736(21)02724-0

Urinary tract infection (UTI)

- UTIs are caused by enteric bacteria
- main causative bacteria (uropathogens):
 - *Escherichia coli* *UPEC
 - *Klebsiella pneumoniae*
 - *Enterococcus faecalis*

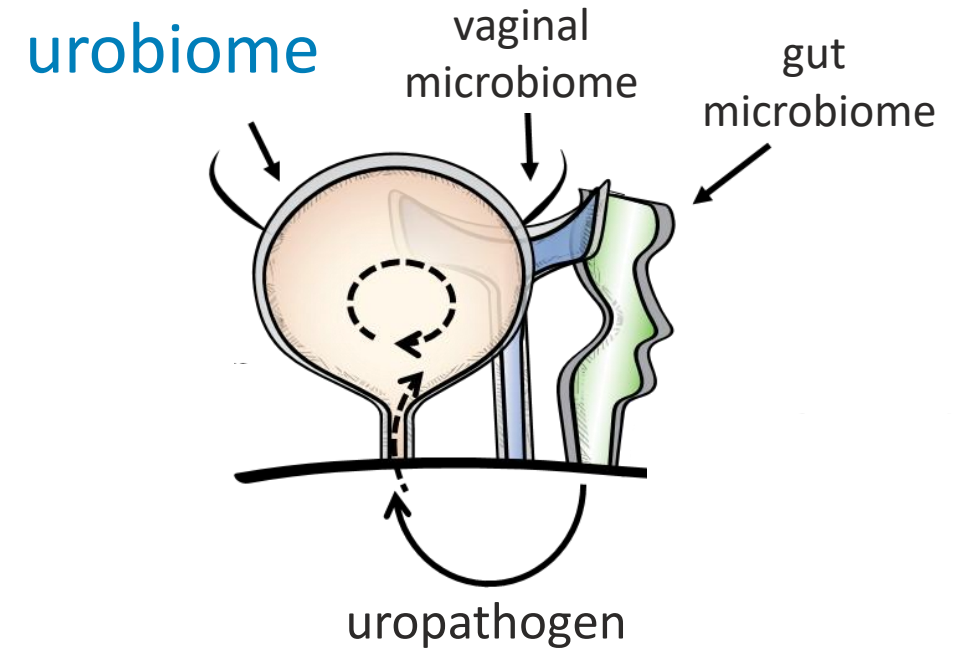


Drug resistance in uropathogens



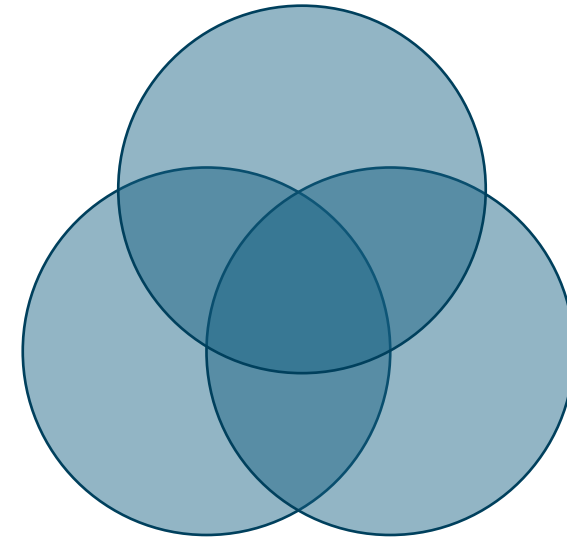
Urinary microbiome and UTI

- Urine is not sterile
- Bladder microbiome contains a variety of bacteria



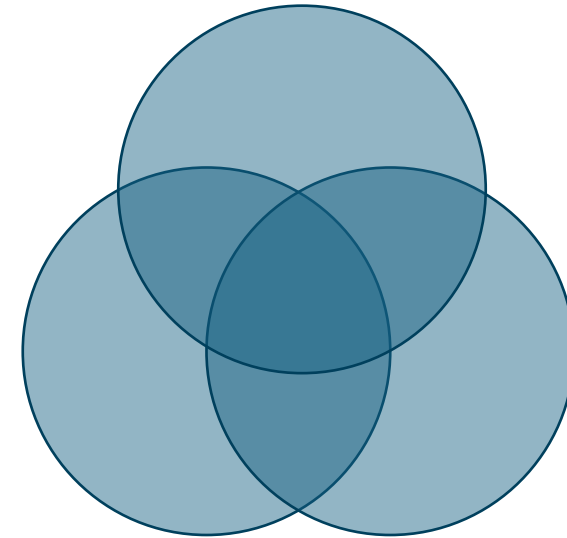
Urobiome as ecological niche

- Host (genetics, development, immune system)
- Urobiome community (residents and invaders)
- Environment (stress, drugs, diet, etc)



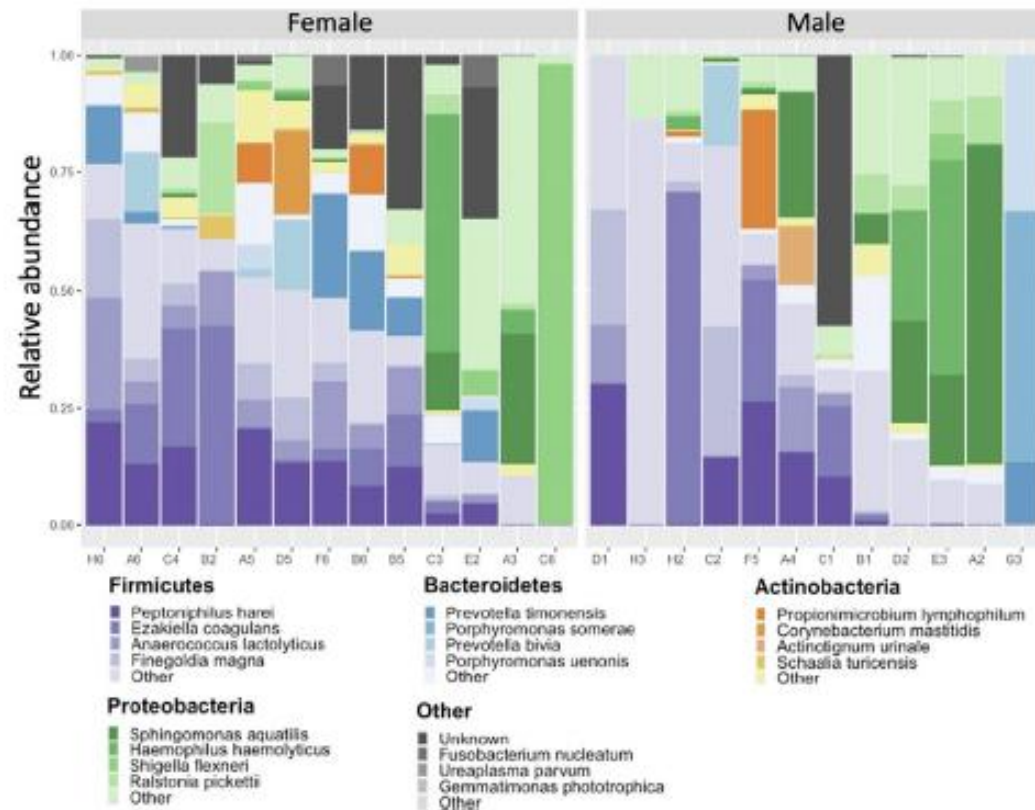
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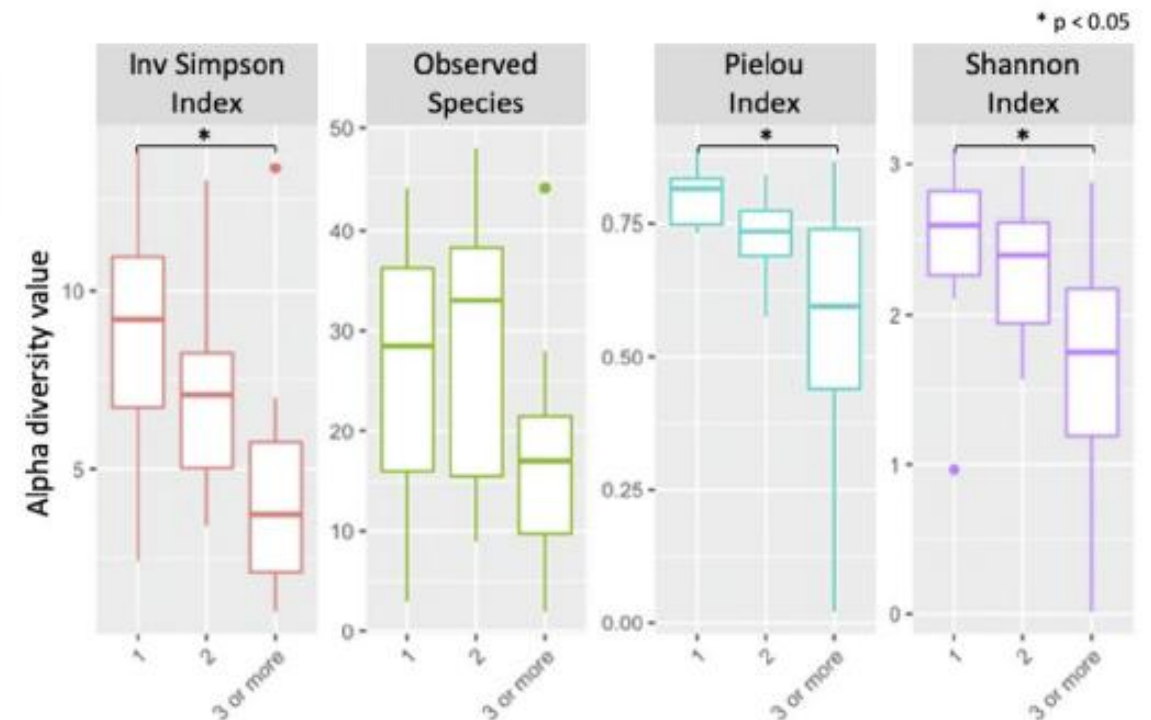


Development of urobiome in children

A. The pediatric bladder microbiome varies between individuals and displays sex-related differences.



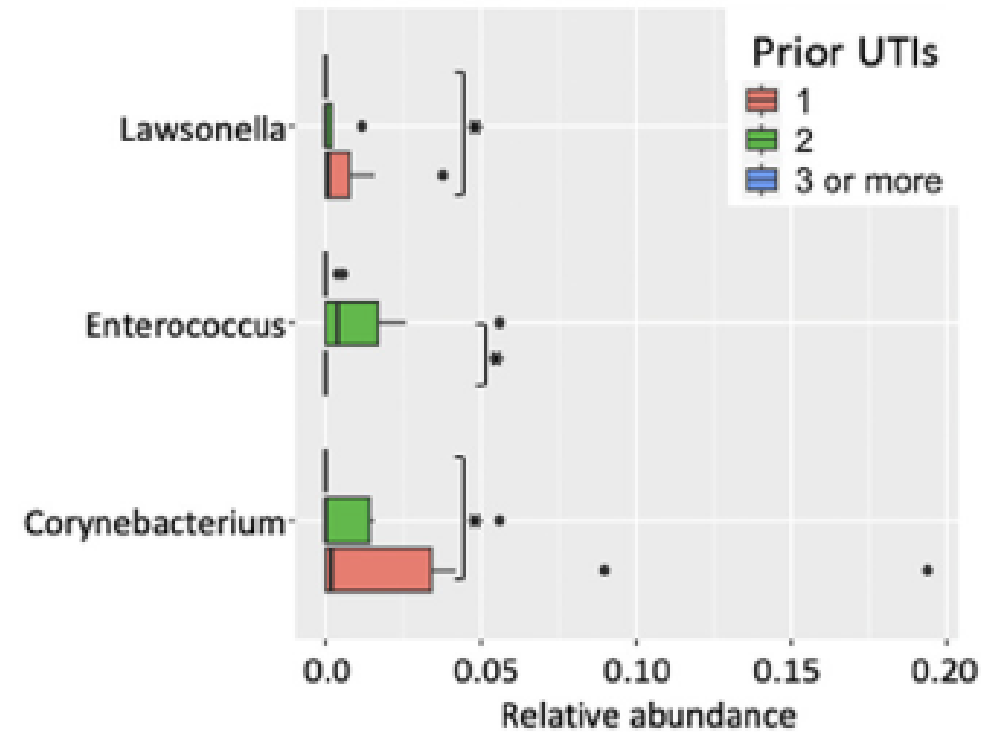
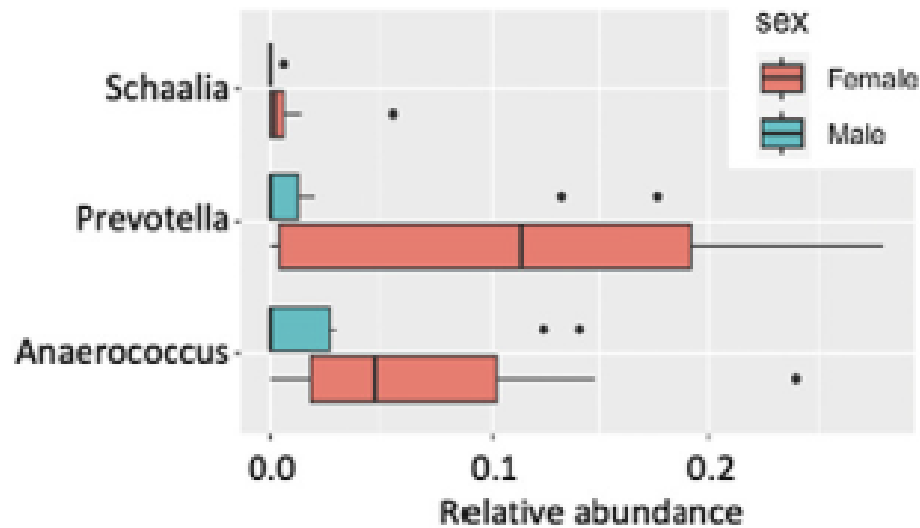
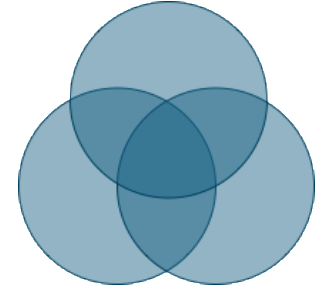
B. Microbial diversity decreases as the number of UTIs increase.



Kelly et al, 2024

Development of urobiome in children

- Species and strain resolution is important
- Urobiome composition differ by sex, age, UTI history

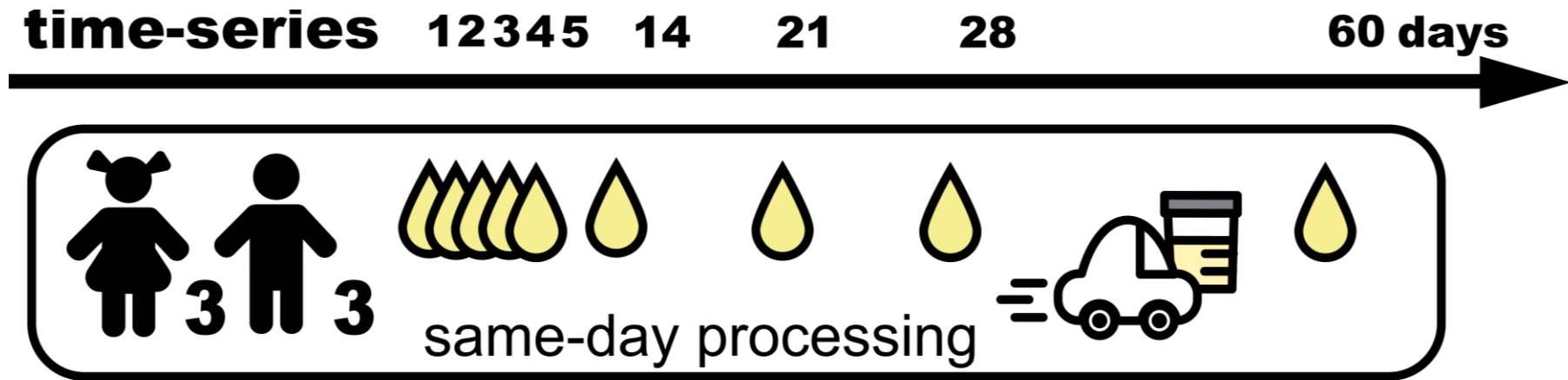


Kelly et al, 2024

Development of urobiome in children



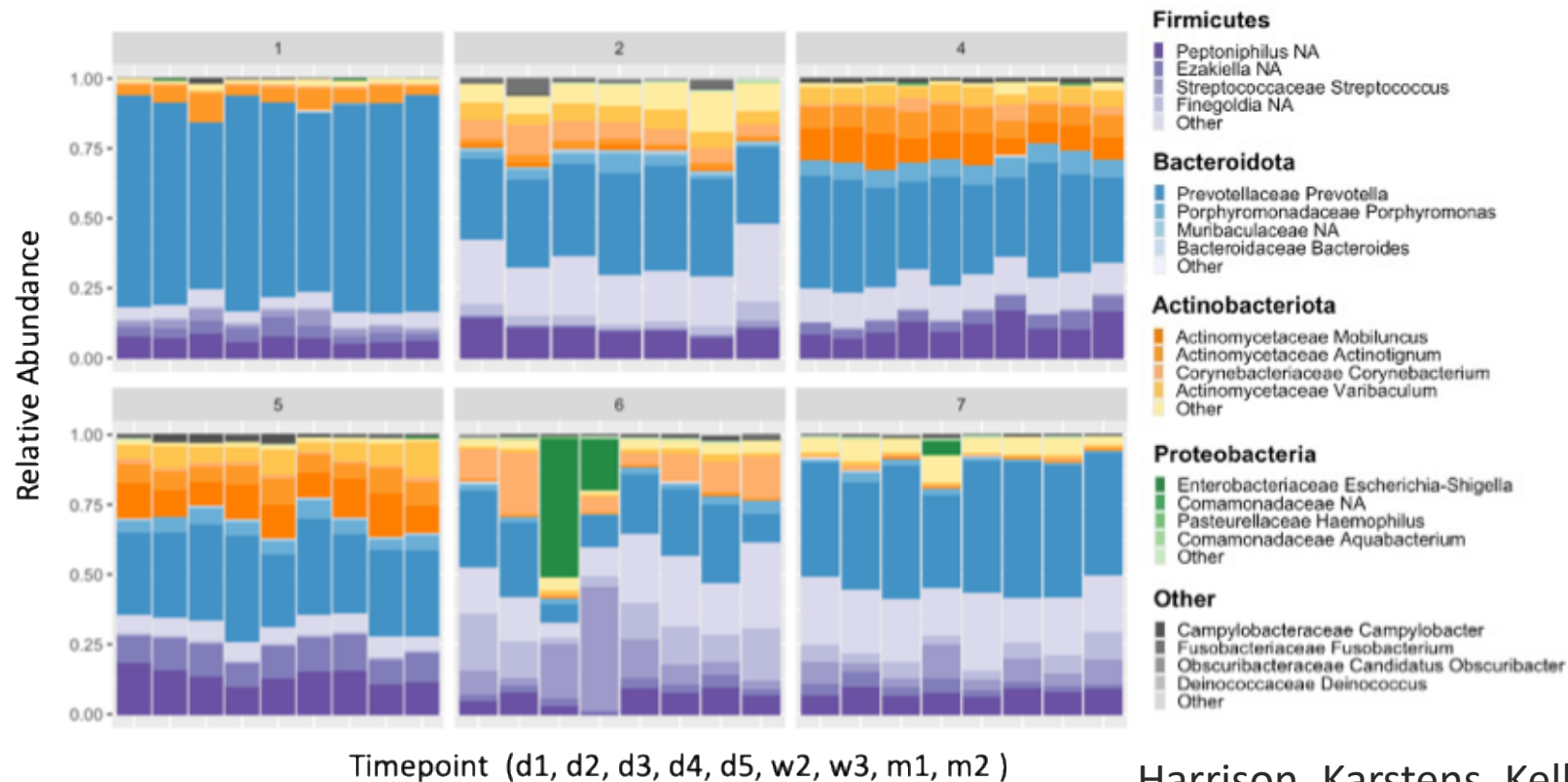
- are cross-sectional studies enough?
- longitudinal collection with days, weeks, months



Harrison, Karstens, Kelly, Sysoeva, unpublished

Development of urobiome in children

- Pediatric urobiome profiles and diversity metrics are individualized and stable over months-range



Harrison, Karstens, Kelly, Sysoeva, unpublished

Development of urobiome in children

- Pediatric urobiome profiles and diversity metrics are individualized and stable over months-range

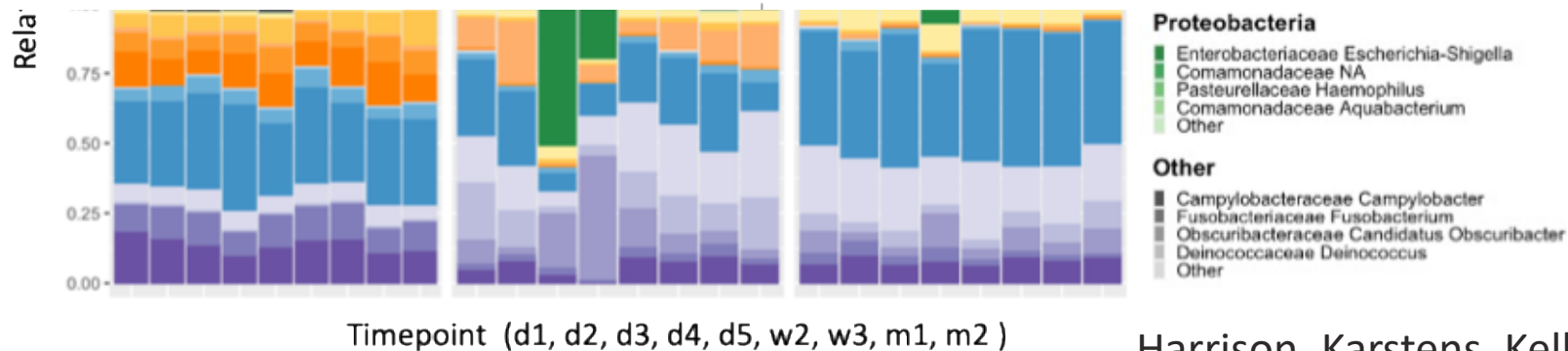


Comparing Sequencing Approaches and Temporal Dynamics to Elucidate the Clinical Utility of the Pediatric Urobiome

Keisha Harrison, PhD¹; Tatyana A. Sysoeva, PhD²; Maryellen Kelly, DNP^{3,4}; Lisa Karstens, PhD^{1,5,6}



1. Division of Oncological Sciences, Knight Cancer Institute, Oregon Health Sciences University, Portland, OR, USA.,
2. Department of Biological Sciences, The University of Alabama in Huntsville, Huntsville, AL, USA.,
3. Healthcare of Women and Children, School of Nursing, Duke University, Durham, NC, USA
4. Department of Urology, Duke University, Durham, NC, USA.,
5. Division of Informatics, Clinical Epidemiology, & Translational Data Science, Oregon Health & Science University, Portland, OR, USA,
6. Division of Urogynecology, Oregon Health & Science University, Portland, OR, USA.



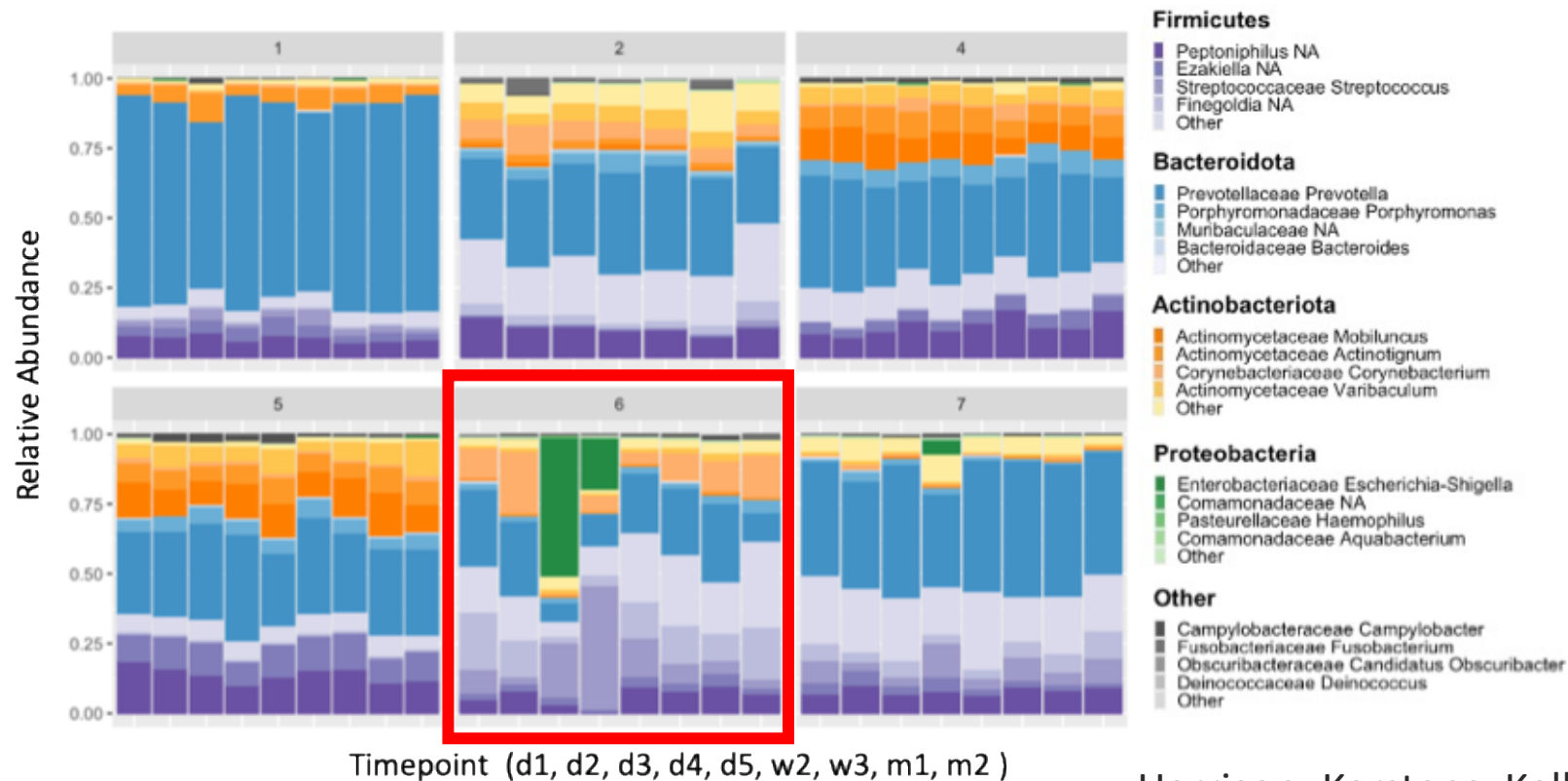
Harrison, Karstens, Kelly, Sysoeva, unpublished



THE UNIVERSITY OF
ALABAMA IN HUNTSVILLE

Development of urobiome in children

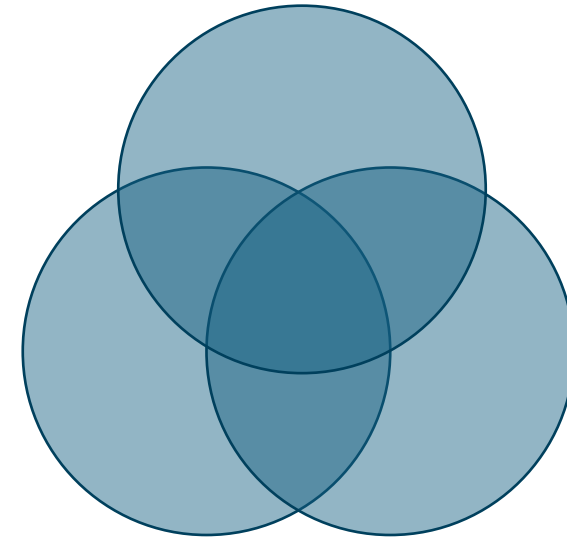
- Examples of invasion of possible pathogen and restoring the community



Harrison, Karstens, Kelly, Sysoeva, unpublished

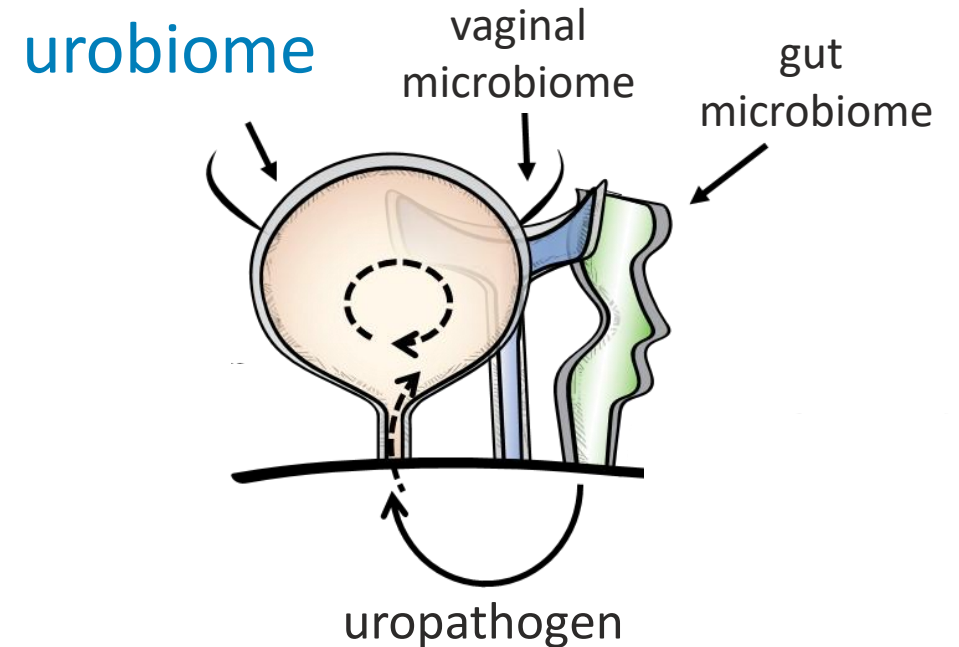
Urobiome as ecological niche

- Host (genetics, development, immune system)
- **Urobiome community (residents and invaders)**
- Environment (stress, drugs, diet, etc)



Urinary microbiome and UTI

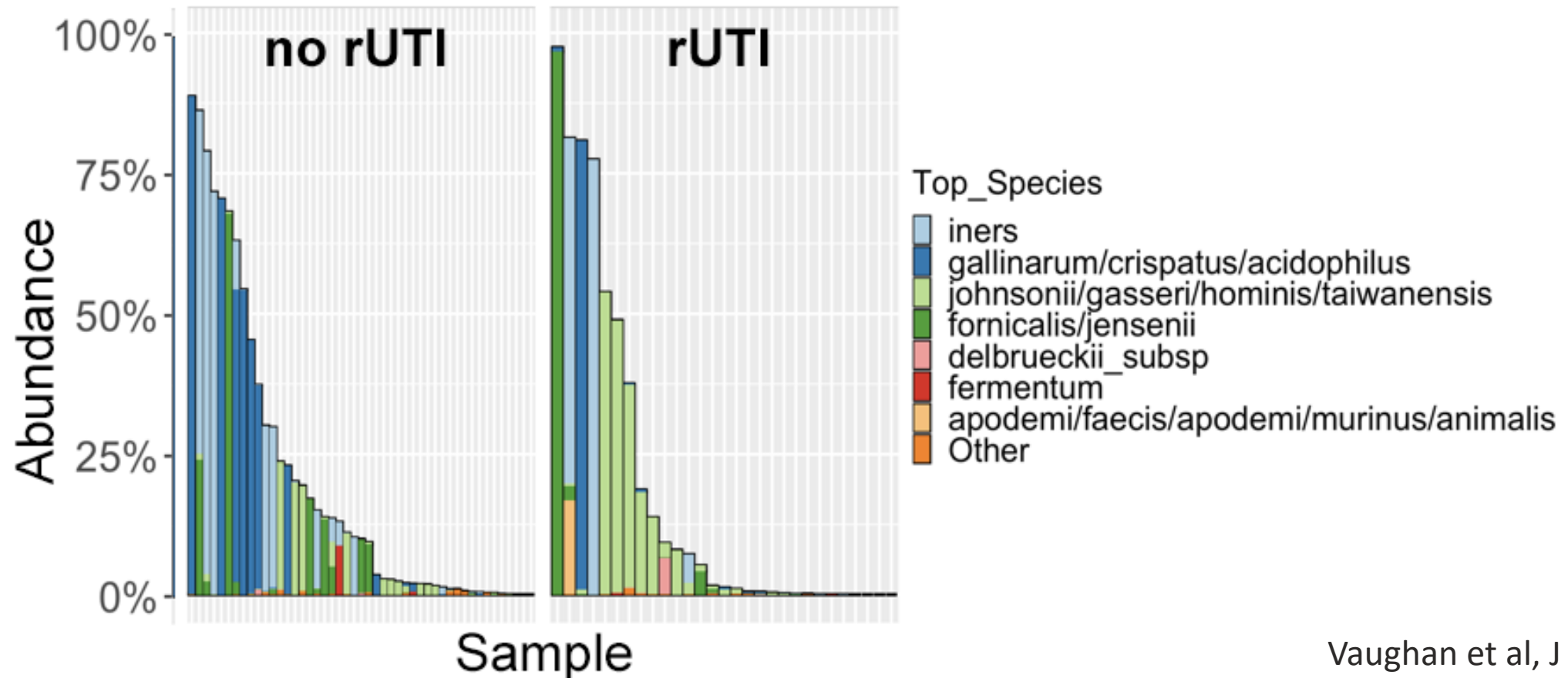
- Urine is not sterile
- Bladder microbiome contains a variety of bacteria
- In women, **lactobacilli** frequently dominate
- Predisposition to UTIs might depend on urinary microbiome – presence or abundance of specific lactobacilli



Vaughan et al, J Urol, 2021

Urinary microbiome and UTI

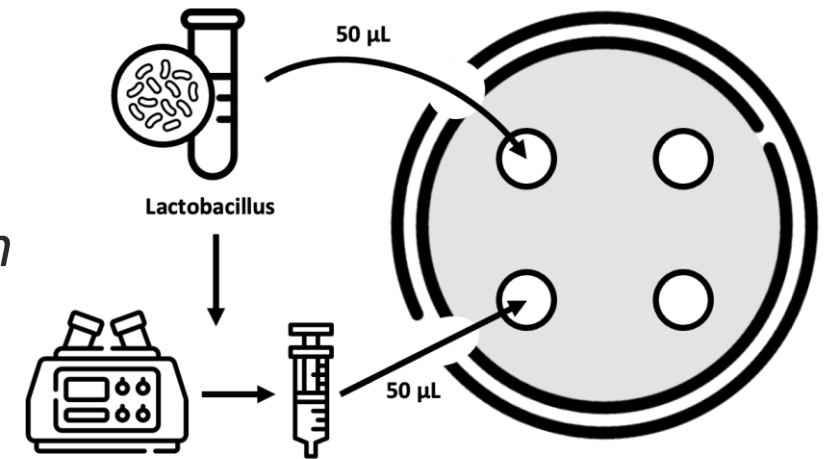
- **lactobacilli** found include *L. crispatus*, *L. gasseri*, *L. jensenii*, *L. rhamnosus*, *L. iners*



Vaughan et al, J Urol, 2021

Urinary Lactobacilli as Microbial Defenders

- Repository of urinary lactobacilli species (20 strains)
 - Postmenopausal women
 - Healthy or SUC-backed rUTI diagnoses
- Observed inhibition of UPEC and other uropathogens *in vitro*
 - Well-diffusion inhibition and co-cultures

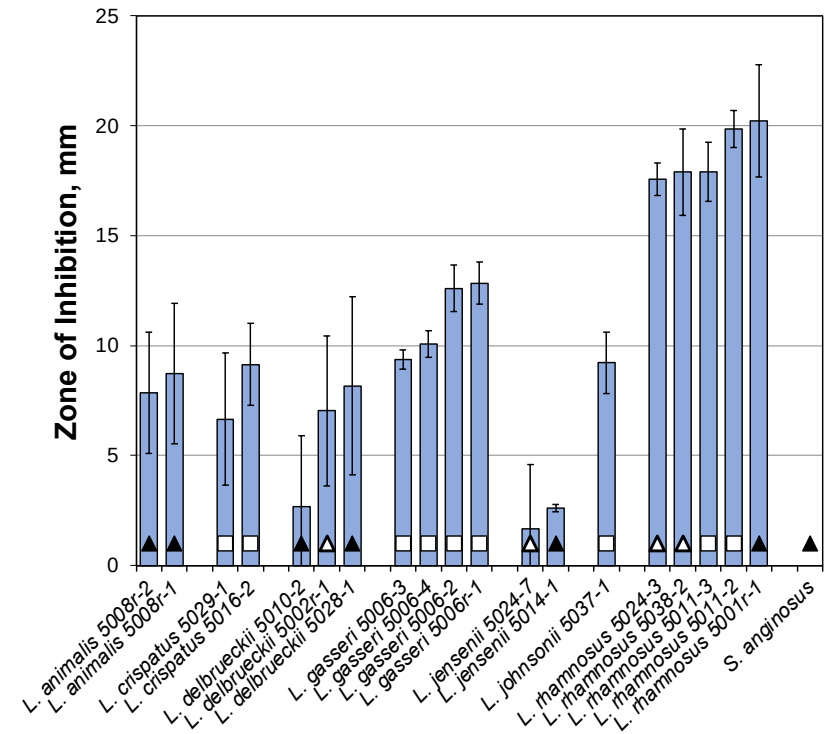


Vaughan et al. 2021

Johnson et al, 2022

Urinary Lactobacilli as Microbial Defenders

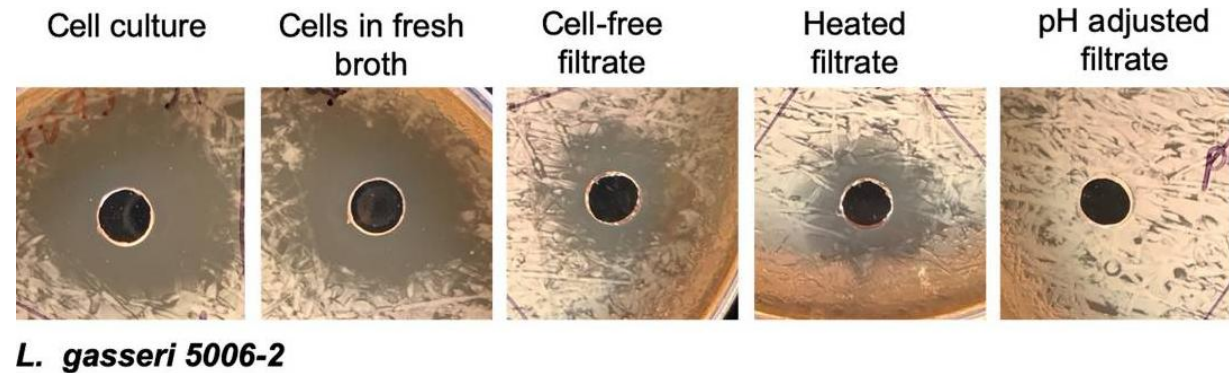
- Majority of urinary lactobacilli inhibit the growth of major uropathogens *E. coli*, *K. pneumoniae* *in vitro* and some inhibit *E. faecalis*. This inhibition is working of multidrug resistant uropathogens as well.
- There is species- and strain- level variability among lactobacilli phenotypes.



Johnson et al, 2022

Urinary Lactobacilli as Microbial Defenders

- Majority of urinary lactobacilli inhibit the growth of major uropathogens *E. coli*, *K. pneumoniae* *in vitro* and some inhibit *E. faecalis*. This inhibition is working of multidrug resistant uropathogens as well.
- There is species- and strain- level variability among lactobacilli phenotypes.
- Acidity- and cell- dependent inhibition - unknown mechanism



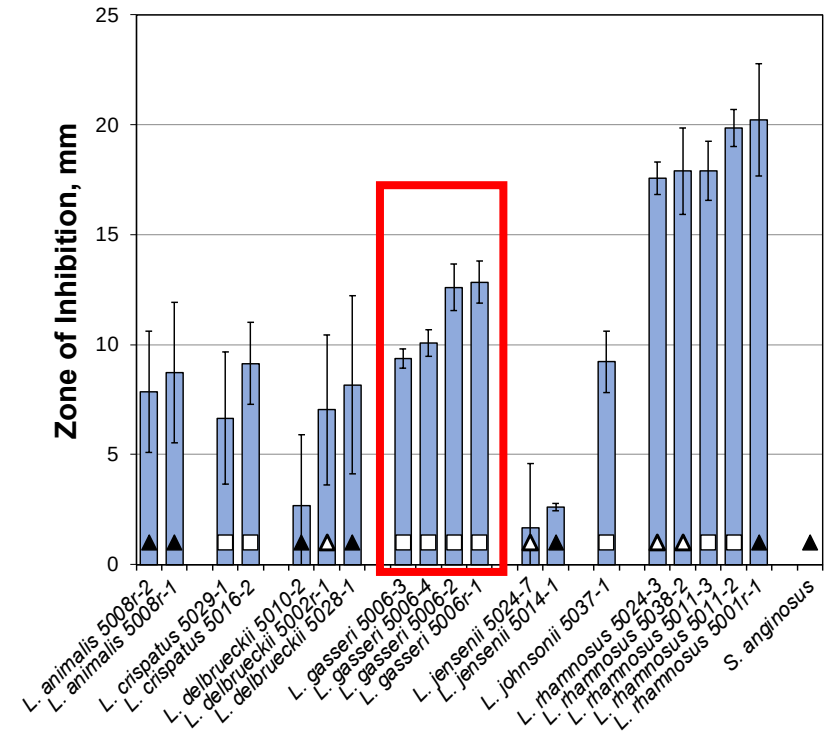
Johnson et al, 2022

L. gasseri inhibits UPEC *in vitro*

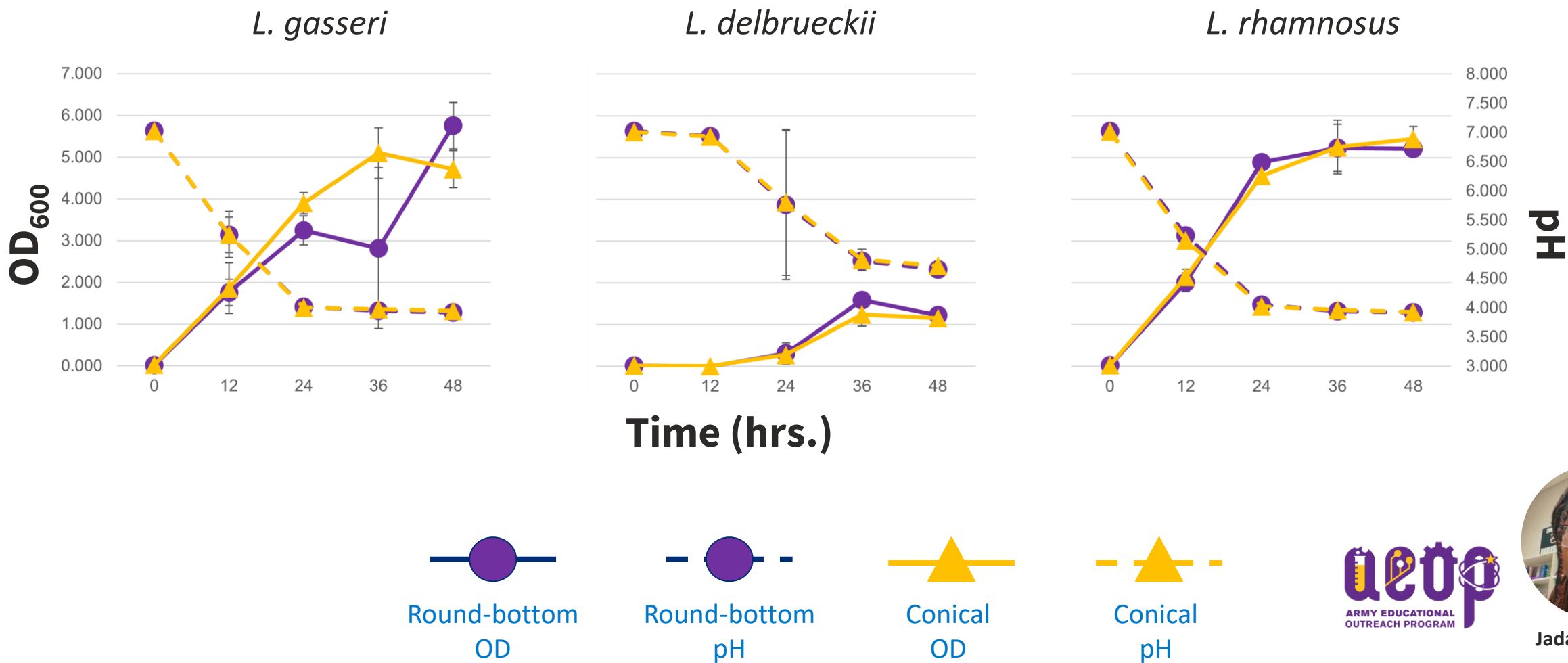
- What is the mechanism of this inhibition?



Layla Jeries



Acidification of media by lactobacilli

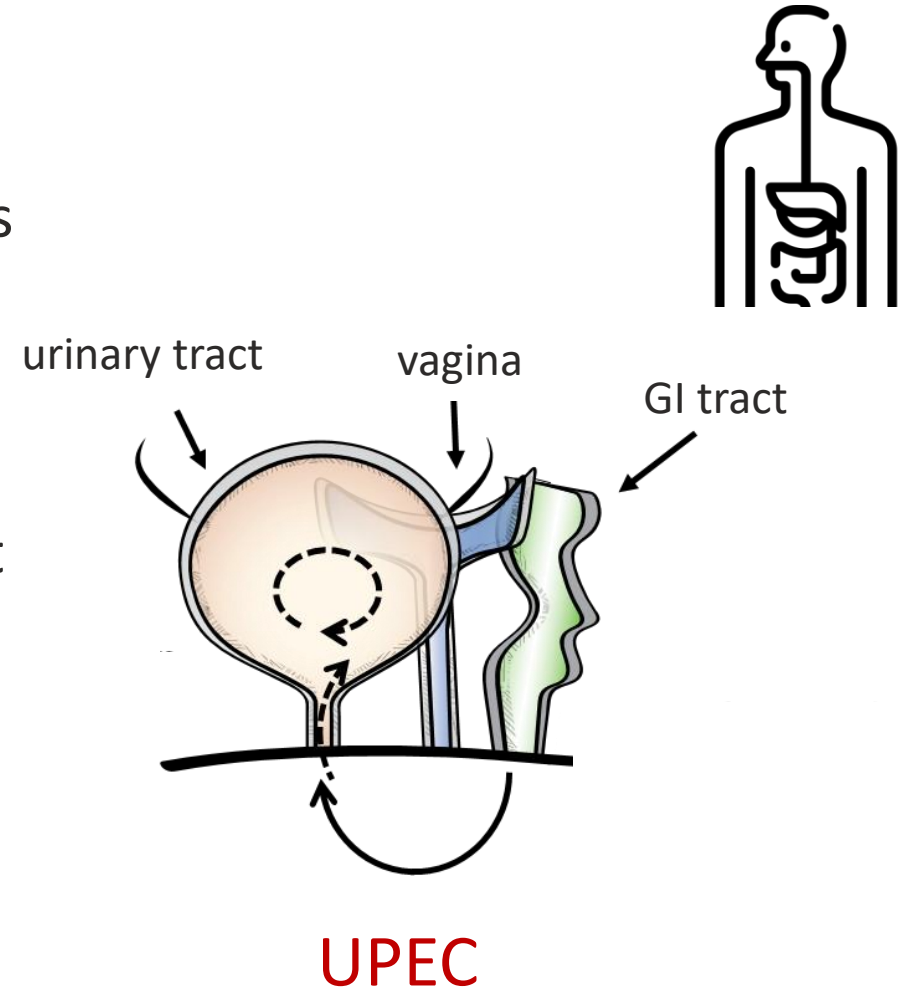


Jada McElroy



Acid Response (AR) in UPEC

- Must survive fluctuating pH of various environments
 - GI tract (gastric: 1.0 – 2.5; intestines: 6.4 – 7.0)
 - Vaginal opening (3.8 – 5)
 - Urine (4.5 – 8)
- Must survive low pH of phagolysosomes during host cell colonization



Acid Response (AR) in UPEC

- *E. coli* has several characterized acid-response (AR) systems to withstand acidic stress

System	Input↓ Output↑	Antiporter	PLP-decarboxylase	Regulation
AR2	Glu↓ GABA↑	GadC	GadA, GadB	RpoS
AR3	Arg↓ Agmatine↑	AdiC	AdiA	AdiY
AR4	Lys↓ Cadaverine↑	CadB	CadA	CadC
AR5	Ornithine↓ Putrescine↑	PotE	SpeF	GTP levels
System	Input↓ Output↑	Antiporter	Deaminases	Regulation
AR6	Ser↓ NH ₃ ↑	SdaC	SdaA SdaB	unknown

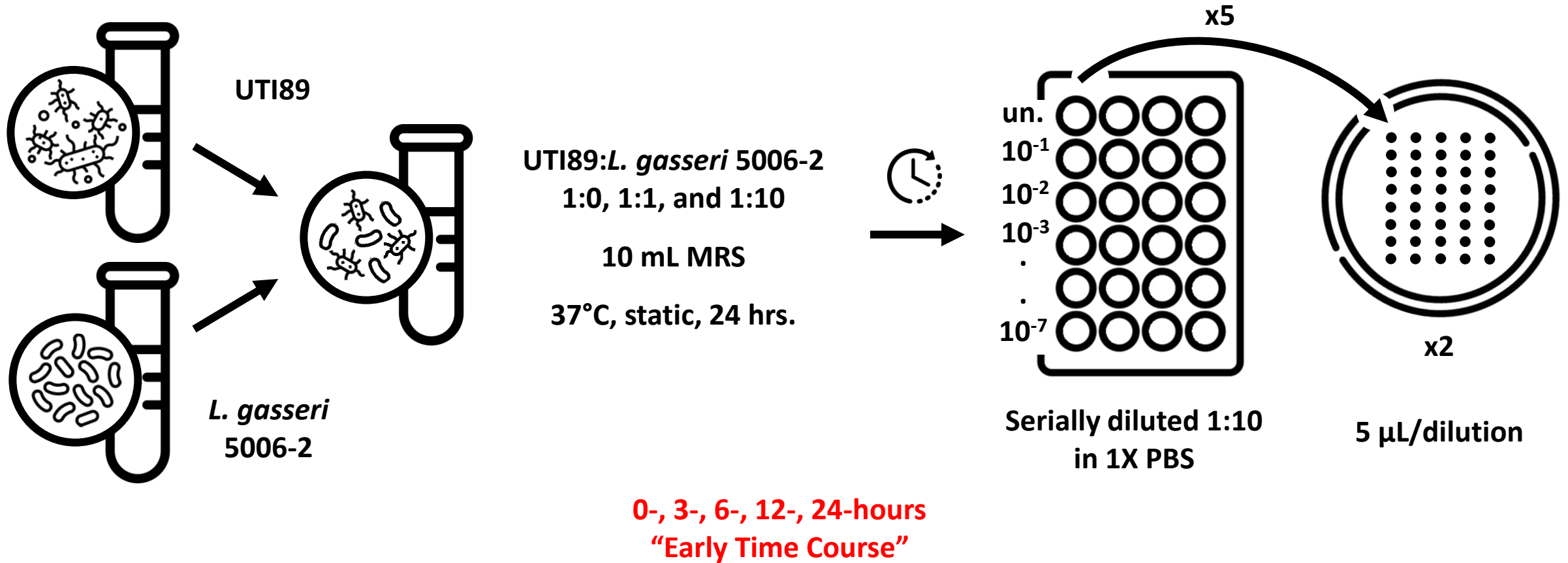
- **Hypothesis:** Disrupting AR will sensitize UPEC to inhibition by lactobacilli

Disruption of Acid Response in UPEC

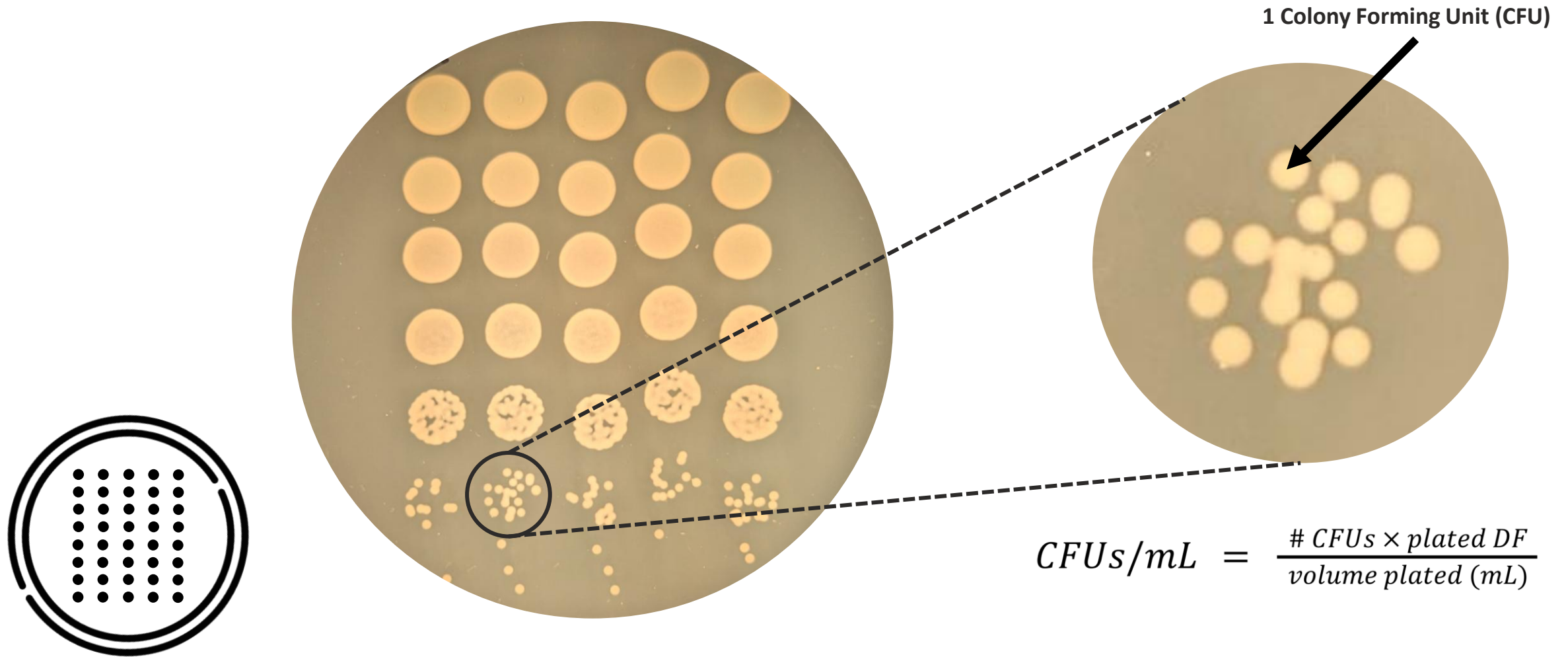
- AR mutants of UTI89 *E. coli* strain created by Michelle Wiebe in [Maria Hadjifrangiskou lab, Vanderbilt University](#)

UTI89 Strain Shorthand ID	Genotype/Description	AR
UTI89-WT	UPEC model, cystitis isolate, wildtype	-
UTI89 $\Delta btsT$	$\Delta btsT \Delta ypdB \Delta phoPQ \Delta evgAS$	AR2, AR6,
UTI89 $\Delta gad \Delta cad$	$\Delta gadA \Delta gadB \Delta cadA$	AR2, AR4
UTI89 Δsda	$\Delta sdaA \Delta sdaB$	AR6

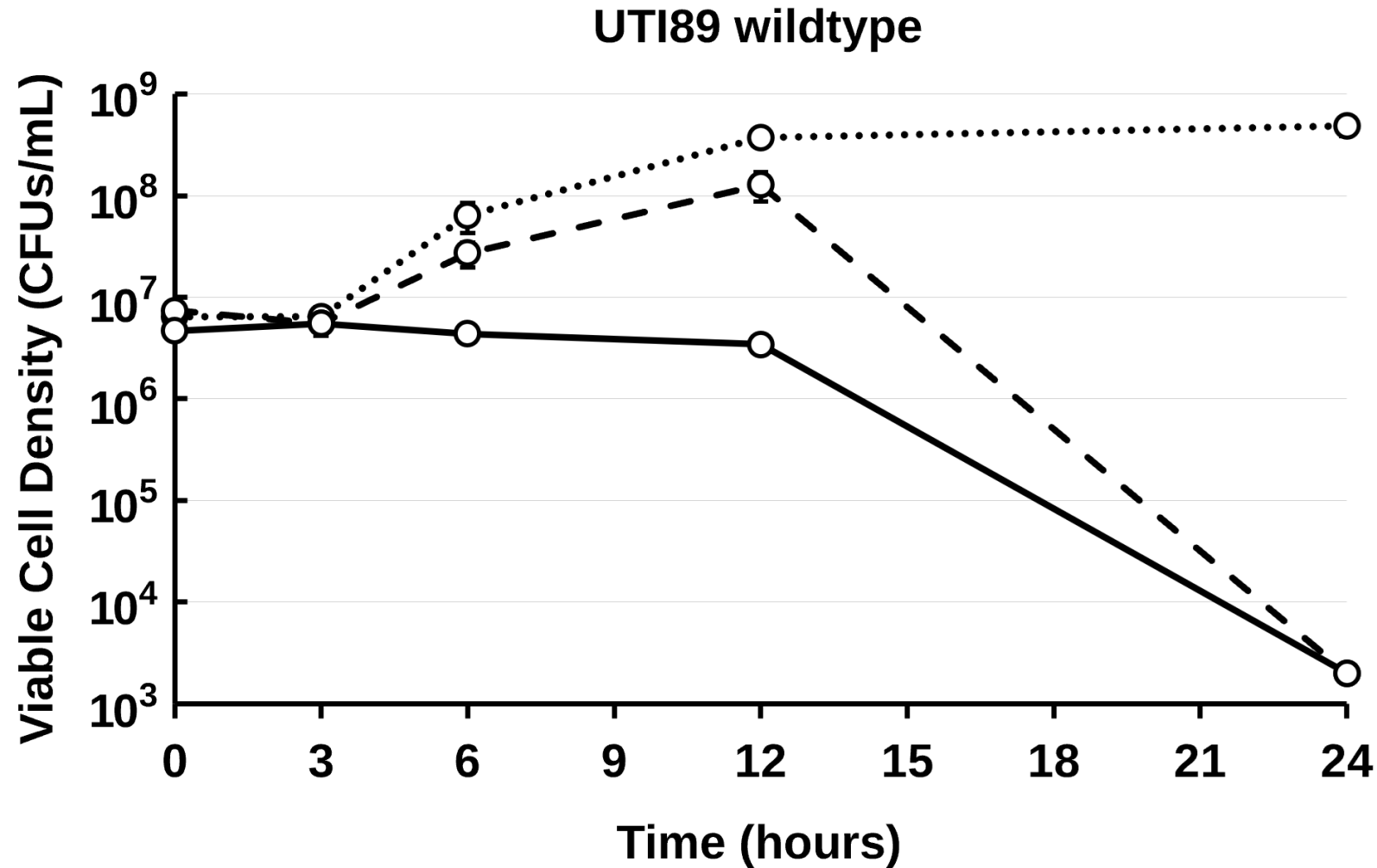
Co-culture conditions and quantification



Quantification – CFUs/mL



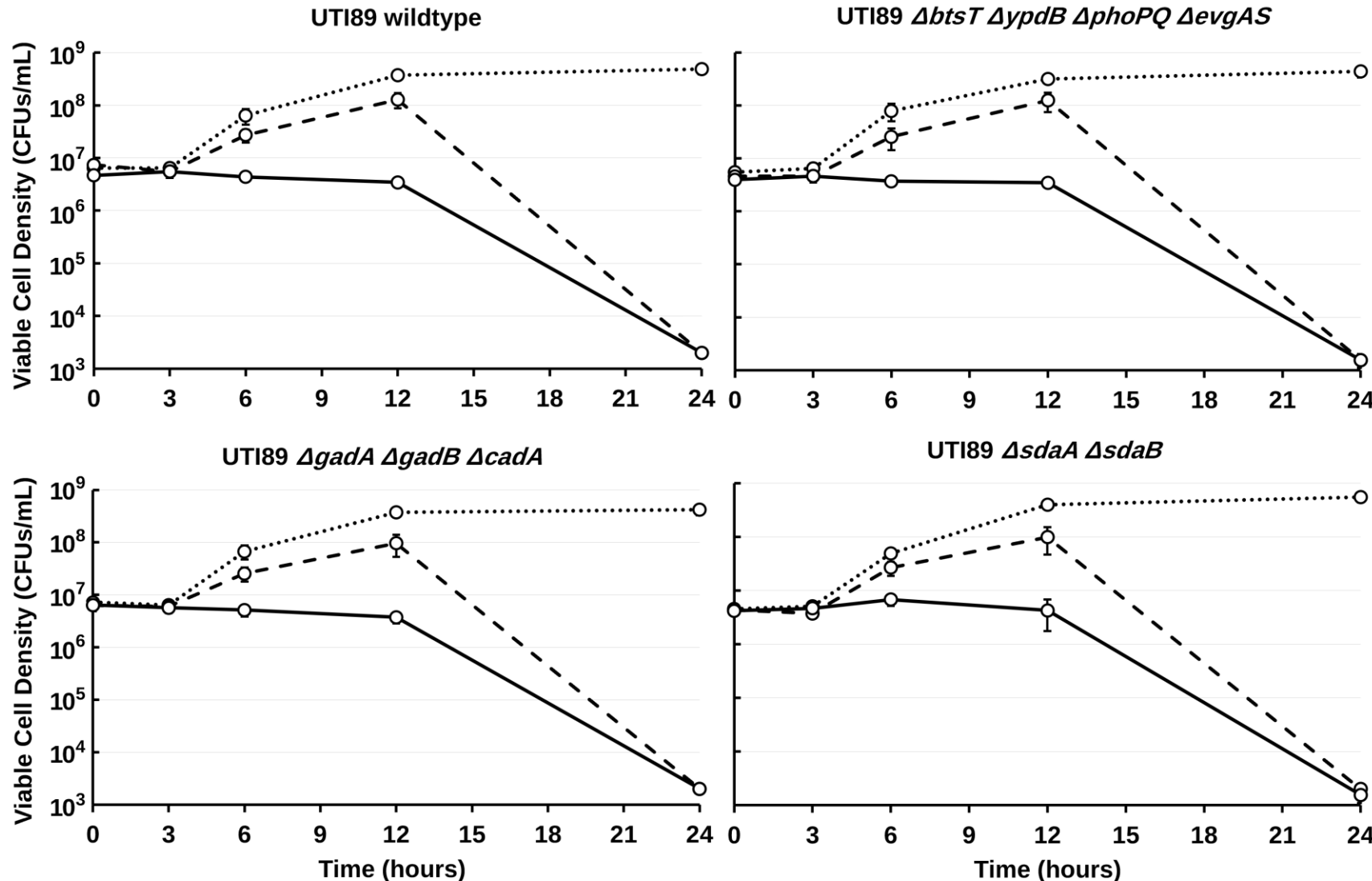
UTI89 is inhibited by *L. gasseri* over time



- Inhibition of UTI89 is sensitive to ratio of UTI89:*L. gasseri* 5006-2
- Viability differences starting at 6 hours
- Limit of detection = 10³ CFUs/mL
- Both ratios drop below LOD by 24-hours

UTI89 strain : *L.gasseri* 5006-2 ratio
··○·· 1:0 -○- 1:1 —○— 1:10

Disruption of Acid Response in UPEC



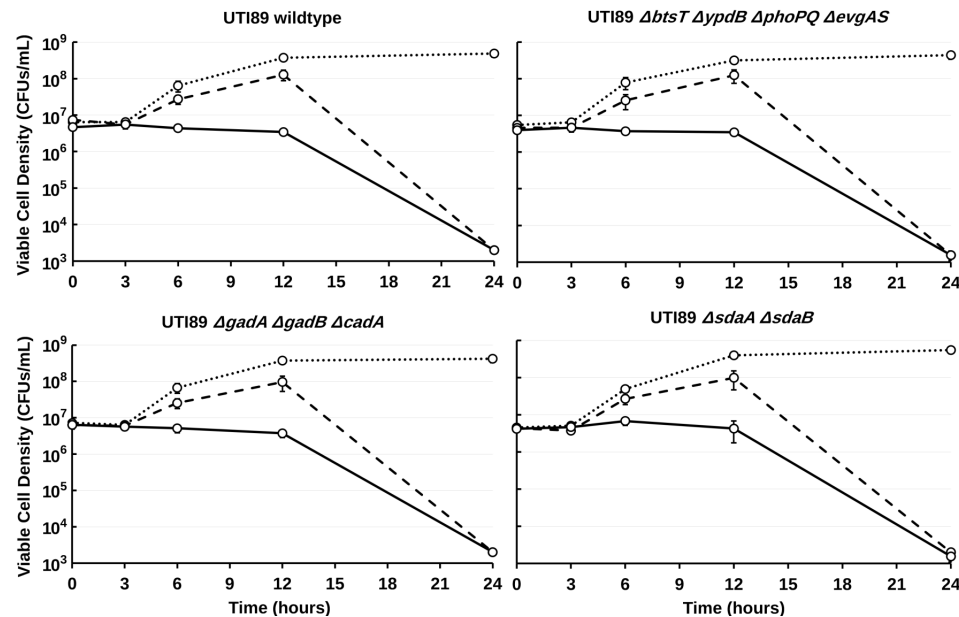
- No difference between AR mutant UTI89 strains

UTI89 strain : *L.gasseri* 5006-2 ratio
 ···○·· 1:0 -○- 1:1 —○— 1:10

Disruption of Acid Response in UPEC

- Are UTI89 strains compensating for missing AR systems?
 - Upregulating others?
 - Employing other systems of stress response?

- No difference between AR mutant UTI89 strains

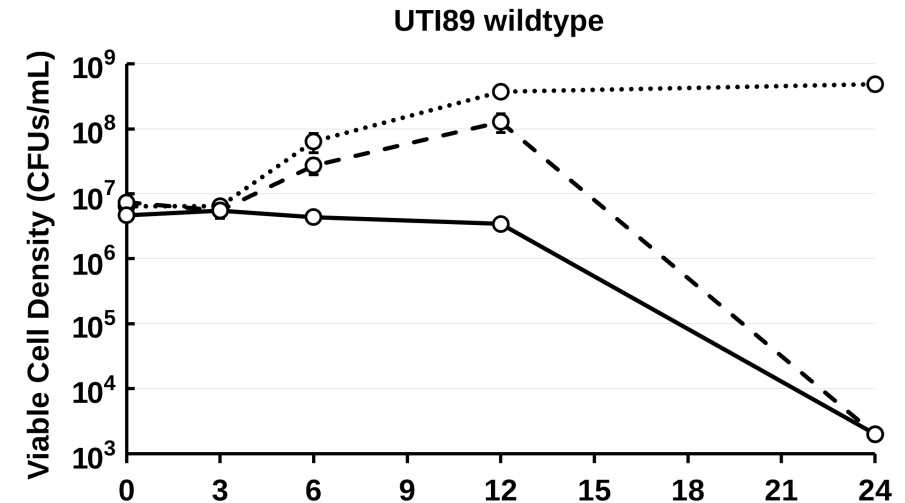


UTI89 strain : *L.gasseri* 5006-2 ratio
··○·· 1:0 -○- 1:1 —○— 1:10

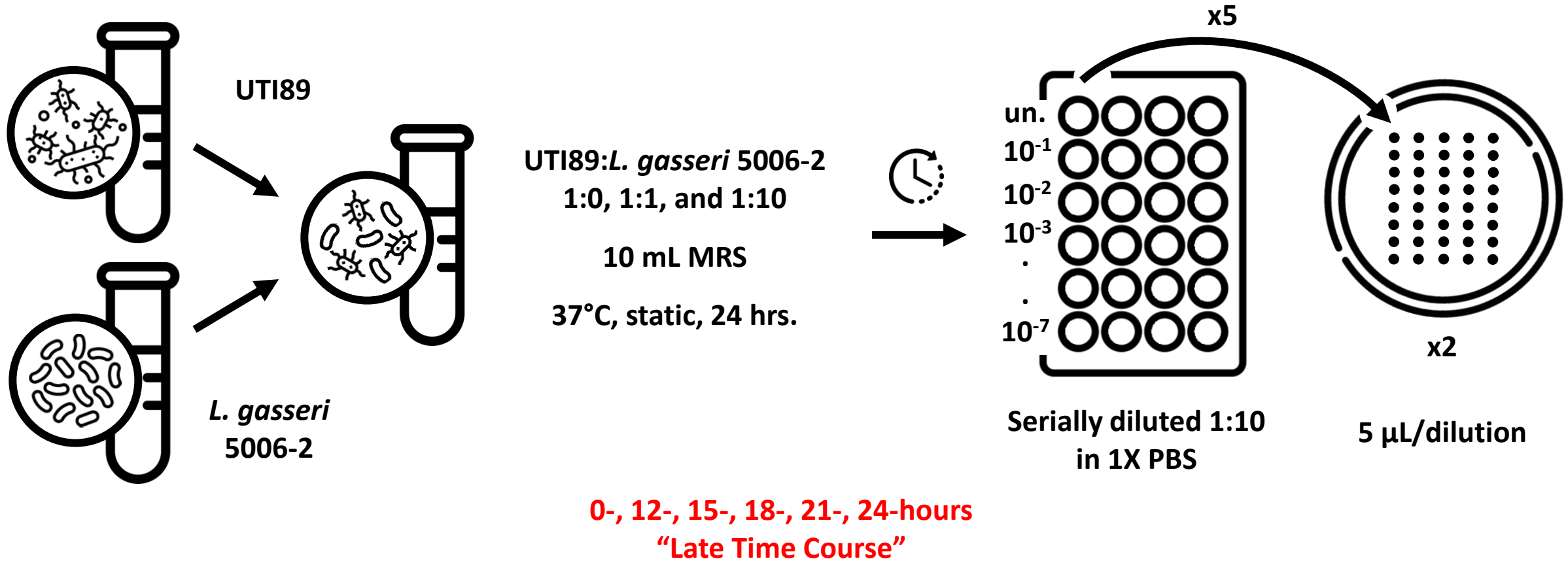
Characterization of late-phase UTI89 viability

- Both treatments pass LOD by 24-hours
- At what rate do death phases occur?

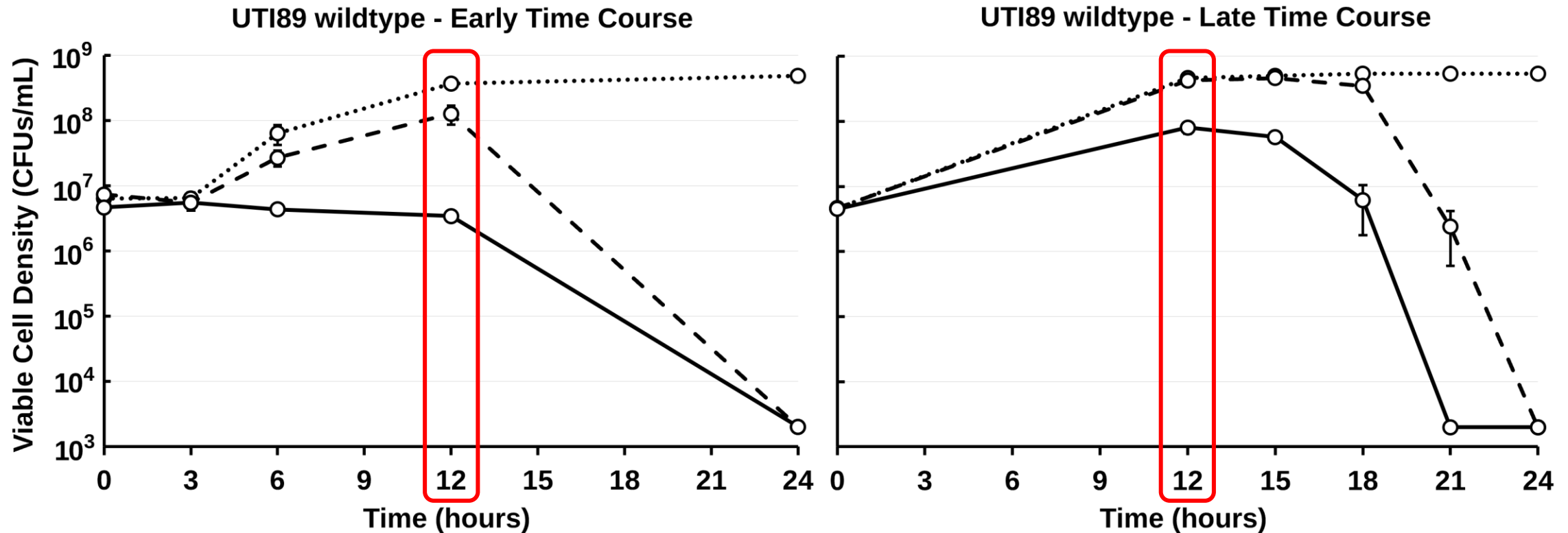
If UTI89 viability drops below the LOD by 24-hours, then viability should rapidly decrease between 12- and 24-hours.



Co-culture conditions and quantification



UTI89 is inhibited by *L. gasseri* 5006-2 differently in the early and late time courses

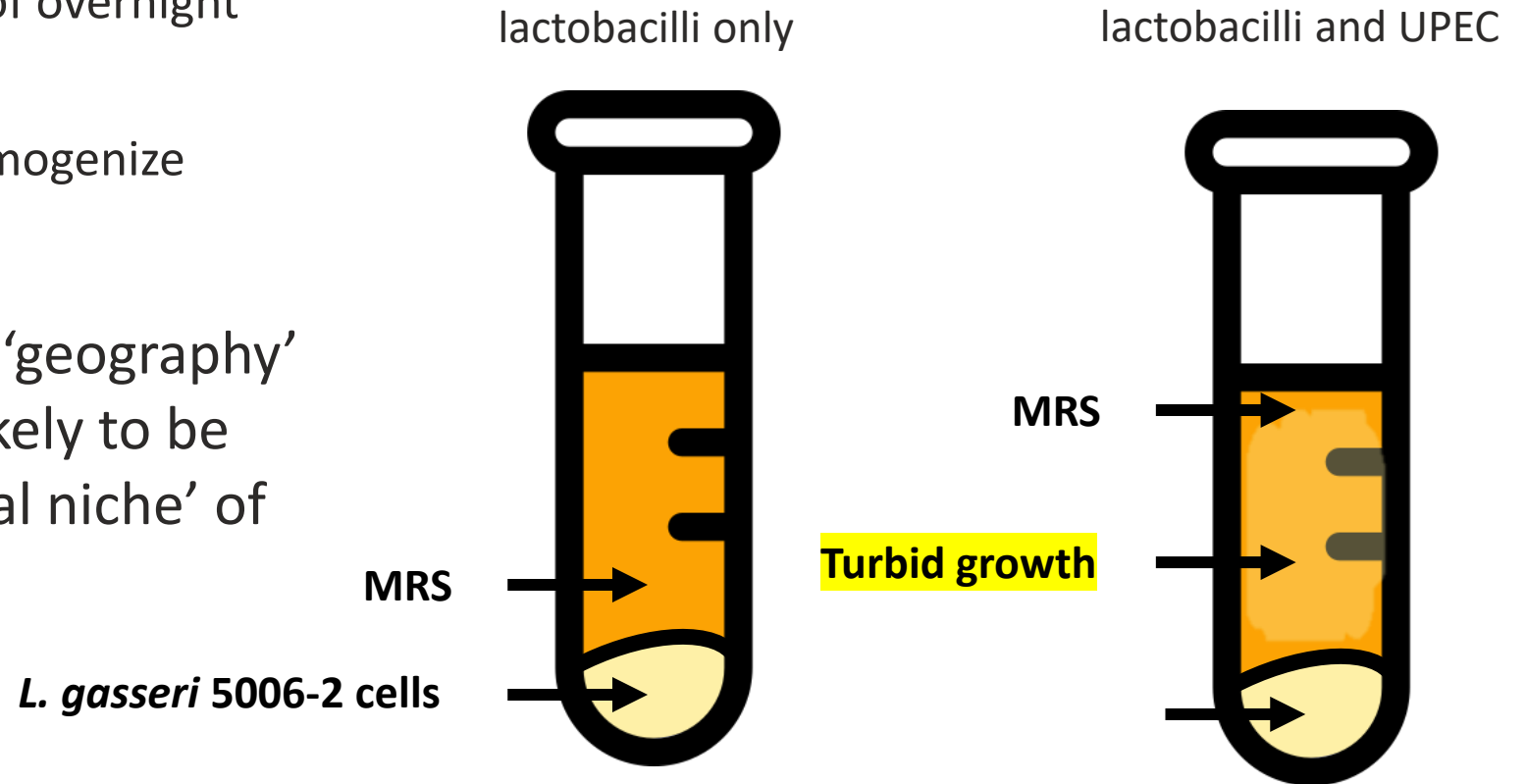


- No significant difference in viability between 1:0 and 1:1 treatments
- 1:10 passed LOD one time point before 1:1
- Death phase for both treatments was rapid

UTI89 strain : *L.gasseri* 5006-2 ratio
··○·· 1:0 -○- 1:1 —○— 1:10

Evaluation of close interactions of UTI89 and *L. gasseri* in co-culture

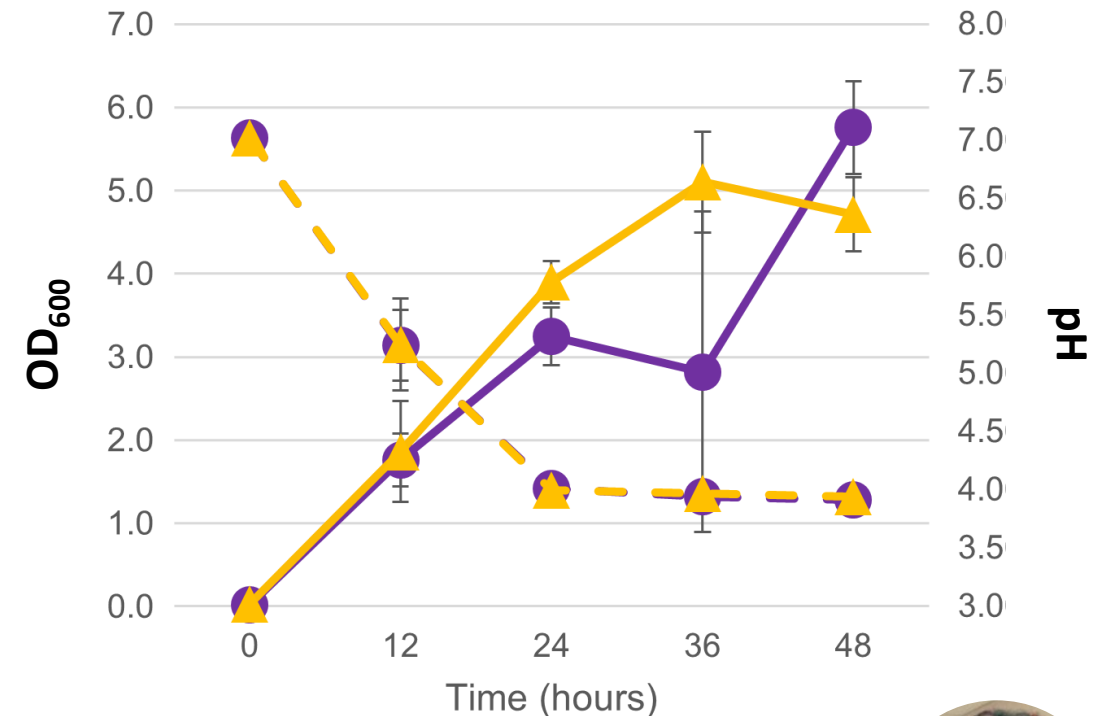
- *L. gasseri* 5006-2 is “sticky”
 - Large pellet at 48 hours of overnight culture
 - Vortexing >10 sec. to homogenize
- Highlight importance of ‘geography’ and cell state which is likely to be present in real ‘ecological niche’ of urinary tract



pH kinetics in mono- and co-cultures

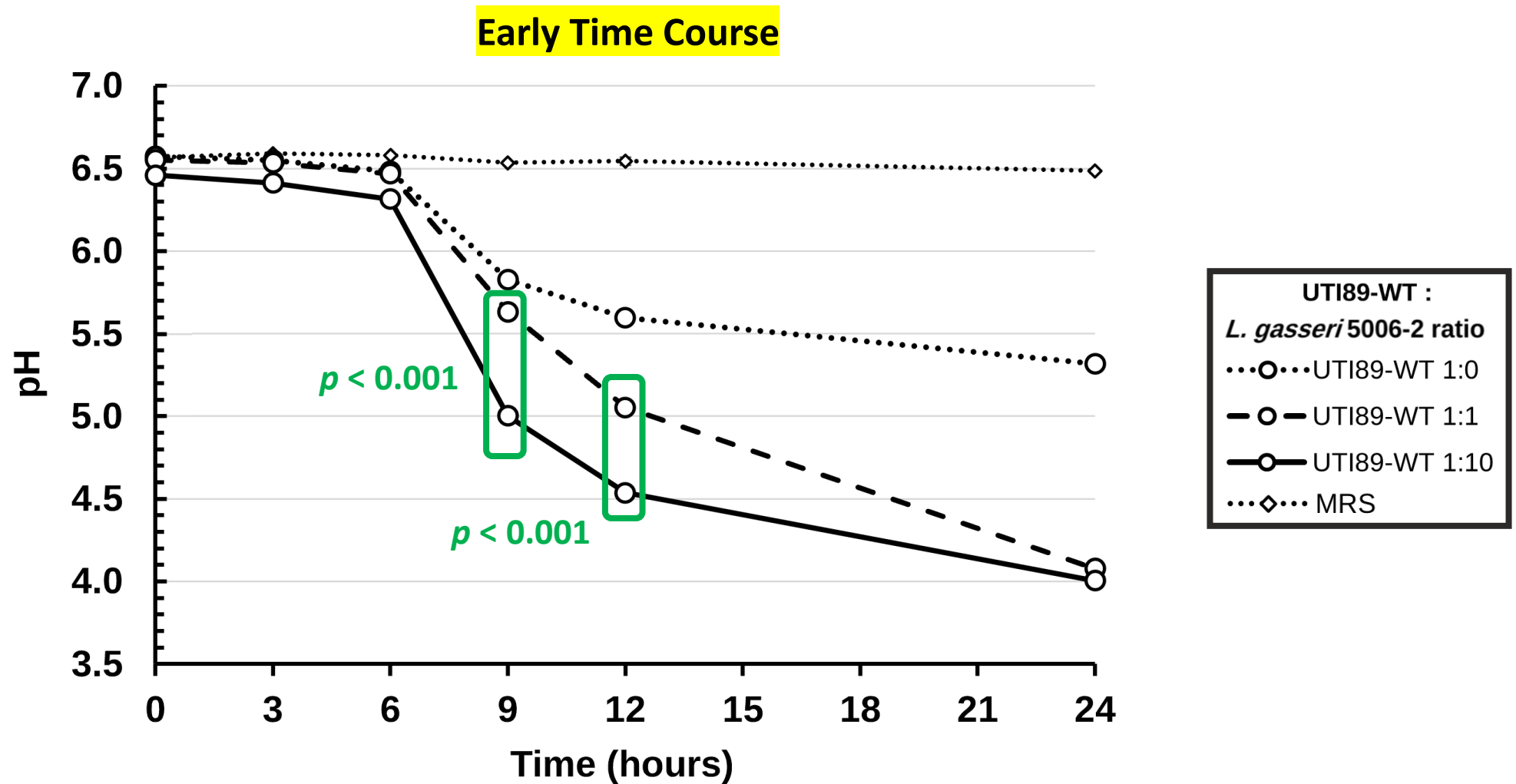
- Are UTI89 strains compensating for missing AR systems?
 - Upregulating others?
 - Employing other systems of stress response?
- Do co-cultures reach required pHs to activate AR systems?

If acidification is significant in UTI89 inhibition, then acidic shifts in pH should correlate with previously measured viability.

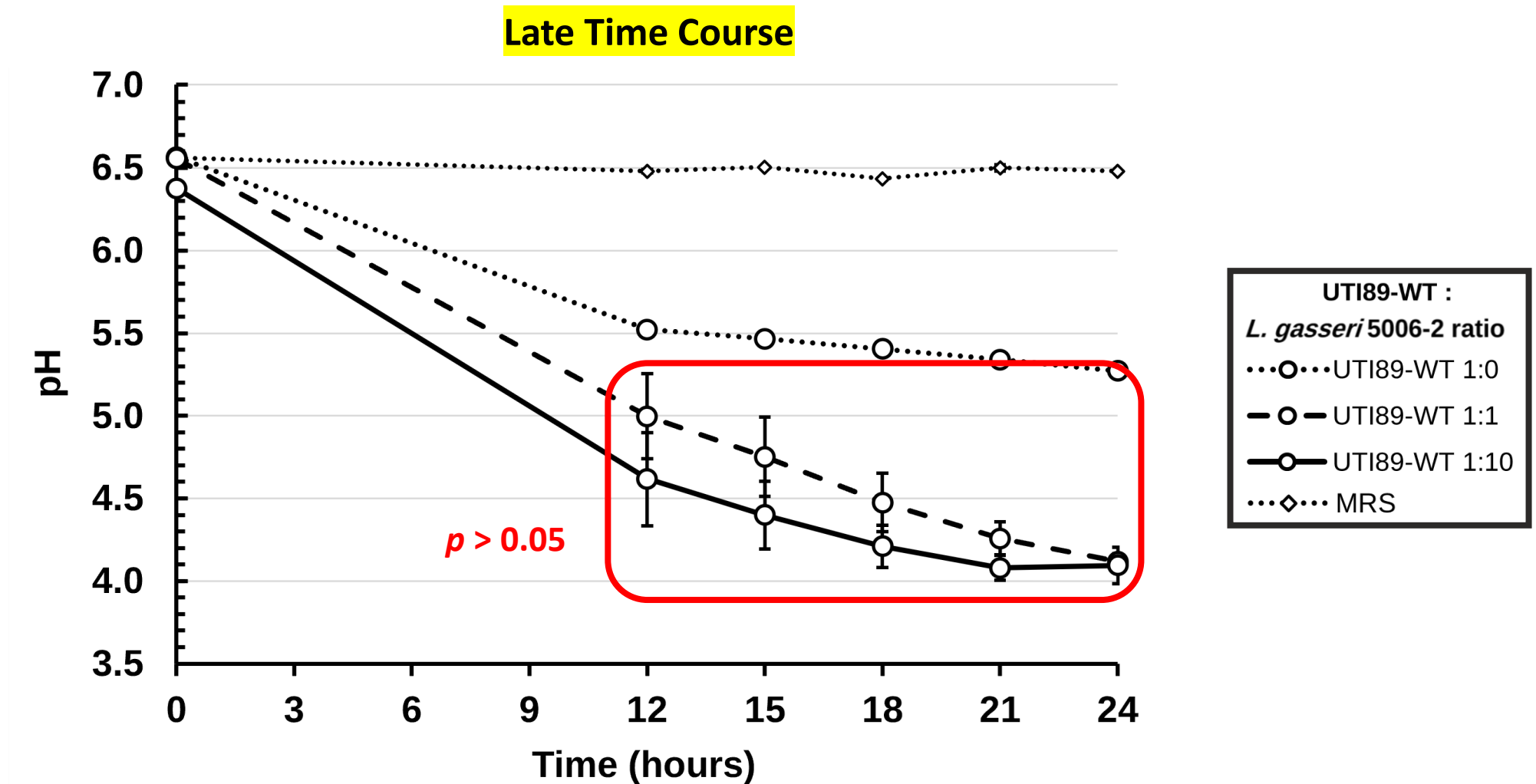


Jada McElroy

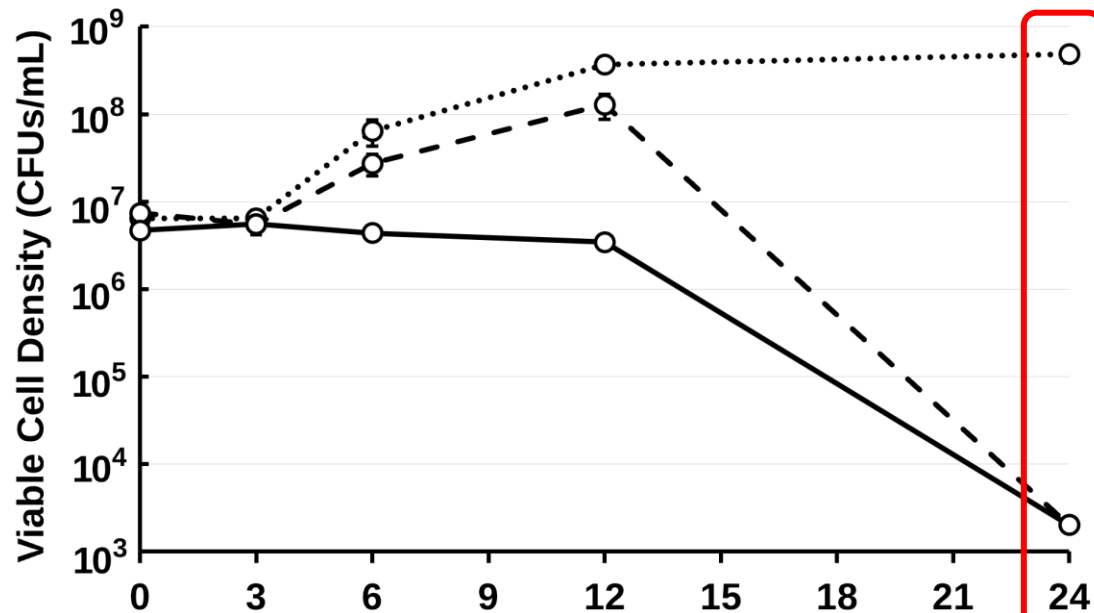
UTI89 mono-cultures and treatments acidify distinctly over time



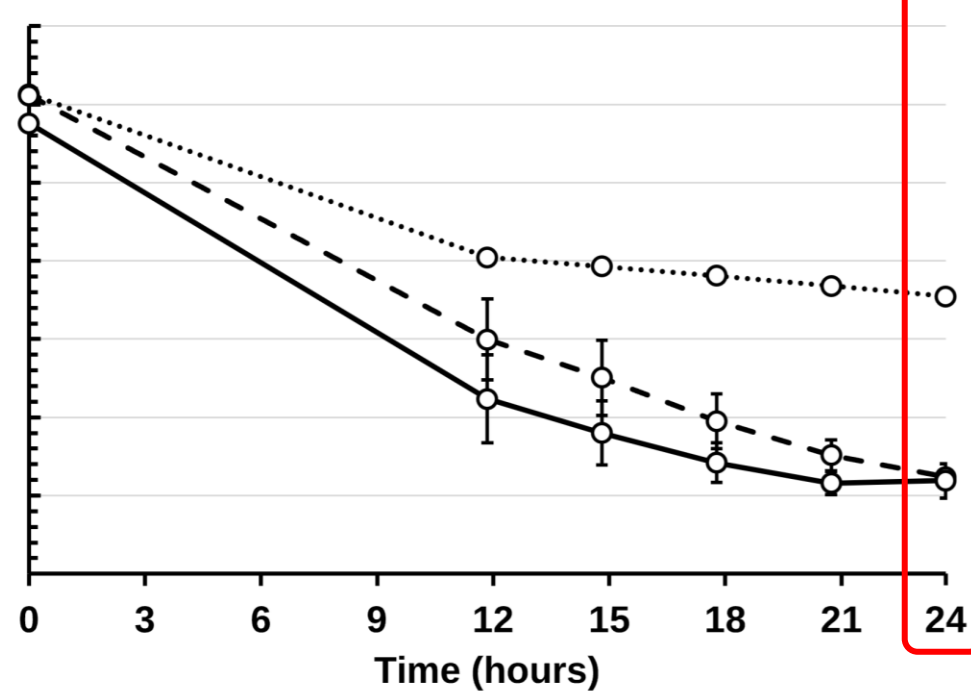
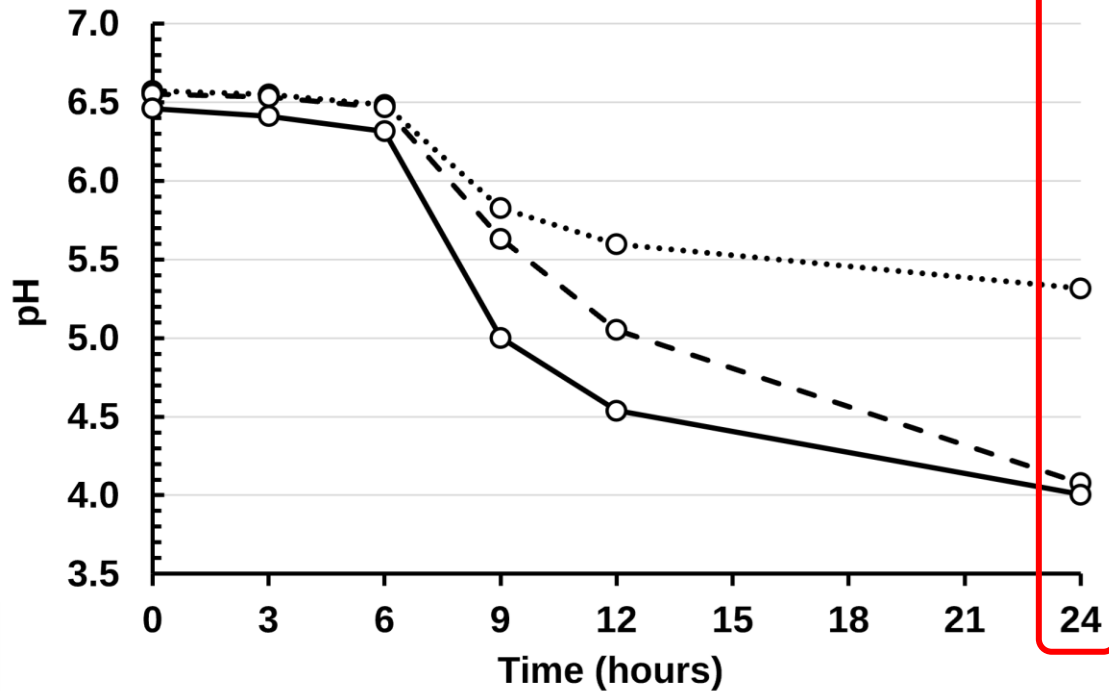
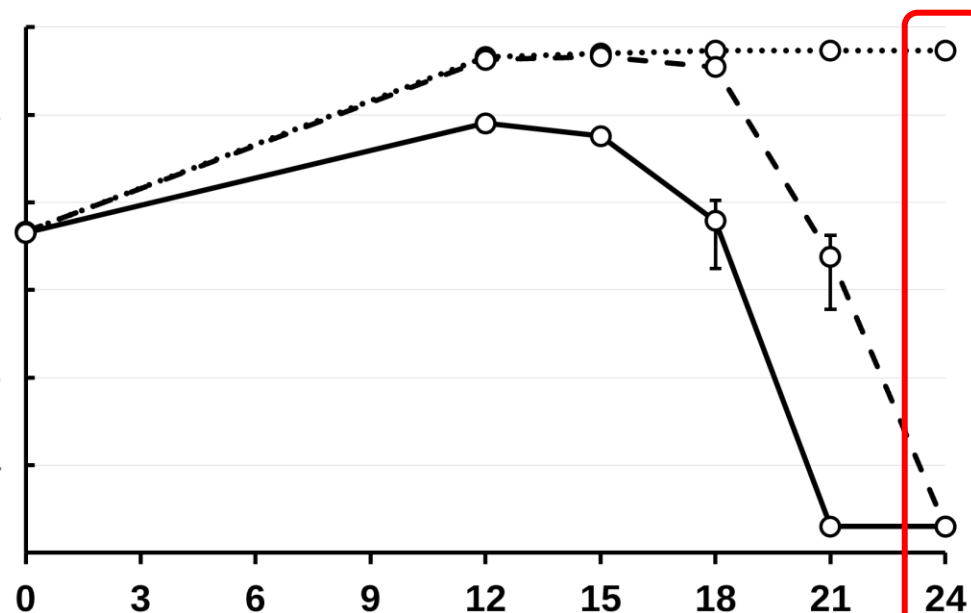
UTI89 mono-cultures and treatments acidify distinctly over time



UTI89 wildtype - Early Time Course



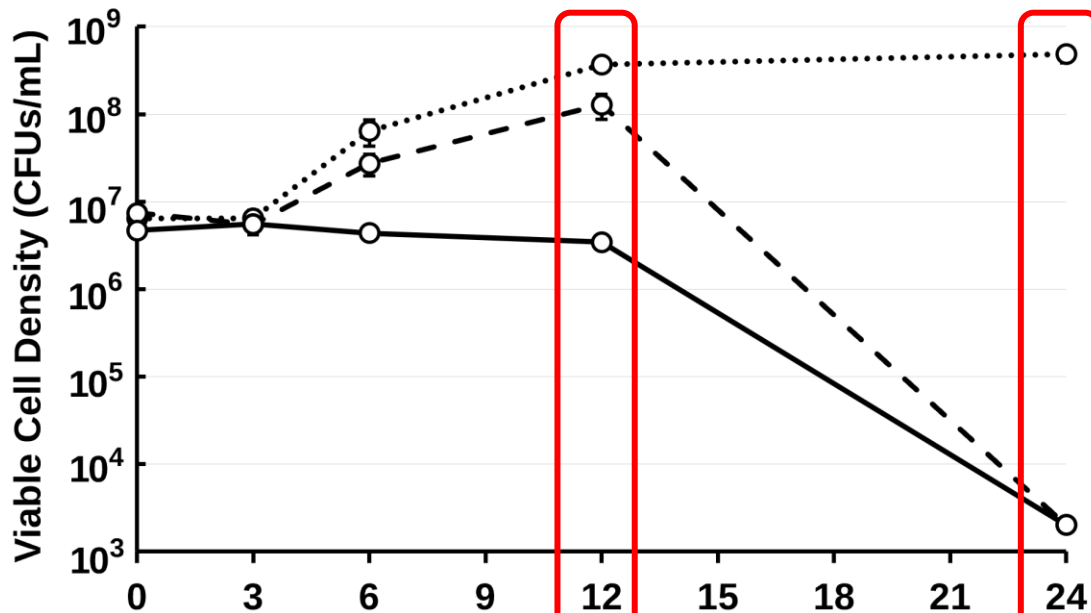
UTI89 wildtype - Late Time Course



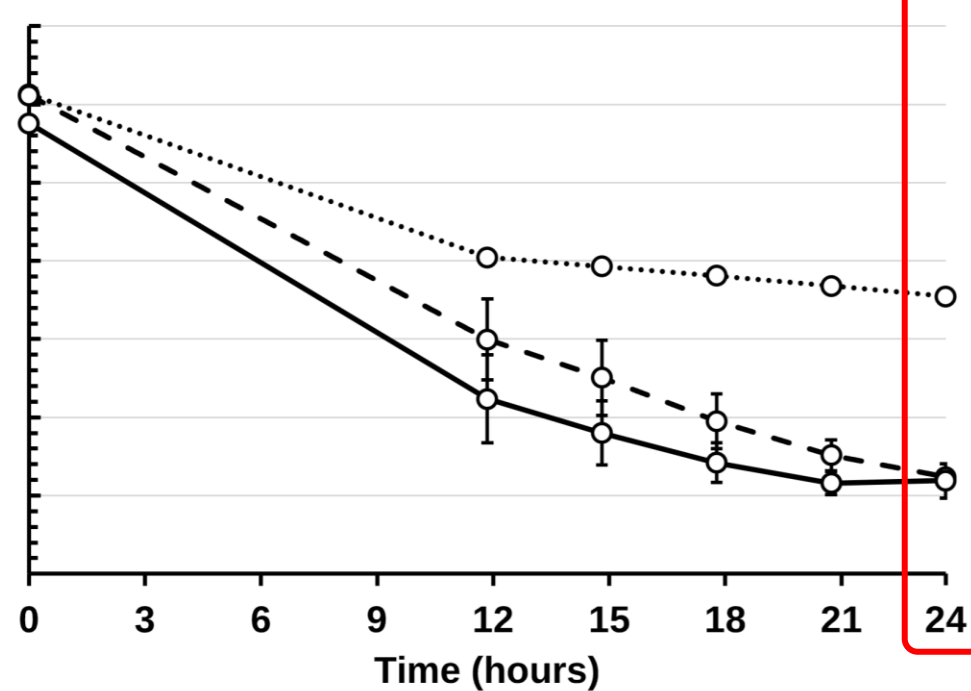
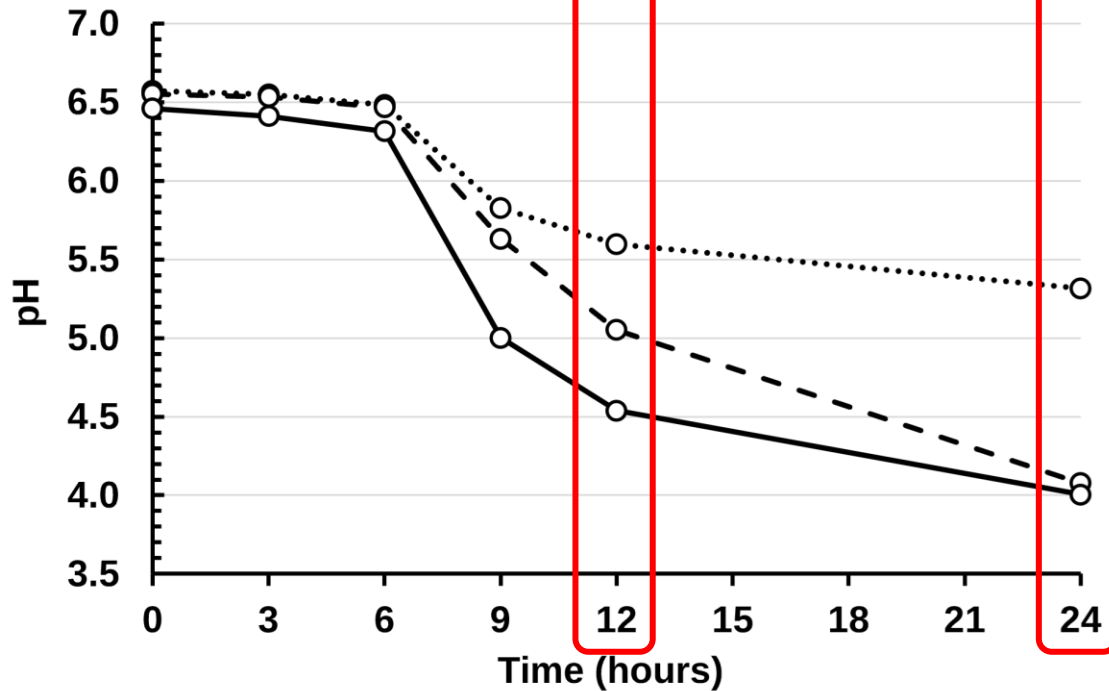
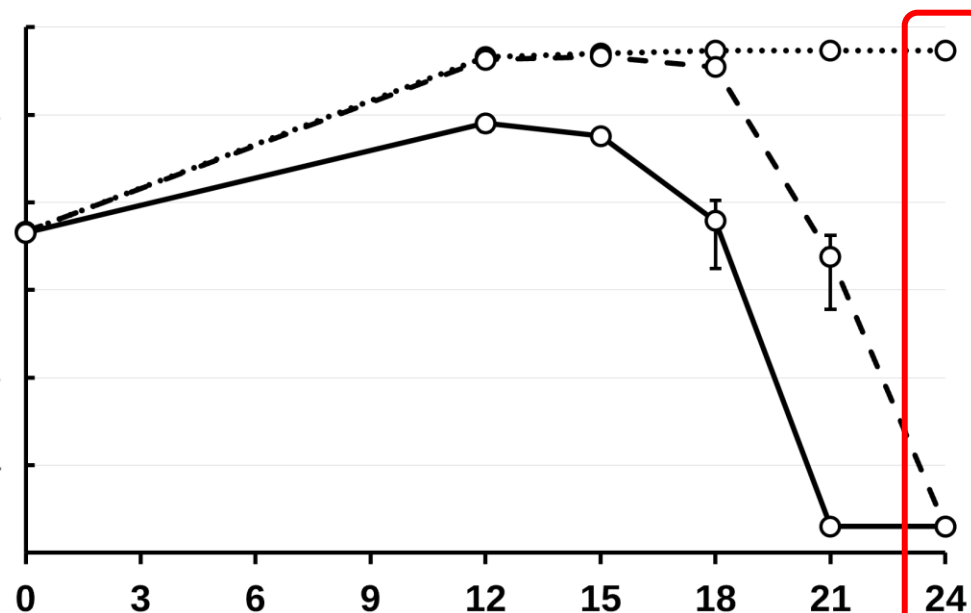
UTI89-WT :
L. gasseri 5006-2 ratio

- UTI89-WT 1:0
- UTI89-WT 1:1
- UTI89-WT 1:10

UTI89 wildtype - Early Time Course

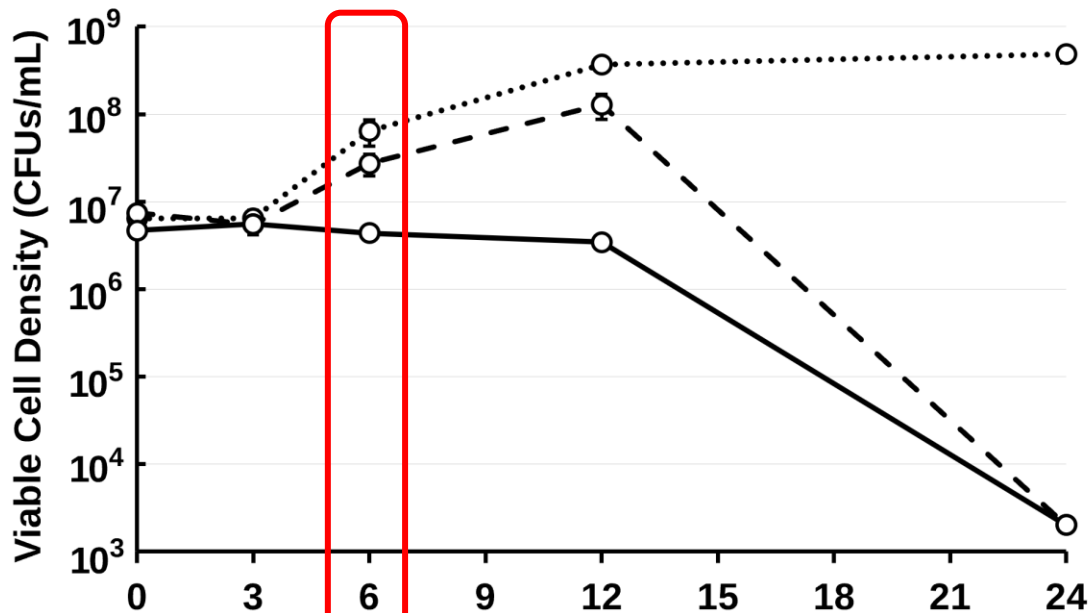


UTI89 wildtype - Late Time Course

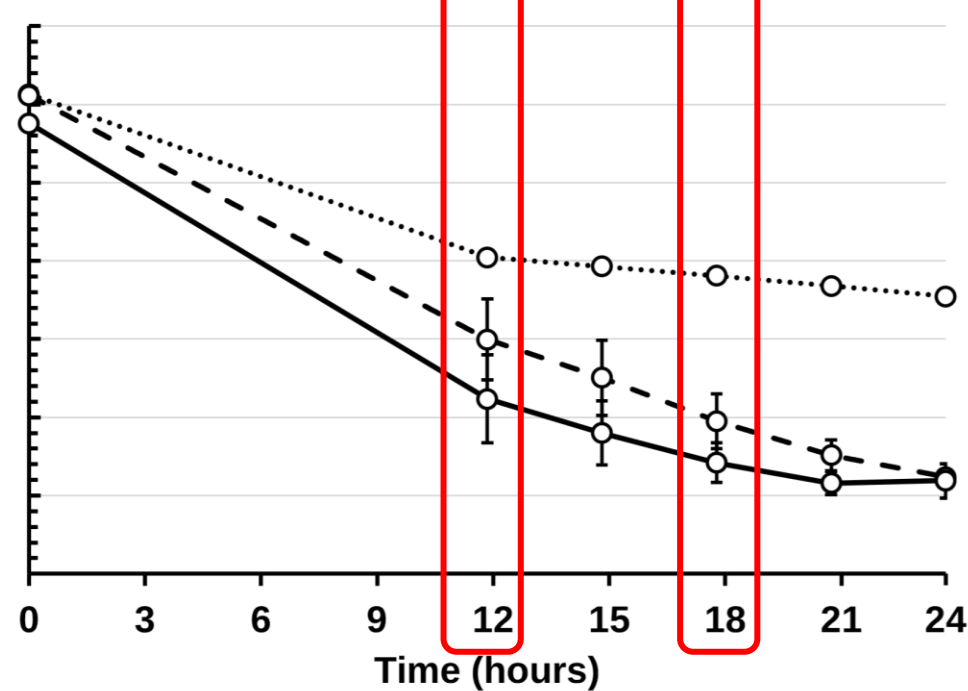
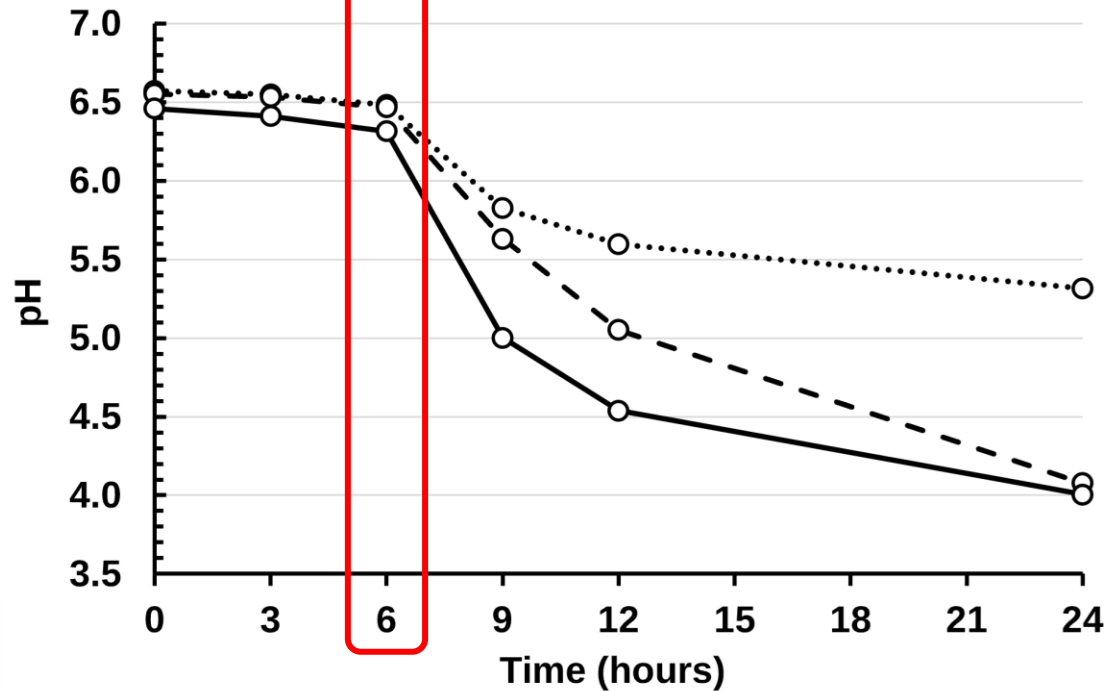
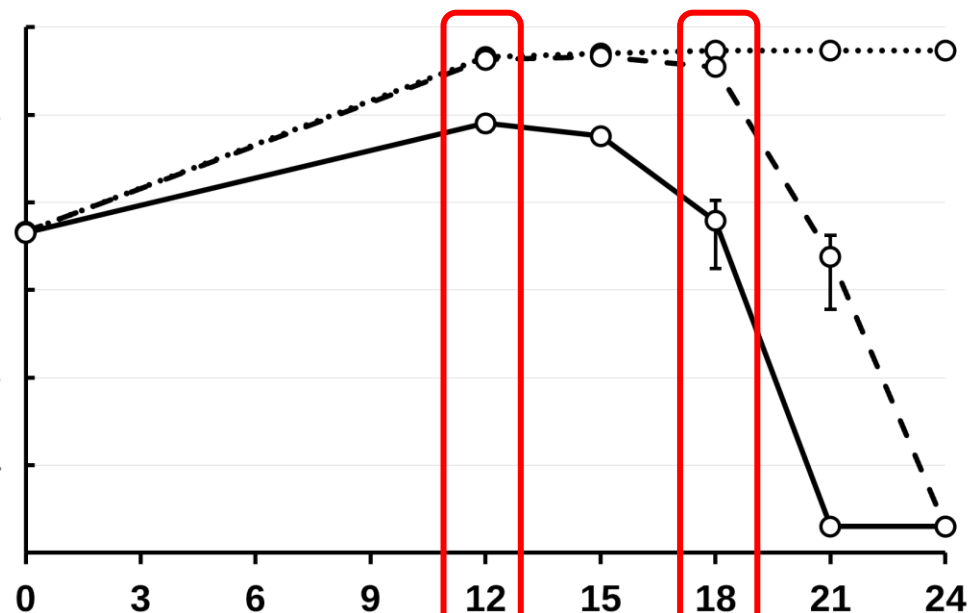


UTI89-WT :
L. gasseri 5006-2 ratio
 ···○··· UTI89-WT 1:0
 -○- UTI89-WT 1:1
 —○— UTI89-WT 1:10

UTI89 wildtype - Early Time Course



UTI89 wildtype - Late Time Course



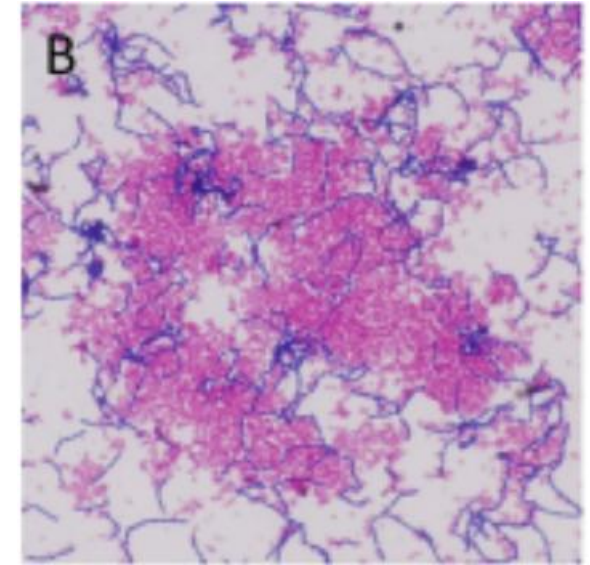
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L. gasseri 5006-2 ratio

- UTI89-WT 1:0
- UTI89-WT 1:1
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Evaluation of close interactions of UTI89 and *L. gasseri* in co-culture

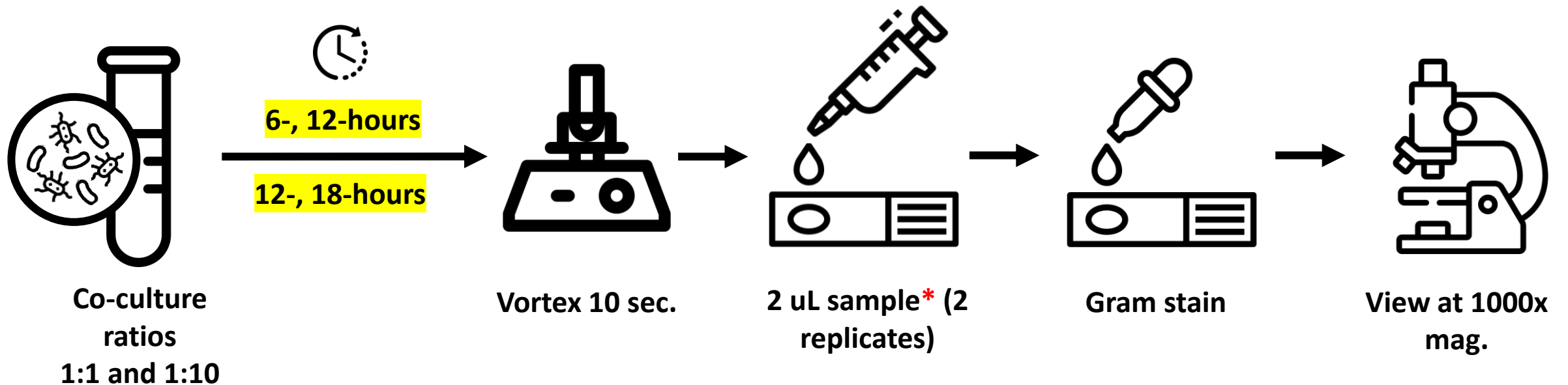
- *L. casei* NBRC 3831 embedded in micro-scale clusters of *E. coli* BW25113 in co-culture (Mizuno et al. 2017)
- **Is cell-to-cell contact necessary for “activation” of inhibition?**

If agitation of co-cultures promotes UTI89-*L. gasseri* 5006-2 contact and aggregation, then more aggregates should be observed for the early time course compared to the late time course.

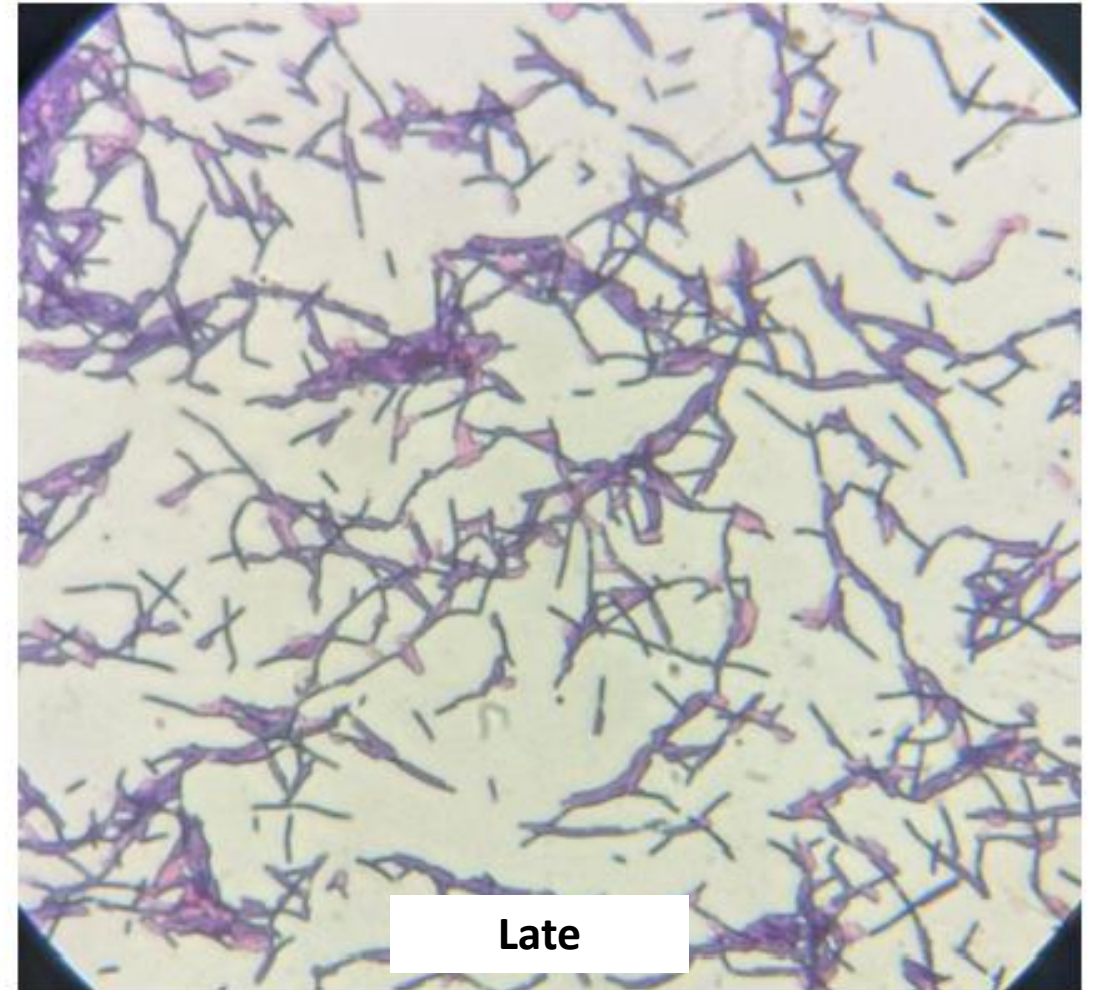
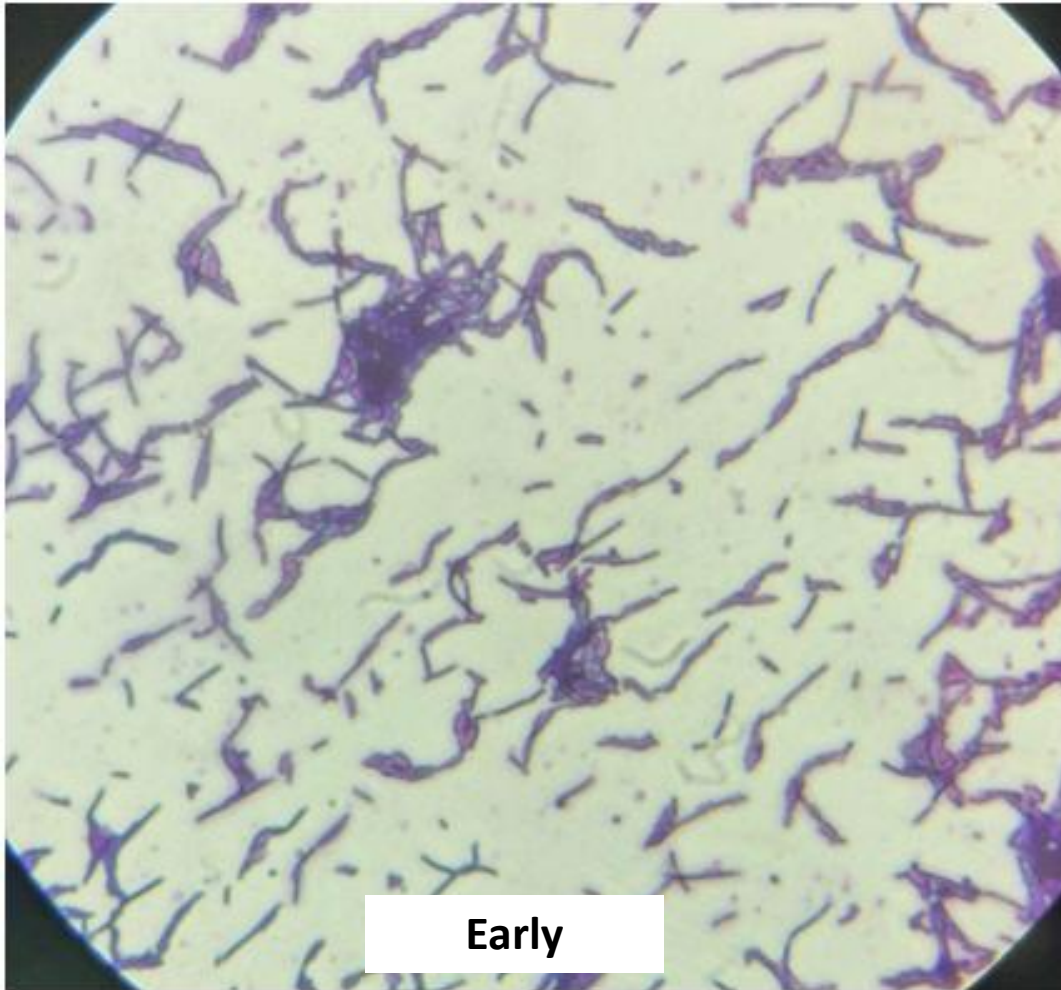


(Mizuno et al. 2017)

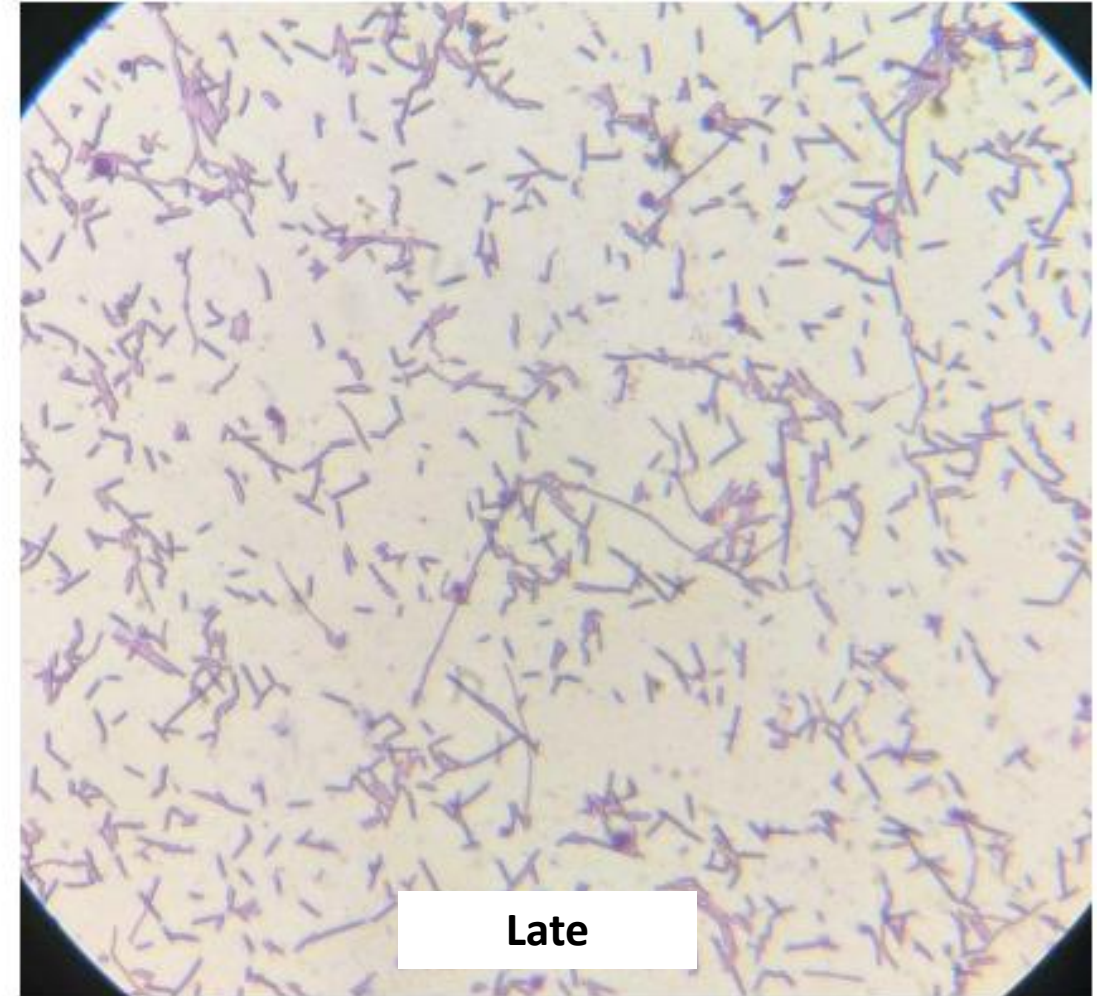
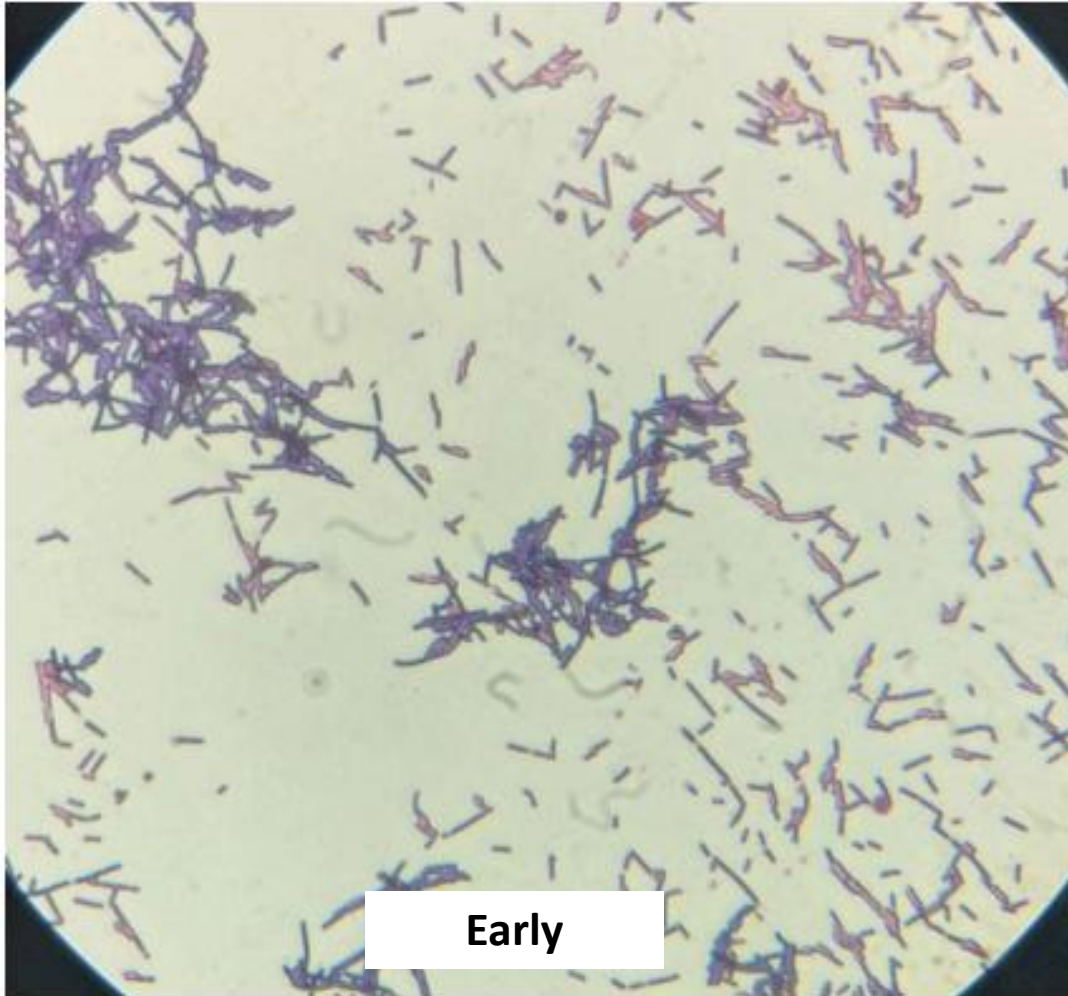
Co-cultures and sampling for microscopy



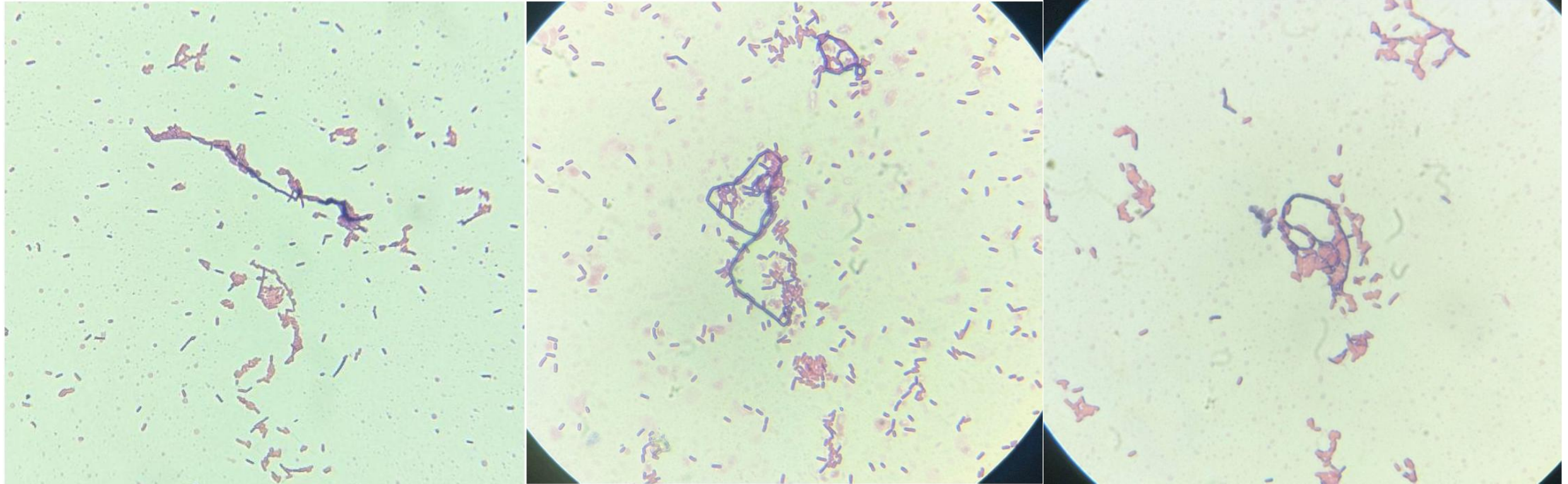
Comparison of coaggregation at 12-hours in time courses: 1:1 co-cultures



Comparison of coaggregation at 12-hours in time courses: 1:10 co-cultures

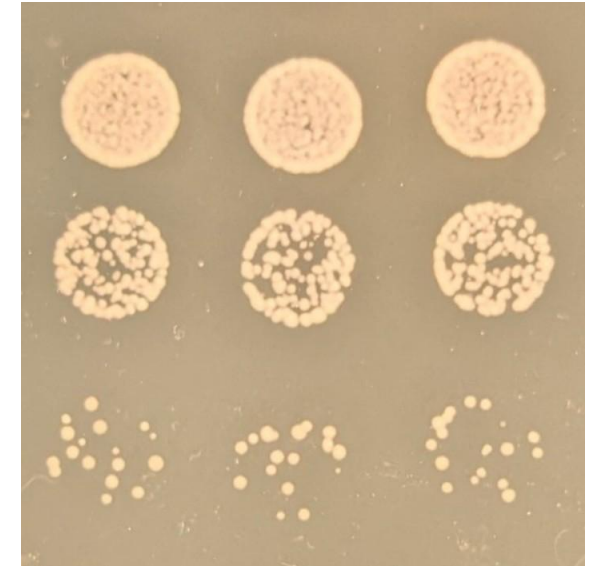


L. gasseri 5006-2 chaining in 1:1 co-culture at 6-hours



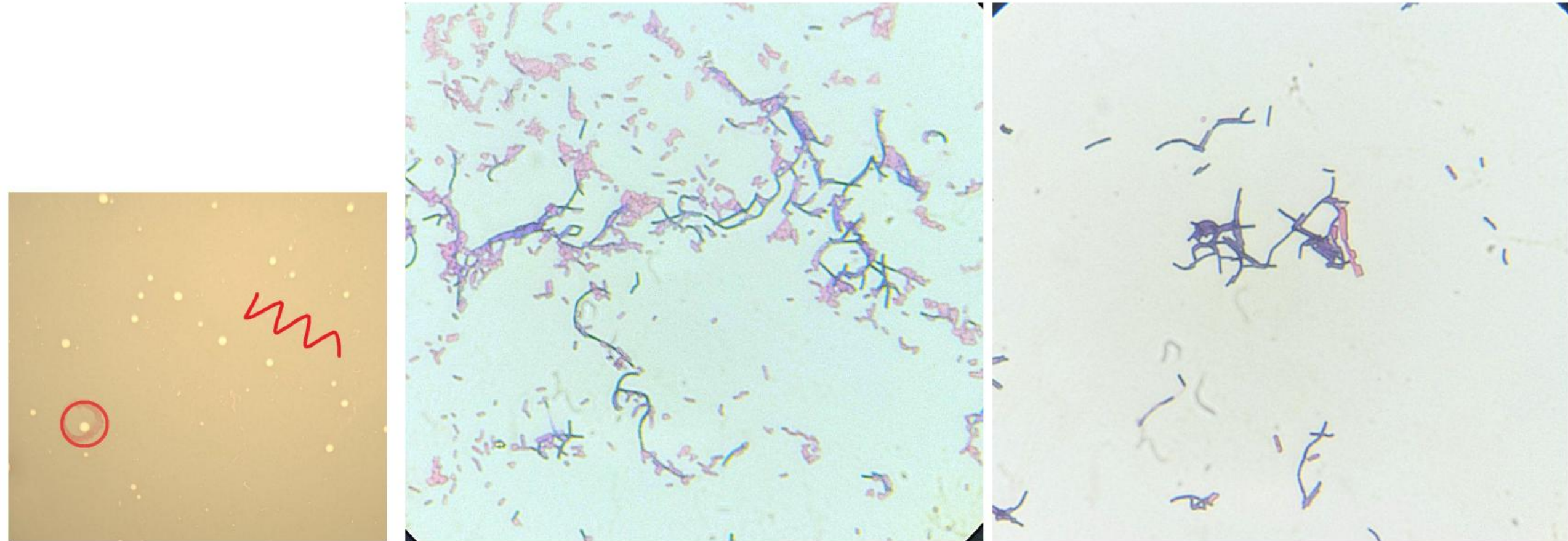
Investigate morphology of small UTI89 colonies

- *E. coli* can take coccus-like morphology in stressful conditions
 - Invasion of bladder epithelium within acidic phagolysosomes
(Pokhrel et al. 2024) (Mueller, Westfall, and Levin 2020)
 - Other AR mechanisms include morphological changes
- Potential response to inhibitory effect of *L. gasseri* 5006-2?
- Are small colonies a phenotypic or genotypic response?

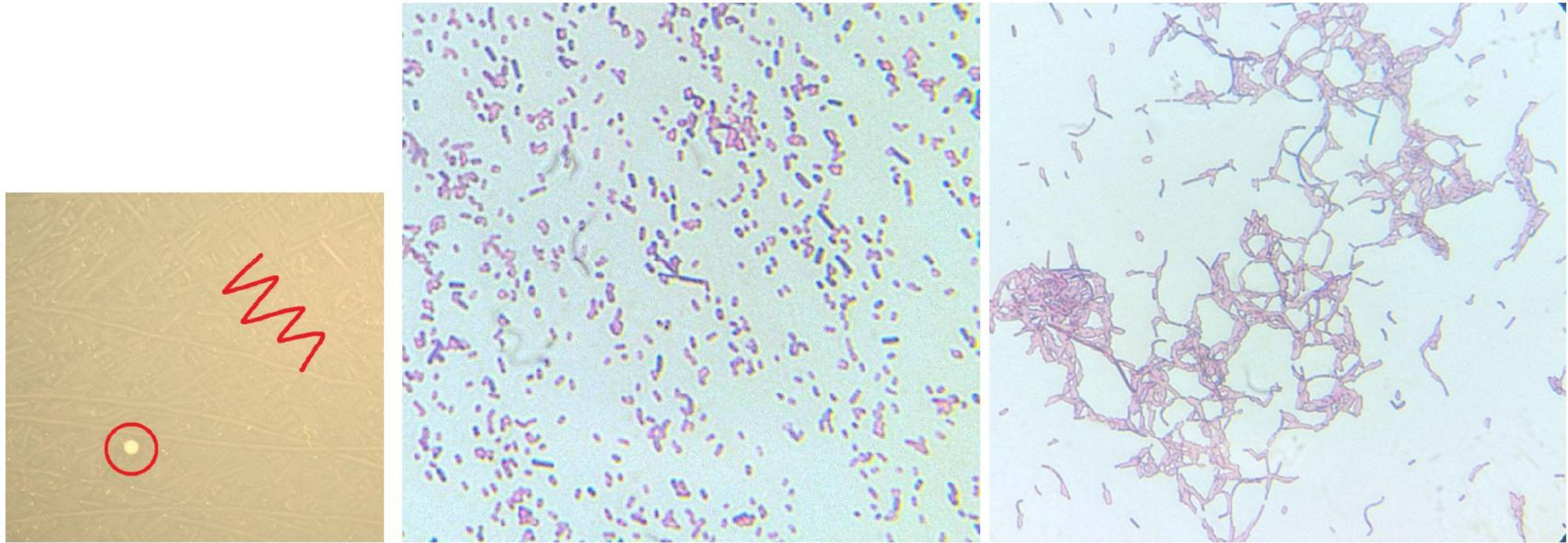


If inhibition causes a morphological stress response, then small colonies should exhibit differences in morphology of cells.

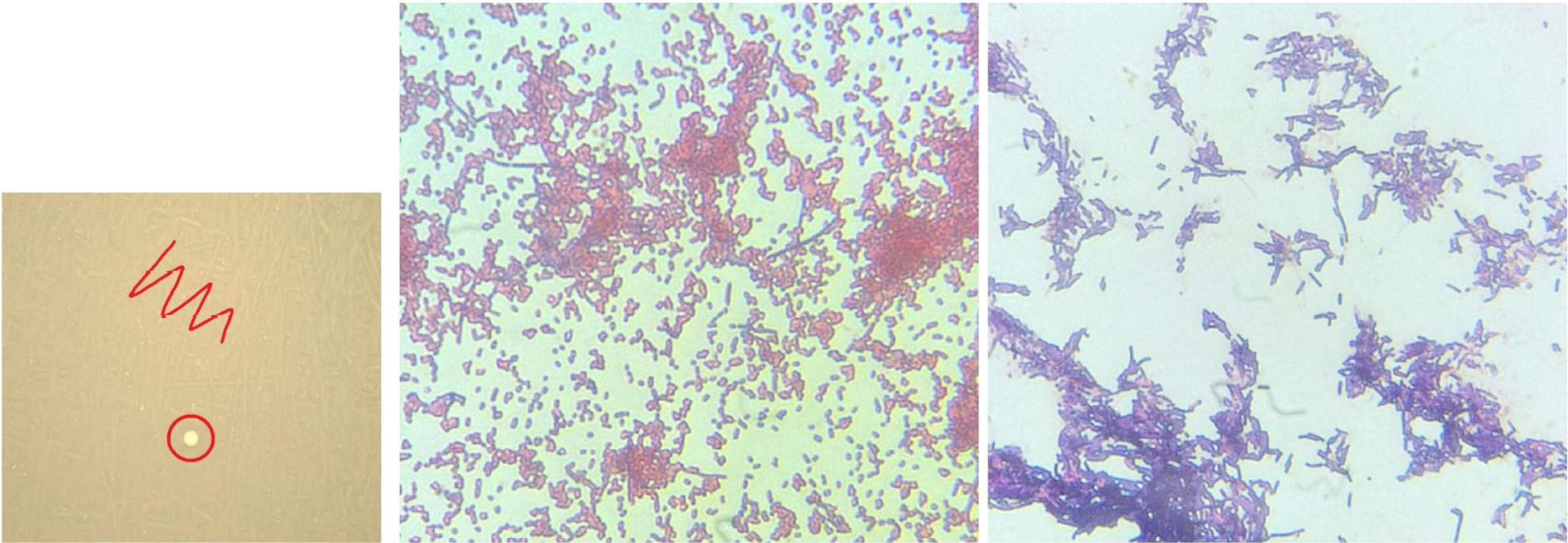
L. gasseri is embedded in small UTI89 colonies and present on plates



L. gasseri is embedded in small UTI89 colonies and present on plates



L. gasseri is embedded in small UTI89 colonies and present on plates

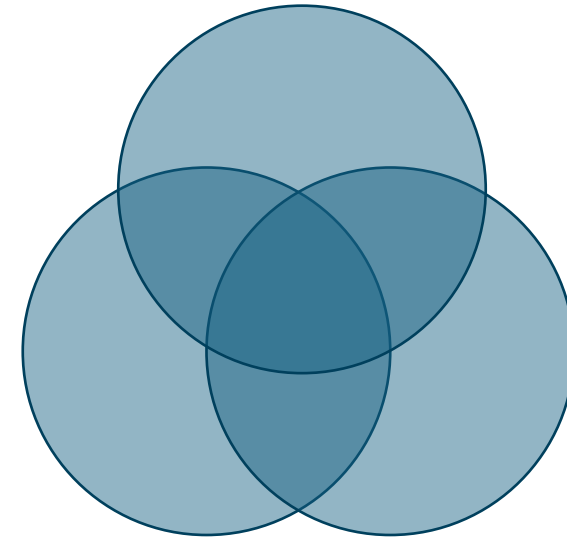


Summary for *L. gasseri* interaction with UPEC

- Inhibition correlates with pH decrease but does not depend on it
- Disruption of acid responses in UPEC does not prevent growth inhibition
- *L. gasseri* and UPEC form some aggregates in liquid broth and on surface of agar
- Cell-contact and 'geography' appears important for inhibition

Urobiome as ecological niche

- Host (genetics, development, immune system)
- **Urobiome community** (**residents** and invaders)
- Environment (stress, drugs, diet, etc)



What's next?

- Commensals of the postmenopausal bladders might not exhibit strong resistance which can be present in pre-menopausal microbiomes
- Urinary microbiome is complex and, therefore, binary interactions might not recapitulate the physiological state
- Host-derived or host-microbiome interactions might shape predisposition and outcome of UTIs – therefore the UTI should be studied with the ecological perspective in mind



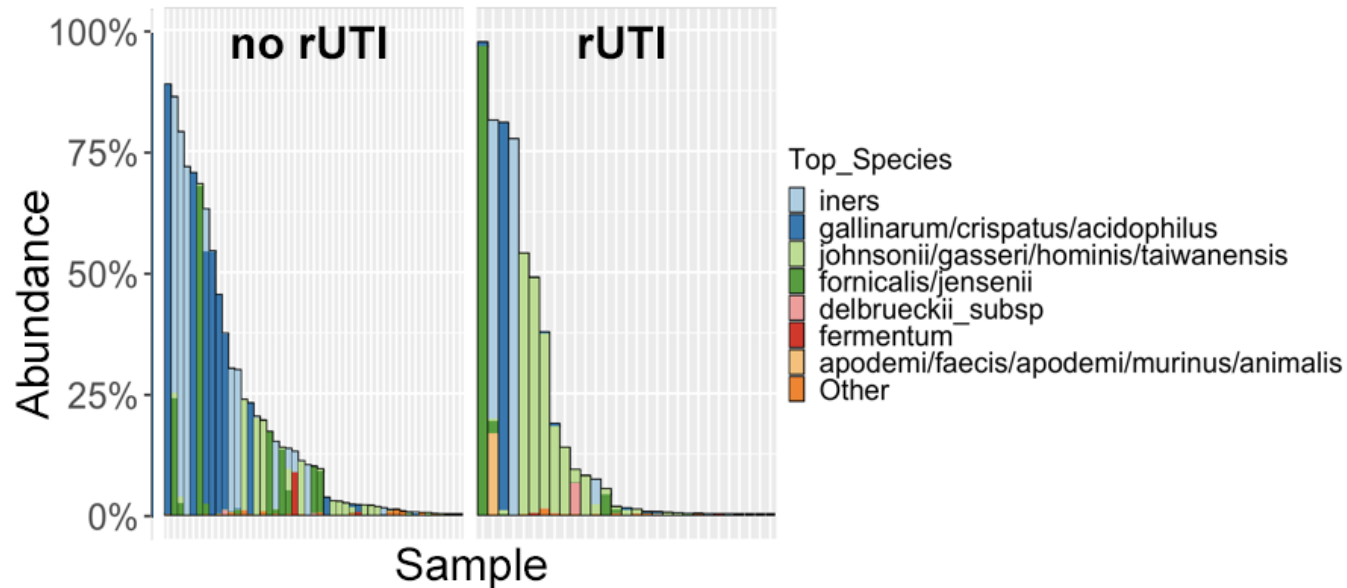
What is a good probiotic?

- Provides colonization resistance to major uropathogens
- Survives delivery to the niche
- Colonizes the urinary tract (stable survival; adhesion)
- It is 'invader' for transient interactions or new 'resident'?



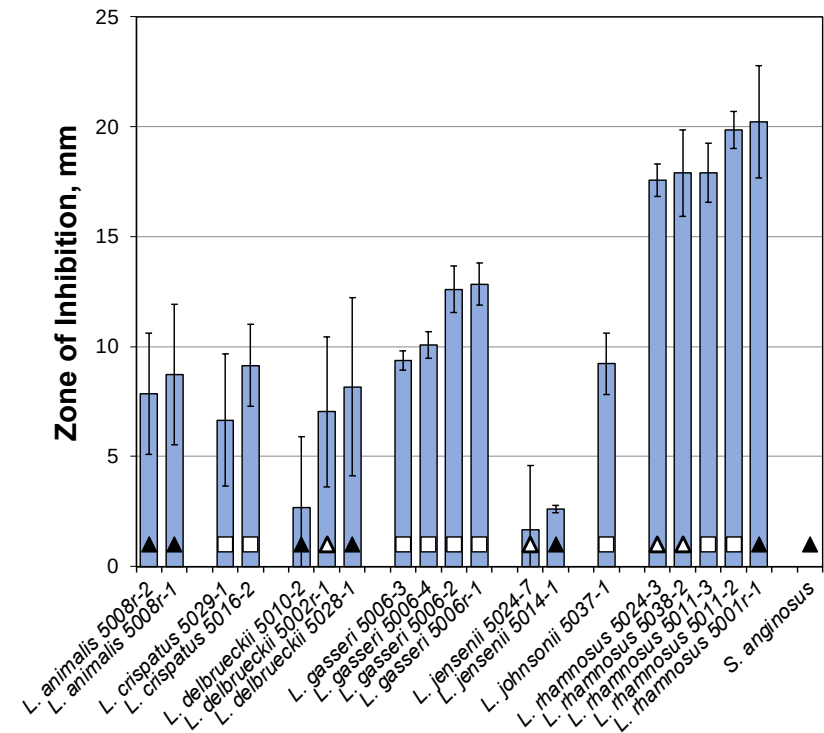
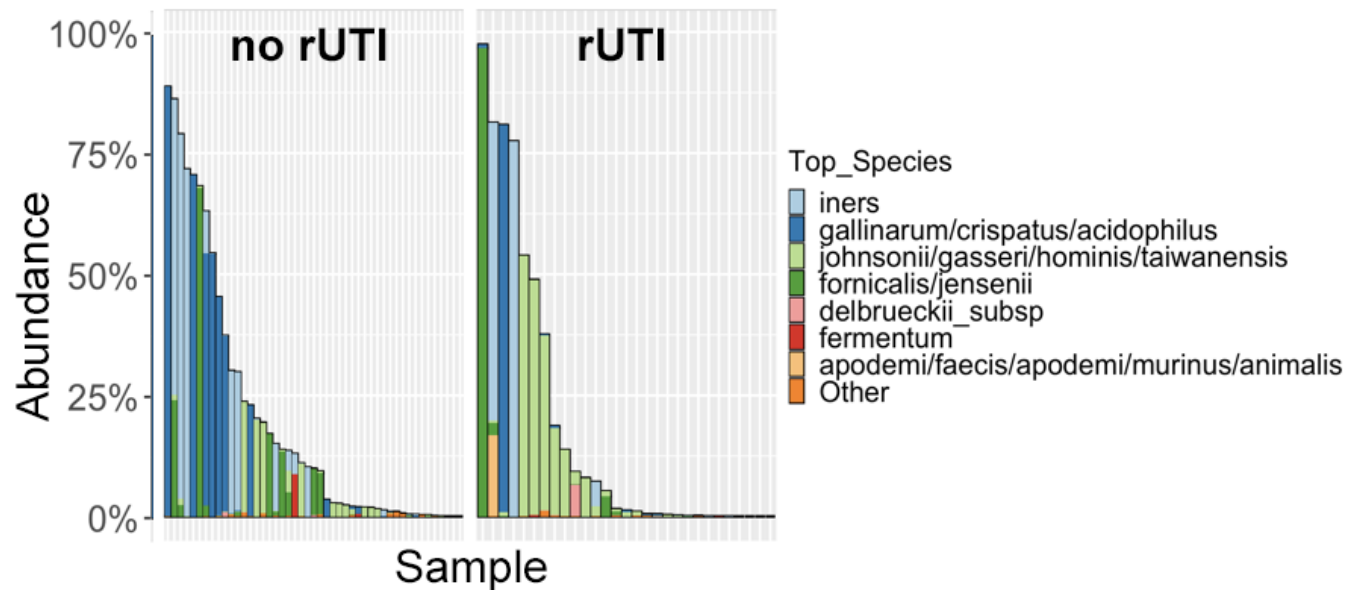
Interbacterial competition of lactobacilli

- Low number of lactobacilli species dominate
- if a new strain is added – will it be stable and successful?



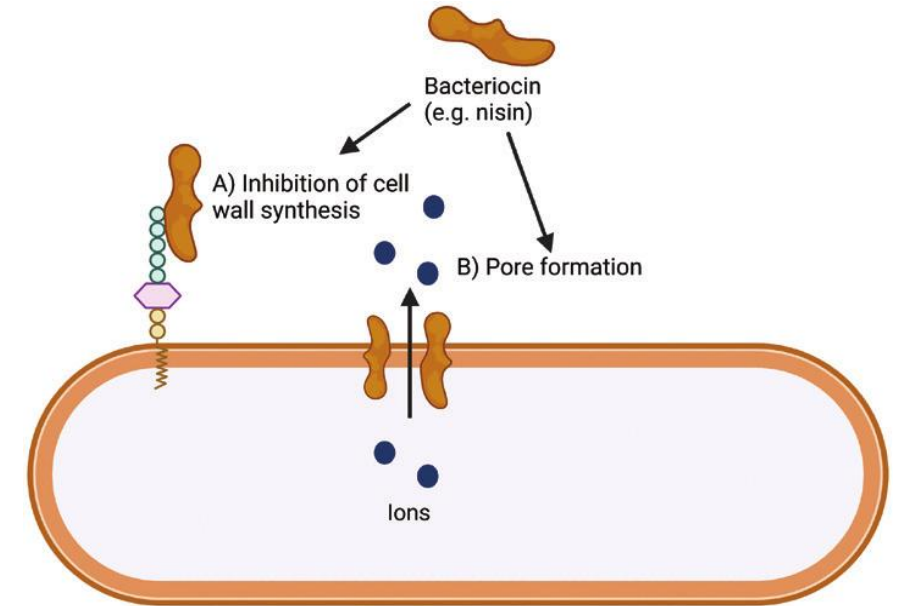
Interbacterial competition of lactobacilli

- Low number of lactobacilli species dominate
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Lactobacilli as competitors - Bacteriocins

- Bacteriocins are small antimicrobial peptides or proteins
- Many bacteriocins interfere with a bacteria's cell wall for instance disrupting cell wall biosynthesis or by creating a pore in the target bacteria
- Bacteriocins are classified depending on size and certain structural characteristics



Example of Bacteriocin (e.g. nisin) mode of action against gram positive bacteria.

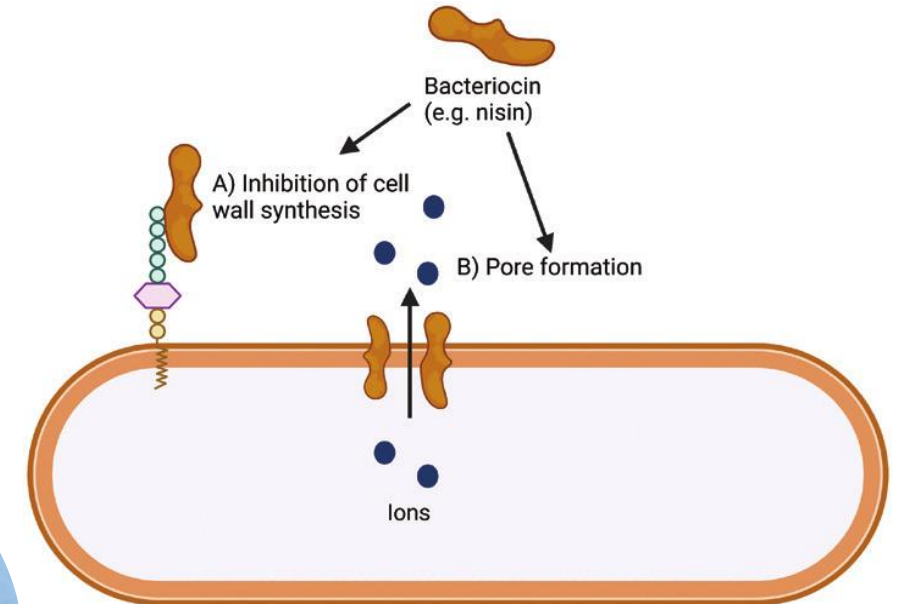
Image taken from Webb et al. 2022

Sequencing and genome analysis for bacteriocins

- Preparing total genomic DNA
- Using long- and short-sequencing reads in hybrid assembly by Unicycler
- Searching databases using tools like Bagel



Kaylie Hintze



Example of Bacteriocin (e.g. nisin) mode of action against gram positive bacteria.

Image taken from Webb et al. 2022

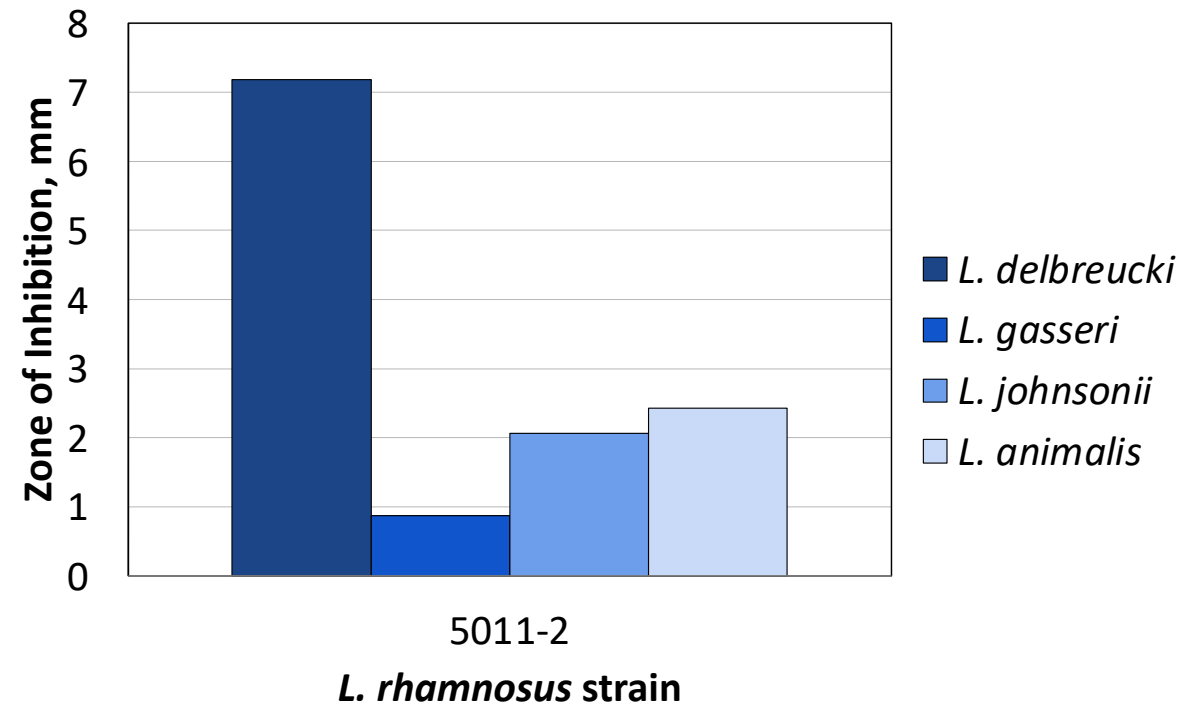
van Heel et al, 2018 - <http://bagel4.molgenrug.nl/>

Sequencing and genome analysis for bacteriocins

Strain #	Species Name	Results from BLASTP
SL101/SL102/SL105/SL175	<i>L. rhamnosus</i>	bacteriocin-like peptide, LSEI_2386 family [Lacticaseibacillus rhamnosus]
		class IIb bacteriocin, lactobin A/cerein 7B family [Lacticaseibacillus rhamnosus]
		hypothetical protein HMPREF0539_2049 [Lacticaseibacillus rhamnosus LMS2-1]
		bacteriocin [Lacticaseibacillus rhamnosus]
SL 103	<i>L. rhamnosus</i>	bacteriocin [Lacticaseibacillus rhamnosus]
		class IIb bacteriocin, lactobin A/cerein 7B family [Bacteria]
		bacteriocin [Lacticaseibacillus rhamnosus]
		hypothetical protein HMPREF0539_2049 [Lacticaseibacillus rhamnosus LMS2-1]
SL 96	<i>L. gasseri</i>	class III bacteriocin [Lactobacillus]
SL 97/SL104/SL107	<i>L. delbrueckii</i>	bacteriocin [Lactobacillus delbrueckii]
		M23 family metallopeptidase [Lactobacillus delbrueckii]
		helveticin J family class III bacteriocin [Lactobacillus delbrueckii]
SL114	<i>L. johnsonii</i>	class III bacteriocin [Lactobacillus johnsonii]
		lactococcin 972 family bacteriocin [Lactobacillus]
		bacteriocin [Lactobacillus johnsonii]
		Blp family class II bacteriocin [Lactobacillus johnsonii]
		bacteriocin immunity protein [Lactobacillus johnsonii]

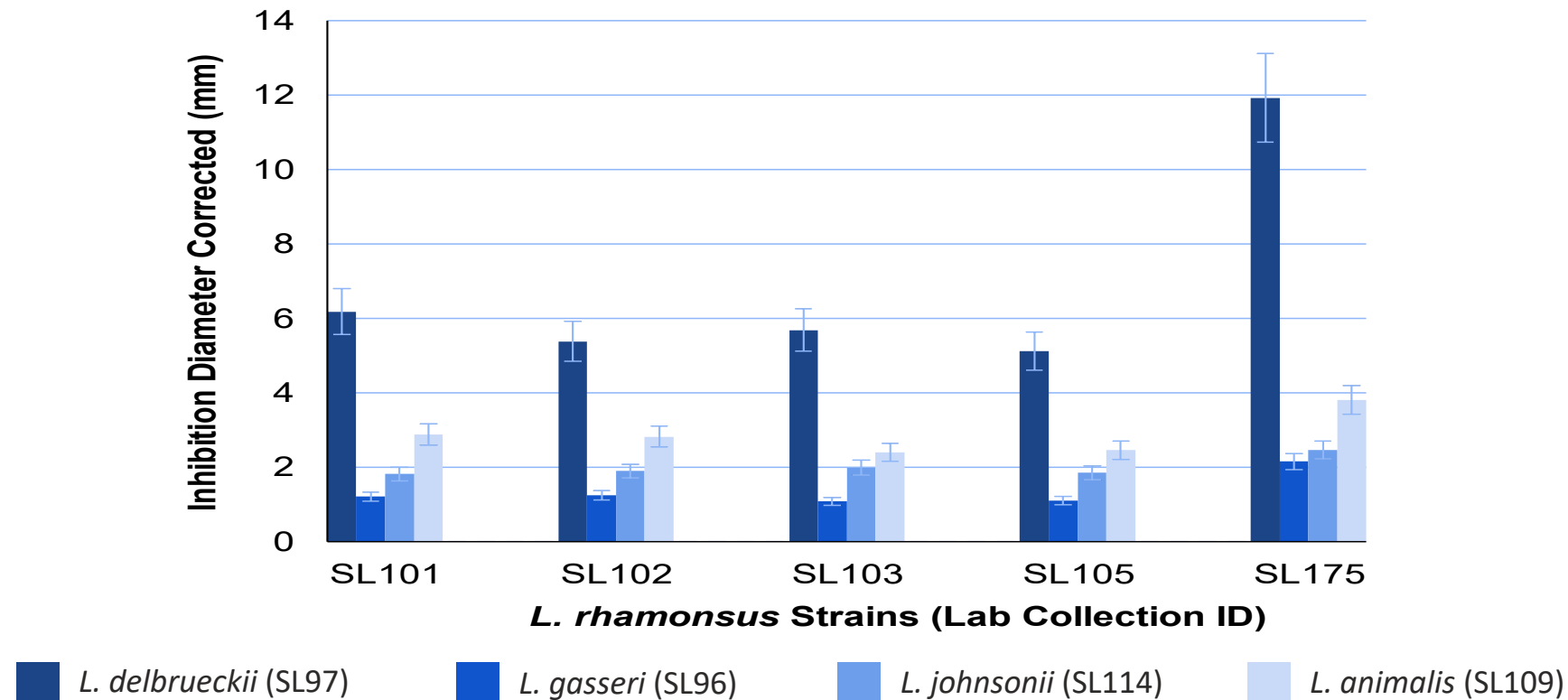
Interbacterial competition of lactobacilli

- Screen lacto-lacto interactions using well-diffusion assay
- *L. rhamnosus* 5011-2 inhibit representatives of three other species

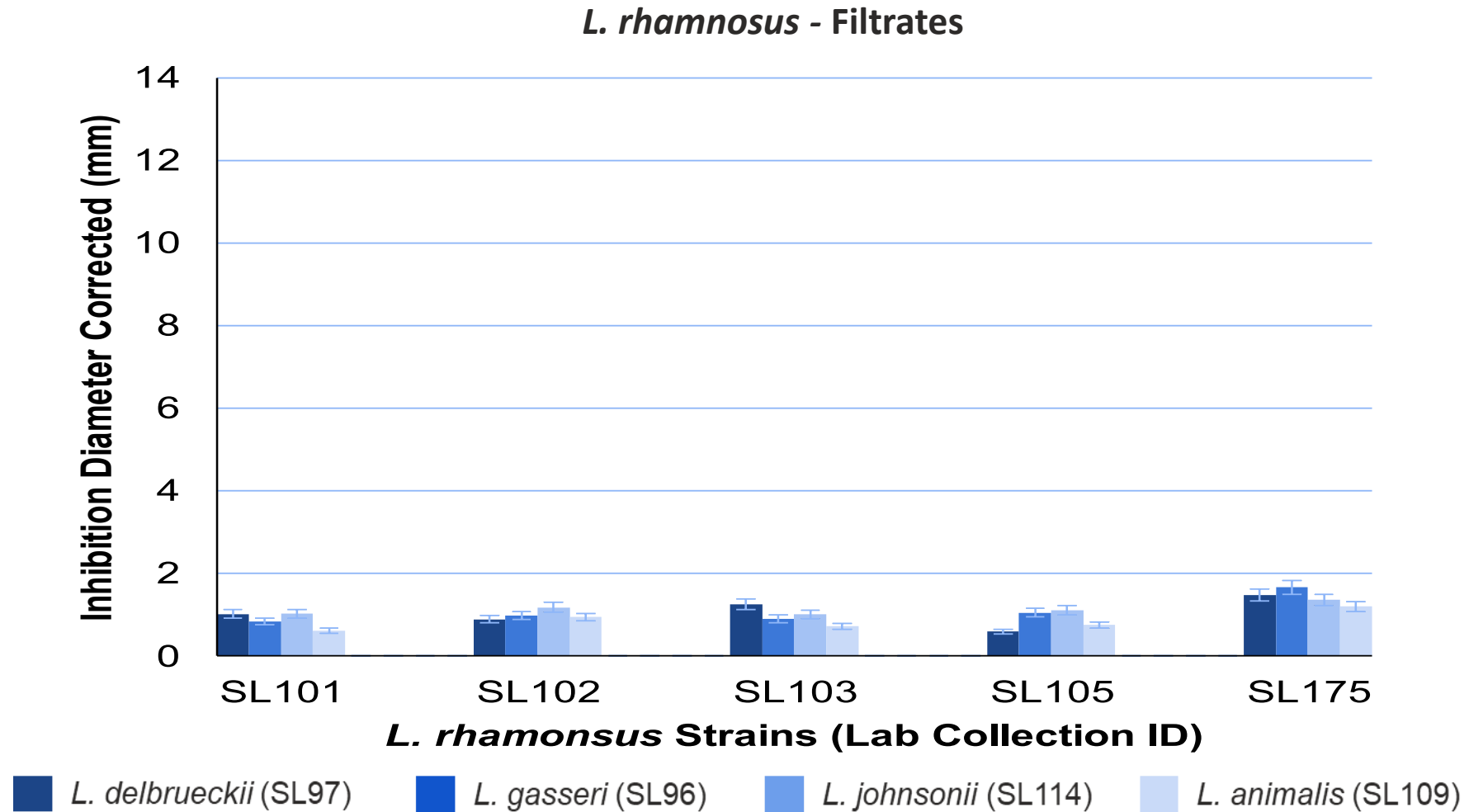


Interbacterial competition of lactobacilli

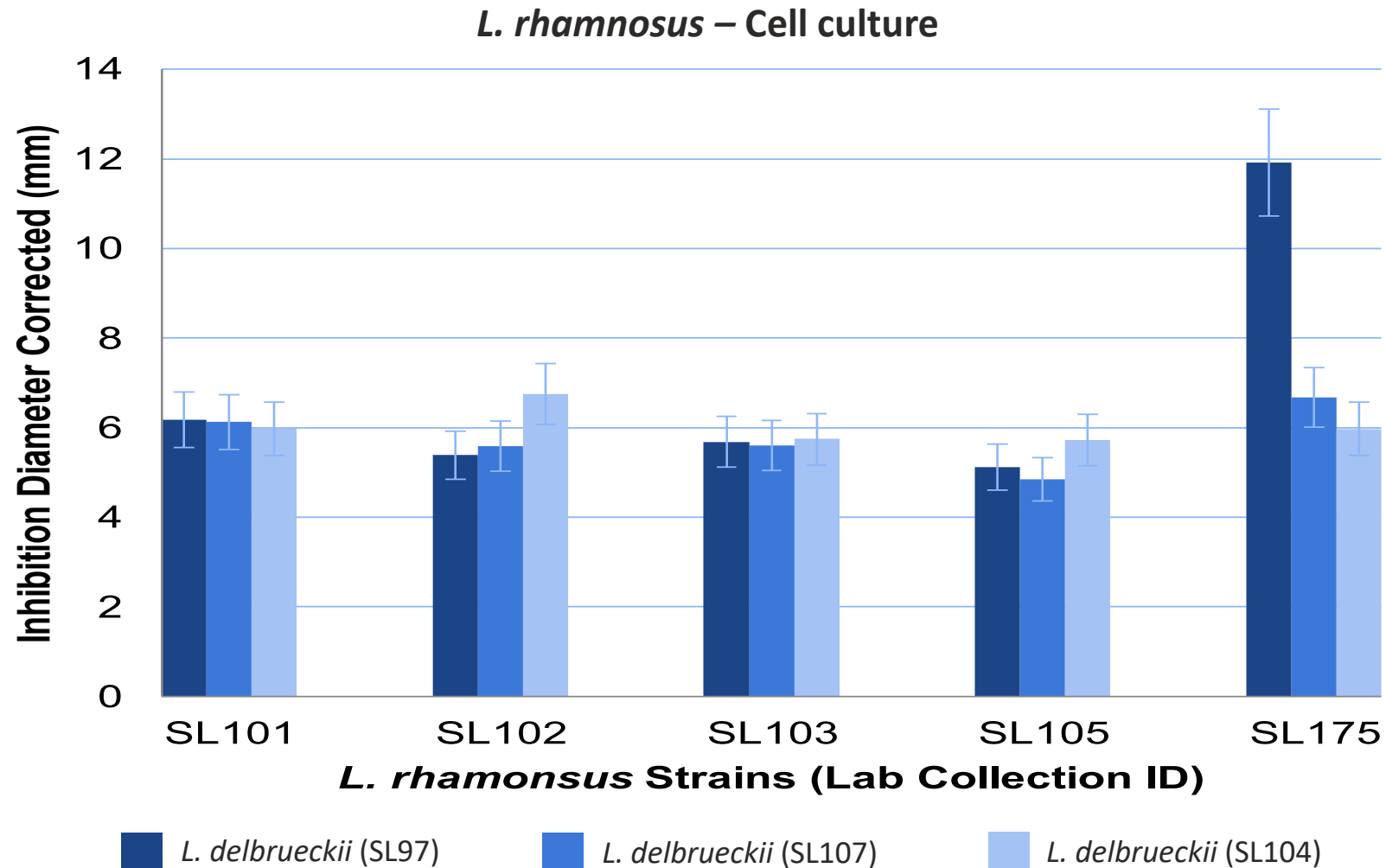
- *L. rhamnosus* strains from three patients act similarly
- This inhibition is cell-dependent, not relying on pH/acidity



No inhibition with cell-free filtrates



Testing other urinary *L. delbrueckii* isolates

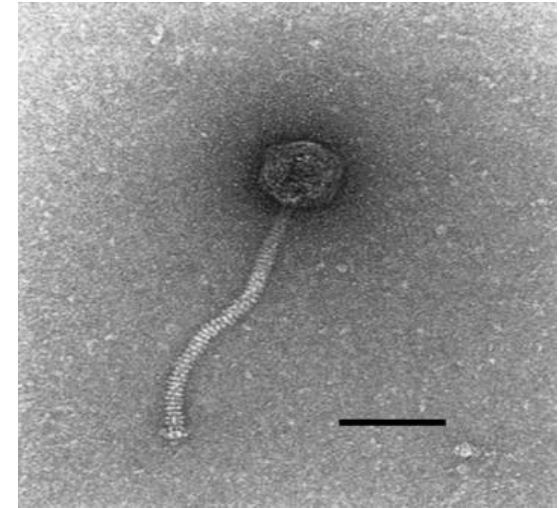


Why urinary *L. delbrueckii* susceptible?

- *L. delbrueckii* strains come from three patients
- Genomes contain plasmids
 - SL97 – 57,988 bp
 - SL104 – 57,987 bp
 - SL107 – 57,987 bp
- Plasmid-form of known *L. delbrueckii* prophage JCL1032 previously found in dairy samples

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Siphoviridae phage Image

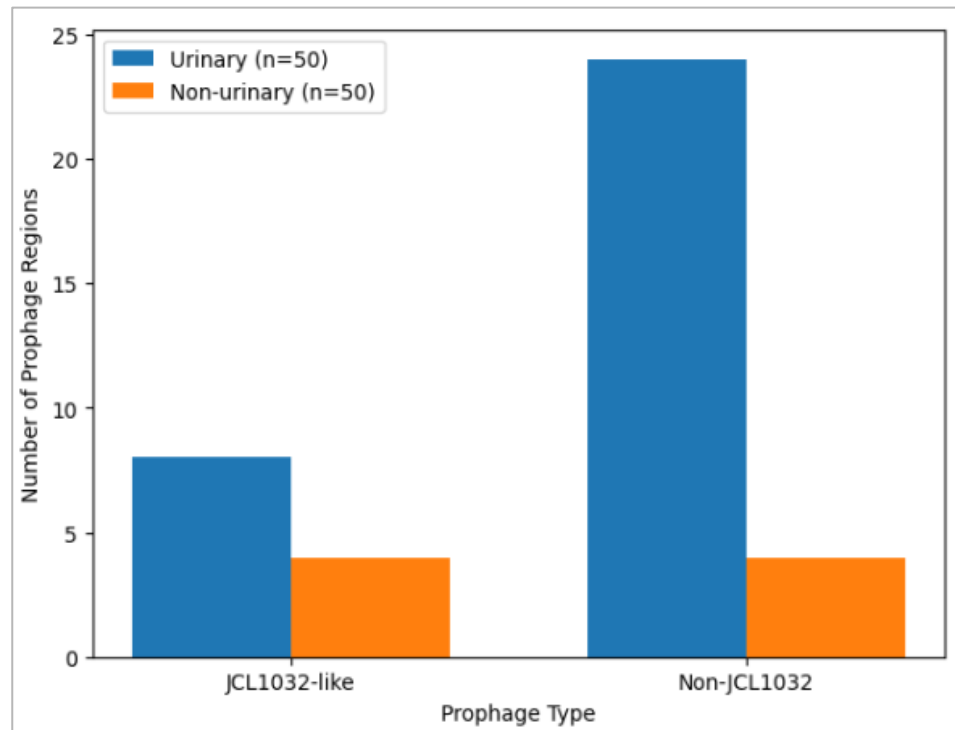
Taken from K. Porter et al 2008

JCL1032 and other prophages in *L. delbrueckii*

- Distribution of prophages in Urinary vs Non-urinary *L. delbrueckii* isolates



Anisha Bhandari



Why urinary *L. delbrueckii* susceptible?

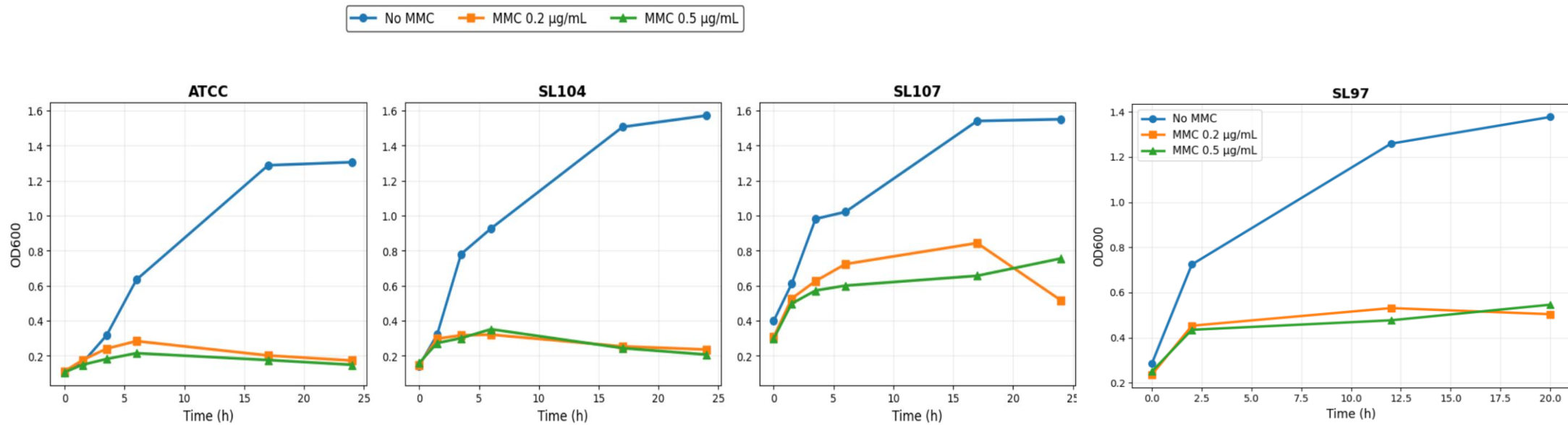
- Hypothesis: Phage is induced by *L. rhamnosus*, high density stress?



Anisha Bhandari

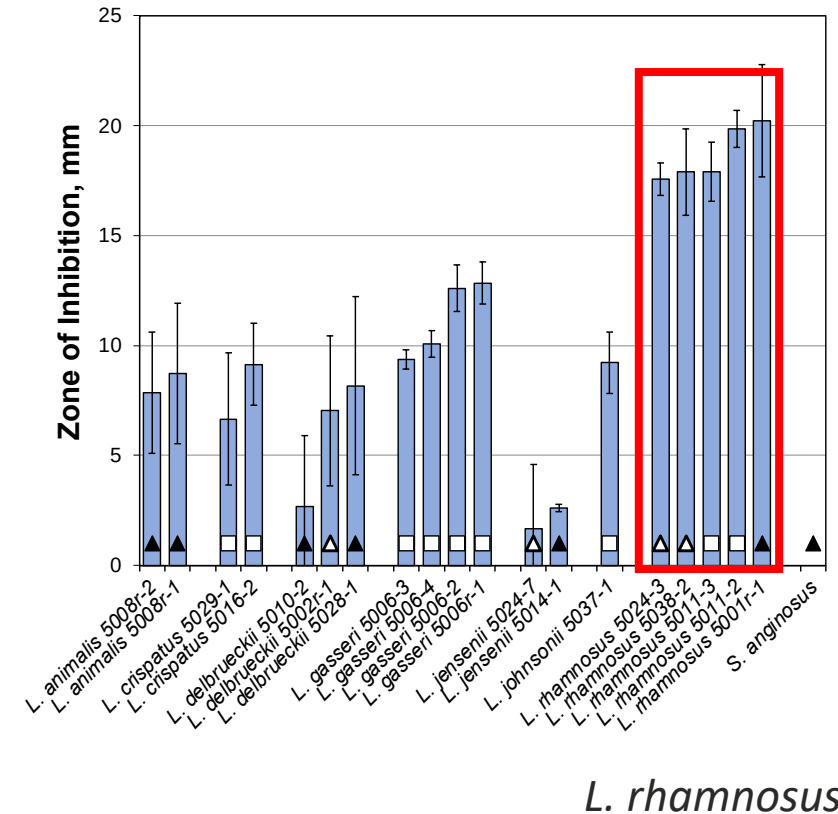
Why urinary *L. delbrueckii* susceptible?

- Attempted to induce using Mitomycin C using ATCC11842 dairy-derived strains with no prophage as control

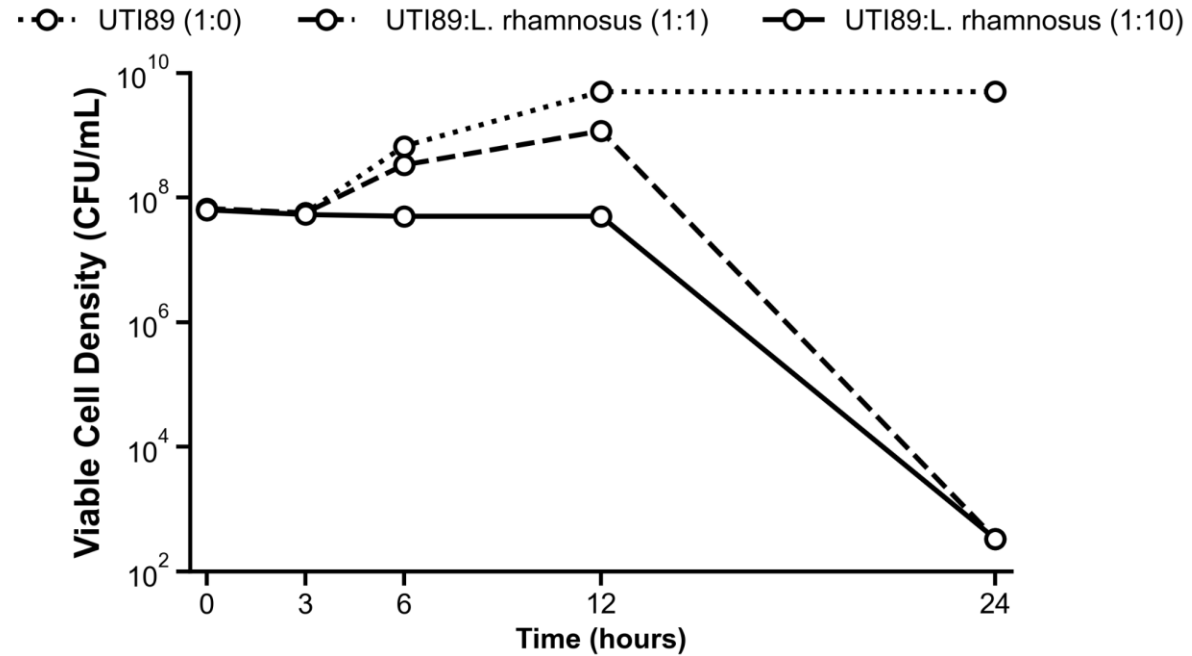


Urinary *L. rhamnosus* isolates

- Some of the strongest UPEC inhibition in well-diffusion assays
- Inhibition of *L. delbrueckii* in well assays
- Genomes contain 1-2 plasmids; some known adhesion factors

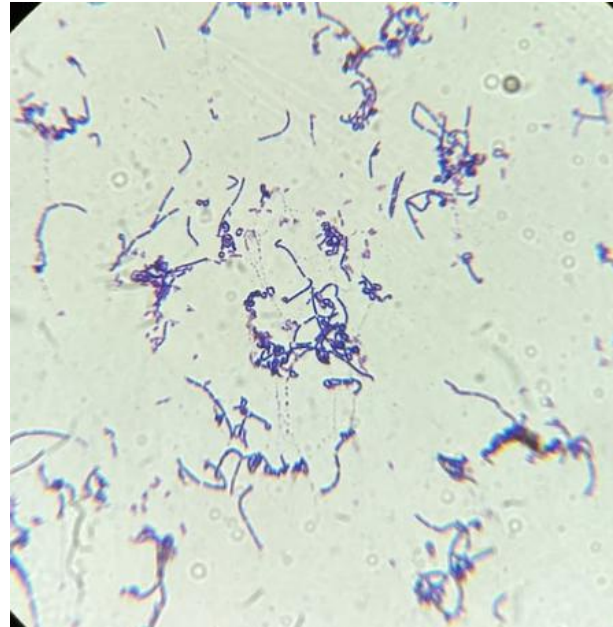


Co-culture of *L. rhamnosus* and UPEC



- similar inhibition to *L. gasseri* 5006-2

Co-culture of *L. rhamnosus* and UPEC



- possibly binds with UPEC cells

Summary – Interlactobacilli interactions

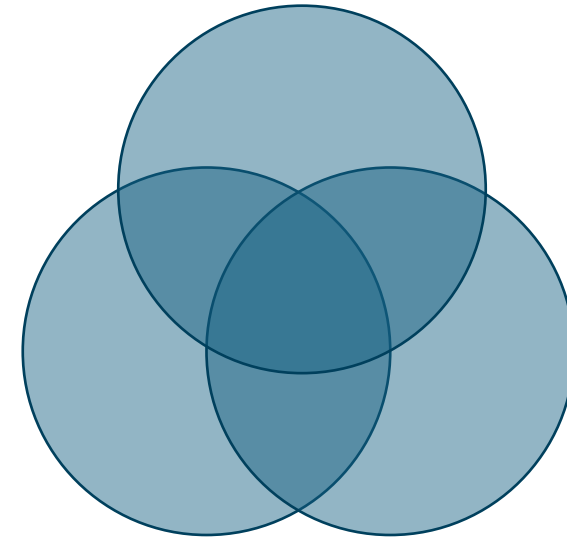
- Urinary lactobacilli can specifically interact amongst themselves
- Urinary lactobacilli contain many prophages
- These and other interactions in urinary microbiome are important in development of probiotics and treatments

Conclusions

- Urobiome in children can be characterized at higher taxonomic resolution using long-read sequencing methods
*can do
- Urobiome in children shows unique composition for individuals and that composition remains stable up to two months but shifts at longer time scales and with perturbations such as recurrent UTIs
*amaze amaze
- UPEC and at least two urinary lactobacilli species have complex interactions including inhibition, metabolic interference and aggregation
*it's complicated
- Urinary lactobacilli compete *in vitro* suggesting that resident urobiome composition and properties of probiotic candidates should be considered in search of anti-UTI interventions
*one size does not fit all

Urobiome as ecological niche

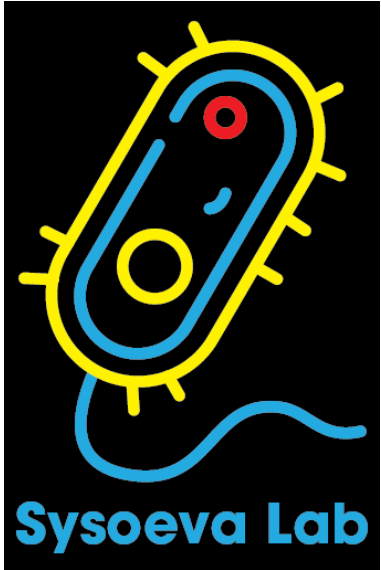
- Host (genetics, development, immune system)
- Urobiome community (residents and invaders)
- Environment (stress, drugs, diet, etc)



Acknowledgments

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Science University

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Thank you!

