



Survival of the fittest in the sweetest proteins...

Exploration of the Bladder Bacteria Metabolism toward Host Glycoproteins

Jean-Philippe Gourdine, PhD ("Dr JP")

Assistant Professor, Lewis & Clark College

Chemistry Department/BCMB program

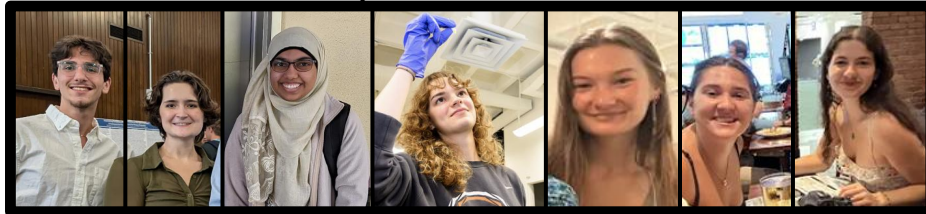
CAIRIBU URobiome Interest Research Group Summer - July 30th 2026

<https://bit.ly/DrJPG>



Acknowledgements

- **Lisa Karstens**, PhD, MSc, (Oregon Health & Science University (OHSU) & Nathan Boyer, MSc (OHSU)
- **Alan Wolfe**, PhD (Loyola University of Chicago)
- **René Fuanta**, PhD (East Stroudsburg University)
- **Chemistry & Biochemistry students**
Lewis & Clark College Undergraduate Research
 - **Elie Al Khoury***
 - Jessica Martin
 - Nuzhat Hoque
 - Malia Heien
 - **Jules Tri***
 - **Stella Davis***
 - **Monique Bittencourt-Dias**
 - Biochemistry Class of 2025 & 2026



Funding - Grants - Travel Awards



1)BACKGROUND

Urine is not sterile...



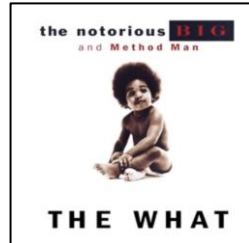
When?



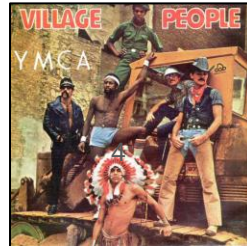
Who?



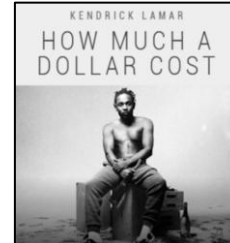
Where?



What?

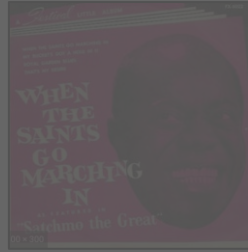


Why?



How?

Urine is not sterile...so



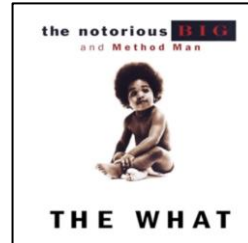
When?



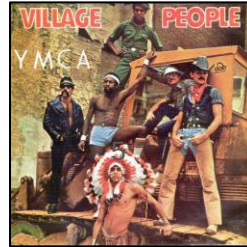
Who?



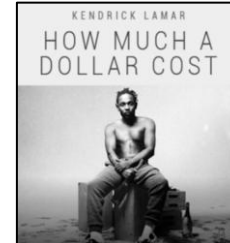
Where?



What?



Why?

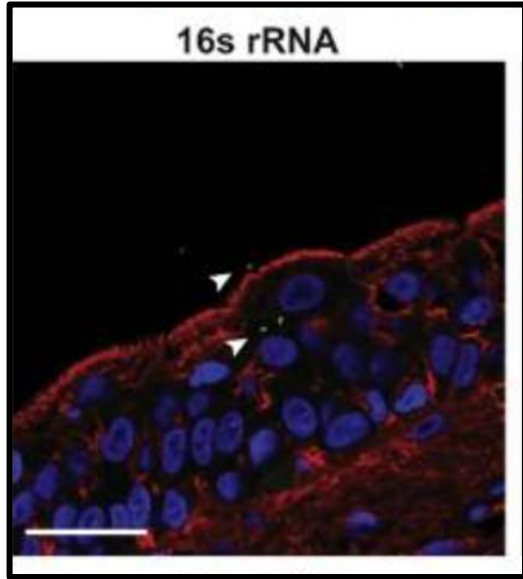


How?

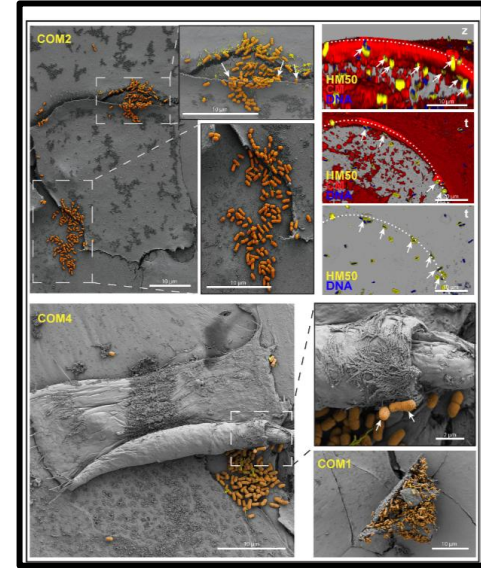
Where are bladder bacteria located ?



Where?



Urothelium for non-inflamed
Patient with recurrent Urinary
Tract Infection (rUTI)
de Nisco et al. J Mol Biol 2019



Urothelial microtissue model:
filamentation chaining, exfoliating
cells for commensal *E. coli*
Flores et al. Science Advances 2023

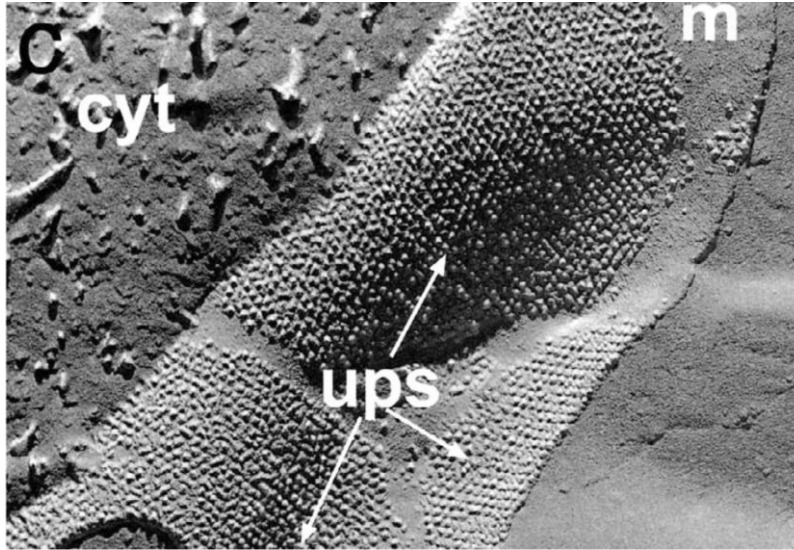
What biological structure is located at the cell surface of the urothelium ?

Glycocalyx

Glycocalyx

Mammalian Bladder Glycocalyx

Glycoproteins: Uroplakins - Mucins
Glycosaminoglycans - Proteoglycans



"Protective and interactive **molecular coating** on cell surfaces, composed of proteoglycans, **glyco**saminoglycans, mucins, **glyco**proteins, and **glyco**lipids, essential for cell communication, stability, and defense"

Roles of Glycans (Sugars) in Glycocalyx

1) Structure

(e.g., cell walls and extracellular matrices)

2) Energy

(e.g., carbohydrates as carbon sources for storage)

3) Information carriers

(e.g., blood group ABO system)

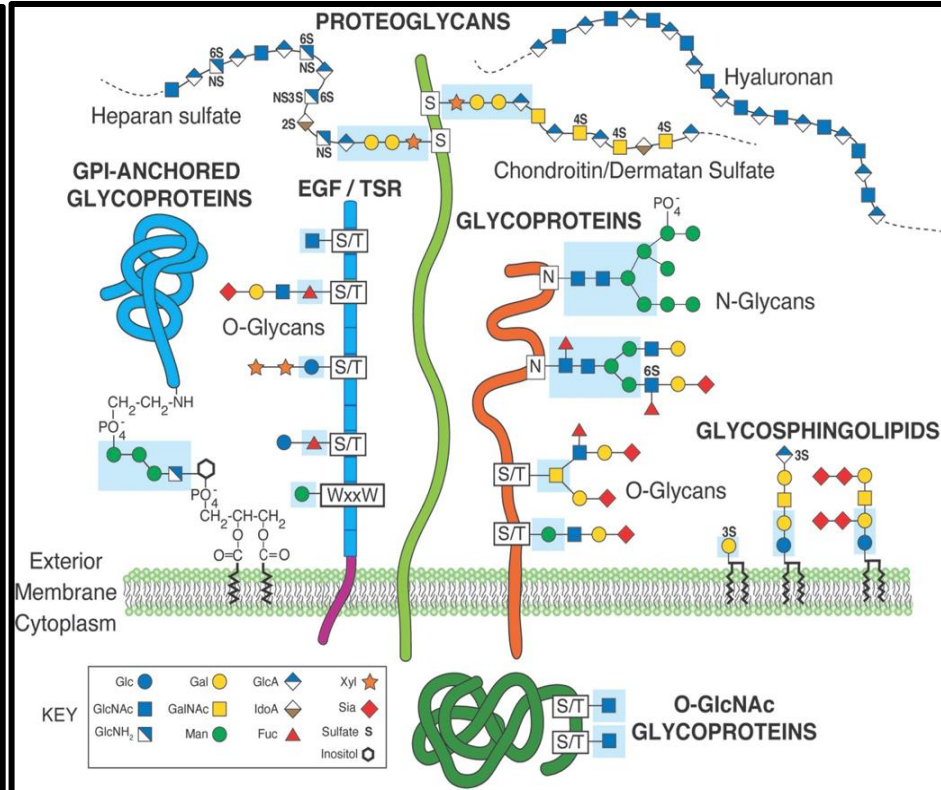
Attached:

Proteins (Glycoproteins)

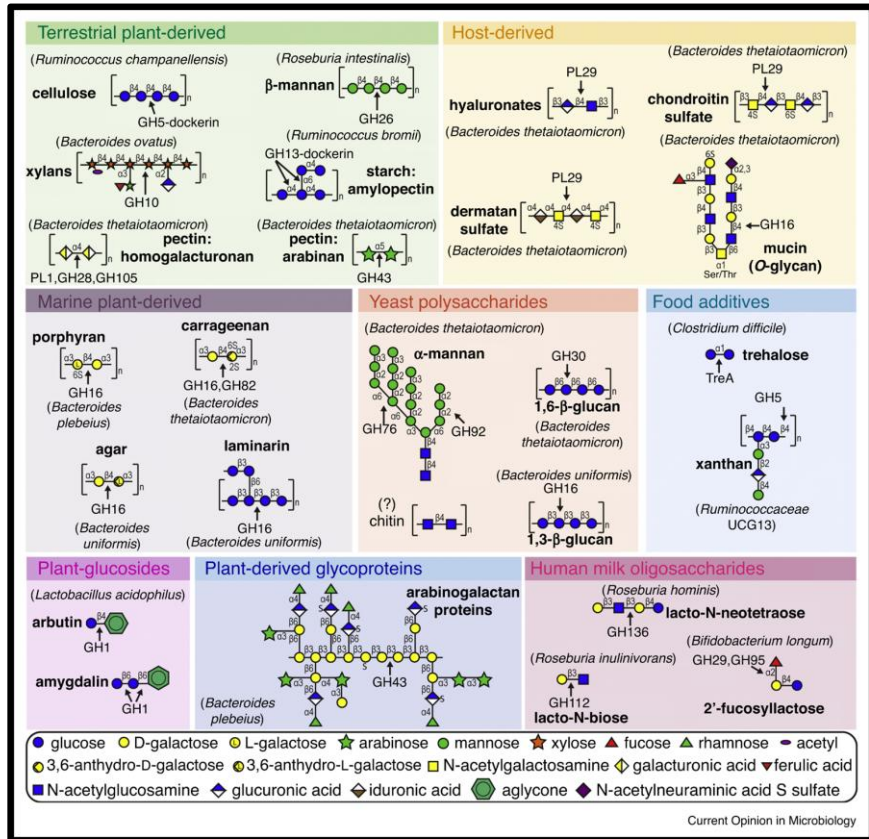
- Asparagine residue → *N*-glycans
- Serine/Threonine residue → *O*-glycans

Lipids (Glycolipids)

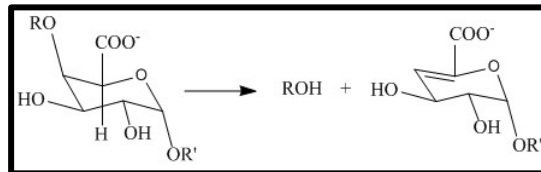
Free: Glycosaminoglycans (e.g., heparin)



In other body sites (e.g. gut and oral cavities) Bacteria Can Digest Complex Sugars with Specialized Enzymes

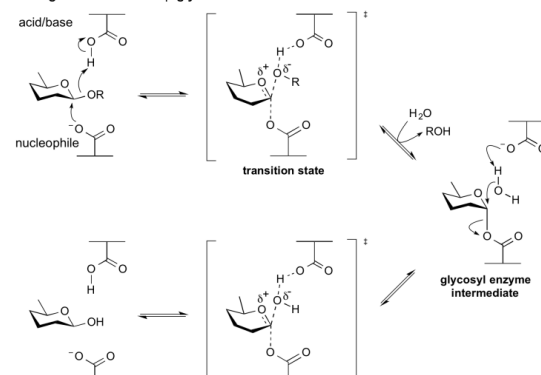


Polysaccharide Lyase (PL)



Glycosyl hydrolase (GH)

Retaining mechanism for a β-glycosidase:



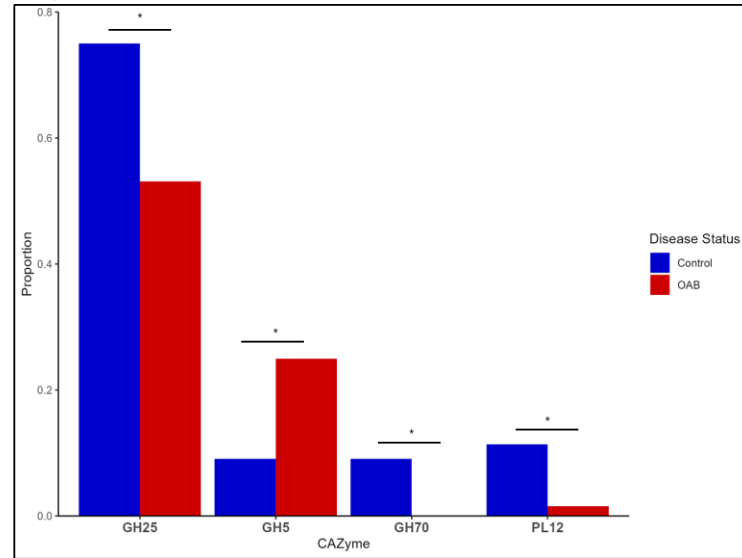
CAZY DATABASE

Bioinformatics Analysis of Bladder Bacterial Genomes Suggest that Bladder Bacteria Can Digest Host Glycans

Statistically significant differences between the numbers of GHs & PLs in Overactive Bladder Syndrome (OAB) and control bladder microbiomes (N> 1,000 genomes)



Nathan Boyer,
Karstens Lab OHSU



Nuzhat Hoque ,
BCMB
Lewis & Clark College
'24

Genomes published by:
Thomas-White et al, Nature Commun. 2018
Du et al. Genome Biology, 2024

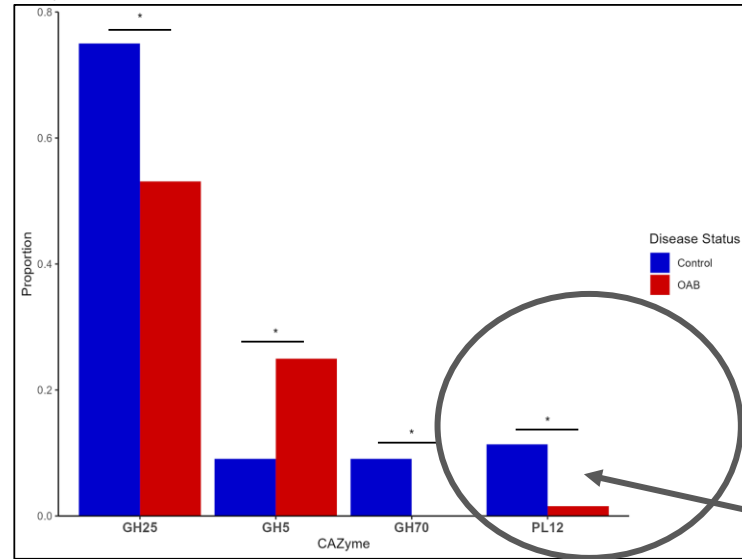
Gourdine et al. Biorxiv 2025
Gourdine & Karstens Labs manuscript in process

Bioinformatics Analysis of Bladder Bacterial Genomes Suggest Urobiome Can Digest Host Glycans

Statistically significant differences between the numbers of GHs & PLs in Overactive Bladder Syndrome (OAB) and control bladder microbiomes (N> 1,000 genomes)



Nathan Boyer,
Karstens Lab OHSU



Nuzhat Hoque ,
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Lewis & Clark College '24

**Bacterial PL12
that can digest
Host Glycosaminoglycans**

Genomes published by:
Thomas-White et al, Nature Commun. 2018
Du et al. Genome Biology, 2024

Gourdine et al. Biorxiv 2025
Gourdine & Karstens Labs manuscript in process

2) THE QUESTIONS?

Questions

1) Can bladder microbes also use host glycoproteins as a carbon source similarly to the oral & gut microbiota ?

Questions

- 1) Can bladder microbes also use host glycoproteins as a carbon source similar to the oral & gut microbiota ?
- 2) If yes, how do they proceed biochemically?

Questions

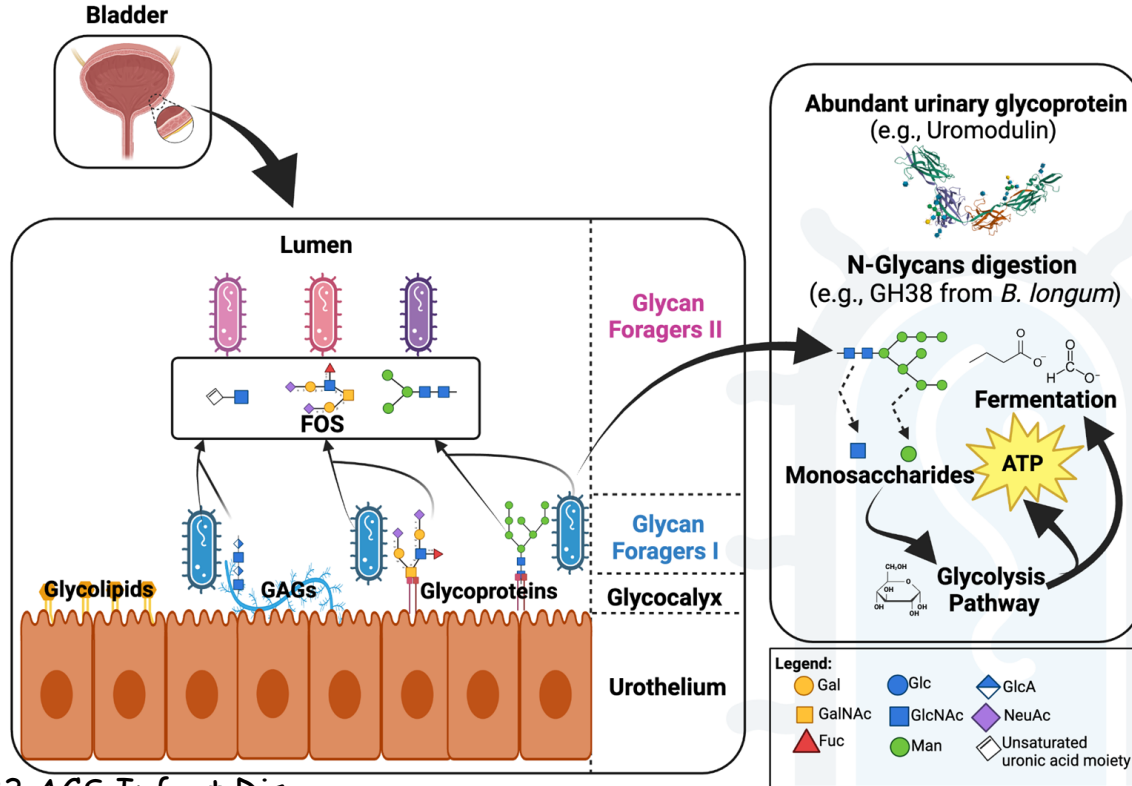
- 1) Can bladder microbes also use host glycoproteins a carbon source similarly to the oral & gut microbiota ?
- 2) If yes, how do they proceed biochemically?
- 3) Are there differences between glycan degradation of health-associated microbes and disease-associated bladder microbes?**

Questions

- 1) Can bladder microbes also use host glycoproteins as carbon source like oral & gut microbiota ?
- 2) If yes, how do they proceed biochemically?
- 3) Are there differences between glycan degradation of health-associated microbes and disease-associated bladder microbes?
- 4) If yes, could this provide valuable knowledge on glycan-based prebiotics?**

3) WORKING MODEL

Working Model



Neugent *et al.* 2023 ACS Infect Dis.

Nguyen *et al.* 2022, Front. Cell. Infect. Microbiol. 2022

Gourdine *et al.* Biorxiv 2025

**Urobiome metabolism
Vis-à-vis host-glycans**



**Biochemical
Approaches**

Host Urinary Glycans



**Bioinformatics
Approaches**

**Free Oligosaccharides
(FOS)**

**Secreted
Glycoproteins
(UMOD)**

**Membrane-associated
Glycans
(e.g., Glycoproteins,
Uroplakins, MUC1)**

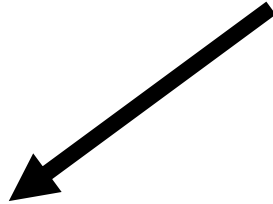
**Urobiome metabolism
Vis-à-vis host-glycans**



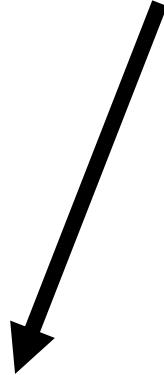
Biochemical
Approach



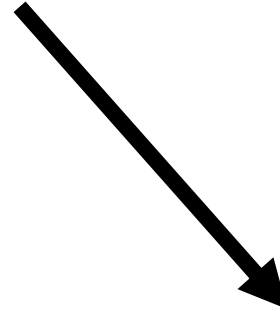
Host Urinary Glycans



**Free Oligosaccharides
(FOS)**



**Secreted
Glycoproteins
(Uromodulin, UMOD)**

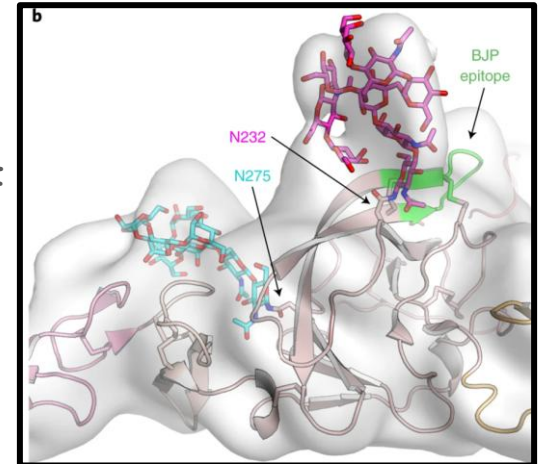
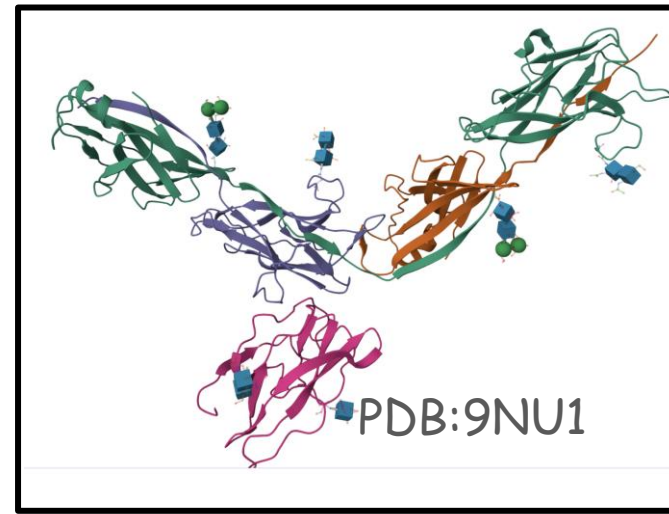


**Membrane-associated
Glycans
(e.g., Glycoproteins,
Uroplakins, MUC1)**

4) UMOD
THE SWEETEST?

UMOD

- Uromodulin, Tamm-Horsfall Protein (THP)
- The most abundant urinary protein (~50 mg daily)
- Produced by renal epithelial cells
- High-molecular weight filaments
- Matrix of hyaline casts
- ~30% of its mass is glycan-based
- **Glycans:**
 - Protects from kidney stones
 - Role in hypertension
 - Forms filaments that antagonize the adhesion of uropathogens through glycan-protein interactions with bacterial FimH



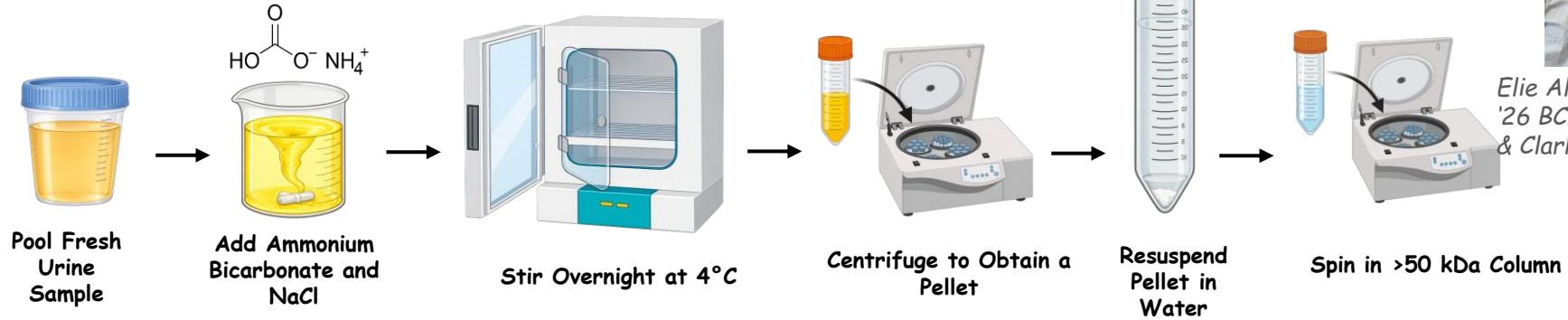
Many gut & bladder bacteria
(e.g., *Bifidobacterium longum*) possess specific
enzymes that digest N-glycoproteins.

Could they also digest UMOD?

5) METHODS & RESULTS

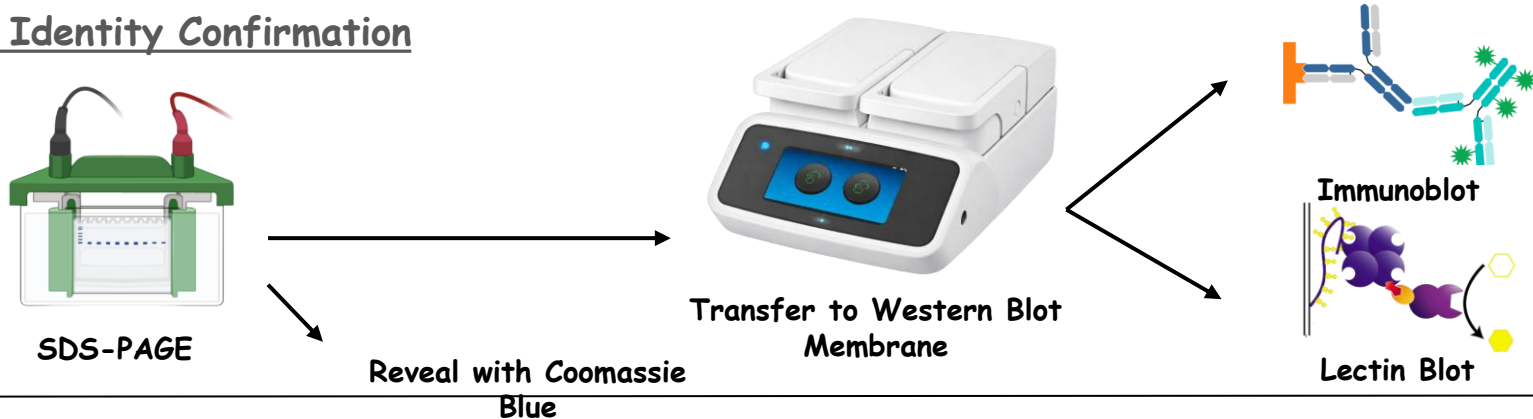
A)UMOD Degradation Assay Isolated Bladder Bacteria

UMOD Purification

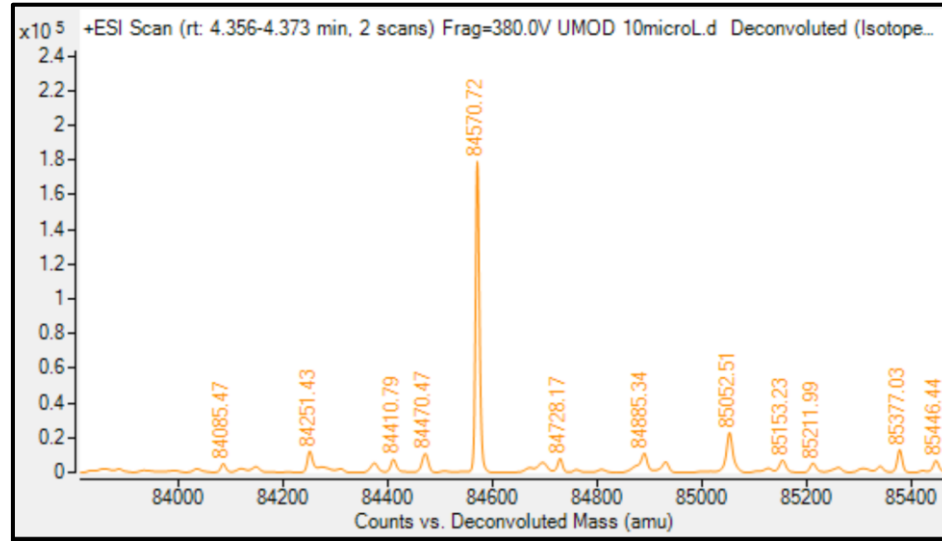
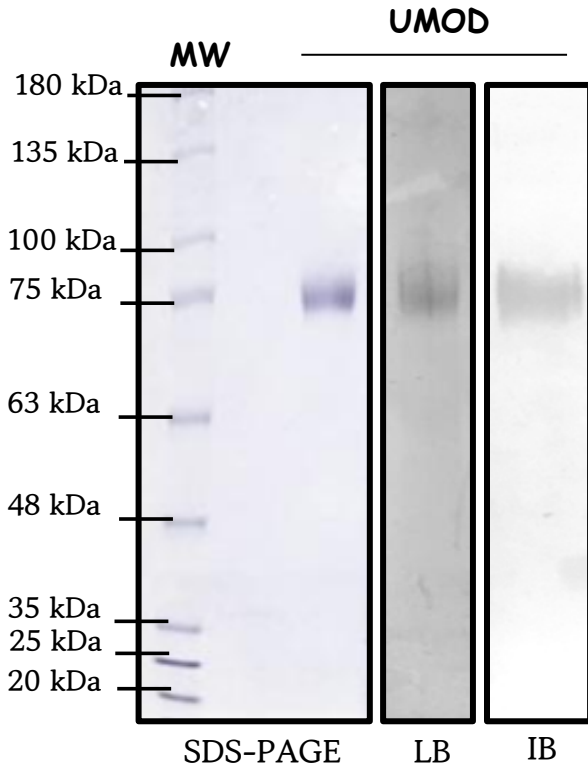


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& Clark College

UMOD Identity Confirmation



UMOD Purification



*MW = Molecular Weight

LB = ConA Lectin Blot

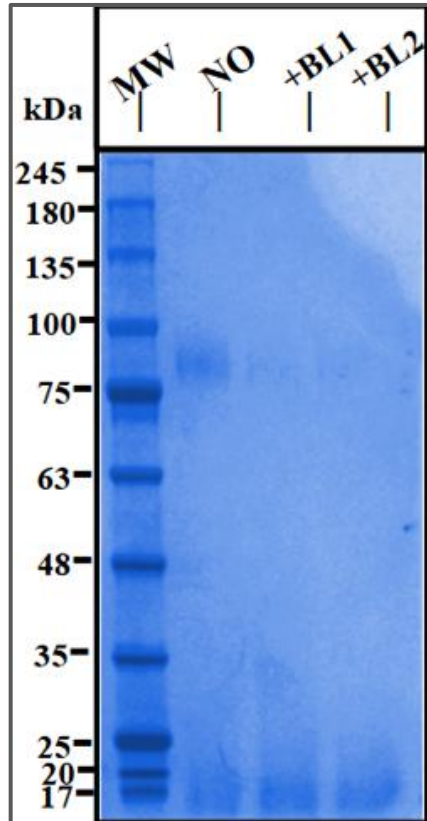
IB = Immunoblot

- Pure UMOD
- Remains Monomeric & Glycosylated
- Mass Spectrometry Data Confirms glyco-heterogeneity (cc:R. Fuanta)



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UMOD degradation Assay with *B. longum*



- Probiotic strain of *Bifidobacterium longum* is a bladder commensal, also present in the gut
- Can *B. longum* digest UMOD ?
- Test similar followed Garrido et al. Molecular & Cellular Proteomics 2012

- 1) *B. longum* added 16h at 37°C in MRS broth with Thioglycolate (200 μ L) +/- UMOD
- 2) Spin
- 3) SDS-PAGE

* **MW:** Molecular Weight

NO: UMOD alone, No bacteria

+ BL1 and BL2 : *Bifidobacterium longum* added 16h at 37°C in MRS broth with Thioglycolate (200 μ L)

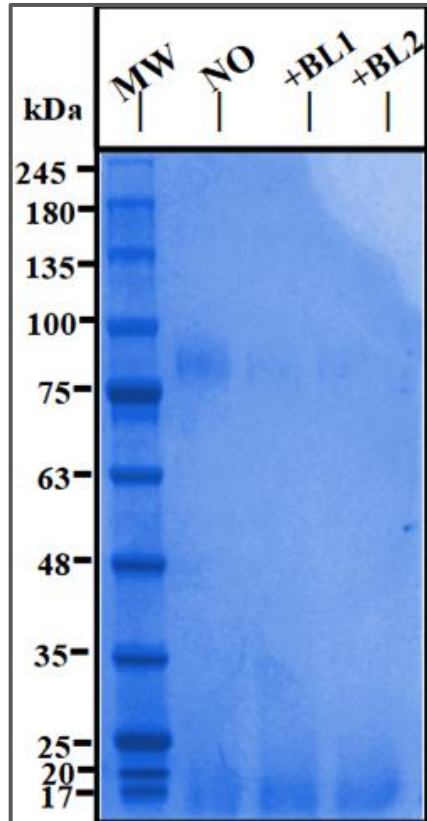


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Jessica Martin ,
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UMOD degradation Assay with *B. longum*



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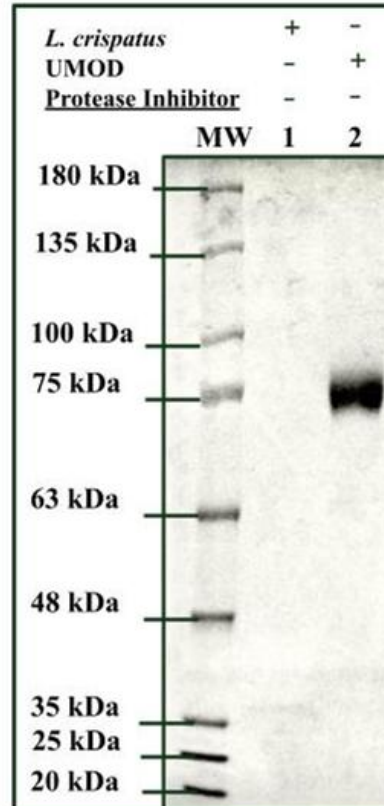
***B. longum* can digest UMOD**

* MW: Molecular Weight
NO: UMOD alone, No bacteria
+ BL1 and BL2 : *Bifidobacterium longum* added 16h
at 37°C in MRS broth with Thioglycollate (200 μ L)

UMOD degradation Assay with *Lactobacillus crispatus*



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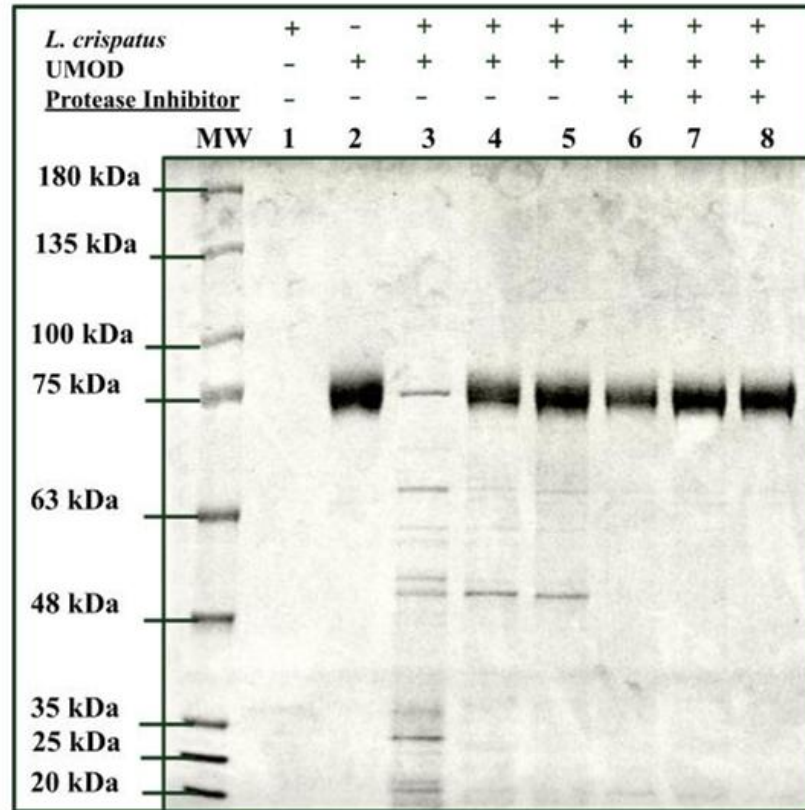


(Al Khoury's thesis, 2026 Gouridine Lab)

UMOD degradation Assay with *Lactobacillus crispatus*



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& Clark College



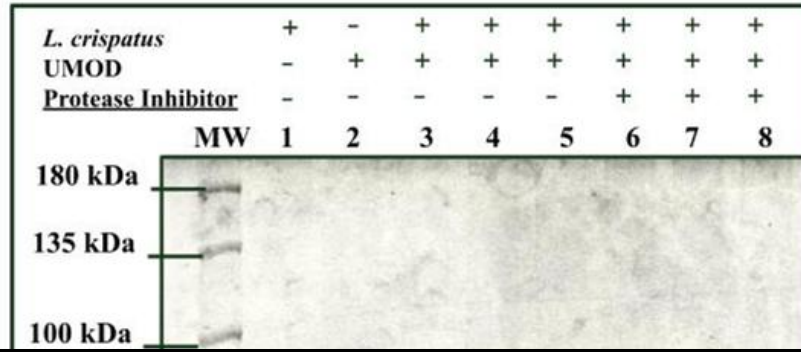
Sharp bands instead of
diffuse indicate
Proteolytic cleavage
Of UMOD

(Al Khoury's thesis, 2026 Gouridine Lab)

UMOD degradation Assay with *Lactobacillus crispatus*



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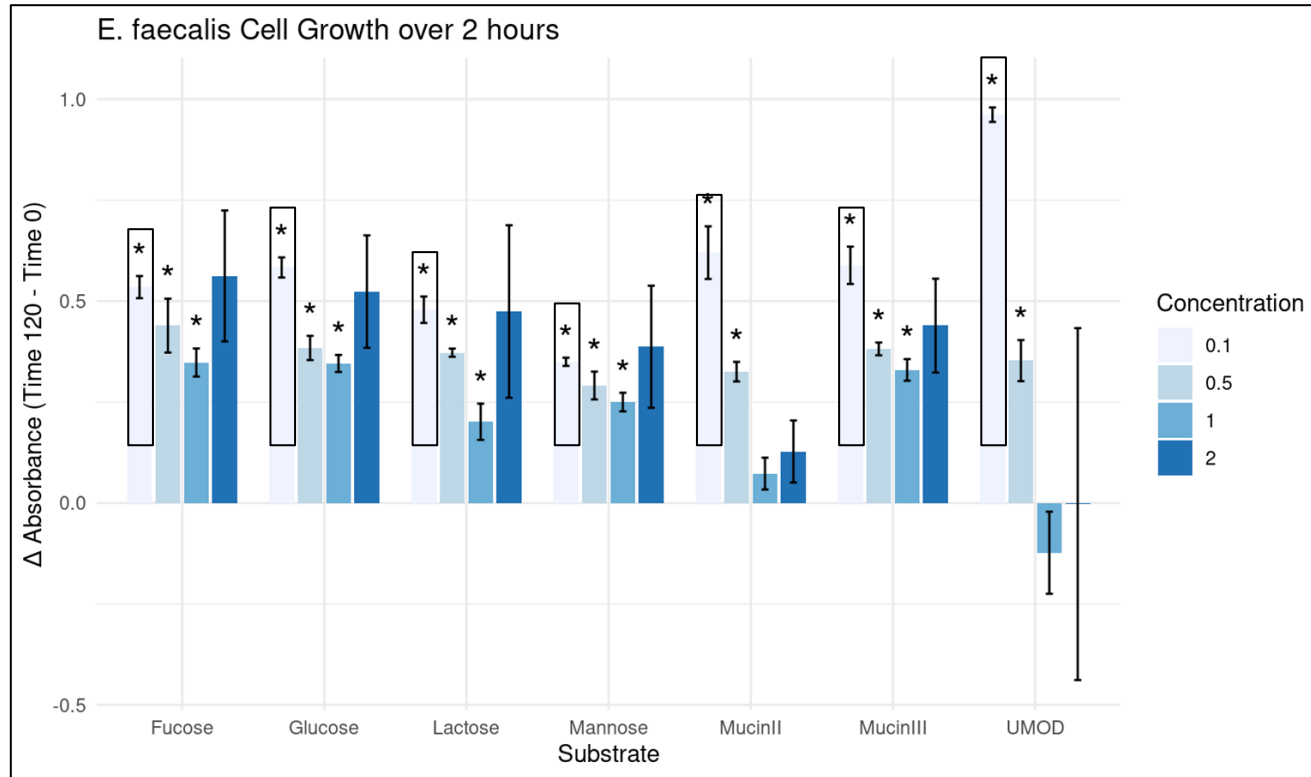
L. crispatus have proteolytic activity against UMOD but not a glycosyl hydrolase activity



(Al Khoury's thesis, 2026 Gouridine Lab)

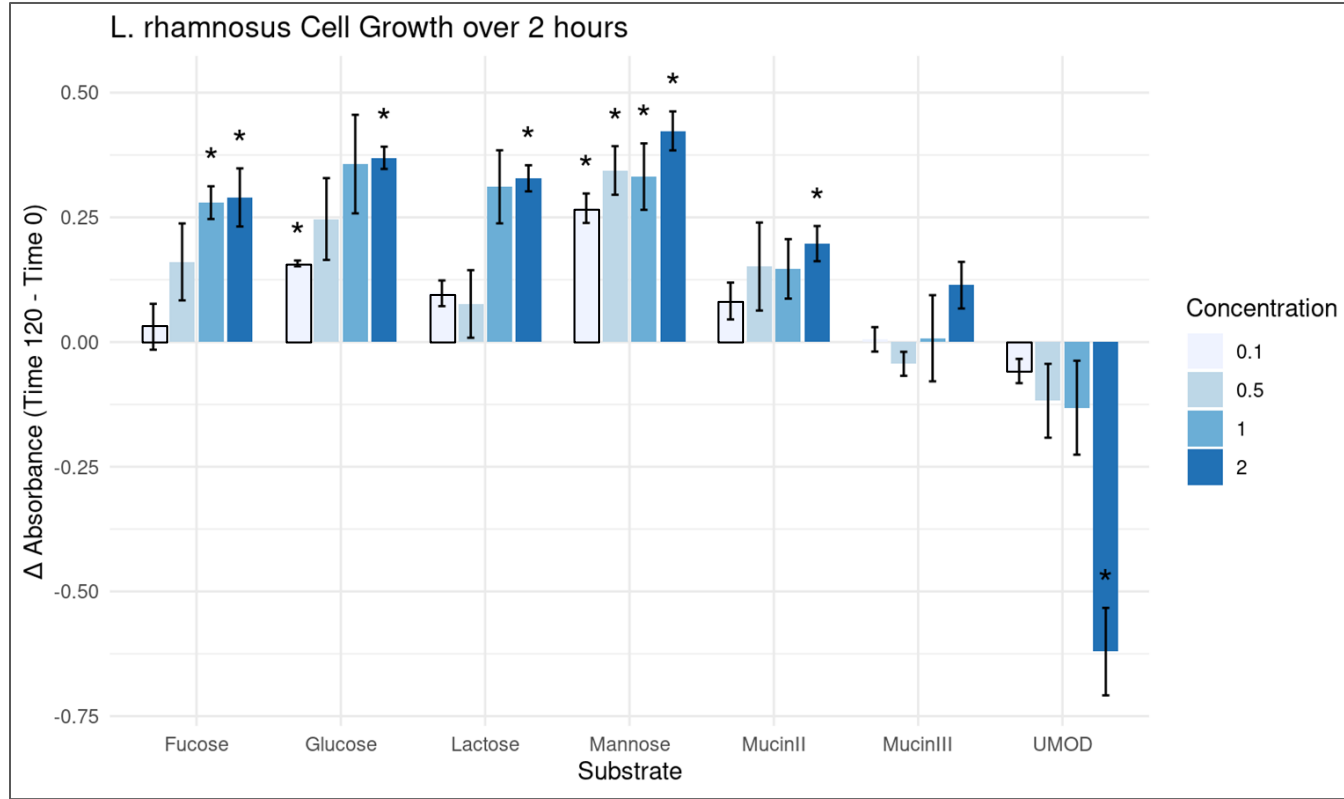
**B) Free Glycans, UMOD &
Mucins Growth Assay in BHI
broth Aerobically +
Isolated Bladder Bacteria**

UMOD - Mucin - Free Monosaccharides



Aerobically, E. faecalis (UMB1018) can grow with UMOD (0.1% - 0.5%) as its only substrate

UMOD - Mucin - Free Monosaccharides



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L. rhamnosus UMB0004
cannot grow on UMOD
at all but can on Mucin
and free sugars

While both *E. faecalis* and *L. rhamnosus* can grow on free saccharides & mucins,

Only *E. faecalis* can grow on UMOD up to 2%

38

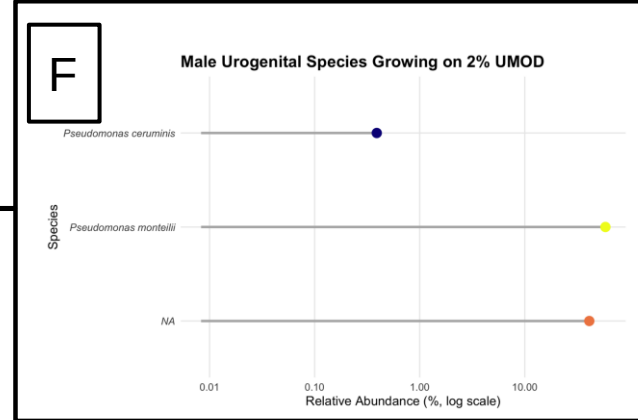
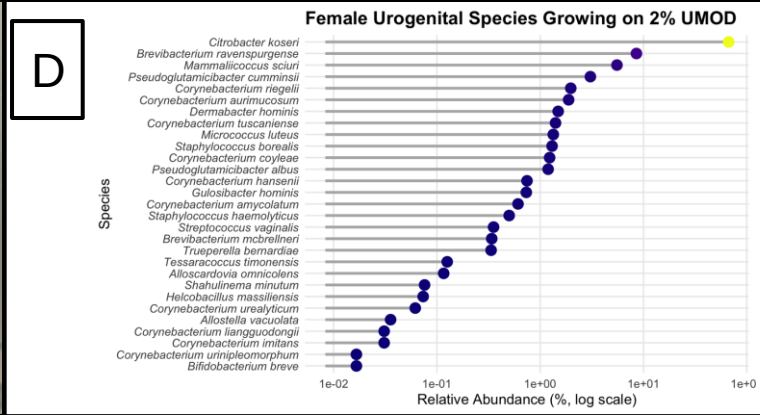
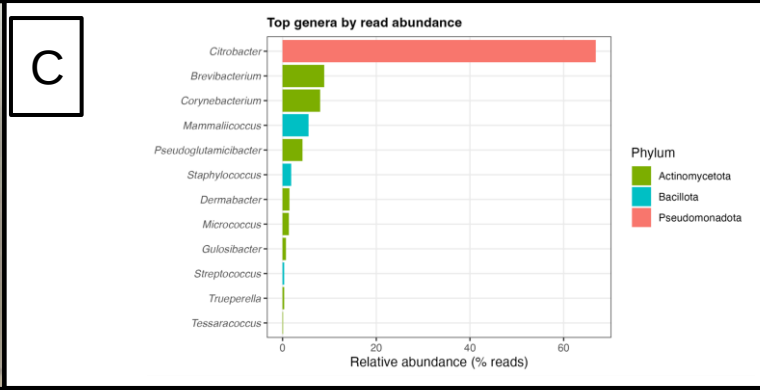
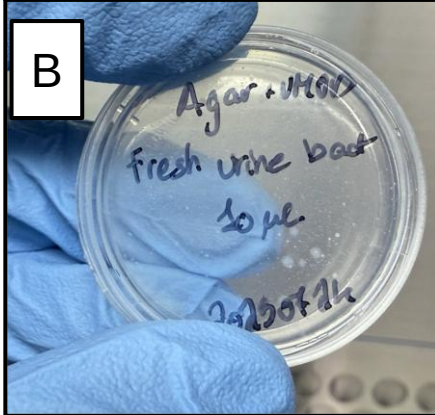
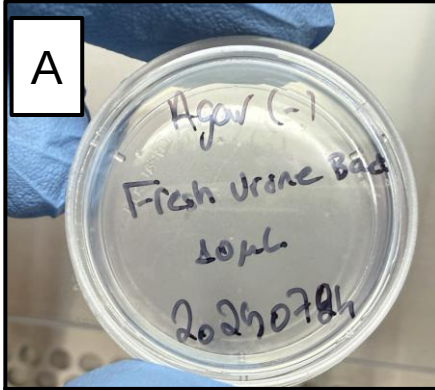
C) Pilot study #1 (10% CO_2)
"Survival of the sweetest"
= Agar + UMOD
+Urogenital Bacteria pellets

Pilot study #1 Aerobic

(N=2; triplicate)



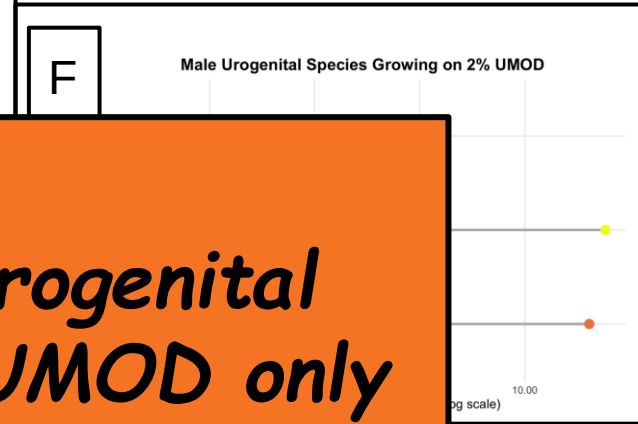
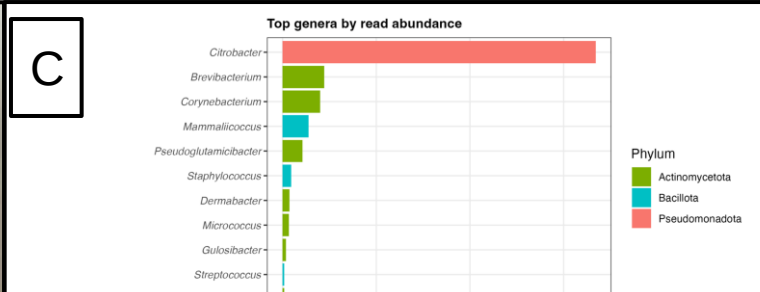
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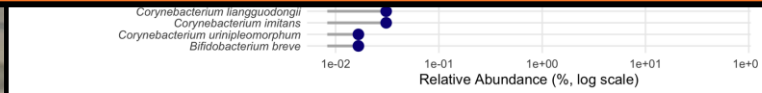
Pilot study #1 Aerobic (N=2; triplicate)



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*Different subsets of urogenital
bacteria can survive on UMOD only*



D) Pilot study #2 (Anaerobic)

"Survival of the sweetest"

= Agar + Minimum Media+ UMOD
+Female Urogenital Bacterial Pellets

Pilot study #2 Anaerobic

Urogenital bacteria from female donors

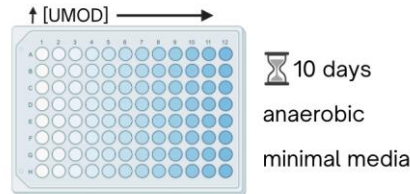


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POSTER #18

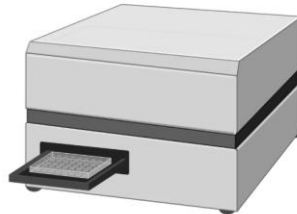
Urinary Bacteria Collection:

- Clean-catch voided urine samples from healthy donors
- Bacterial pellets were obtained through centrifugation at 4,000 rpm

Absorbance Assay: ↓



Absorbance at
600 nm ↓

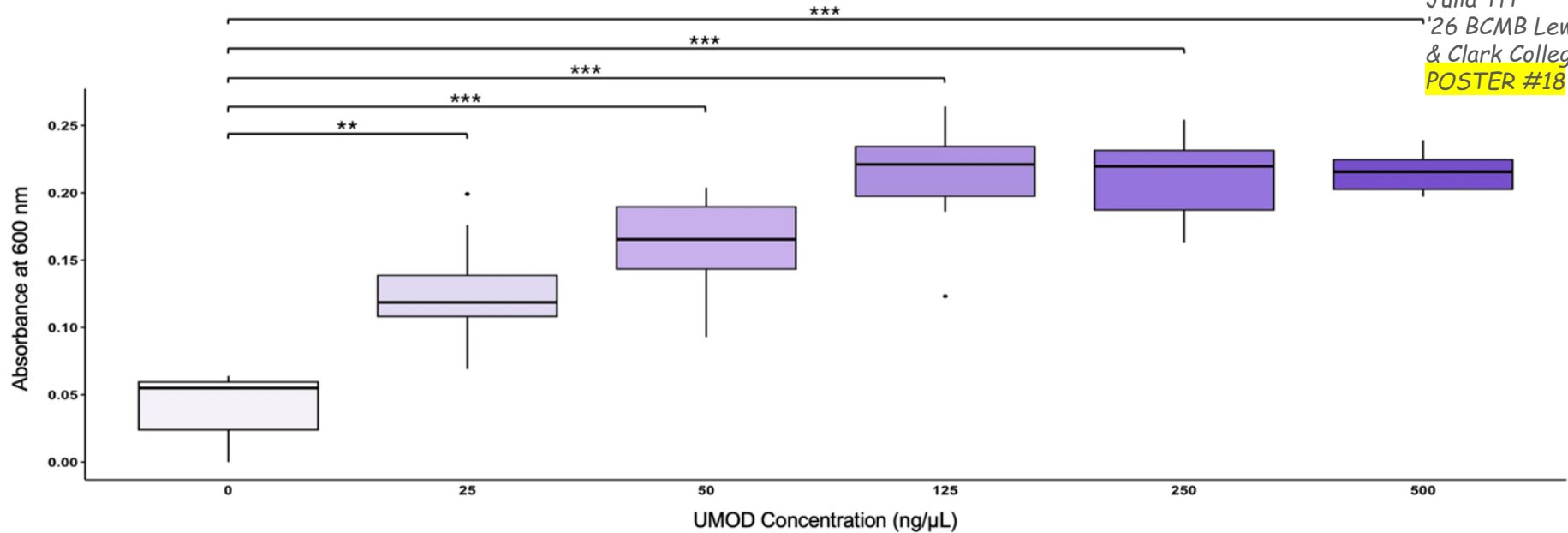


(Gourdine Lab, Unpublished)

Increase of OD600nm suggest Urogenital Bacteria can grow anaerobically on UMOD



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POSTER #18



Increase of OD600nm suggest Urogenital Bacteria can grow anaerobically on UMOD



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'26 BCMB Lewis
& Clark College
POSTER #18



Are they really growing?
Is the increase of OD due
to cell clumping with
increase [UMOD] ?

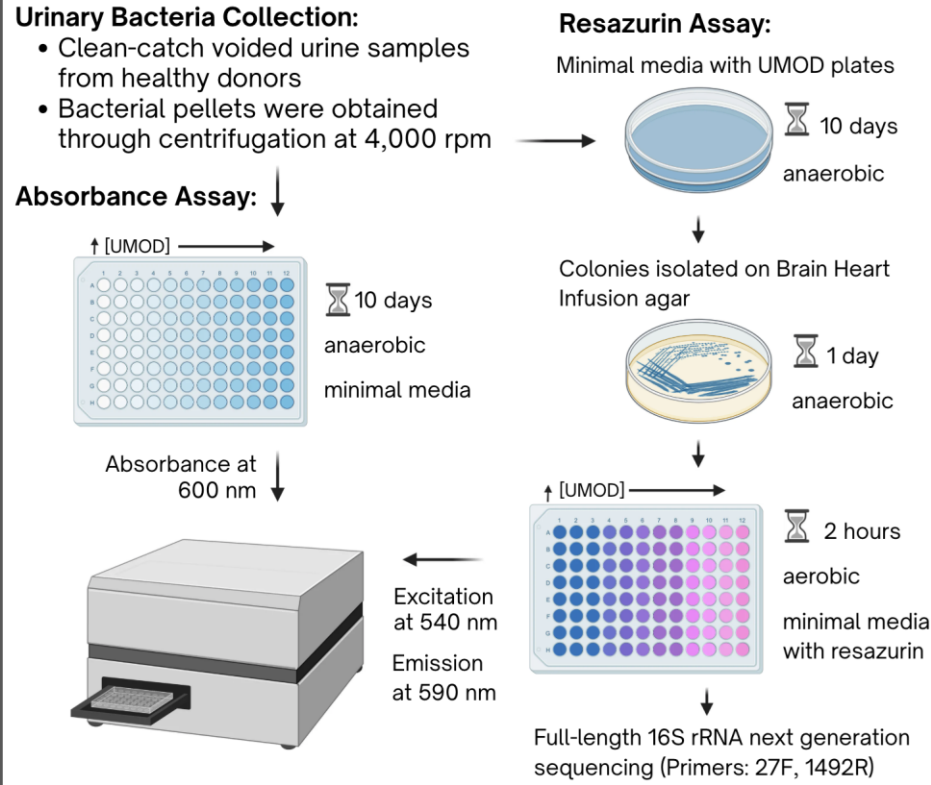
Pilot study #2 Anaerobic

Urogenital bacteria

from female donors



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POSTER #18



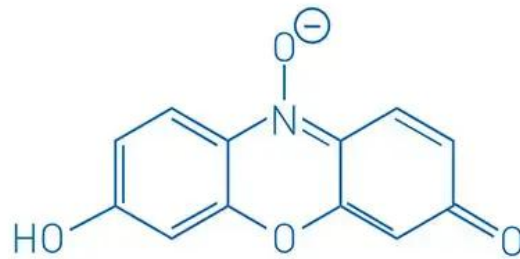
(Gourdine Lab, Unpublished)

Resazurin Assay:

7-Hydroxy-3H-phenoxazin-3-one 10-oxide

Fluorescent, nontoxic, cell-permeable & redox-sensitive Assay

Excitation 540nm - Emission 590 nm

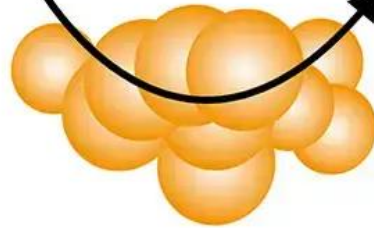


Resazurin

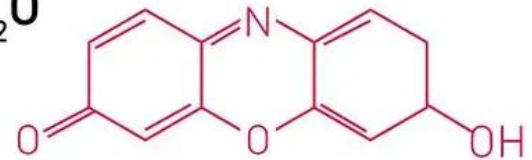
Blue and low
fluorescence

NADH/H⁺

NAD⁺/H₂O



Viable bacteria



Resorufin

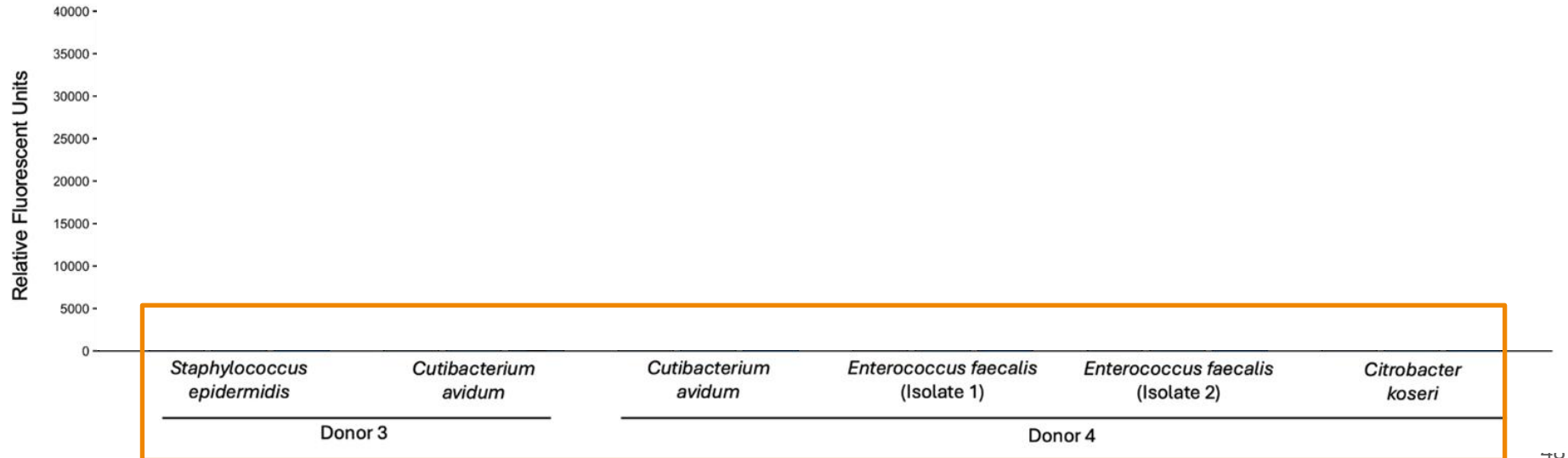
Pink and high
fluorescence

Selected Female Urogenital Bacteria can grow on UMOD



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UMOD-growing isolates identified by 16S rRNA metagenomics
2h growth then 17h (N=44 in octuplet)



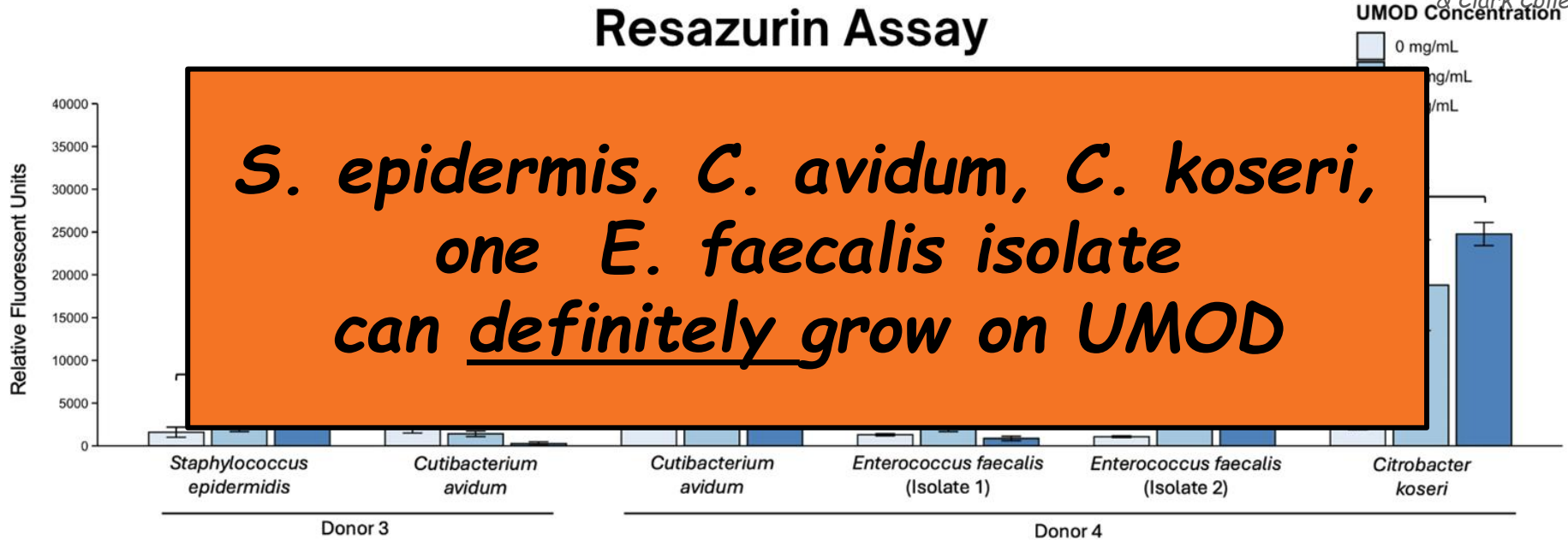
Female Urogenital Bacteria can grow on UMOD anaerobically



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Resazurin Assay

S. epidermis, *C. avidum*, *C. koseri*,
one *E. faecalis* isolate
can definitely grow on UMOD



6) CONCLUSION

Conclusion - Some Answers

1) Can bladder microbes also use host glycoproteins as a carbon source like in the oral & gut microbiota ?

Yes UMOD is a also a carbon source and not only antibacterial!

(See also Poster #18)



2) If yes, how do they proceed biochemi

See Poster #10



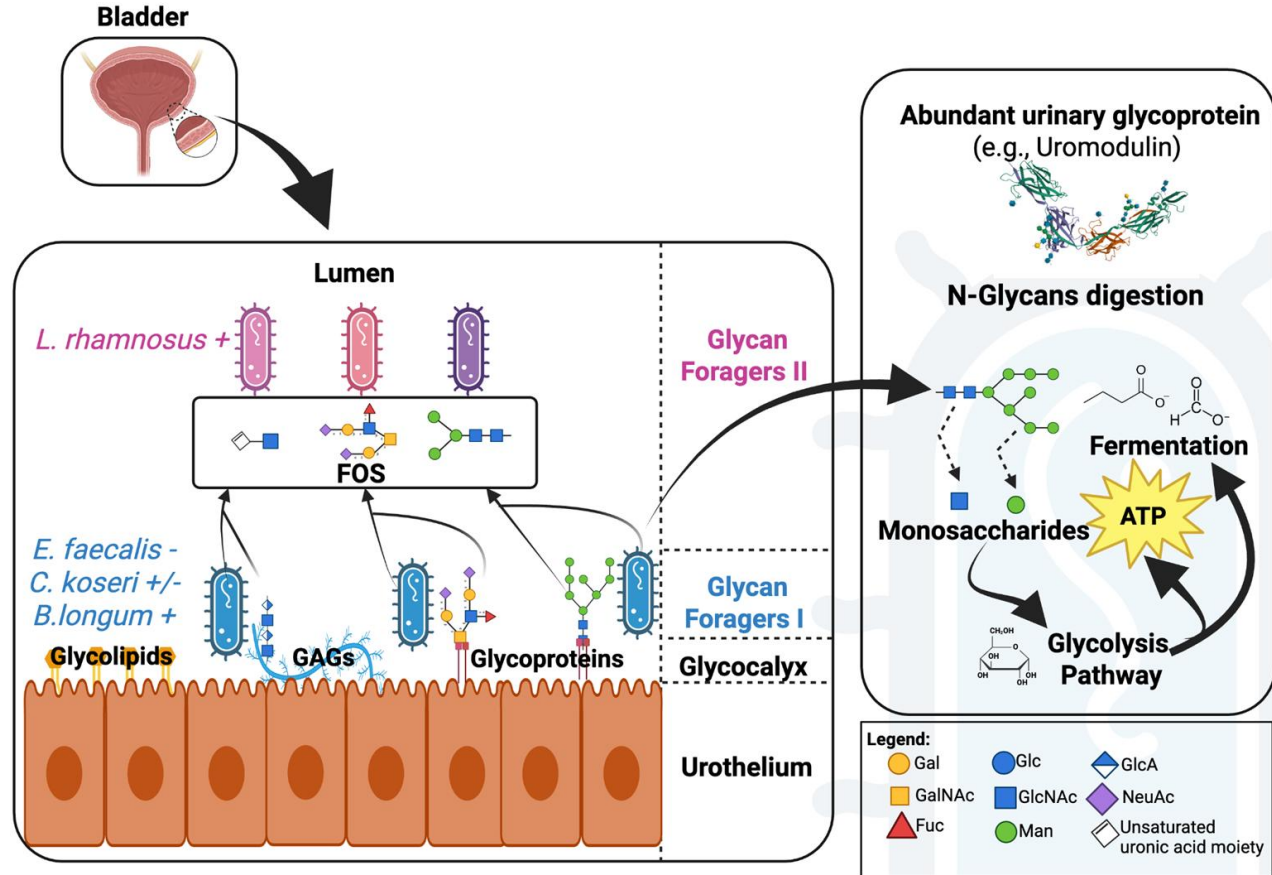
3) If yes, are there differences between glycan degradation of health-associated microbes and disease-associated bladder microbes?

It's complicated!

Revised Model Female Urobiome

Health
Associated

Disease
&
Health
Associated





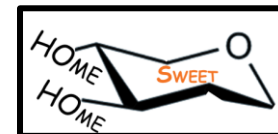
Undergraduate Research Students

2 full months per
year

Over the last
3 years



Thank you for your attention



Funding sources:

- Murdock Charitable Foundation Funding Source
- Dean's Office Startup Fund (L&C)
- NSF-LEAPS 2532889 (2025-2027)

<https://jpgourdine.github.io/>
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