Lipidomic Profiling Reveals Interactions Between Uropathogenic *Escherichia coli* And *Actinotignum schaalii*

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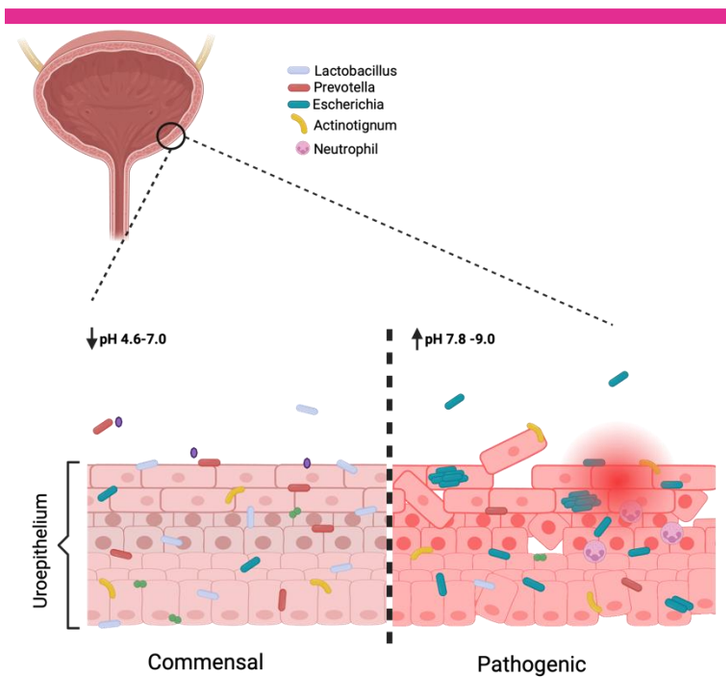


Introduction

The urobiome—the complex and dynamic microbial consortium residing within the urinary tract—plays a crucial role in maintaining urinary health and in the pathogenesis of urinary diseases. Recent research has expanded our understanding of its microbial diversity beyond classical uropathogens, uncovering complex interactions that may modulate susceptibility to infection, pathogen persistence, and therapeutic response. While uropathogenic *Escherichia coli* (UPEC) remains the most prevalent etiological agent of urinary tract infections (UTIs), its ecological behavior within the polymicrobial urobiome environment is not yet fully elucidated.

Purpose

Identify and explore metabolic interactions between urobiome member *Actinotignum schaalii* and the well-known uropathogen *Escherichia coli*. The aim is to understand how these bacteria may influence each other's presence, growth, and pathogenicity within the urinary tract environment

Survey Of The Urobiome

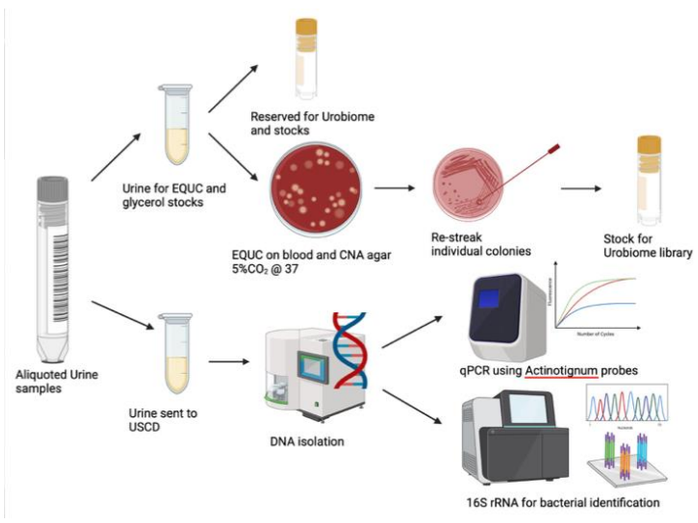


Figure 1. Study design and methods to determine the presence of *Actinotignum species* at VUMC.

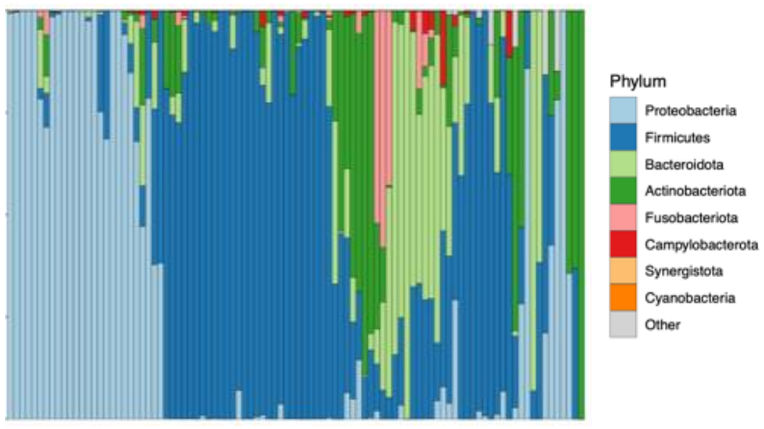


Figure 2: 16S rRNA amplicon sequencing of the VUMC urobiome cohort (N=100) demonstrates that the urinary microbiota is predominantly composed of members of the Firmicutes and Proteobacteria phyla, with notable interindividual variation in relative abundance. A total of 21 samples tested positive for Actinotignum, among which 20 also harbored Escherichia species (not shown).

Actinotignum Growth Is Promoted With The Addition Of Specific Host Fatty Acids

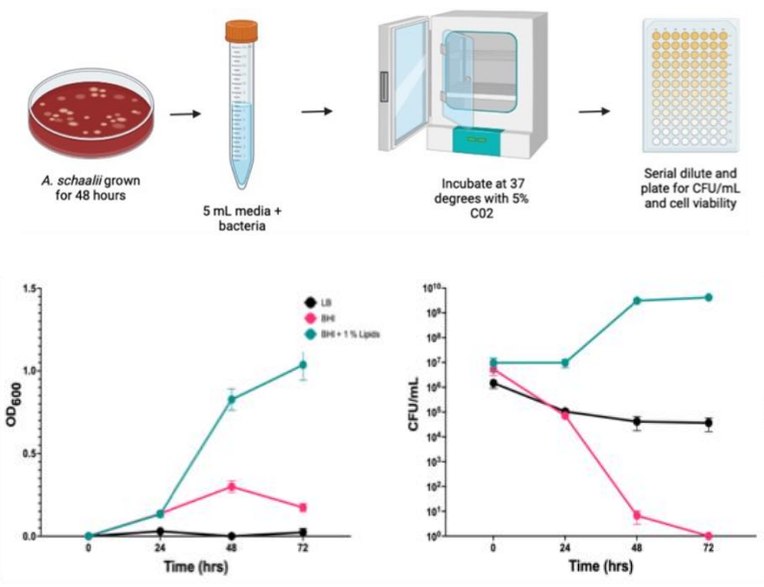


Figure: 3. Workflow for *Actinotignum* growth **B.** *A. schaalii* inoculated into common laboratory media used for bacterial propagation: BHI, LB, or BHI supplemented with 1% lipid mixture.

Lipidomic Profiling Of *Actinotignum* Cultivated In The Presence Of Host-derived Fatty Acids.

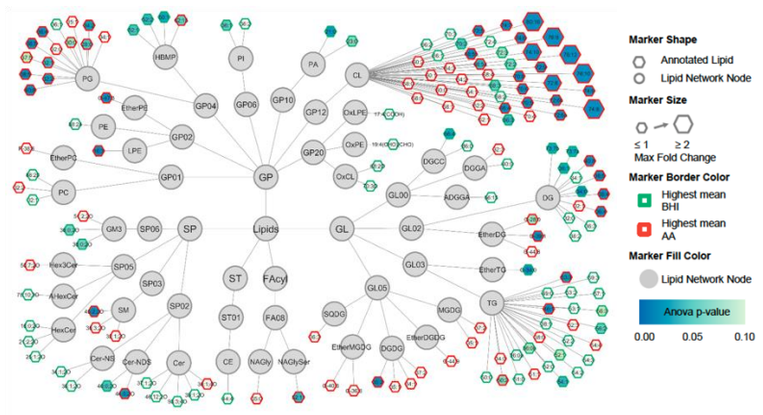


Figure: 4. The Cytoscape lipid network map reveals that phosphatidylglycerol and cardiolipin nodes are upregulated in response to arachidonic acid supplementation, indicating enhanced functional activity within these lipid pathways.

Actinotignum Growth Is Enhanced In The Presence Of UPEC

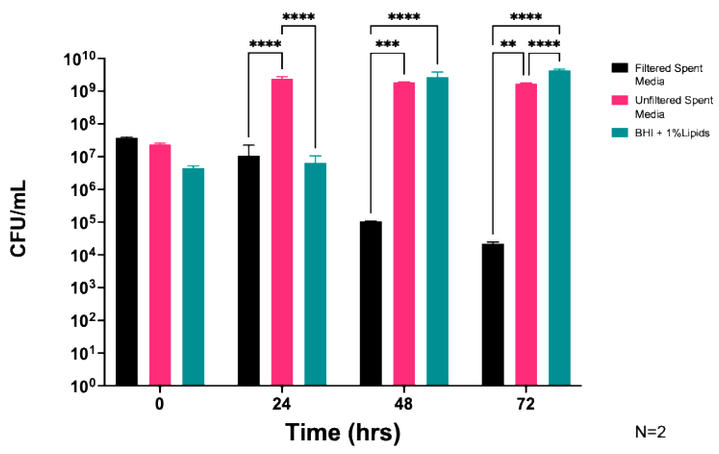


Figure 5: *Actinotignum* inoculated into spent UPEC LB medium. Filtered spent medium (sterilized, removing cells or debris), Unfiltered spent medium (contains soluble factors and cellular material), and BHI + Lipid medium. This setup facilitates the comparison of media effects on Actinotignum's growth and responses in spent UPEC medium

Lipidomic Profiling Of *Actinotignum* Grown In The Presence Of UPEC

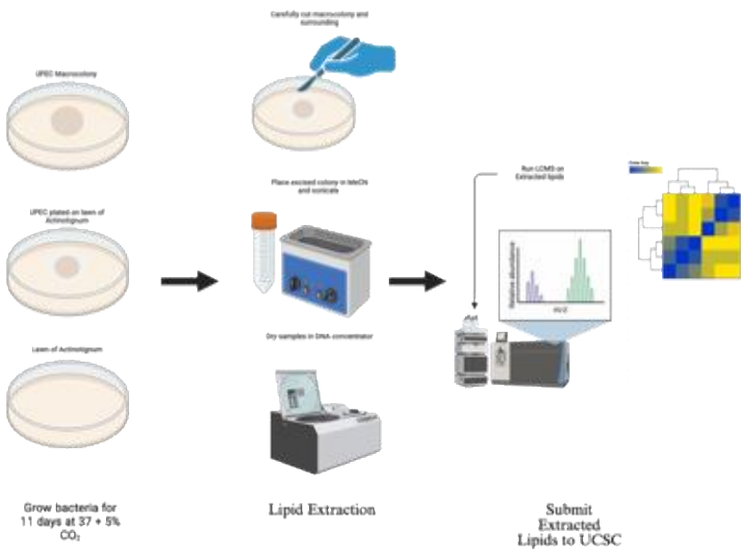


Figure 6. A detailed study design and methods to investigate potential metabolic interactions between *Actinotignum* and UPEC.

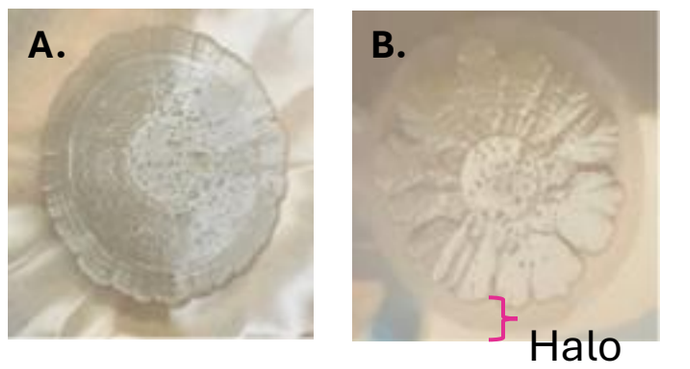


Figure 7. UPEC macro-colony +/- the presence of A. schaalii. **A.** UPEC macro-colony grown without lawn of *Actinotignum* at 37 in 5% CO_2 **B.** Halo Observed around UPEC macrocolony grown in the presence of *Actinotignum* at day 11 grown at 37 in 5% CO_2.

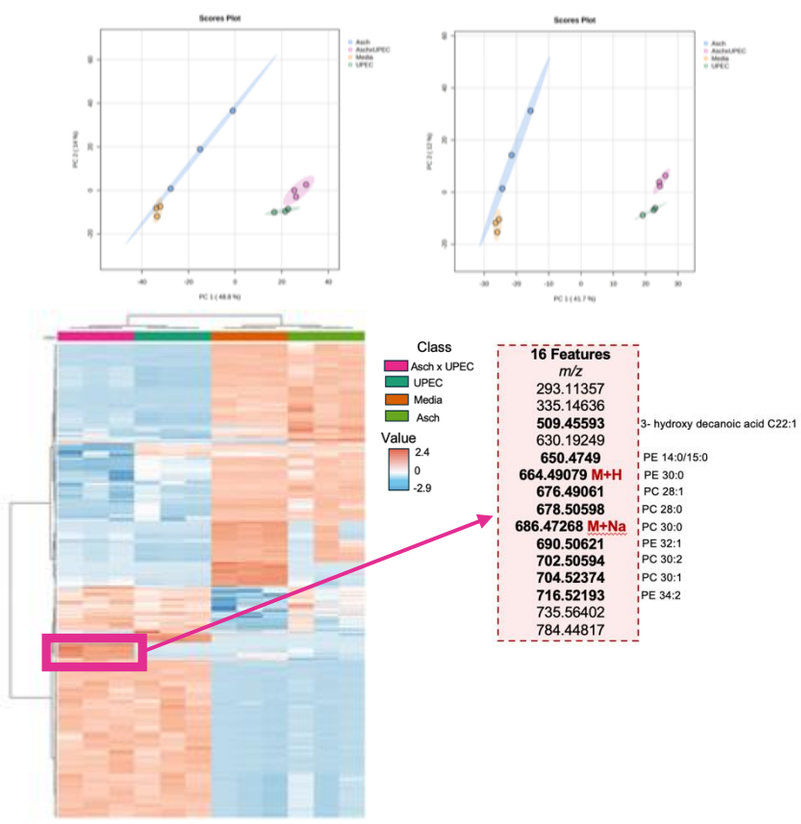
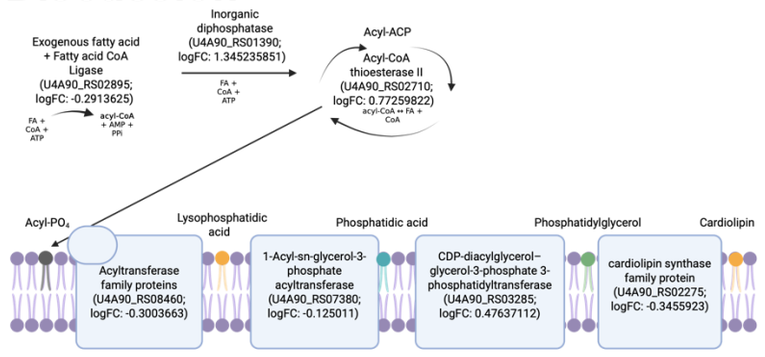


Figure 8. Principal component analysis (PCA) score plot derived from untargeted lipidomic profiling of samples obtained via both positive and negative electrospray ionization modes. Left + mode, right - negative mode. Heatmap showing clustering of lipid species from untargeted lipidomics between conditions in + mode. 16 distinct lipid features, including multiple species of phosphatidylcholine, phosphatidylethanolamine, and 3-hydroxy decanoic acid.

Discussion



Actinotignum utilizes a CoA-dependent lipid biosynthesis pathway. Exogenous fatty are enzymatically activated to acyl-CoA. These activated fatty acids are then incorporated into membrane phospholipids via acyl-CoA–dependent acyltransferases. The incorporated fatty acids can either be directly integrated into membrane structures or be metabolized into lipid intermediates such as acyl-CoA or acyl-ACP for subsequent lipid synthesis. This process is crucial for promoting bacterial growth and cell survival.