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Unveiling Bacterial Hitchhikers in the Urobiome

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ABSTRACT

Microbial communities of bacteria, viruses, and fungi form complex interactions in nature. However, our understanding of these communities is limited to those that can be cultured in the lab. Enhanced culture methods have enabled the isolation of numerous fastidious species from urinary tract, definitively proving that the urinary tract of asymptomatic individuals is not sterile. In working with these isolates, our group has observed several instances in which a "purified" isolate actually harbors more than one species. We refer to these additional members as bacterial hitchhikers, a term previously used to describe similar occurrences in soil communities. I hypothesized that under a given culture condition, these bacterial hitchhikers would proliferate such that they could be isolated. Here, I focus on identifying biological hitchhikers in the urinary microbiota and investigating why these bacteria may hitchhike. First, I sequenced and identified the species of bacteria present in our samples using 16S rRNA gene sequencing. Next, I observed frequently co-occurring species and assessed the fitness of strains grown in isolation as well as strains grown in co-culture with other species. Finally, I examined the metabolic profiles from bacterial genomes of a given participant's urine sample. By understanding complex interactions in the urobiome, researchers will understand the challenges of manipulating a microbial community.

CHAPTER ONE

INTRODUCTION

The Human Microbiome

Microorganisms in and on the human body affect life in ways we are only starting to understand. Scientists are dedicated to studying microbes in virtually every niche in the human body to understand the interconnectedness of humans and their microbial inhabitants. These organisms, which include bacteria, archaea, fungi, and viruses, contribute to microbe richness and diversity in every body site. Our microbial community, also known as the human microbiota, has a critical role in major biological processes such as stimulating immune responses (Cosseau et al., 2008), aiding in digestion (De Angelis et al., 2021; Gunzburg et al., 2020), and modulating brain activity (Tillisch et al., 2013). While human-inhabiting microbiota are recognized as a factor in human disease, it is equally important to recognize the role these microbes play in maintaining health (Huse et al., 2012).

The colonization of the human body by microorganisms begins at birth and develops during infancy. The instantiation of the human microbiota, and the human microbiota in general, is best understood within the gut (Davenport et al., 2014; Greenblum et al., 2015; Krych et al., 2013). Identifying bacteria present in stool samples has provided a glimpse into the diversity and complexity of the gut microbiota. Dysbiosis or disruption of the "normal" microbiota has been associated with unfavorable health issues and disease. For example, a study on a cohort of 43 US infants concluded that antibiotic exposure depleted various bacterial genera in the gut, including

Clostridiales and Lachnospiraceae (Bokulich et al., 2016). In another study, evidence suggests that certain chronic illnesses such as type 2 diabetes, obesity, and metabolic syndrome are associated with a disequilibrium in the gut microbiome (Wampach et al., 2017).

16S rRNA gene sequencing

A critical process to understanding the human microbiota involves sequencing the microbiota in and on the human body. The Human Microbiome Project (HMP) set forth to identify these microbes, originally focusing on the bacterial members of the microbiota in five anatomical sites: the gastrointestinal tract, the mouth, the vagina, the skin, and the nasal cavity (Kolde et al., 2018; Peterson et al., 2009). The HMP, which was the largest 16S rRNA survey study (Conlan et al., 2012a), produced the first reference of bacterial diversity in healthy humans. There are an estimated 500-1000 bacterial species inhabiting humans at any given time (Locey & Lennon, 2016). The HMP was established with the goal of describing the extent to which these microorganisms are shared between individuals and how they contribute to human health (Jumpstart Consortium Human Microbiome Project Data Generation Working Group, 2012). The most significant component of this project was cataloguing microbial species through sequencing of the 16S ribosomal ribonucleic acid subunit (rRNA) gene (Conlan et al., 2012b). The publicly available datasets from 16S rRNA gene amplicon sequencing in addition to ample participant data serve as a basis for present and future human microbiome research (Schiffer et al., 2019).

The 16S rRNA gene is highly conserved and ubiquitous among bacterial species (Jay & Inskeep, 2015; Ritari et al., 2015; K. Thomas-White et al., 2016), which is why it is routinely used for bacterial taxa identification (Tran et al., 2017). Each species of bacteria has roughly 5-10 copies of the 16S rRNA gene, although this number is highly variable among different

bacterial taxa (Louca et al., 2018; Větrovský & Baldrian, 2013). The interrogation of bacterial species using 16S was first proposed by Carl Woese and George Fox in 1977, and since then, classifying 16S genes has been a microbiology research standard for decades (Mizrahi-Man et al., 2013). This evolutionary marker contributed to the unveiling of 2 domains of life (Bacteria and Archaea), which catapulted taxonomy and phylogeny studies. The entire sequence is roughly 1,500-1,600 base pairs (bp). The gene consists of highly conserved regions and 9 hypervariable regions (Figure 1) that are divergent between bacterial species (Chakravorty et al., 2007). The first 500 bp, which includes 3 variable regions (V1, V2, and V3), can distinguish between many bacterial taxa to the genus and species level (Chakravorty et al., 2007). Targeting variable regions is ideal for high-throughput, short-read sequencing given their length, making taxonomic profiling of complex bacterial communities like those that live in the human body both logistically and economically feasible. Although this gene does not provide any functional information, it remains a powerful tool to identify bacterial taxa.

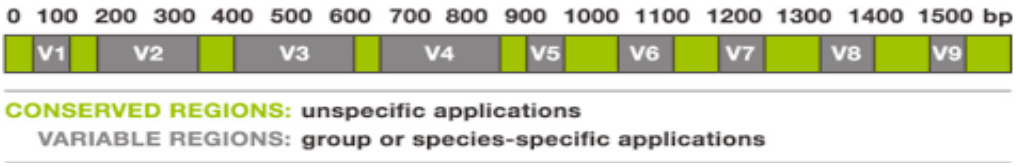

Figure 1. Conserved and variable regions of the 16S rRNA gene (Fasesan et al., 2020).

What allows for taxonomic identification of bacteria are public 16S rRNA sequence databases (Benson et al., 2010). One of the most widely used and highly curated sequence databases is GenBank, which is maintained by the National Center for Biotechnology Information (NCBI). Recent estimates state that GenBank contains 15.3 trillion base pairs and over 2.5 billion nucleotide sequences for 504,000 species (Sayers et al., 2021). The GenBank

database houses 16S rRNA sequences as well as other sequences. As of September 22nd 2022, NCBI's 16S sequence database contained over 22,000 sequences from bacterial and archaeal samples. Other 16S rRNA gene sequence databases routinely used in microbiome studies include the curated databases SILVA (Quast et al., 2013) and Greengenes (McDonald et al., 2012), although the latter is no longer maintained. Our knowledge of new bacterial strains and taxa is constantly expanding due to the addition of new16S rRNA sequences daily (Park & Won, 2018).

The initial goal of human microbiome studies was to define the composition of bacterial taxa (Dixit et al., 2021). While targeting the 16S rRNA gene does not provide insight into what the microbes are doing (their functional potential), it does capture the phylogenetic diversity (Gu et al., 2016). 16S rRNA gene surveys have been used, not only in the HMP, but also in studies of different organs and health statuses. For instance, DiGuilio *et al.* performed a detailed 16S rRNA gene survey of the vagina, distal gut, saliva, and tooth/gum microbiome of 40 women during pregnancy. The results of this study found the microbiota composition remained constant over gestational time, with few disturbances (see results in DiGiulio et al., 2015). Thanks to high-throughput sequencing technologies, scientists can efficiently capture the phylogenetic diversity of bacterial organisms in any given ecological niche.

Bacteria of the Urinary Tract

The bladder was originally omitted from the early HMP, which focused on microbes in the healthy human body. This was because the urinary tract of healthy individuals was believed to be sterile. The standard method for detecting microbes in urine was developed in the 1950s and is optimized to detect known uropathogens that cause acute urinary tract infections (UTIs), namely *E. coli* (Corbett et al., 2017). UTIs are not the only symptom/disease of the urinary tract. There are a number of factors that influence bladder function, which include diet (Lohsiriwat et al., 2011), social influences (Boyt, 2005), age, and gender. An unhealthy bladder is characterized by one or symptoms or diseases that can impair an individual's quality of life (Coyne et al., 2009). Common lower urinary tract symptoms (LUTS) include overactive bladder (OAB), bladder outlet obstruction (BOO), bladder pain syndrome/interstitial cystitis (BPS/IC), urinary tract infection (UTI), and bladder cancers. In 2008 approximately 1.9 billion individuals worldwide experienced at least one LUTS (Irwin et al., 2011). Additionally, the incidence of LUTS is more prevalent in women (13.9%) than men (8.5%) (Maserejian et al., 2013). Given the high prevalence of bladder conditions and diseases among individuals globally, it is important to promote optimal bladder health.

Urine in the bladder was often poorly described based upon the lack of identifiable microbes through traditional urine cultures. However, this assumption was dismissed as scientists first identified bacterial DNA via high-throughput sequencing of urine samples (Fouts et al., 2012; Siddiqui et al., 2011; Wolfe et al., 2012) and subsequently living bacterial species (Hilt et al., 2014; Khasriya et al., 2013). Bacteria from culture negative urine samples were isolated by performing an enhanced culture method called the Enhanced Quantitative Urine

Culture (EQUC) protocol (Hilt et al., 2014). Furthermore, most of the bacterial organisms identified through high-throughput 16S rRNA gene surveys could be grown and cultured in the lab (Hilt et al., 2014; Pearce et al., 2014). Although species of bacteria are currently detectable in urine samples, the urinary microbiota have not been as extensively studied compared to the study of densely populated microbial niches such as the human gut. Because it was excluded from early HMP efforts, studies of the urinary microbiome are years behind more comprehensively studied microbiomes in humans (Thomas-White et al 2016). Nonetheless, scientists are continuing to study organisms present in the bladder to demonstrate that this niche contains a diverse array of microbial species.

The term ‘urobiome’ refers to the characterization of microbes and their genomes in the human urinary tract (for a review, see (Brubaker et al., 2021)). These microbes include bacteria and other microbes such as viruses and fungi. While the greatest knowledge of the urobiome concerns is the bacterial species, much less is known about the viral or fungal species. Additionally, the metabolic processes of microbes within a microbiome are important for understanding the regulatory functions that are necessary for the organisms themselves and the host (Koeth et al., 2014). These metabolic processes have not been extensively studied in the urobiome yet, largely because the past decade has been focused on simply determining who is there, a critical first step in microbiome research. There are several urobiome studies that have focused on characterizing the bacterial composition in healthy and symptomatic individuals. This work has relied on conducting 16S surveys of bacteria in the bladder. These surveys are often taken from adult females, as urinary conditions and infections disproportionately affect more women than men (Irwin et al., 2006; Magliano et al., 2012; Wu et al., 2015). With regards to the

male urobiome, there are investigations that characterize the urobiome in healthy men and men with a sexually transmitted infection (STI) (Dong et al., 2011; Nelson et al., 2010). Studies of the female urobiome are more comprehensive with regards to what species reside in health and disease. Studies of the urobiome in healthy women tend to reveal communities that are relatively low in diversity, which is indicative of being asymptomatic (K. Thomas-White et al., 2018a).

Studies of the urobiome in healthy women continue to capture a wide range of bacterial taxa. Much of this knowledge stems from 16S surveys and advanced culturing of urine. Several 16S studies have identified taxa known to colonize the urinary tract, e.g., *Actinotignum, Aerococcus, Corynebacterium, Escherichia, Gardnerella, Klebsiella, Lactobacillus, Staphylococcus,* and *Streptococcus* (S. L. Chen et al., 2006; Nelson et al., 2010; Pearce et al., 2014; T. K. Price, Wolff, et al., 2020; Siddiqui et al., 2011; K. Thomas-White et al., 2016; K. J. Thomas-White et al., 2018). Moreover, culture-based techniques have allowed for the identification of fastidious organisms in the urobiome. Figure 2 displays an example of the bacterial diversity in the female urobiome (Pearce et al., 2015). In this study, live bacteria were isolated using the EQUC method and 16S surveys were conducted in women with and without urgency urinary incontinence (UUI). This study found that many of the taxa identified via the 16S survey were found when the urine sample was cultured under the EQUC conditions. It also highlights differences in the urobiome taxa between healthy women and women with UUI.

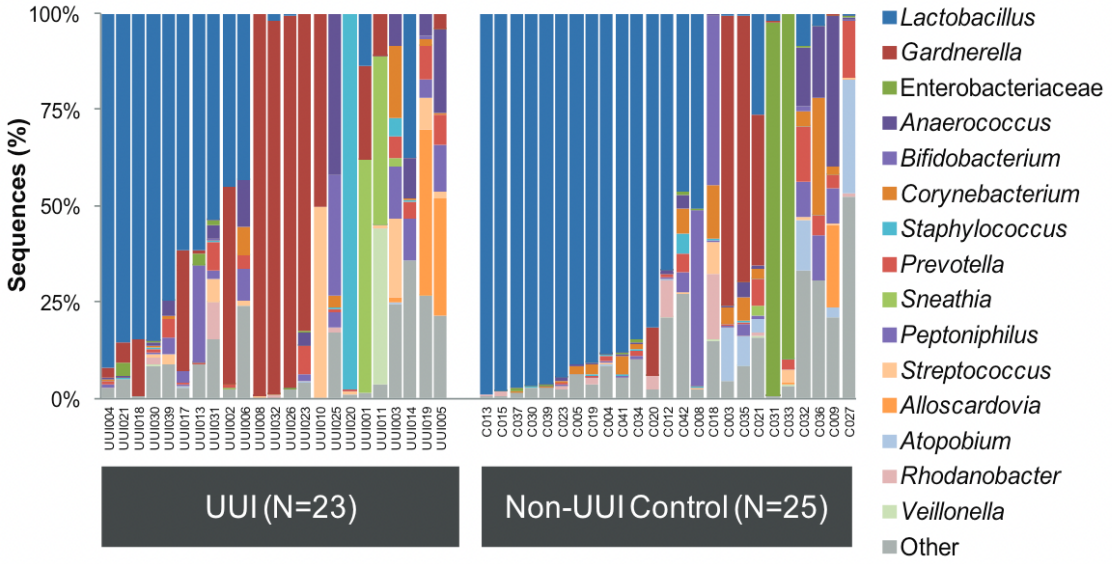

Figure 2. Urobiome survey of the female urobiome based on 16S sequencing of the V4 region. Each bar represents participant samples (UUI and non-UUI). 15 abundant genera were identified (Pearce et al., 2015).

Urobiome studies typically capture species diversity during one period of time in an individual that is either healthy or symptomatic. *Lactobacillus* and *Gardnerella* species often dominate a healthy female urobiome. This is illustrated in Figure 2 where *Lactobacillus* is shown in blue and *Gardnerella* in maroon. Pathogenic bacteria characteristic of urinary tract infections (UTIs) include *Escherichia coli, Klebsiella pneumoniae, Proteus mirabilis, Pseudomonas aeruginosa*, and *Staphylococcus aureus*. (S. L. Chen et al., 2006; Kammili et al., 2020; Pearce et al., 2015; K. J. Thomas-White et al., 2018). A study by Price *et al.* investigated how healthy women and women with urinary incontinence disorders differ in microbial diversity and abundance, with a more diverse array of bacteria being detected with urinary incontinence (T. K. Price, Lin, et al., 2020). Yet, the microbiota of the urinary tract changes over time, and it varies greatly between people. Health is a major factor in determining bacterial composition of the urobiome. Another study focused on the longitudinal stability of the urobiome in adult females

experiencing LUTS. This demonstrated that over a 3 month period, lower urinary tract microbiomes captured via voided urine samples varied in composition and diversity due to several factors including intercourse and menstruation (T. K. Price, Wolff, et al., 2020). Additional studies involving urobiome stability and variability are needed to comprehensibly describe community dynamics as well as interactions between members of this community.

While advanced culture identification techniques are useful in recognizing bacterial species, they do not capture every species present in the urobiome. Much of our understanding of bacteria – be it a resident of the human body or any ecological niche – is limited to those that can be cultured in the lab due to several factors such as temperature, media requirements, and oxygen exposure (Zingue et al., 2018). In fact, many of the bladder organisms identified by 16S rRNA surveys are unable to grow under standard laboratory conditions (Wolfe et al., 2012). For instance, fastidious bacteria can bypass standard urine cultures (Sathiananthamoorthy et al., 2019; Siddiqui et al., 2011). Furthermore, the urobiome houses a unique environment for bacteria; it has low oxygen levels and low nutrient availability, which make it difficult for species to grow in conditions other than the urinary tract (Shannon et al., 2019). Despite limitations in bacterial culturing techniques, culture protocols are continually improving in the urobiome in order to characterize this unique microbiome (Coorevits et al., 2017; Hilt et al., 2014; T. K. Price, Dune, et al., 2016).

As proven by numerous studies of human microbiomes, bacteria do not exist in isolation. They are often members of a complex community. To illustrate this, a study by Zhang *et al.* revealed negative inter-phylum bacterial interactions that shape soybean microbiome community dynamics and function. For instance, these interactions significantly reduce the populations of

nitrogen-fixing bacteria, which in turn decreases energy investment in nitrogen fixation (Camenzind et al., 2018; Zhang et al., 2021). Bacteria are often isolated in different conditions for scientific research purposes or to diagnose a bacterial infection, but they spend the majority if not all their time with other species. Based upon previous knowledge of bacterial communities in other human body sites (e.g., gut, skin, vagina) and from previous knowledge of bladder bacterial communities (K. Thomas-White et al., 2018b), we hypothesize that bacteria within the urobiome interact with each other.

Bacterial Hitchhiking

There are several possible ways in which bacteria form associations with other species and how they navigate densely populated environments. A particularly intriguing observation of how bacteria form communities is through physical association. This physical association, known as "hitchhiking," has been observed in both prokaryotic cells and eukaryotic cells (Muok & Briegel, 2021). This behavior involves non-motile and motile species of bacteria in which non-motile bacteria attach to bacterial cells of motile species in order to navigate their environment. This phenomenon has been observed in several instances such as between different species in controlled laboratory environments (Samad et al., 2017) and in the oral human microbiome (Shrivastava et al., 2018).

A similar phenomenon of bacterial hitchhiking has been observed in soil isolates, where researchers modeled how soil microbial communities interact. They isolated or co-isolated bacterial species found in soil that likely interact under natural conditions and named this model microbiome THOR (the hitchhikers of the rhizosphere) (Lozano et al., 2019). They selected bacterial species that tend to carry hitchhikers (*Bacillus cereus, Flavobacterium johnsoniae* and

Pseudomonas koreensis) and grew them either in isolation or with other bacteria in the rhizosphere to see how the colonies interacted. THOR members interacted in several ways that are common in microbial communities. For example, *B. cereus* increases *F. johnsoniae* growth through nutritional enhancement and protects it from growth inhibition by *P. koreensis*, illustrating how pairwise interactions can be modulated by other members of the community. Growth interference and enhancement in communities reinforce the importance of microbial community modulation to capture the impact of multi-organism interactions. It is through this prior study of soil microbes that the term "bacterial hitchhiking" was coined, referring to a physical, rather than a genetic, association between cells.

Motivation for this Thesis

While bacterial hitchhiking has been observed within the rhizosphere, it has yet to be studied in human microbiotas, including the community within the urinary tract. Although the urobiome has now been taxonomically profiled, the interactions and effects of these bacteria with/on each other has yet to be fully investigated. Here I present a study focused on characterizing bacterial hitchhikers in samples isolated from the female bladder. Chapter 2 describes the methods implemented in this study. Chapter 3 reports my results from both experimental and computational work as well as a discussion of their implications on urobiome knowledge. Lastly, Chapter 4 provides concluding remarks and areas for further investigation.

CHAPTER TWO

METHODS

Patient Recruitment

Patient sampling for this study was approved by Institutional Review Boards at either Loyola University Chicago or the University of California, San Diego. Participants gave verbal and written consent for chart abstraction and urine collection with analysis for research purposes. In total, samples were selected from 60 participants, all recruited as part of separate studies (Hilt et al., 2014; Pearce et al., 2014, 2015; T. K. Price, Mehrtash, et al., 2016; K. Thomas-White et al., 2016, 2018a).

Urine Collection and EQUC Bacterial Culturing

Urine was collected aseptically via transurethral catheter and was placed in BD Vacutainer Plus C&S preservative tubes for culturing. This technique bypasses the vulva, vagina, and urethra, resulting in samples from the bladder niche. All samples underwent EQUC as part of the aforementioned studies (Hilt et al., 2014; Pearce et al., 2014, 2015; T. K. Price, Mehrtash, et al., 2016; K. Thomas-White et al., 2018a; K. J. Thomas-White et al., 2016). EQUC was performed in the Wolfe lab as described previously (Hilt et al., 2014). Briefly, 100 µL of urine was grown under five conditions with BD BBL® prepared plated media: (1) Blood Agar Plates (BAP) in CO_2 for 48 h, (2) chocolate agar (CHOC) in CO_2 for 48 h, (3) colistin and nalidixic acid (CNA) agar in CO_2 for 48 h, (4) CDC aerobic blood agar (CDC) in an anaerobic jar for 48 h, and (5) BAP in aerobic conditions (BD GasPak Anaerobe Sachets) for 48 h. The detection level

was 10 colony forming units (CFU) per mL, represented by 1 colony of growth on any of the plates.

Plates were inspected visually, and each unique colony morphology was sub-cultured on a plate, using the same media as the plate from which it was originally isolated. This plate was divided into 6 wedges, with morphologically identical colonies streaked on each wedge. This plate was then incubated for 24 h under the atmospheric and temperature conditions used for the media. Plates were inspected visually a second time. In the case that each wedge had colonies with the same morphology, a toothpick was used to pick a colony and perform Matrix-assisted laser desorption/ionization-time of flight (MALDI-TOF) mass spectrophotometry. The MALDI Biotyper 3.0 software program (Bruker Daltonics, Billerica, MA) was used to identify bacterial strain species (see (Hilt et al., 2014) for specific protocol). If the colony morphologies were not the same across the 6 wedges, each morphology was re-streaked until each wedge had identical morphologies. Wedges were then scraped and stored in glycerol at -80°C at the Wolfe Lab.

Each frozen isolate was streaked onto either anaerobic blood agar (ANA), BAP, CHOC, CNA, or tryptone soy agar (TSA), depending upon the genus identified by MALDI-TOF. Each plate was incubated for 48 h in 5% CO_2 at 35°C and then transferred to the Putonti lab. The plates were then scraped and added to 1 mL of liquid media. The media was selected based upon the MALDI-TOF predicted genus and includes: Lysogeny broth (LB), Actinomyces (Sigma-Aldrich), Brain Heart Infusion (BHI) (BD) + 1% Tween 80 (BHI+Tween), De Man, Rogosa, and Sharpe (MRS) (Millipore) + 1% Tween 80 (MRS+Tween), New York City III (NYC III), Tryptic Soy broth (TSB) +5% sheep blood, or TSBYE (TSB+0.5% w/v yeast extract). After samples grew for 2 days at 35°C with 5% CO_2, bacterial glycerol stocks were created using 1 mL

liquid culture and 1 mL 50% (v/v) glycerol. Stocks were then frozen at -80°C until further processing.

Bacterial strains were streaked from freezer stock onto 6 different types of 1.7% agar plates (LB, Actinomyces, TSB, NYC III, BHI+Tween, MRS+Tween) and incubated for 48 h in 5% CO_2 at 37°C. These 6 media were chosen as they represent the diversity of media that can support most of the taxa that have been identified in the urinary microbiome (K. Thomas-White et al., 2018b). After incubation, colony morphologies of plates were examined under a light microscope. Morphologically distinct colonies were identified, and a single colony was picked from each plate. Colonies were grown in 1 mL of the liquid media of the plate from which it was derived (LB, Actinomyces, TSB, NYC III, BHI+Tween, MRS+Tween). This liquid culture was then incubated for 48 h in 5% CO_2 at 37°C. For plates that did not have any colonies after 48 h, the freezer stock was streaked again and incubated for another 48 h.

DNA Extraction and 16S PCR

DNA was extracted from the liquid culture of the colony. First, the liquid culture was centrifuged to produce bacterial pellets. Supernatant was removed and DNA was extracted from the bacterial pellets using the Qiagen DNeasy UltraClean Microbial Kit following the manufacturer's protocol. DNA concentration was quantified using the Qubit fluorometer. The 16S rRNA gene sequence was amplified using the 63f and 1387r primers (Marchesi et al., 1998). 25 µL reactions were performed with 5 µL of bacterial DNA, 0.5 µL of 63f primer, 0.5 µL of 1387r primer (both at 10mM concentration),12.5 µL of GoTaq Master Mix (Promega), and 6.5 µL of NFH_20 (Nuclease Free water). The following thermocycler conditions were used: Initial denaturing at 94°C for 5 m; 30 cycles of 90°C for 1 m, 55°C for 1 m, and 72°C for 1 m 30 s. The

final extension step occurred at 72°C for 5 m. Following amplification, 5 µL of PCR reaction underwent agarose gel electrophoresis. Bands were confirmed at around 1,325bp using the Axygen 100bp DNA Ladder. PCR products were purified from the reaction mixture using the EZNA Cycle Pure Kit, following the manufacturer's protocol, and quantified using the Qubit fluorometer. PCR was repeated for products without quantifiable DNA. 16S rRNA gene amplicons were sequenced via Sanger sequencing by Genewiz (New Brunswick, NJ, United states) using one 16S primer per sequencing reaction thus producing 2x coverage of the amplified sequence.

16S Sequence Identification

Sanger sequenced amplicons were manually trimmed and paired in Geneious v. 2021.0.3 (Biomatters, Ltd., Auckland, New Zealand). Pairwise alignments were performed in Geneious to derive the consensus sequence representative of the amplified region of the16S rRNA gene sequence. The consensus sequence was then queried against the NCBI 16S ribosomal RNA database via megablast to identify the bacterial species. All 16S sequences were organized by sample ID to identify samples in which the same bacterial taxon was isolated on different media plates and/or presumed different colony morphologies. Each pair was manually inspected using Geneious v. 2021.0.3 (Biomatters, Ltd., Auckland, New Zealand) to assess quality. Quality sequences contained few or no ambiguous bases (Ns). Sequences were filtered out if they contained 30 or more N's. and/or gaps. To identify unique 16S sequences, all quality sequences for an individual sample ID were aligned to each other using MAFFT v. 7.490 (Katoh & Standley, 2013) through Geneious Prime. In the event that two or more identical sequences were generated for isolates from the same sample ID (signifying that the same species was isolated on

2 or more medias and/or 2 or more colonies that were perceived to be morphologically distinct), only one was kept for further analysis.

Field Guide to the Urobiome Database Creation

A 16S rRNA gene sequence database was created by combining unique 16S rRNA gene sequences from our sample collection and all 16S sequences from Loyola's publicly available urobiome genome sequences. All rna_genomic files for genome assemblies in the BioProject PRJNA316969 were downloaded on April 18th, 2022. This BioProject includes all urobiome genome assemblies from the Wolfe lab's collection. Complete 16S rRNA gene sequences were extracted from the genomes. For construction of this database, we first compared all of the 16S sequences from the publicly available genomes and the 16S rRNA gene sequences from our sequenced isolates. Multiple sequence alignment was performed using MAFFT v. 7.490 through Geneious Prime. Duplicate sequences were removed as were sequences that were subsequences of a longer representative sequence. The final set of sequences were aligned, again using MAFFT, and a phylogenetic tree was derived for these sequences using FastTree v. 2.1.11 (M. N. Price et al., 2010). The tree was visualized using iTOL v. 6 (Letunic & Bork, 2021).

Co-Occurrence in Samples

A matrix was constructed using Python v. 3.8.1 to calculate the number of times each species (per BLAST 16S rRNA gene sequence identification) was identified in the samples examined as well as the number of times a pair of species was observed in the same sample. For each pair of co-isolated species, a 2-by-2 contingency table was created. This contingency table includes the number of times that the two species were isolated from the same sample, the total number that each species was isolated in the samples tested, and the total number of strains

isolated. Fisher's exact or Chi-square association tests were performed in R v 4.1.2 for each pair. Fisher's exact tests were applied on contingency tables where one or more values was less than 5. Otherwise, Chi-square association tests were performed**.** Lastly, multiple testing correction (Bonferroni adjustment) was applied for each pair of species to control the family-wise error rate.

Comparing Fitness of Strains Grown in Isolation and with Frequent Hitchhikers

Samples containing *E. coli* or *K. pneumoniae* and their hitchhikers were streaked on agar plates of the media that they were isolated from (LB, Actinomyces, TSB, NYC III, BHI+Tween, MRS+Tween). Plates were incubated for 24 h in 5% CO_2 at 37°C. After incubation, colony morphologies were examined under a light microscope. Once colony morphologies were confirmed to be uniform across the plate, a single colony was selected and grown in 1 mL liquid LB for 24 h in 5% CO_2 at 37°C. Freezer stocks were created by adding 700 µL of glycerol to the culture which were then stored at -80°C.

Colony counts were used to compare bacterial growth of strains in isolation and growth in co-culture with another strain. Isolate freezer stocks were streaked on LB agar plates and incubated for 24 h in 5% CO_2 at 37°C. After confirming uniform colony morphologies on plates, a single colony was grown in 10 mL of liquid LB and incubated for 24 h in 5% CO_2 at 37°C. The Optical Density (OD) for each culture was measured at 600 nm using a spectrophotometer. Cultures were diluted using LB such that the same OD was used for both strains in the co-culture. Control lines (strains grown in isolation) were also grown from this diluted sample. The dilution equation $M_1V_1 = M_2V_2$ was utilized to calculate V_2 (in mL or µL) to dilute each sample based on their OD measurement. Cultures of strains in isolation or in pairs were incubated for 24

h in 5% CO_2 at 37°C. Each culture was conducted with three replicates. Next, a serial dilution was carried out for each replicate, diluting samples by a factor of 10. Dilutions 10^{-5}, 10^{-6}, 10^{-7}, and 10^{-8} were plated on LB agar plates and spread using 3-4 sterile glass beads. Plates were incubated for 24 h in 5% CO_2 at 37°C. Following incubation, colony-forming units (CFU) were counted for each plate. For all plates in which CFUs were distinct and thus able to be counted, the CFU.Ai application **(**Version 1.4; Medixgraph Inc.**)** was used. This application takes a picture of the plate, automates CFU identification, and counts all CFUs in the picture. To verify this number, plates and pictures were visually inspected, and colonies were manually counted. Sample names with their respective dilutions and CFU counts were recorded. To compare all CFU's in each group, all CFU counts were converted to 10^{-7}.

Relative CFU counts were analyzed in the R programming language (R version 4.1.2), where the following analyses were performed. A Shapiro-Wilks normality test was done on the dataset to check for normality. A Multiple Linear Regression model was fit for species grown in isolation and in pairs. This type of statistical model allows for the examination of relationships to check if they have a significant contribution to the model, thus verifying if growing species together is better than, worse than, or has no significant difference with growing them individually.

Genome Assembly, Annotation, and Metabolic Pathway Prediction

Bacterial genomes from participants that have multiple bacterial isolates sequenced were assembled using SPAdes v 3.15.2 (Bankevich et al., 2012) via the Pathosystems Resource Integration Center (PATRIC) v. 3.6.12 (Davis et al., 2020). Genome assemblies were also annotated using PATRIC. The features.txt file produced from each annotation were parsed via

Python v. 3.8.5 to extract the enzyme codes (EC). Following this, a python wrapper was created (https://github.com/sang-15/Mixing-Metabolomes) to generate metabolic maps using KEGG Mapper-Color (Kanehisa, 2019; Kanehisa et al., 2021; Kanehisa & Goto, 2000); one per patient per significant metabolic pathway.

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CHAPTER THREE

RESULTS AND DISCUSSION

Culturing Urinary Isolates

We sought out to culture urinary isolates from the female bladder to detect hitchhiking bacteria in urinary species by a means of characterizing distinct colonies followed by 16S sequencing. We selected 203 urinary isolates. These isolates had previously been purified as part of the EQUC procedure (see Chapter 2 for details). Each urinary isolate was streaked on plates of 6 different medias: LB, TSB, Actinomyces, NYC III, BHI+Tween, MRS+Tween. Of the 203 isolates plated, 155 had growth on at least one media. Figures 3-6 show four different isolates streaked on each of the six plates. For each plate, colony morphologies were examined.

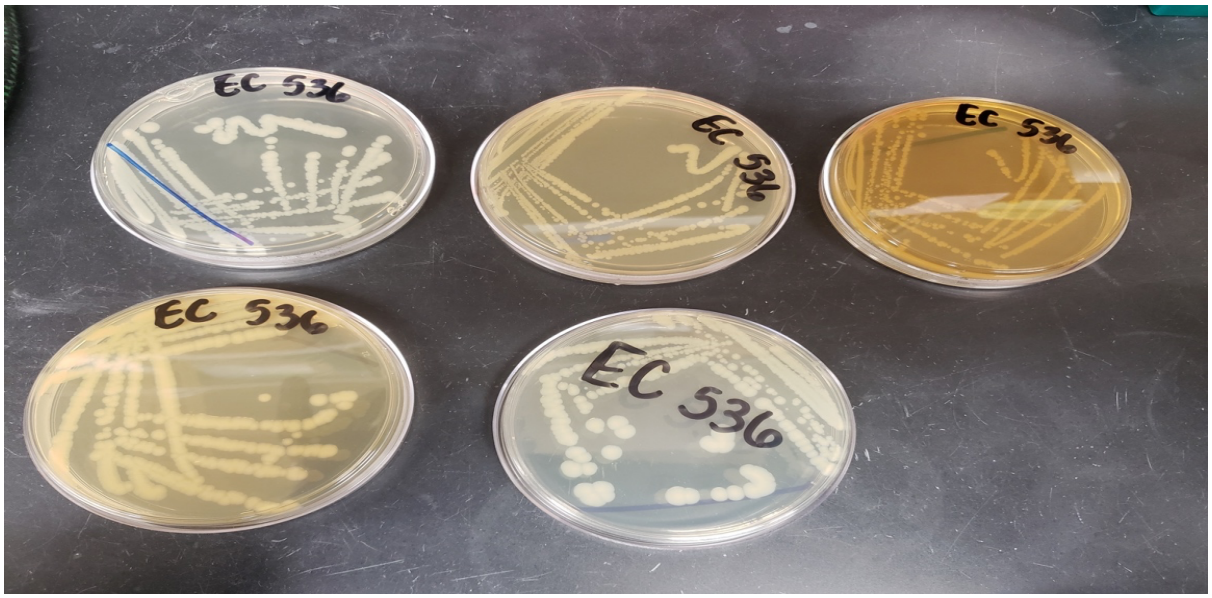

Figure 3. Sample Isolate *E. coli* (EC) 536 colonies streak results. Growth was observed on LB (upper left), TSB (upper center), Actinomyces (upper right), NYCIII (lower left), and BHI+Tween (lower center) plates.

Figure 4. Isolate EC648 colonies. Growth was observed on plates of all 6 medias: LB (upper left), Actinomyces (upper center), TSB (upper right), NYCIII (lower left), BHI+Tween (lower center), and MRS+Tween (lower right).

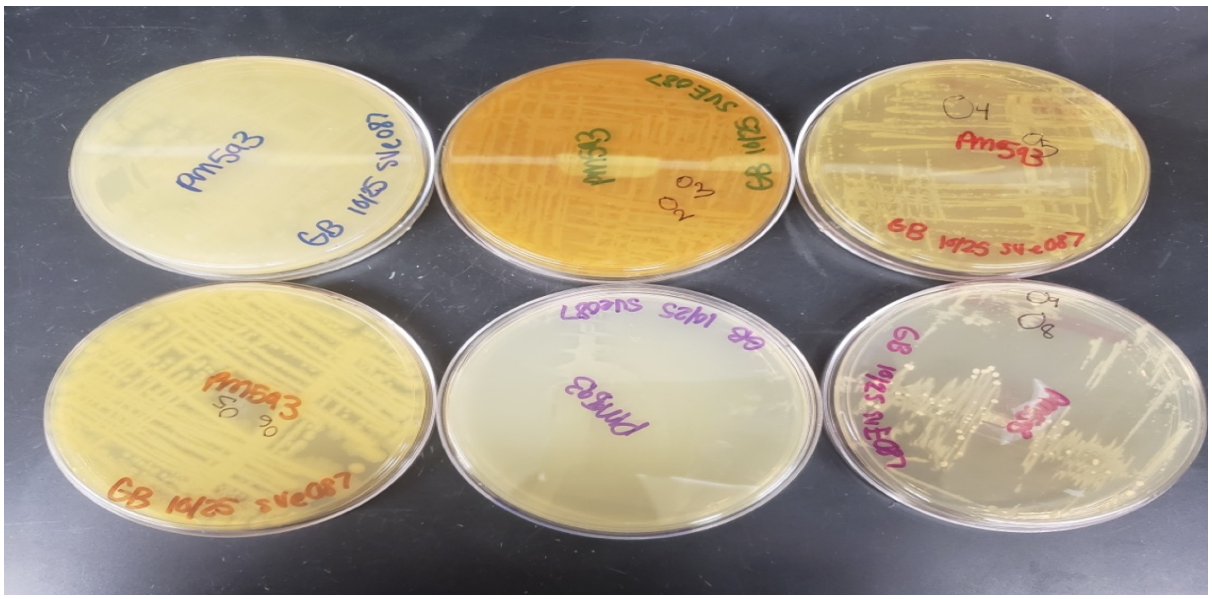

Figure 5. Isolate *P. mirabilis* (PM) 593 colonies. Growth was observed on plates of all 6 medias: LB (upper left), Actinomyces (upper center), TSB (upper right), NYCIII (lower left), BHI+Tween (lower center), and MRS+Tween (lower right).

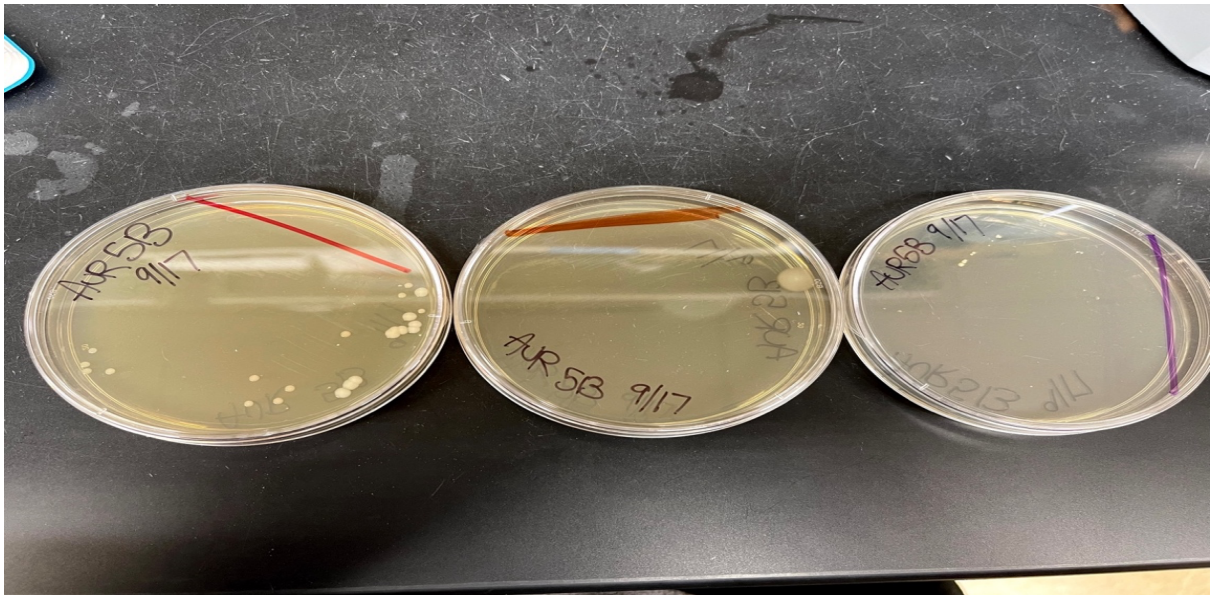

Figure 6. Isolate *A. urogenitalis* (AUR) 513 colonies. Growth was observed on TSB (left), NYCIII (center), and BHI+Tween (right) plates.

Several different scenarios were observed when culturing these isolates. First, there were plates that had uniform and similar colony morphologies regardless of what media they were streaked on, for instance the plates from sample *E. coli* (EC) 536 (Figure 3). Even though the colony morphologies between plates appeared identical, a single colony was picked from each plate to confirm their species via 16S rRNA gene sequencing. Second, we observed isolates in which the colony morphologies between plates looked different. An example is shown in Figure 4. Here, isolate EC648's colonies are not uniform across all 6 agar plates. The BHI+Tween plate colonies (lower center) are distinct from the colonies on the other 5 plates, as they are all small and yellow in color. Third, we observed instances in which more than one colony morphology was observed on the same plate. For example, EC648 colonies on NYC III had two different

morphologies; larger opaque colonies and smaller yellow colonies (Figure 4, lower left). An additional example is shown in Figure 5. Here, the LB and BHI + Tween plates each contain only one distinct morphology. However, the Actinomyces, TSB, NYC III, and MRS+Tween plates had more than one observed colony morphology, which are indicated by the markings on each plate. Given that distinct colonies were found on the 4 medias, we suspected that these are hitchhiking bacteria found in the presence of *P. mirabilis*. Fourth, we observed distinct colony morphologies on all plates with growth. As an example, Figure 6 shows the three plates with growth for *Actinomyces urogenitalis* (AUR) AUR513. The TSB plate (left) had a combination of white and light brown raised colonies. The NYC III plate (center) had just a single colony, which differed from those observed on the other plates. The small yellow colonies seen on the BHI+Tween plate (right) did not resemble the colonies of the other plates.

Identifying the Taxonomy of Isolates

To determine the species of the bacteria isolated from the samples, DNA was extracted from the 1,008 single colonies identified. The 16S rRNA gene was amplified and sequenced (see Chapter 2). As a result, 831 of these colonies had quality 16S rRNA gene sequences. Quality sequences were defined as having < 30 ambiguous nucleotides. Additionally, their sequencing chromatogram peaks are distinct, evenly spaced, and most importantly, there is only one peak per nucleotide. These sequences indicate that there is likely a single species sequenced.

On the other hand, 177 sequences were classified as low quality. These sequences had ≥ 30 ambiguous bases. Because of this, their sequence chromatograms were of poor quality (e.g., multiple peaks per nucleotide, overlapping peaks, poorly defined peaks). There are several possible causes for a low quality 16S rRNA gene sequence amplicon. Reasons include DNA or

primer concentration that is too high or too low, left and/or right primer not binding to the DNA, and/or contaminants such as extra primers, buffers, and salts. Thus, either the PCR reaction, PCR cleanup, or sequence reaction preparation were repeated. Despite this, similar results arose when viewing the sequencing results. Upon further inspection, we hypothesize that the morphologically distinct single colonies picked for these sequences were not pure, i.e., more than one species is present. We thus excluded these low quality sequences from further analysis.

While 83% of our sequences were of high quality, the low quality sequences hint at the complexities of bacterial relationships within our isolates. The main problem we encountered was the poor nucleotide identity in the 16S rRNA gene sequences. It is important to note that all DNA extractions that preceded PCR amplification had quantifiable DNA. Even with repeated PCR and sequence preparation, the issue remained. Given that each low quality sequence was produced from a single colony, we suspected that multiple species were present within that visible single colony. We hypothesize that bacterial hitchhikers are present. Separating species in these colonies may be impossible. While rounds of colony isolation and restreaking is the common practice for purifying bacterial species, it does not guarantee that an isolate is pure.

The 831 quality 16S sequences were derived from 150 of the samples grown. Based upon BLAST sequence searches, these sequences represented 67 different species and 31 genera. 78 of the 150 samples contained more than one species (determined by quality 16S sequences), signifying that the purified samples tested were not in fact pure. Because we found additional species isolated within these samples, these are notably considered as suspected hitchhiking bacteria. The remaining 72 samples only contained a single species. In 49 of these 72 pure samples, we isolated the species that was originally identified via MALDI-TOF. It is important

to note that in 96 of the 150 samples, we did isolate the MALDI-TOF species. Thus, many of the samples, including the ones harboring putative hitchhikers, did contain the species predicted (see Appendix A). In Table 1, we have listed the species that were most frequently identified in pure samples. For instance, only one *K. aerogenes*, *L. rhamnosus*, and *P. mirabilis* sample contained a hitchhiker; the majority of samples only contained the MALDI-TOF identified species.

Species	# pure samples matching the MALDI-TOF species	# samples predicted to be this species by MALDI-TOF
K. aerogenes	2	3
L. rhamnosus	2	3
E. faecalis	3	4
K. pneumoniae	7	10
P. mirabilis	8	9
E. coli	11	15

Table 1. Species most frequently identified in pure samples. Number of samples in which MALDI-TOF identification matched 16S rRNA gene sequence identification.

We next evaluated the 831 quality 16S rRNA gene sequences to identify unique 16S rRNA gene sequences. 120 of the 150 samples contained more than one 16S rRNA gene sequence, meaning that we isolated and sequenced more than one colony from the sample. These could be “duplicate” sequences. For instance, we produced 8 separate 16S rRNA gene sequences for *P. mirabilis* (PM) 267. However, all 8 sequences were identical thus signifying that the isolates from different plates and those thought to represent different colony morphologies were in fact the same species. The different 16S rRNA gene sequences for a given sample could also represent different 16S rRNA gene sequences. This could represent different copies of the 16S rRNA gene sequence within the same strain or different strains of the same species. For instance, for samples EC536 (Figure 3) and EC648 (Figure 4) 2 unique sequences were produced for each.

For both of these samples, both of the 16S rRNA gene sequences were identified via BLAST as *E. coli*. Alternatively, samples with more than one 16S rRNA gene sequence could represent more than one species. Sample AUR513 (Figure 6) included 3 different species per high quality 16S rRNA gene sequences. As previously mentioned, the TSB plate had two different observed colony morphologies. Sequencing, however, revealed that both were *Staphylococcus hominis.* The colony on NYCIII and BHI+Tween were identified as *Bacillus tropicus* and *Kocuria palustris*, respectively. None of the species identified were the species identified via MALDI-TOF. This was not a unique result. For example, three unique sequences were identified in sample 276. MALDI-TOF had identified the sample as *Lactobacillus iners*. Yet, *Enterococcus faecalis* and *Lactobacillus gasseri* were isolated; *L. iners* was not. In total, 162 unique 16S rRNA gene sequences were identified from our samples.

The most intriguing results from our plated samples came from plates that had different colony morphologies. As mentioned earlier, these plates were suspected to have hitchhiking bacteria. As with plates that had uniform colony morphologies, we confirmed each varied colony morphology by 16S sequencing. In the example shown in Figure 5, despite the different morphologies on four of the medias tested, every colony was identified as *P. mirabilis*. In fact, 7 of the 8 samples identified via MALDI-TOF as *P. mirabilis* were pure; only 1, sample 593, also contained *Shigella flexneri* (isolated from the MRS+Tween plate). This informs us that urobiome strains of *P. mirabilis* have a diverse array of colony phenotypes. *P. mirabilis*, a known uropathogen and primary cause of catheter-associated urinary tract infections (Jacobsen et al., 2008), is known to have variation in its colony morphology; it is capable of swarming as well as

persisting in a vegetative state (Rauprich et al., 1996). Differences in phenotype have been linked to *P. mirabilis*' quorum sensing system (Armbruster & Mobley, 2012).

By utilizing different medias here, we were able to separate different species on different medias. In the case of AUR513, three species were identified in this sample, suggesting that these species may be forming a type of dependence on one another under the conditions in which the original isolate was "purified" via the EQUC procedure. The same can be said for the samples in which the MALDI-TOF species was not recovered, but another species was. The media in which these potential hitchhikers were isolated may provide the resources necessary for the hitchhiker's colonization but not the MALDI-TOF identified species. Likewise, the EQUC media used to isolate and purify the sample may not have provided the resources necessary for the hitchhiker's colonization. Thus, expanding the medias used during the EQUC process may expand the species that can be identified and improve purification.

Determining Media that Support Urobiome Species

With the taxonomies for the individual colonies now identified, we could now ascertain what medias each species was able to be isolated from. Table 2 summarizes these results. A full list of the results can be found in Appendix B. Interestingly, there were 25 of 67 species that were isolated from all 6 media. These species include *Aerococcus urinae*, *Enterococcus faecalis*, *E. coli*, *P. mirabilis*, *Klebsiella species* (n=2), *Lactobacillus species* (n=3), *Staphylococcus species* (n=6), *Streptococcus species* (n=2). These results show the magnitude of urobiome species that can be isolated from a variety of medias.

Species	LB	Actinomyces	TSB	NYC III	BHI + Tween	MRS + Tween
Acinetobacter radioresistens	+					
Actinomyces neuii	+	+	+	+	+	
Actinotignum schaalii	+	+	+		+	
Aerococcus sanguinicola		+	+	+	+	
Aerococcus urinae	+	+	+	+	+	+
Bacillus aryabhattai				+		
Bacillus infantis	+					
Bacillus mobilis			+			
Bacillus nealsonii		+				
Bacillus subtilis				+		
Bacillus tropicus			+	+		
Brevibacterium frigoritolerans		+				
Citrobacter koseri		+	+	+		
Corynebacterium amycolatum	+		+	+	+	
Corynebacterium coyleae	+	+	+	+		
Corynebacterium aurimucosum					+	
Corynebacterium imitans	+		+		+	
Corynebacterium simulans	+	+	+			
Corynebacterium urealyticum		+		+	+	
Curtobacterium citreum			+			
Candida lustitaniae		+	+			
Dermabacter jinjuensis				+		
Dermacoccus nishinomiyaensis					+	

Enterobacter cloacae				+			
Enterococcus faecalis	+	+	+	+	+	+	
Escherichia coli	+	+	+	+	+	+	
Escherichia fergusonii		+	+				
Facklamina hominis	+			+	+		
Granulicatella adiacens	+		+				
Klebsiella aerogenes	+	+	+	+	+	+	
Klebsiella oxytoca	+						
Klebsiella pneumoniae	+	+	+	+	+	+	
Kocuria marina			+				
Kocuria palustris					+		
Lactobacillus crispatus				+	+	+	
Lactobacillus delbrueckii	+	+	+	+	+	+	
Lactobacillus gasseri	+	+	+	+	+	+	
Lactobacillus reuteri		+	+			+	
Lactobacillus rhamnosus	+	+	+	+	+	+	
Micrococcus aloeverae				+			
Micrococcus yunnanensis	+		+	+			
Morganella morganii	+	+	+	+	+		
Paenibacillus pabuli	+						
Paenibacillus taichungensis		+					
Paenibacillus taiwanensis				+			
Pantoea eucrina				+			

Proteus mirabilis	+	+	+	+	+	+
Pseudoclavibacter alba	+		+	+	+	
Pseudomonas aeruginosa			+	+	+	+
Pseudomonas oryzihabitans			+			
Rothia mucilaginosa		+	+	+	+	
Shigella boydii	+	+			+	+
Shigella flexneri	+	+				+
Sphingomonas aeria	+					
Staphylococcus aureus	+	+	+	+	+	+
Staphylococcus capitis	+	+	+	+	+	+
Staphylococcus condimenti			+			
Staphylococcus epidermidis	+	+	+	+	+	+
Staphylococcus haemolyticus	+	+	+	+	+	+
Staphylococcus hominis	+	+	+	+	+	+
Staphylococcus pasteuri			+		+	
Staphylococcus saccharolyticus			+			
Staphylococcus warneri	+	+	+	+	+	+
Streptococcus agalactiae	+	+	+	+	+	+
Streptococcus anginosus	+	+	+	+	+	+
Streptococcus oralis		+				
Streptococcus pasteurianus				+	+	

Table 2. Summary of the media in which each species identified was isolated from. Species were identified by 16S rRNA gene sequencing.

Creating a Database Representative of Urobiome Bacterial Species

All unique 16S sequences from our collection were combined with 16S sequences from Loyola University Chicago's urobiome genome collection (NCBI BioProject PRJNA316969). Combined, these sequences represent the diversity of species that have been isolated from the female urobiome to date. We refer to this sequence collection as a "Field Guide to the Urobiome." As previously mentioned, we identified 162 unique 16S rRNA gene sequences in our samples. Additionally, 495 unique 16S rRNA sequences were parsed from the 424 genomes downloaded from PRJNA316969. These 495 complete 16S rRNA gene sequences were added to our collection of 162 unique sequences. In total, 292 unique 16S rRNA sequences were identified. These 292 unique 16S rRNA sequences represent 123 species and 61 genera.

131 of the 292 unique sequences were not in our collection. These sequences either represented species that were not included in our samples or species variants that were not represented in our samples. There were also 35 species that were unique to our culture collection, meaning that a complete genome has not been generated for the species as part of the BioProject. Recent sequencing efforts in the Wolfe lab have produced genomes for some of these species, although they have yet to be added to the BioProject. Taking these unpublished species into consideration, we can definitively say that we have identified 23 species that have yet to be sequenced by Loyola urobiome researchers. These species include: *Acinetobacter radioresistens, Bacillus aryabhattai, Bacillus mobilis, Bacillus nealsonii, Bacillus subtilis, Bacillus tropicus, Brevibacterium frigoritolerans, Corynebacterium imitans, Curtobacterium citreum, Dermabacter jinjuensis, Dermacoccus nishinomiyaensis, Kocuria palustris, Micrococcus*

aloeverae, Paenibacillus pabuli, Paenibacillus taichungensis, Paenibacillus taiwanensis, Pantoea eucrina, Pseudoclavibacter alba, Pseudomonas oryzihabitans, Sphingomonas aeria, Staphylococcus condimenti, Staphylococcus saccharolyticus, and *Staphylococcus warneri*. Prior to our work here, only one species from the genus *Bacillus* had been isolated from the urobiome, *Bacillus infantis.* Here we have added 5 additional species.

It is important to note that ascertaining if new species were identified required taking into consideration that the naming of bacterial taxa is ever changing. For instance, *Lactobacillus rhamnosus* is now called *Lacticaseibacillus rhamnosus* (Petrova et al., 2021) and *Micrococcus yunnanensis* was reclassified in 2019 as *Micrococcus luteus* (Huang et al., 2019). Furthermore, some of the 16S rRNA gene sequences produced here were identical to the 16S rRNA gene sequences of sequenced urobiome isolates assigned to different genera. For instance, the 16S rRNA gene sequence of our isolate identified via blast analysis as *Enterobacter cloacae* is the same sequence as a 16S from one of the urobiome *Klebsiella pneumoniae* strains. Similar observations were made for the following species, e.g., *Streptococcus oralis* (identical to a urobiome *S. mitis* strain) and *Kocuria marina* (identical to *K. rhizophila* strain). Furthermore, there was some ambiguity in the species identified. For instance, there is noted variation between strains in the species *E. coli* (Parks et al., 2021). The 16S rRNA gene is not an ideal gene marker for distinguishing between these variants. Thus, some of our own 16S rRNA gene sequence amplicons from *E. coli* samples had greater sequence similarity to records deposited as *Escherichia fergusonii* or *Shigella flexneri*. Here, we decided to refer to all of these variants as *E. coli*.

All 16S sequences in our database were next aligned and a phylogenetic tree was derived. Figure 7 shows this tree. The most sequences within this tree are from the order *Lactobacillales* (n=93). The abundance of different sequences from this order indicates that we are frequently isolating species of this order. These species include *Aerococcus urinae*, *Lactobacillus gasseri*, *Lactobacillus jensenii, Streptococcus mitis,* and *Streptococcus anginosus*, among others. This outcome is particularly interesting, as we are able to isolate species that are known to be part of a healthy urobiome population (T. K. Price, Hilt, et al., 2020; K. Thomas-White et al., 2018b; Wolfe et al., 2012). Additionally, 60 sequences are from members of the order *Enterobacterales*. This order includes frequent uropathogens such as *Proteus mirabilis*, *Escherichia coli*, and *Klebsiella pneumoniae*. We were also regularly isolating known UTI causing species (Imirzalioglu et al., 2008; Jacobsen et al., 2008; Neugent et al., 2020; Sathiananthamoorthy et al., 2019). Overall, we found that the female urobiome houses a diverse array of bacteria. These findings indicate that there is vast array of bacteria (non-pathogenic and pathogenic) that can be isolated from urobiome samples.

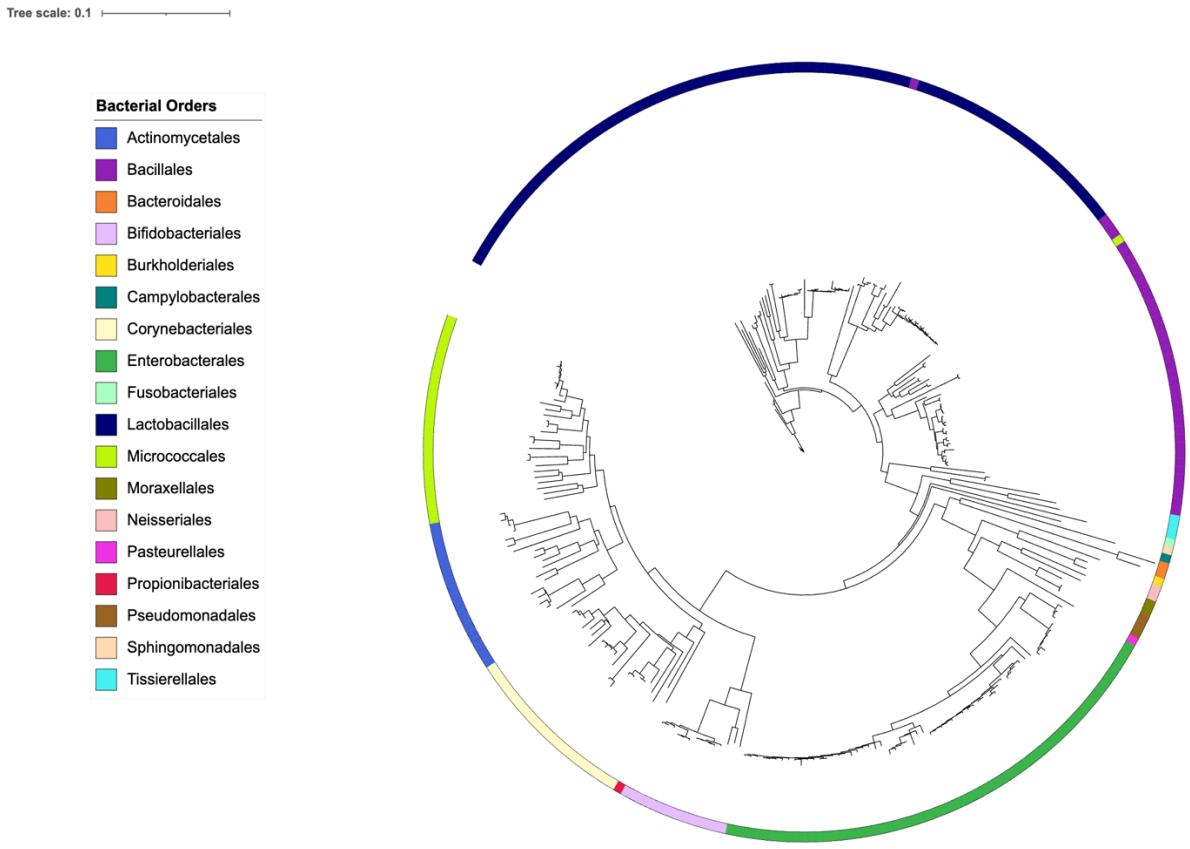

Figure 7. Diversity of bacteria included in the "Field Guide to the Urobiome" database. 18 bacterial orders were identified.

Making this 16S rRNA gene sequence database captures the species diversity found in the female urinary tract of both healthy and symptomatic individuals. Our samples come from both asymptomatic females as well as females with recurrent UTI (rUTI) and acute UTI symptoms. The 16S rRNA gene sequences retrieved from the BioProject also include isolates from females with UUI, OAB, and stress urinary incontinence (SUI) (K. Thomas-White et al., 2018b). The biggest significance of this resource is its utility to aid in identifying taxa for high-throughput 16S rRNA gene surveys of the urobiome. High-throughput 16S rRNA gene sequencing of urine samples identified that the bladder of "healthy" individuals was not sterile, and that bacterial DNA was present (Nelson et al., 2010; Siddiqui et al., 2011; Wolfe et al.,

2012). This method is routinely used for identifying bacterial species in the urinary tract (Karstens et al., 2016; Komesu et al., 2018; Pearce et al., 2014; T. K. Price, Hilt, et al., 2020; T. K. Price, Wolff, et al., 2020), often targeting the 16S V4 region (Hoffman et al., 2021). As these studies are aimed at identifying the taxa present within the sample, they necessitate 16S rRNA gene sequences representative of the diversity found within the microbiota of the urinary tract. Most databases of 16S rRNA gene sequences lack a representation of diversity in urobiome species (Brubaker et al., 2021). Thus, the sequences generated as part of this study could be used to aid in taxonomic profiling of 16S rRNA gene data sets from urine.

Identifying Potential Hitchhikers

Returning to our samples, we next investigated the frequency in which a given species was co-isolated with another species. Based upon the 16S rRNA gene sequence identification, the number of times each species was detected and the number of times each species was co-isolated with another species (from the same sample) was recorded. From the 150 samples tested, 25 different pairs of species were co-isolated. As previously mentioned, 78 of the 150 samples were found to contain two or more species. Thus, many of the same pairs of species were retrieved.

Table 3 reports the number of samples in which each species pair was observed as well as the raw and adjusted p-values. As shown in the 3rd column, only 2 pairs are statistically significant: S*taphylococcus condimenti* & *S. hominis* and *Micrococcus yunnanensis* & *S. hominis*. Although these pairs were significant, we did not observe them often. *Staphylococcus condimenti* was found only 2 times, *Micrococcus yunnanensis* was found 5 times, and *Staphylococcus hominis* was found 12 times. Given the relative number of times each of these

species was found among all samples, their co-occurrence was more frequent than expected by chance. *S. condimenti* and *S. hominis* were found together twice. In fact, *S. condimenti* was only found with *S. hominis*. *Micrococcus yunnanensis* and *S. hominis* were found together three times. It is to be noted that when performing multiple testing correction on the 25 hypothesis tests (i.e. species pairs), none of the pairs are significant. As Table 3 reports, the most frequently co-isolated species were *K. pneumoniae* and *E. coli* (n=8). However, their co-occurrence was not statistically significant as we had 31 samples with *K. pneumoniae* and 29 with *E. coli*. In fact, these were the two most frequently isolated species from our samples. While these two species were identified with other species, these were two species that were (per MALDI-TOF identifications) most represented in the 150 samples. It is thus not too surprising that they were most frequently isolated.

Bacterial Species	# Occurrences	p-value	adjusted p-value Bonferroni
L. gasseri & S. hominis	2	0.2785	1
S. capitis & E. faecalis	3	0.4223	1
E. coli & K. pneumoniae	8	0.4417	1
L. gasseri & S. epidermidis	3	0.7102	1
K. pneumoniae & S. epidermidis	3	0.1986	1
E. coli & P. mirabilis	2	1	1
K. pneumoniae & P. mirabilis	2	0.7354	1
S. hominis & S. capitis	2	0.2785	1

S. epidermidis & S. capitis	2	1	1
E. faecalis & S. capitis	3	0.4223	1
E. faecalis & L. gasseri	2	1	1
S. epidermidis & S. anginosus	2	0.2339	1
S. boydii & E. faecalis	2	0.06149	1
S. haemolyticus & E. faecalis	3	0.1685	1
S. aureus & E. faecalis	3	0.1421	1
E. coli & E. faecalis	3	0.5693	1
K. pneumoniae & E. faecalis	3	0.4118	1
S. epidermidis & E. faecalis	5	0.9043	1
S. condimenti & S. hominis	2	0.005906*	0.14765
M. yunnanensis & S. hominis	3	0.003632*	0.0908
S. epidermidis & S. hominis	3	0.6979	1
E. coli & S. aureus	2	0.6854	1
E. coli & S. boydii	2	0.09574	1
K. pneumoniae & A. schaalii	2	0.1849	1
E. coli & S. agalactiae	2	0.2475	1

Table 3. Assessment of the co-isolated species pairs observed in the samples tested. * indicates that the raw p-values < 0.05 and are significant. In the corrected p-values, no pair is significant.

Assessing Fitness of Frequently Co-Occurring Bacterial Species

Although the *E. coli* and *K. pneumoniae* pair was not statistically significant (Table 3), it was the most frequently observed species pairing. We thus decided to further explore these two species and the effects of hitchhiking on them. We chose four of the 8 samples in which both *E. coli* and *K. pneumoniae* were both found in the sample. These include samples 359, 541, 542, and 564. We also selected two samples for each species in which the original sample was found to be pure (i.e., no hitchhikers were detected); 510 and 511 were pure isolates of *K. pneumoniae* and 527 and 529 were pure isolates of *E. coli*. One strain of *P. mirabilis* was also tested. This strain was also isolated from sample 564, which contained *E. coli* and *K. pneumoniae*. Based upon our analysis of co-isolated species, we observed that both *E. coli* and *K. pneumoniae* were also commonly co-isolated with *E. faecalis* and *S. epidermidis* (Table 3). *E. faecalis* was co-isolated with *E. coli* three times and with *K. pneumoniae* three times. *S. epidermidis* was co-isolated with *E. coli* once and with *K. pneumoniae* three times. Because *S. epidermidis* could also grow on the same media as *E. coli* and *K. pneumoniae* (Table 2), we selected *S. epidermidis* for further empirical tests.

To assess the effects that community members may have on other members of their community, I assessed the fitness of bacterial strains grown in isolation as well as bacterial strains grown in co-culture with another species. To quantify bacterial fitness, colony forming units (CFU/mL) were used. All relative CFU counts for each replicate are included in Appendix C. The resulting colony counts compare fitness between bacterial species in isolation and in co-culture.

First, we evaluated the growth of different strains of the same species for our hitchhiking bacteria. As shown in Figure 8, not all strains of the same species had the same measured fitness. As is clearly shown, replicates of the 7 *E. coli* strains and 8 *K. pneumoniae* strains were highly variable in their relative CFU counts (y-axis). The *E. coli* strain replicates were relatively similar, with the exception of EC283, in which one of the three replicates had a much higher relative CFU (Figure 8, left panel). As shown in Figure 8's right panel, the *K. pneumoniae* strain replicates are more variable in their relative CFU measurements.

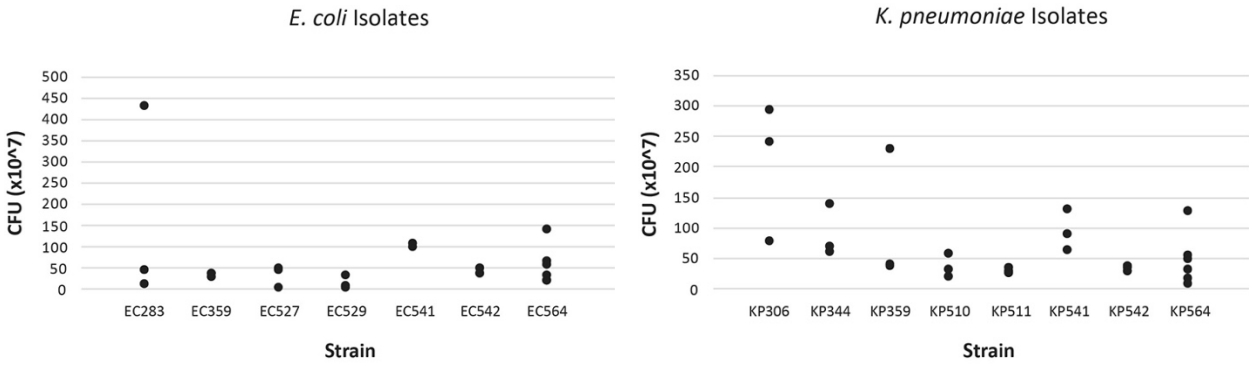

Figure 8. Strain Variation. 7 strains of *E. coli* (left) and 8 strains of *K. pneumoniae* (right) were compared to each other. All strains have 3 replicates (3 dots) except EC564 and KP564, which have 6 replicates (6 dots).

When examining just these two species, we can draw several conclusions. First, there is often significant variation between the relative CFU values for each strain. This is of course inherent variation since colonies were counted. In the case of EC283, there was one replicate that had a significantly higher CFU than the other two replicates. It is important to note that increasing the number of replicates for each strain may reduce this significant variation. Second, some strains grew better (> relative CFU) than other strains of the same species. For example, Figure 8's right panel shows that two KP306 replicates grew much better in isolation than any

other strain of *K. pneumoniae*. Based on this, we can infer that KP306 *in vitro* is better adapted to the lab environment and LB media than other strains.

Our tests next compared strains grown in isolation and grown in co-culture. Figures 9-11 summarize the bacterial fitness from strains that grew similarly, better, or worse in co-culture. There were several strain pairings that had relatively similar CFU counts to strains grown in isolation, e.g., EC359 & KP359, EC283 & SE283, EC564 & PM564 (Figure 9). For instance, EC359+KP359 replicates (35.25, 22.84, and 21.15 relative CFU/mL) were similar to the relative CFU of the two species grown in isolation: EC359 (28.13, 33.76, and 33.76 relative CFU/mL) and KP359 (40.25 and 35.22 relative CFU/mL). These strain's CFUs were similar with the exception of a single KP359 replicate (228.93 CFU/mL). A similar instance was seen in EC283 & SE283 (Figure 9, center panel) and EC564 & PM564 (Figure 9, right panel).

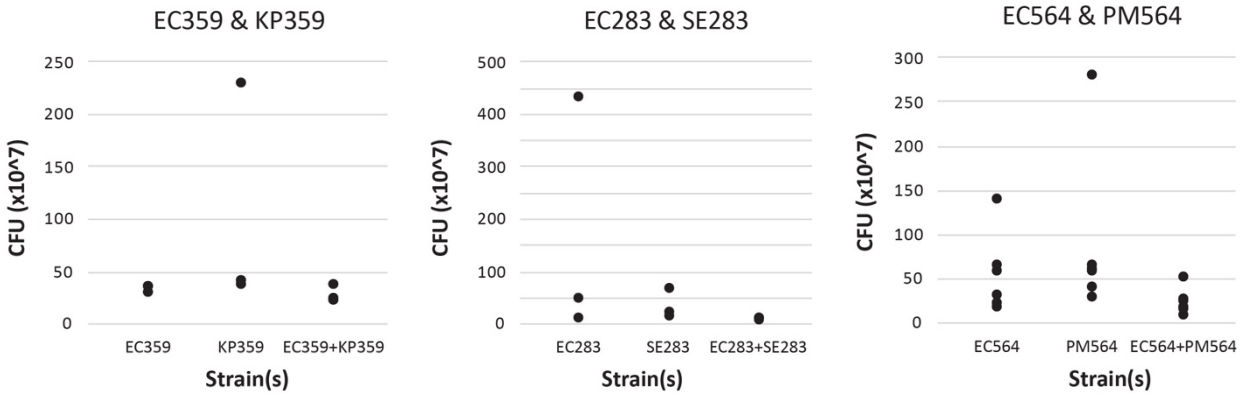

Figure 9. Samples with similar strain growth in isolation and in co-culture. EC359 & KP359 and EC283 & SE283 graphs have 3 replicates (dots) per strain(s). The EC564 & PM564 graph has 6 replicates (dots) each.

For several of the strain pairings, CFU counts observed for co-cultures surpassed CFUs from strains grown in isolation. For example, EC359 & SE283, KP306 & SE344, and KP511 & EC529 were samples that clearly show higher CFU counts in co-culture (Figure 10). As shown in

Figure 10's right panel, all 3 replicates of KP511+EC529 are higher (56.54, 102.94, and 97.14 relative CFU/mL) than the 3 replicates of KP511 (26.05, 31.84, and 23.15 relative CFU/mL) and EC529 (30.5, 1.53, and 7.63 relative CFU/mL) alone. In KP306+SE344, 2 replicates (3,175.07 and 1,275.92 relative CFU/mL) are significantly higher than KP306 (239.47, 76.05, 292.11 relative CFU/mL) and SE344 (17.32, 139.66, and 184.36 relative CFU/mL) replicates (Figure 10, center panel). Lastly, in EC359+SE283, all 3 replicates have higher CFUs (47.20, 86.08, and 63.86 relative CFU/mL) than each strain individually, with the exception of one SE283 replicate (65.79 relative CFU/mL) (Figure 10, left panel).

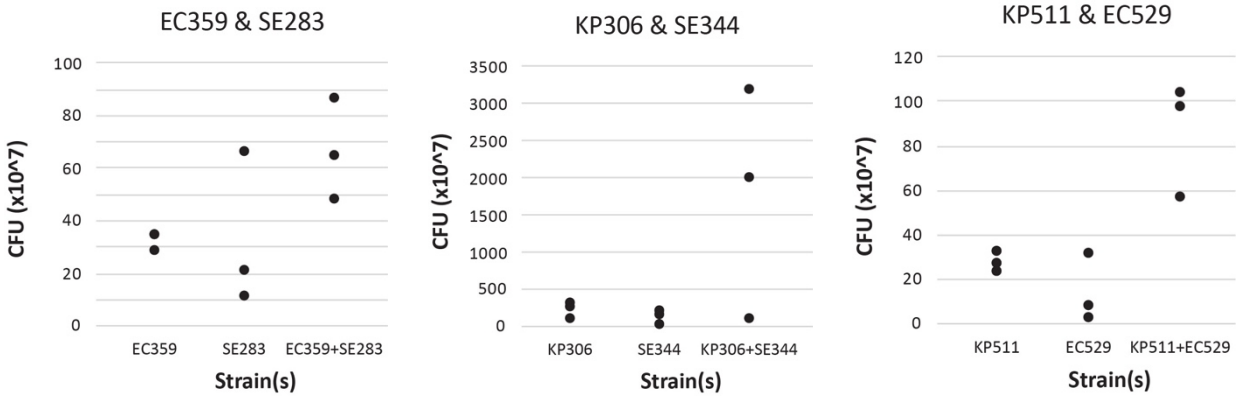

Figure 10. Samples with higher CFU's in co-culture. Each graph has 3 replicates per strain(s).

There also were cases where the observed co-culture relative CFU count is less than the relative CFUs of the strains grown in isolation. As shown in Figure 11, the growth of EC542+KP542, EC541+KP542, and EC564+KP564 was often worse than the growth of one of the strains in isolation. In the EC542+KP542 graph (Figure 11, left panel), every replicate for the strain pair (7.79, 19.48, and 18.18 relative CFU/mL) was lower than the EC542 and KP542 replicates. This is also seen in the EC541+KP542 graph, with the exception of one replicate (42.51 relative CFU/mL). Lastly in the EC564 & KP564 graph (Figure 11, right panel), most of

the 6 replicates were low compared to EC564 and KP564 replicates. Each replicate for EC364+KP365 was lower than 20 relative CFU/mL, with the exception of a few EC564 (16.27 and 19.75 relative CFU/mL) and KP564 (7.84 and 14.47 relative CFU/mL) replicates.

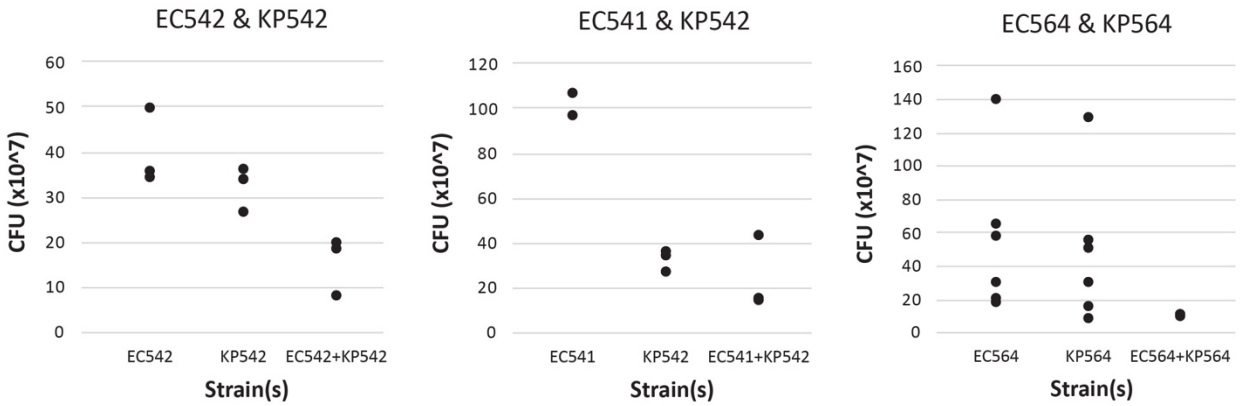

Figure 11. Samples with lower CFUs in co-culture. EC542 & KP542 and EC541 & KP542 graphs have 3 replicates (dots) per strain(s). The EC564 & KP564 graph has 6 replicates (dots) each.

We next assessed the statistical significance of these observations. 19 linear regression models were fit for each species pair (see Appendix D for model results). We identified 4 pairs of species that were statistically significantly better (n=2) or worse (n=2) together than their strains grown in isolation. Interestingly they are all 4 of the KP & SE pairings. KP306 & SE306 are significantly better together (p-value=0.04738). The same is true for KP344 & SE306 (p-value=$1.19*10^{-11}$). KP344 & SE344 are significantly worse together (p-value=0.001989). This is also the case for KP306 & SE344 (p-value= 0.005982). The sign of the "Estimate" indicates if they grew significantly better (positive) or worse (negative) tother. Apart from this group, all other groups (whether they grew better or worse) had no statistical significance and thus had no major differences between their pairings.

Where 2 of the 4 significant strain pairs were found to grow together better, it is important to note that when measuring strains in co-culture, we do not know if just one strain grew better or if both strains did. This is a critical requirement in future studies studying the co-culture of urobiome strains. Quantifying each strain could be conducted via qPCR with species specific primers or via high-throughput sequencing. It's crucial to note that our growth assays utilized species with similar morphologies. Future work could utilize organisms with distinctly different morphologies grown in co-culture such that they can be visually distinguished or the strains being tested could be genetically manipulated with different gene markers to easily identify species. Nevertheless, increased relative CFU/mL in co-culture suggests that certain strains may in fact be better colonizers in the presence of another species. It is important to note that variation was seen between co-cultures of the same species when different strains were tested. For instance, the 4 pairs that were statistically significant explored different strains of *K. pneumoniae* and *S. epidermidis*. SE306 and a *K. pneumoniae* strain did better together than SE344 and a *K. pneumoniae* strain.

We also saw several cases in our tests where strains had decreased growth in co-culture. This suggests that specific strains could be inhibiting the growth of other strains. This phenomenon is best exemplified in Figure 11, where pair replicate CFU counts were mostly lower than individual replicate CFU counts. EC542+KP542 replicates were all considerably lower than these strains in isolation. Based on this observation, we hypothesize that KP542 inhibited the growth of EC542 (or vice versa).This could be due to several mechanisms such as nutrient competition (Momose et al., 2008).

While there are many facets of these co-cultures that we did not measure beyond relative strain abundance, e.g., mechanisms for interactions, our assays were a first step for future studies examining the effect of interactions between urobiome isolates. Overall, our empirical experiments for these groups of bacterial strains support our hypothesis that strains are likely interacting in the urobiome. It has been previously reported that many urobiome bacterial interactions are either slightly positive, slightly negative, or neutral. In particular, interactions between *E. coli* and *K. pneumoniae* were found to be negative, meaning that there was a decreased population size. (Zandbergen et al., 2021). However additional studies are required to understand interaction dynamics between these species and other urobiome species.. It is important to note that the experiments conducted here were under controlled laboratory conditions. LB medium was used to grow and plate all strains throughout the CFU count experiments. LB is a nutritionally rich media commonly used to grow most bacterial species (Ezraty et al., 2014). This media is much more nutrient rich than in the bladder (Hashemi et al., 2016), and while these experiments do not aim to represent growth in the bladder, they do aim to capture relationships between different strains of bacteria isolated from the bladder environment. The experiments performed here are certainly not the best way to capture bacterial relationships, but nonetheless we were able to optimally assess the fitness of urobiome strains. Thus, future experiments that are carried out under environmental conditions similar to the bladder will be more informative about potential interactions.

Examining Pathways of Bacterial Isolates from the Same Community

To examine urobiome communities and their potential interactions computationally, we next predicted the pathways of all members of a given participant's urine sample. To do so, we needed genomic sequences for all members from the participant's urine sample. We were thus limited in the number of participants that we could examine. Purified samples from participants in which we isolated a new species or a strain that was not previously found for that participant's community were excluded as the genome was not available. To complement our experimental work looking at *K. pneumoniae* and *E. coli*, we preferentially selected participants that included one of these two species in their sample. Four participants met these criteria: RUTISD6, RUTISD9, RUTISD018, and RUTISD25. All four participants have a clinical diagnosis of rUTI. For these four participants, there are 22 bacterial genomes (see Table 4).

AWS ID #	UMB ID #	Patient ID #	Species
512	8077	RUTISD6	*Staphylococcus epidermidis*
610	8694	RUTISD6	*Klebsiella pneumoniae*
946	8695	RUTISD6	*Klebsiella pneumoniae*
612	8696	RUTISD6	*Lactobacillus gasseri*
615	8699	RUTISD6	*Lactobacillus delbrueckii*
617	8701	RUTISD6	*Enterococcus faecalis*
441	7779	RUTISD9	*Klebsiella pneumoniae*
442	7780	RUTISD9	*Enterococcus faecalis*
443	7781	RUTISD9	*Staphylococcus epidermidis*
444	7782	RUTISD9	*Streptococcus agalactiae*
445	7783	RUTISD9	*Klebsiella pneumoniae*
446	7784	RUTISD9	*Lactobacillus jensenii*
479	8022	RUTISD018	*Escherichia coli*
102	8024	RUTISD018	*Enterococcus faecalis*

482	8026	RUTISD018	*Lactobacillus jensenii*
483	8027	RUTISD018	*Lactobacillus gasseri*
530	8144	RUTISD25	*Escherichia coli*
531	8145	RUTISD25	*Streptococcus anginosus*
532	8146	RUTISD25	*Actinotignum sanguinis*
533	8147	RUTISD25	*Aerococcus urinae*
535	8149	RUTISD25	*Aerococcus sanguinicola*
536	8150	RUTISD25	*Escherichia coli*

Table 4. Genomes sequenced from strains isolated from 4 participant samples.

For each participant sample's genomes, we computationally predicted the pathways/functionality of the members via RAST. From this, multiple genes were identified that are associated with one or more metabolic pathways. While there are many metabolic pathways available for analysis, the vast majority showed little or no information. In other words, the genomes examined did not encode for a significant number of gene products that were part of that pathway or genes that were part of a given metabolic pathway were not identified by gene annotation tools. Thus, the analysis presented here focuses on just four pathways: (1) arginine biosynthesis pathway, (2) folate biosynthesis pathway, (3) glycine, serine, and threonine metabolism pathway, and (4) TCA cycle. All four pathways include multiple genes associated with the pathway and/or multiple species have genes associated with the pathway.

First, we analyzed the urobiome strains for participant RUTISD6. Six strains were isolated and sequenced from the urine sample of this participant: *Klebsiella pneumoniae* 946, *Lactobacillus delbrueckii* 615, *Lactobacillus gasseri* 612, *Klebsiella pneumoniae* 610, *Enterococcus faecalis* 617, and *Staphylococcus epidermidis* 512. For the arginine biosynthesis pathway, the majority of the identified genes are from the two *K. pneumoniae* strains (green strips, Figure 12). In the folate biosynthesis pathway (Figure 13), evidence of functionality

encoded by the *Klebsiella* strains as well as *E. faecalis* is detected. Furthermore, the *Klebsiella* strains and *Lactobacillus* strains encode for dihydrofolate reductase [EC:1.5.1.3]. For the glycine, serine, and threonine metabolic pathway, functionality associated with transforming phosphoserine to serine, serine to glycine, and L-Aspartate 4-semialdehyde to threonine are only encoded by the two *Klebsiella* and *Lactobacillus* strains (Figure 14). Based on our analysis, only the two *K. pneumoniae* strains encode the TCA cycle (Figure 15).

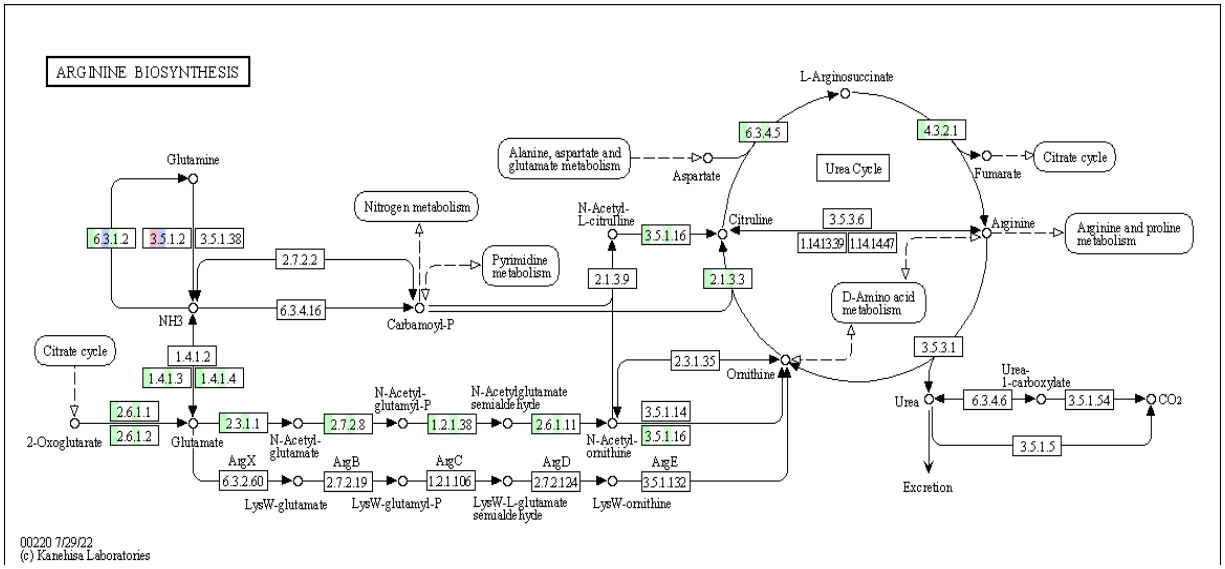

Figure 12. RUTISD6 Arginine biosynthesis metabolic pathway. The presence of a gene product is indicated by colored bars in the following order, left to right: (1) KP946, (2) LD615, (3) LG612, (4) KP610, (5) EF617, and (6) SE512.

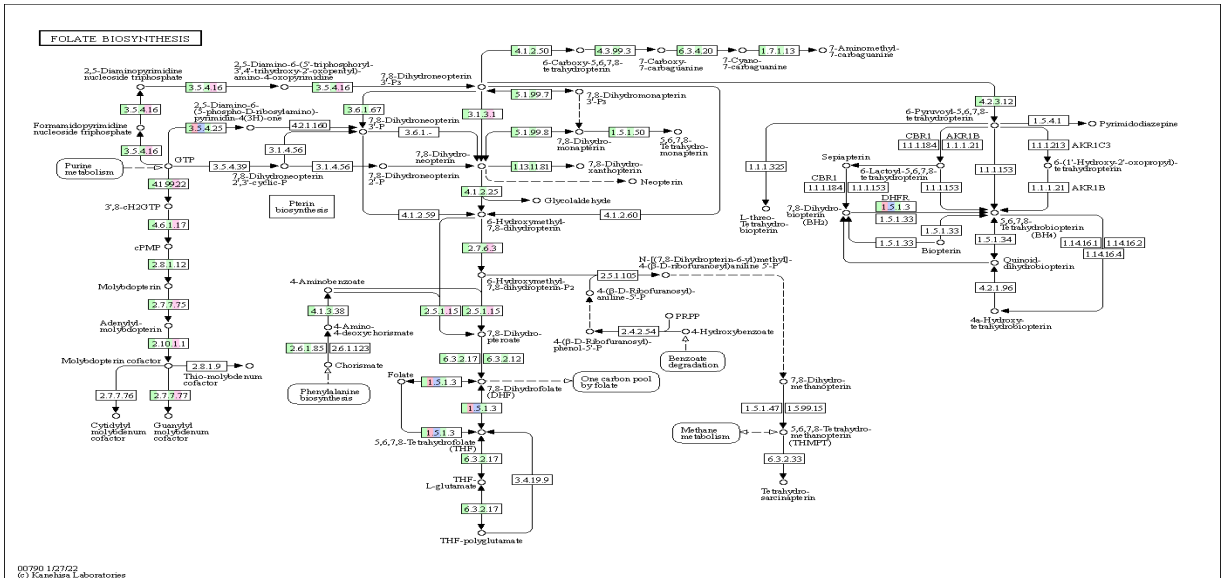

Figure 13. RUTISD6 Folate biosynthesis metabolic pathway. The presence of a gene product is indicated by colored bars in the following order: (1) KP946, (2) LD615, (3) LG612, (4) KP610, (5) EF617, and (6) SE512.

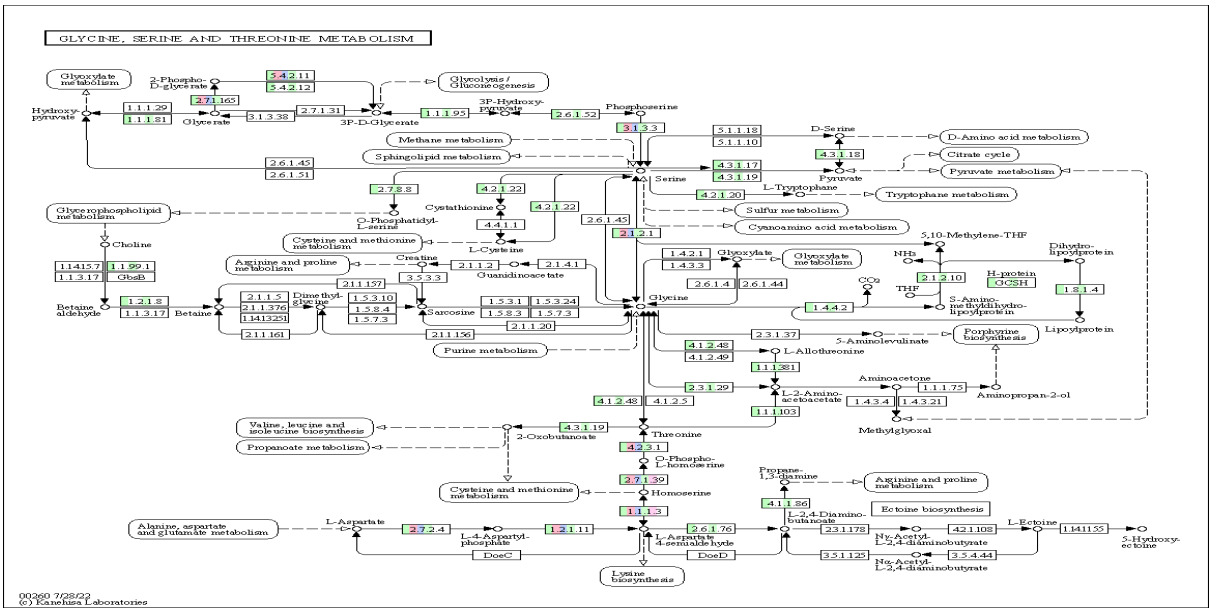

Figure 14. RUTISD6 Glycine, serine, and threonine metabolic pathway. The presence of a gene product is indicated by colored bars in the following order: (1) KP946, (2) LD615, (3) LG612, (4) KP610, (5) EF617, and (6) SE512.

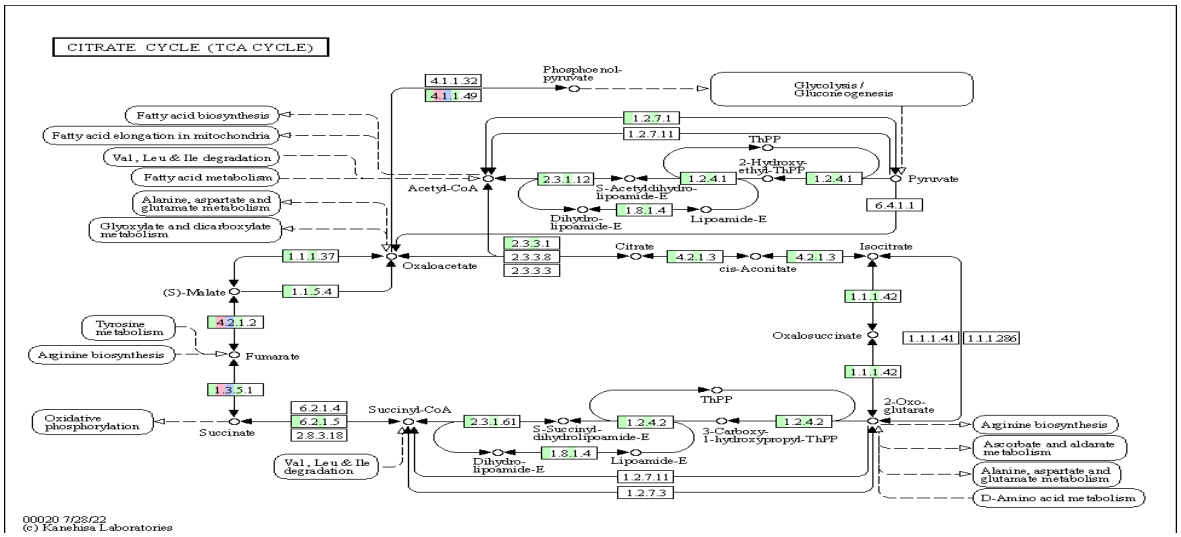

Figure 15. RUTISD6 TCA cycle. The presence of a gene product is indicated by colored bars in the following order: (1) KP946, (2) LD615, (3) LG612, (4) KP610, (5) EF617, and (6) SE512.

Next, we inspected the pathways for the genomes from the sample from participant RUTISD9. Six strains were isolated and sequenced from the urine sample of this participant: *Lactobacillus jensenii* 446, *Streptococcus agalactiae* 444, *Enterococcus faecalis* 442, *Klebsiella pneumoniae* 445, *Klebsiella pneumoniae* 441, and *Streptococcus epidermidis* 443. In the arginine synthesis pathway, most of the genes identified come from the two strains of *Klebsiella pneumoniae,* which is similar to participant RUTIDS6 (green and pink strips, Figure 16). Three species of bacteria (*Lactobacillus jensenii*, *Streptococcus agalactiae*, and *Klebsiella pneumoniae*) are involved in producing folate. Additionally, pathways that utilize guanosine triphosphate (GTP) have genes encoded by *Enterococcus faecalis* and *Klebsiella pneumoniae* (blue, green, and pink strips, Figure 17). The glycine, serine, and threonine pathway has genes encoded for the 2 strains of *Klebsiella pneumoniae* for the majority of enzyme codes that contained genes from bacterial DNA (Figure 18). This finding also corresponds to the TCA

cycle, where the majority of enzyme codes identified are from the 2 strains of *Klebsiella pneumoniae* (Figure 19).

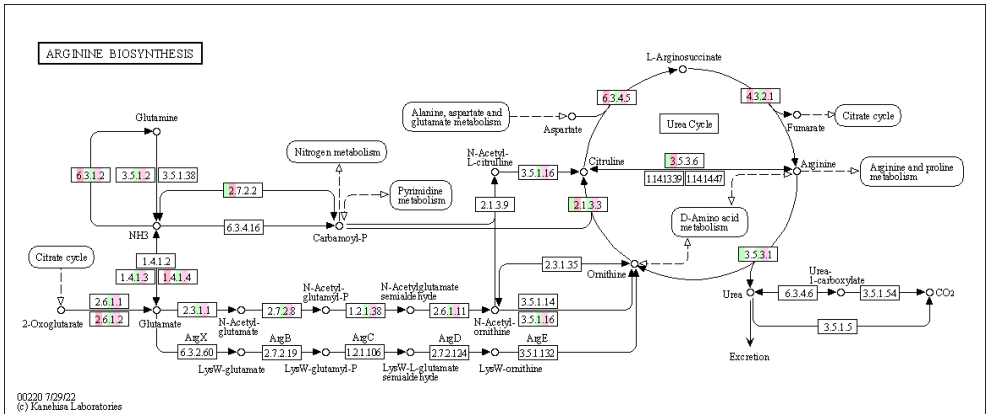

Figure 16. RUTISD9 Arginine biosynthesis metabolic pathway. The presence of a gene product is indicated by colored bars in the following order: (1) LJ446, (2) SAG444, (3) EF442, (4) KP445, (5) KP441, and (6) SE443.

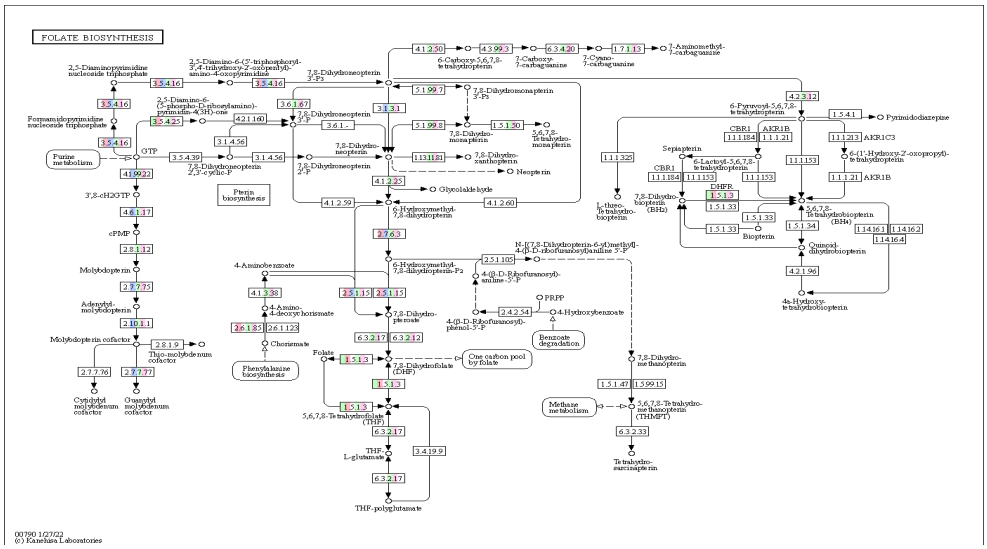

Figure 17. RUTISD9 Folate biosynthesis metabolic pathway. The presence of a gene product is indicated by colored bars in the following order: (1) LJ446, (2) SAG444, (3) EF442, (4) KP445, (5) KP441, and (6) SE443.

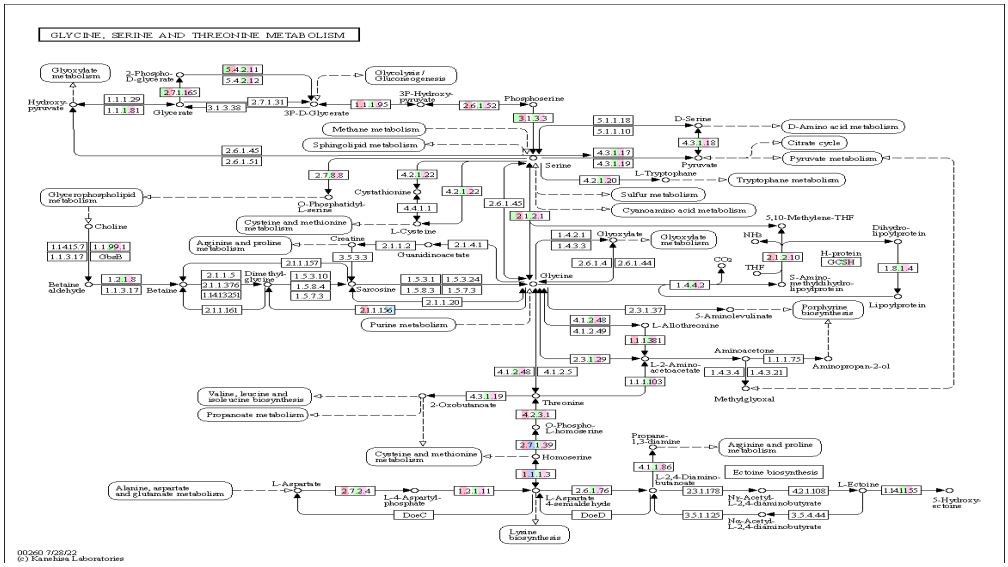

Figure 18. RUTISD9 Glycine, serine, and threonine metabolic pathway. The presence of a gene product is indicated by colored bars in the following order: (1) LJ446, (2) SAG444, (3) EF442, (4) KP445, (5) KP441, and (6) SE443.

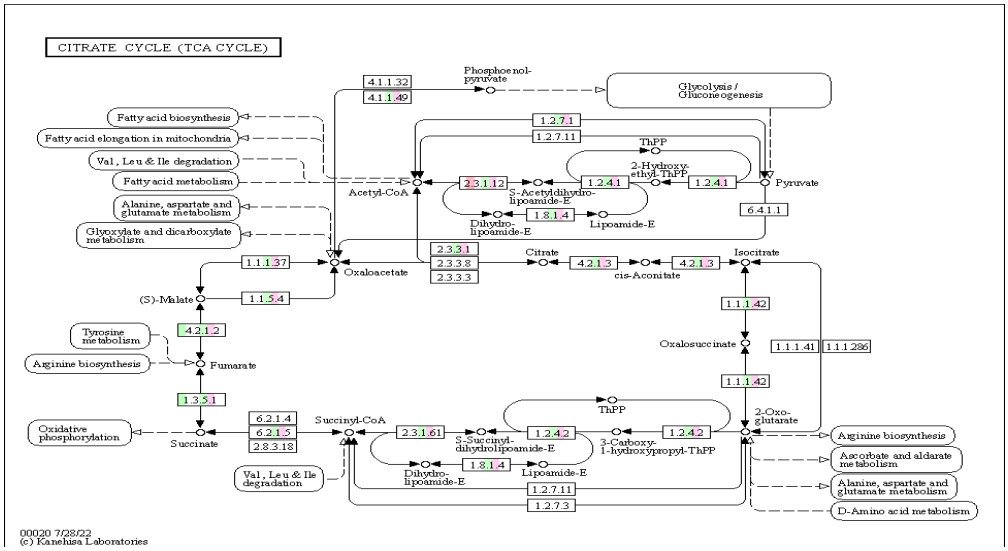

Figure 19. RUTISD9 TCA cycle. The presence of a gene product is indicated by colored bars in the following order: (1) LJ446, (2) SAG444, (3) EF442, (4) KP445, (5) KP441, and (6) SE443.

The next participant that we examined was RUTISD018. Four strains were isolated and sequenced from the urine sample: *Escherichia coli* 479, *Enterococcus faecalis* 102, *Lactobacillus jensenii* 482, and *Lactobacillus gasseri* 483. Most of the genes involved in the urea cycle that were identified are from the *Lactobacillus jensenii* strain (blue strips, Figure 20). However, very few genes in the arginine biosynthesis pathway are identified. In the folate biosynthesis pathway, the *Enterococcus faecalis* strain is included in the initial purine metabolism pathway. The two *Lactobacillus* species are represented in the production of folate (blue and green strips, Figure 21). These species are also represented in the production of serine [EC: 3.1.33 and 2.1.2.1] in the glycine, serine, and threonine metabolism pathway (Figure 22). Again, genes encoded by the two *Lactobacillus* species are part of the tyrosine biosynthesis and arginine biosynthesis within the TCA cycle (Figure 23). Based on our analysis, the *Lactobacillus* species play a key role in several of these metabolic pathways.

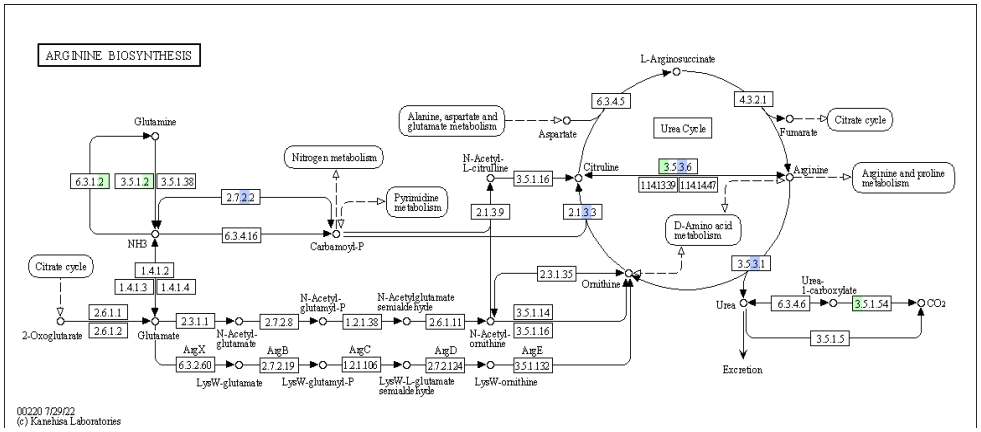

Figure 20. RUTISD018 Arginine biosynthesis metabolic pathway. The presence of a gene product is indicated by colored bars in the following order: (1) EC479, (2) EF102, (3) LJ482, and (4) LG483.

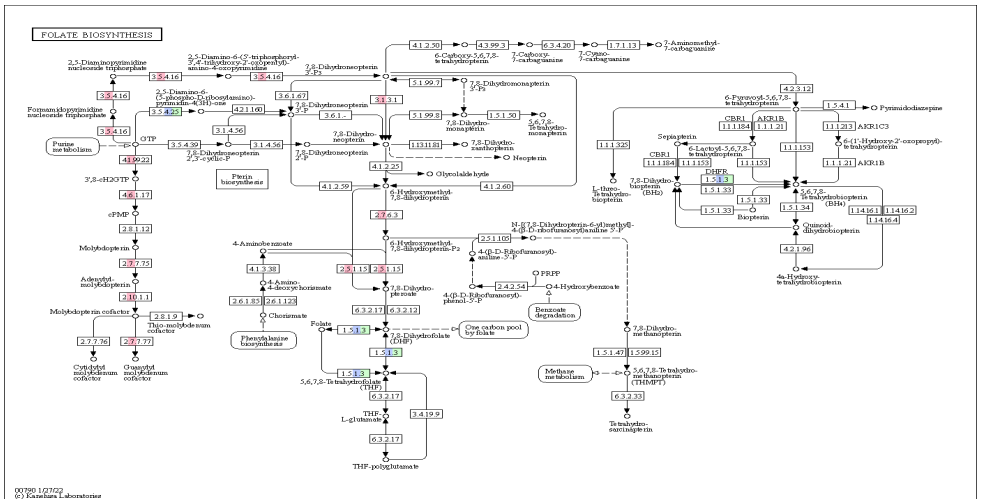

Figure 21. RUTISD018 Folate biosynthesis metabolic pathway. The presence of a gene product is indicated by colored bars in the following order: (1) EC479, (2) EF102, (3) LJ482, and (4) LG483.

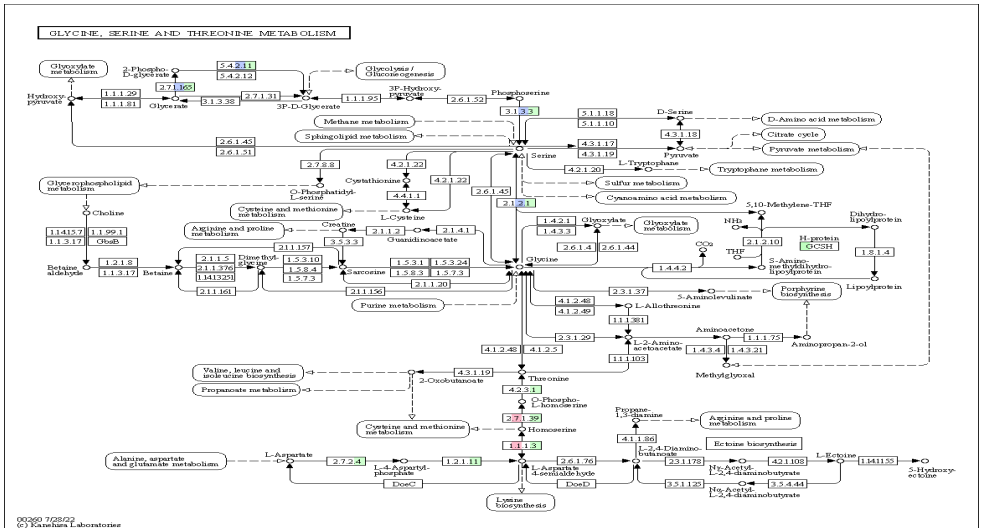

Figure 22. RUTISD018 Glycine, serine, and threonine metabolic pathway. The presence of a gene product is indicated by colored bars in the following order: (1) EC479, (2) EF102, (3) LJ482, and (4) LG483.

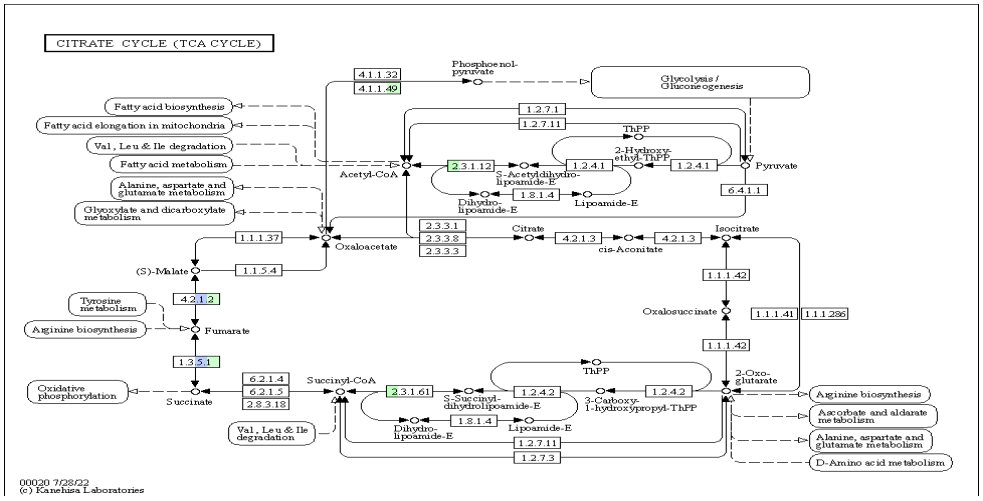

Figure 23. RUTISD018 TCA cycle. The presence of a gene product is indicated by colored bars in the following order: (1) EC479, (2) EF102, (3) LJ482, and (4) LG483.

The final participant that we analyzed was RUTISD25. Six strains were isolated and sequenced from the urine sample: *Escherichia coli* 536, *Aerococcus sanguinicola* 535, *Streptococcus anginosus* 531, *Aerococcus urinae* 533, *Escherichia coli* 530, and *Actinotignum sanguinis* 532. Noticeably, every strain except the two *E. coli* strains is involved in the urea cycle (red, blue, and green strips, Figure 24). In the folate biosynthesis pathway, *Aerococcus sanguinicola, Aerococcus urinae,* and *Actinotignum sanguinis* genes are encoded for folate functionality (red, green and blue strips, Figure 25). Furthermore, genes that encode for *Aerococcus sanguinicola, Streptococcus anginosus,* and *Actinotignum sanguinis* provide evidence of GTP functionality (red, purple, and blue strips, Figure 25). In the glycine, serine, and threonine pathway, all strains except for the *E. coli* strains encode for threonine production (red, purple, green, and blue strips, Figure 26). Lastly in Figure 27, the majority of the gene products in the TCA cycle are encoded by *Actinotignum sanguinis.* Only two gene products [EC: 2.3.1.2 and 2.3.1.61] are from the two *E. coli* strains.

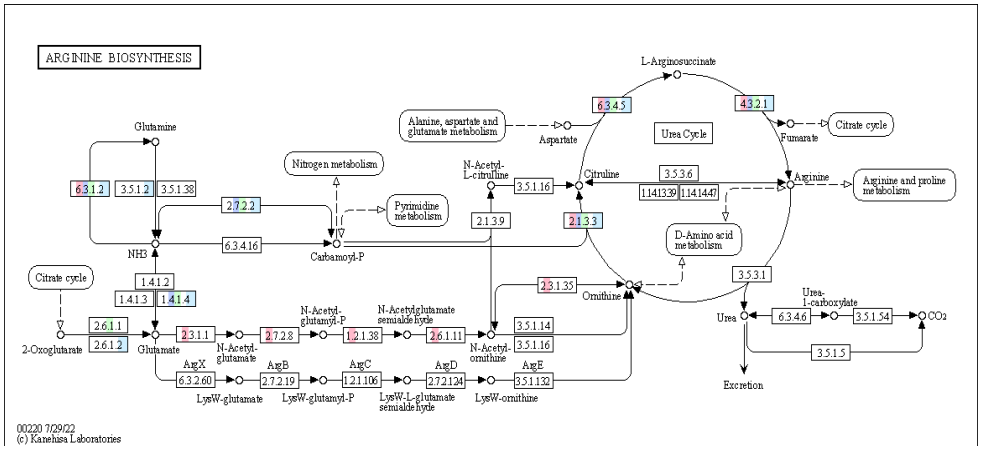

Figure 24. RUTISD25 Arginine biosynthesis metabolic pathway. The presence of a gene product is indicated by colored bars in the following order: (1) EC536, (2) ASAN535, (3) SAN531, (4) AU533, (5) EC530, and (6) AS532.

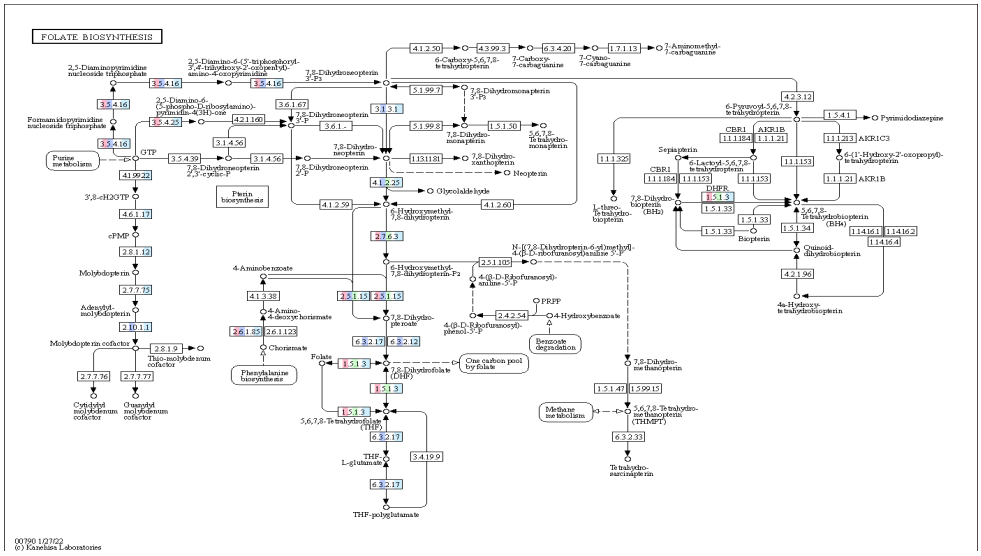

Figure 25. RUTISD25 Folate biosynthesis metabolic pathway. The presence of a gene product is indicated by colored bars in the following order: (1) EC536, (2) ASAN535, (3) SAN531, (4) AU533, (5) EC530, and (6) AS532.

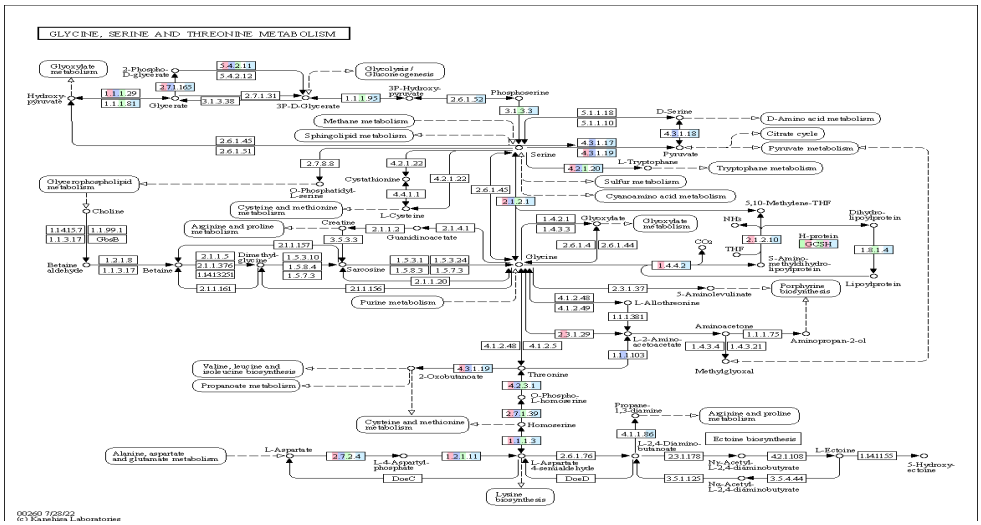

Figure 26. RUTISD25 Glycine, serine, and threonine metabolic pathway. The presence of a gene product is indicated by colored bars in the following order: (1) EC536, (2) ASAN535, (3) SAN531, (4) AU533, (5) EC530, and (6) AS532.

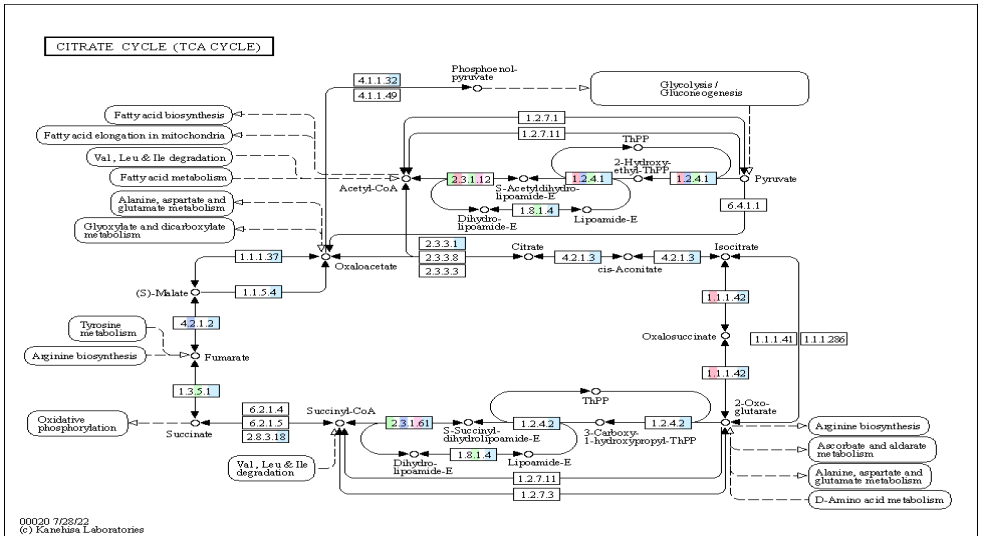

Figure 27. RUTISD25 TCA cycle. The presence of a gene product is indicated by colored bars in the following order: (1) EC536, (2) ASAN535, (3) SAN531, (4) AU533, (5) EC530, and (6) AS532.

Based on our analysis, we were able to identify genes within in the urinary bacterial strains that are involved in several metabolic processes. In participant RUTISD6, the majority of the genes for each pathway were from the *K. pneumoniae* genomes. This species was especially prevalent in arginine biosynthesis and the TCA cycle (Figures 12 and 15, respectively). Similarly in participant RUTISD9, the two strains of *K. pneumoniae* were identified in most genes encoded in arginine biosynthesis (Figure 16) and the TCA cycle (Figure 19). With regards to arginine biosynthesis, *K. pneumoniae* hydrolyzes urea as a source of nitrogen, which allows it to proliferate (Liu & Bender, 2007). *K. pneumoniae* is also known to grow anaerobically by using citrate as a sole carbon source. Citrate is one of the important biproducts of the TCA cycle, and it is one of the only sources of carbon in human urine (Y.-T. Chen et al., 2009). While this species is known to colonize individuals with urinary tract infections, the metabolic mechanisms involved in its pathogenicity are not fully understood.

Interestingly in participant RUTISD018, the majority of genes identified in all four pathways examined here were from the *Lactobacillus jensenii* and *Lactobacillus gasseri* genomes. *Lactobacillus* taxa are known to be part of a healthy urobiome environment, especially in adult females (Hilt et al., 2014; Pearce et al., 2015; K. Thomas-White et al., 2018b). Another notable observation we saw was that EC479 did not have many genes encoded in all four pathways. This was likely due to either E. coli lacking these metabolic pathways or RAST was unable to detect *E. coli*.

Similarly for participant RUTISD25, our two strains of *E. coli* were not detected in KEGG mapper for all four pathways. As mentioned previously, these results could likely be due to the RAST annotation tool not being able to detect genes that encode for our 2 strains of *E.*

coli. To see if this was true, we looked at these four metabolic pathways for the manually curated *E. coli* K-12 MG1665 strain, which is the best studied bacterial species (Riley et al., 2006, p. 12). *E. coli* K-12 MG1665 did have genes encoded in all four pathways. This leads to the conclusion that RAST was not able to properly annotate our *E. coli* strains.

While RAST is a useful bioinformatics resource for annotating bacterial genomes, it is limited by the gene predictions performed. The four metabolic pathways we analyzed provide insights into the metabolic profiles of species from participant urine samples. We sought out to examine urobiome communities and their potential contributions to the community computationally. While we identified gene codes for key species in the urobiome such as *K. pneumoniae* and *Lactobacillus jensenii*, we could not draw conclusions about other species with this analysis alone. This was due to several factors, including the limited number of participants we could examine and the inability of RAST to annotate our *E. coli* strains. Nonetheless, this analysis provides a foundation to investigate mechanisms underlying hitchhiker associations. Further examining of bladder-specific bacterial genome sequences as well as urinary metabolomic studies is necessary to gain key insights into how these bacterial species utilize their environment and potentially interact with each other through resource sharing.

CHAPTER FOUR

CONCLUSIONS

It was only recently that scientists discovered that the urine of healthy individuals was not sterile and that it houses a diverse population of bacterial species. Presently, urobiome studies are carried out by either sequencing all of the DNA present (shotgun metagenomics) or more frequently targeting the 16S rRNA. Alternatively, the urobiome is cultured such that individual strains can be isolated and identified via MALDI-TOF or subjected to whole genome sequencing. These approaches are undoubtedly important for describing what species exist. Neither of these techniques are useful for describing the interactions between different species.

This research project was driven by prior researchers in the Putonti and Wolfe labs' frequent observation of more than one bacterial species present in the whole genome sequencing of purified urobiome isolates. The recent analysis of bacterial community interactions and dynamics in the soil microbiome, the THOR model (Lozano et al., 2019), prompted my investigation into the urobiome for hitchhiking bacteria. In contrast to other complex environmental communities (e.g., soil, water) or other human microbiota(e.g., gut, oral cavity), the urobiome was an ideal niche to conduct bacterial interaction investigations because of the fact that just a few species comprise the entire community.

This study contributed significantly to the knowledge of bacterial diversity in the urobiome. We identified 23 species that have yet to be sequenced by Loyola urobiome researchers. Furthermore, we were able to construct the "Field Guide to the Urobiome" database.

Our database, which contains 292 16S sequences from our own collection and from Loyola University's female urinary microbiota BioProject, captures the species and strain diversity found in the female urinary tract of both asymptomatic and symptomatic individuals. The biggest significance of this resource is its utility to aid in identifying taxa for high-throughput 16S rRNA gene surveys of the urobiome. To our knowledge, some databases of 16S sequences lack a representation of diversity in urobiome species. (Hoffman et al., 2021). Thus, the sequences generated as part of this study are useful in aiding the taxonomic profiling of 16S data sets from urine.

Additionally, our sequencing data allowed us to recognize two bacteria that are likely hitchhikers or have hitchhikers: *E. coli* and *K. pneumoniae. E. coli* is considered to be a motile species while *K. pneumoniae* is non-motile (Yang et al., 2020). However, both are associated with UTIs and are thought to be introduced to the bladder from other niches (S. L. Chen et al., 2006; Imirzalioglu et al., 2008; Neugent et al., 2020). From two samples, I isolated *K. pneumoniae* and *P. mirabilis* and *E. coli* and *P. mirabilis*. *P. mirabilis* is a known motile bacterium (Mushenheim et al., 2014), suggesting that perhaps *E. coli* and *K. pneumoniae* were hitchhiking with *P. mirabilis*. Further investigation is needed for the results of the co-isolated species with regards to the motility of the strains under urinary tract relevant conditions.

We often saw these species with each other and with other species in our collection. This particular observation drove our bacterial fitness tests, which led to us seeing interesting relationship dynamics through my co-culture assays. It also allowed us to capture how distinct bacterial strains grow differently under the same conditions. While only 4 statistically significant strain pairings were identified, it was interesting that all of these pairs came from *K. pneumoniae*

and *S. epidermidis*. While we could not draw conclusions on the relationship between these two species in general, we were able to conclude that two of our KP & SE strain pairs grew significantly better together and the other two pairs grew significantly worse. Noticeably in the pairs that grew better together, the same strain of *S. epidermidis* was present (SE306). Perhaps this *S. epidermidis* strain causes *K. pneumoniae* to grow better. Further analyses need to be carried out to fully understand why specific strain pairs grow better together than in isolation as well as if this observation holds true in bladder-relevant nutrient and atmospheric conditions.

Finally, we sought out to examine urobiome communities and their potential interactions computationally by analyzing the putative functionalities via pathway analysis of the members of a single community. By selecting communities that included *K. pneumoniae* and/or *E. coli*, we hoped to draw connections between this computational analysis and our experimental co-culturing assays. However, we saw that *E. coli* genes were not detected in the annotation process such that many metabolic pathways observed did not include genes from our *E. coli* strains. We proved that this was due to limitations of the RAST annotation tool; pathways that are known to be present in *E. coli*, confirmed by examining the manually curated *E. coli* K-12 MG1665 strain's KEGG pathways, were not represented in our analysis.. What this analysis really highlights is the limitations of computational predictions of the metabolome from genomes alone. Improved algorithms are needed. Future metabolic studies are needed to truly ascertain what these urinary microbes are doing in the urinary tract. Nonetheless, our data does provide strong evidence that hitchhiking likely occurs in the urobiome. This analysis will serve as a foundation to investigate mechanisms underlying the hitchhiker associations in the bladder.

Although our understanding of bacterial hitchhiking in the urobiome undoubtedly remains incomplete, the work presented here provides evidence in support of a complex hypothesis involving an interconnected community of bacteria. It is crucial to acknowledge that the difference between a hitchhiker and a contaminant is not known. It's possible that strains we identified as hitchhikers were introduced during initial purification steps prior to my acquisition of my samples. We conclude that additional sequence-analysis studies, as well as controlled laboratory experiments, are needed to fully establish the dynamics of bacterial hitchhiking in the urobiome.

APPENDIX A

16S rRNA SEQUENCING RESULTS

Sample ID	# Unique 16S sequences	Species	Who it was supposed to be
349	1	KP	BL
267	1	PM	PM
268	2	SAU	SAN
269	2	PM, SAN	SAN
270	2	SAG, SAU	SAN
273	6	EC, PT, EF, SAU	SAU
276	3	LG, EF	LI
275	2	EF	SAN
563	1	SHOM	AS
564	5	EC, PM, KP	SAN
544	2	PM	PM
546	2	EF	SC
545	4	PM, SE	SE
540	2	EC	EC
539	2	KP	KP
419	2	EC, CA	CA
402	2	EC, SF	CAL
430	1	PAL	CJ
421	4	LG, SE	LG
420	4	ENC, PM, KP	PS
418	3	SE	SE
295	1	SE	AC
287	2	SE, AU	AN
282	1	EC	EC
284	2	EF	EF
286	2	LG	LG
296	4	EC, LG, KP, EF	LG
285	1	LR	LR
283	3	EC, SE	SE
298	7	SE, SHOM, SH, SPAU, SCO	SG
307	2	EF, AN	AN
313	2	SHOM, SE	AS
310	4	KP, AS, CI	CI
311	3	CSI, KP	CSI

305	6	KP	KP
312	4	EF, KP, SE	LG
309	3	SH, EF, EFG	SC
306	2	KP, SE	SE
308	7	EC, KP, AN, EFG	SH
324	1	AU	AU
327	8	SCAP, BMO, BAR, KM, DN, SHOM, CU	CL
323	2	LG	LG
322	1	LR	LR
329	3	BN, SAG	SAG
330	4	SH, MA, SO	SI
331	6	PO, SE, MY, CU	CU
332	3	EC, SAG	SAG
335	4	EC, SAG	SAG
333	1	KP	SO
338	2	GA, SAU	GA
339	1	SCAP	LI
336	2	RM	RM
337	3	EC, KP, SAU	TB
342	4	EC, KP	EC
346	3	LC, SAU	LC
343	3	LRT, KP	LP
345	2	SAU	SAN
344	7	KP, SE	SE
351	2	LG	LG
360	2	DJ, AN	AN
359	2	EC, KP	GV
480	1	KP	CG
368	4	SAN, KP	SC
377	3	KA, SF, EF	CAL
378	5	SHOM, LG, SS, SPA	LG
372	2	SE, EF	AT
371	4	EF, SE, SCAP	CKRU
373	3	EF, SCAP	LJ
390	5	LR, KP	KP
452	4	LC, SAE, SE	LC

451	2	SAE, SW	LJ
479	4	EC, CITK, BT, SCAP	EC
483	2	LG, SCAP	LG
482	1	LJ	LJ
748	1	EC	LJ
476	2	SE	SE
746	1	SCAP	SE
478	1	SE	SM
481	3	EF, SAU	SO
477	1	SCAP	SV
415	3	SE	SE
447	2	EF	EF
448	2	LR, EF	LJ
462	5	CCI, BF, SHOM, MY, SCO	SM
505	3	EF, MY, SE, AS	AS
504	2	SHOM, AU	AU
501	2	CC	CC
541	6	EC, KA, KP	EC
503	1	FH	FH
502	3	SAN, SE, EF	SAN
513	3	SHOM, KPAL, BT	AUR
510	5	BI, KP	KP
511	3	KP	KP
522	2	CA	CA
520	2	LR	LG
519	2	LG	LR
516	3	SHOM, LG, SE	SE
521	4	SH, LG, LD	SH
527	3	EC	EC
529	2	EC	EC
528	4	LG, SE	LG
532	1	SAN	ASAN
535	6	SCAP, PE, SHOM, MY, ASAN	ASAN
533	3	AU	AU
530	2	EC	EC
536	2	EC	EC
531	2	AS, SPA	SAN

537	5	SAU, PA, SB, EF, KO	CALB
525	2	EC	EC
411	1	SE	EA
412	3	LG, KA	SE
623	2	EC	EC
624	1	PM	PM
629	3	MM	MM
630	1	PM	PM
795	4	AS, KP, CA	AS
617	5	EC, SB, EF	EF
610	4	KP	KP
611	2	KP	KP
853	3	KP	KP
613	2	KP, LD	LF
512	2	SE	SE
614	3	SE, LD	SE
854	1	SHOM	SHOM
438	1	CAU	CAU
436	1	SW	LI
435	5	AR, SW, BSU, SHOM, MY	LJ
437	4	SW, SAN, SE	SAN
434	4	SE, PTS	SE
445	3	SCAP, AU	AU
442	3	EF	EF
441	1	KP	KP
446	5	PP, SCAP, LJ, EF	LJ
444	3	SAG, SCAP	SAG
443	2	SE, SCAP	SE
488	2	EC	EC
509	2	KA	EA
514	1	PM	PM
408	2	KA	EA
577	3	PM, SF	PM
591	1	EC	EC
648	2	EC	EC
593	3	PM	PM
603	1	ASAN	ASAN

602	2	PM	PM
604	5	EC, SB, PM, SPA	SAN
816	2	ASAN	ASAN
815	1	PM	PM
543	5	SH, KP, EF	KP
542	5	EC, KP	EC

Abbreviation	Species
AN	*Actinomyces neuii*
AR	*Actinomyces radingae*
ASAN	*Aerococcus sanguinicola*
AS	*Actinotignum schaalii*
AU	*Aerococcus urinae*
BAR	*Bacillus aryabhattai*
BF	*Brevibacterium frigoritolerans*
BI	*Bacillus infantis*
BN	*Bacillus nealsonii*
BSU	*Bacillus subtilis*
BT	*Bacillus tropicus*
CA	*Corynebacterium amycolatum*
CAU	*Corynebacterium aurimucosum*
CC	*Corynebacterium coyleae*
CCI	*Curtobacterium citreum*
CITK	*Citrobacter koseri*
CSI	*Corynebacterium simulans*
CU	*Corynebacterium urealyticum*
DJ	*Dermabacter jinjuensis*
DN	*Dermacoccus nishinomiyaensis*
EC	*Escherichia coli*
EF	*Enterococcus faecalis*
ENC	*Enterobacter cloacae*
FH	*Facklamina hominis*
GA	*Granulicatella adiacens*

KA	*Klebsiella aerogenes*
KM	*Kocuria marina*
KO	*Klebsiella oxytoca*
KP	*Klebsiella pneumoniae*
KPAL	*Kocuria palustris*
LC	*Lactobacillus crispatus*
LD	*Lactobacillus delbrueckii*
LG	*Lactobacillus gasseri*
LJ	*Lactobacillus jensenii*
LR	*Lactobacillus rhamnosus*
LRT	*Lactobacillus reuteri*
MA	*Micrococcus aloeverae*
MM	*Morganella morganii*
MY	*Micrococcus yunnanensis*
PA	*Pseudomonas aeruginosa*
PAL	*Pseudoclavibacter alba*
PE	*Pantoea eucrina*
PM	*Proteus mirabilis*
PO	*Pseudomonas oryzihabitans*
PP	*Paenibacillus pabuli*
PT	*Paenibacillus taichungensis*
PTS	*Paenibacillus taiwanensis*
RM	*Rothia mucilaginosa*
SAE	*Sphingomonas aeria*
SAG	*Streptococcus agalactiae*
SAN	*Streptococcus anginosus*
SAU	*Staphylococcus aureus*
SB	*Shigella boydii*
SCAP	*Staphylococcus capitis*
SCO	*Staphylococcus condimenti*
SE	*Staphylococcus epidermidis*
SF	*Shigella flexneri*
SH	*Staphylococcus haemolyticus*
SHOM	*Staphylococcus hominis*
SPA	*Staphylococcus pasteuri*
SPAU	*Streptococcus pasteurianus*
SW	*Staphylococcus warneri*

APPENDIX B

UROBIOME SAMPLE MEDIA GROWTH

Species	AWS	Media
Acinetobacter radioresistens	435	LB
Actinomyces neuii	307	LB; Actinomyces; NYC III; BHI + Tween
Actinomyces neuii	308	TSB
Actinomyces neuii	360	Actinomyces
Actinotignum schaalii	310	Actinomyces
Actinotignum schaalii	505	LB; Actinomyces; TSB
Actinotignum schaalii	531	Actinomyces
Actinotignum schaalii	795	BHI + Tween
Aerococcus sanguinicola	535	Actinomyces; BHI + Tween
Aerococcus sanguinicola	603	Actinomyces; TSB; BHI + Tween
Aerococcus sanguinicola	816	Actinomyces; NYC III
Aerococcus urinae	287	LB; TSB; Actinomyces
Aerococcus urinae	324	Actinomyces; NYC III; BHI + Tween
Aerococcus urinae	445	Actinomyces; BHI + Tween; MRS + Tween
Aerococcus urinae	504	BHI + Tween
Aerococcus urinae	533	Actinomyces; TSB; NYC III; BHI + Tween
Bacillus aryabhattai	327	NYC III
Bacillus infantis	510	LB
Bacillus mobilis	327	TSB
Bacillus nealsonii	329	Actinomyces
Bacillus subtilis	435	NYC III
Bacillus tropicus	479	TSB; NYC III
Bacillus tropicus	513	NYC III
Brevibacterium frigoritolerans	462	Actinomyces
Citrobacter koseri	479	Actinomyces; TSB; NYC III
Corynebacterium amycolatum	419	LB; TSB; NYC III
Corynebacterium amycolatum	522	LB; TSB; NYC III; BHI + Tween
Corynebacterium amycolatum	795	LB; TSB; NCY III; BHI + Tween
Corynebacterium coyleae	501	LB; Actinomyces; TSB; NYC III
Corynebacterium aurimucosum	438	Actinomyces; BHI + Tween

Corynebacterium imitans	310	LB; TSB; BHI + Tween
Corynebacterium simulans	311	LB; Actinomyces; TSB
Corynebacterium urealyticum	327	NYC III
Corynebacterium urealyticum	331	Actinomyces; BHI + Tween
Curtobacterium citreum	462	TSB
Candida lustitaniae	402	Actinomyces; TSB
Dermabacter jinjuensis	360	NYC III
Dermacoccus nishinomiyaensis	327	BHI + Tween
Enterococcus faecalis	273	MRS + Tween
Enterobacter cloacae	420	NYC III
Enterococcus faecalis	275	LB; Actinomyces; TSB; NYC III; BHI + Tween
Enterococcus faecalis	276	TSB; MRS + Tween
Enterococcus faecalis	284	LB; Actinomyces; NYC III; BHI + Tween; MRS + Tween
Enterococcus faecalis	296	Actinomyces
Enterococcus faecalis	309	BHI + Tween; MRS + Tween
Enterococcus faecalis	312	MRS + Tween
Enterococcus faecalis	371	LB
Enterococcus faecalis	372	LB; Actinomyces; TSB; BHI + Tween; MRS + Tween
Enterococcus faecalis	373	Actinomyces; BHI + Tween
Enterococcus faecalis	377	TSB; NYC III
Enterococcus faecalis	442	Actinomyces; TSB; NYC III; BHI + Tween; MRS + Tween
Enterococcus faecalis	446	LB; BHI + Tween
Enterococcus faecalis	447	Actinomyces; NYC III; BHI + Tween; MRS + Tween
Enterococcus faecalis	448	NYC III
Enterococcus faecalis	476	NYC III
Enterococcus faecalis	481	Actinomyces; TSB; NYC III
Enterococcus faecalis	502	MRS + Tween
Enterococcus faecalis	537	BHI + Tween
Enterococcus faecalis	543	BHI + Tween
Enterococcus faecalis	546	LB; Actinomyces; TSB; NYC III; MRS + Tween
Enterococcus faecalis	617	LB; Actinomyces; NYC III; BHI + Tween; MRS + Tween
Escherichia coli	273	LB; NYC III; BHI + Tween

Escherichia coli	282	LB; Actinomyces; TSB; NYC III; BHI + Tween; MRS + Tween
Escherichia coli	283	Actinomyces; TSB; NYC III
Escherichia coli	296	TSB
Escherichia coli	308	Actinomyces; BHI + Tween
Escherichia coli	335	LB; Actinomyces
Escherichia coli	337	TSB
Escherichia coli	342	LB; Actinomyces; TSB; NYC III; BHI + Tween
Escherichia coli	359	LB; TSB; NYC III; BHI + Tween
Escherichia coli	419	BHI + Tween
Escherichia coli	479	Actinomyces
Escherichia coli	488	MRS + Tween
Escherichia coli	525	LB; Actinomyces; TSB; NYC III; BHI + Tween; MRS + Tween
Escherichia coli	527	LB; TSB; NYC III; BHI + Tween
Escherichia coli	529	LB; Actinomyces; TSB; NYC III; BHI + Tween; MRS + Tween
Escherichia coli	530	LB; Actinomyces; TSB; NYC III
Escherichia coli	536	TSB; NYC III; BHI + Tween
Escherichia coli	540	LB; Actinomyces; TSB; NYC III; BHI + Tween; MRS + Tween
Escherichia coli	541	Actinomyces
Escherichia coli	541	LB; Actinomyces; TSB; NYC III; BHI + Tween
Escherichia coli	542	LB; Actinomyces; TSB; NYC III
Escherichia coli	564	Actinomyces
Escherichia coli	591	LB; Actinomyces; TSB; NYC III; BHI + Tween; MRS + Tween
Escherichia coli	604	LB; NYC III; BHI + Tween
Escherichia coli	617	TSB
Escherichia coli	623	LB; Actinomyces; TSB; NYC III; BHI + Tween; MRS + Tween
Escherichia coli	648	LB; Actinomyces; TSB; NYC III; BHI + Tween; MRS + Tween
Escherichia coli	748	LB
Escherichia fergusonii	308	TSB
Escherichia fergusonii	309	Actinomyces
Facklamina hominis	503	LB; NYC III; BHI + Tween
Granulicatella adiacens	338	LB; TSB
Klebsiella aerogenes	377	NYC III

Klebsiella aerogenes	408	LB; Actinomyces; TSB; NYC III; BHI + Tween
Klebsiella aerogenes	412	LB; TSB; NYC III; BHI + Tween
Klebsiella aerogenes	509	LB; Actinomyces; TSB; NYC III; BHI + Tween; MRS + Tween
Klebsiella aerogenes	541	MRS + Tween
Klebsiella oxytoca	537	LB
Klebsiella pneumoniae	296	LB
Klebsiella pneumoniae	305	LB; Actinomyces; TSB; NYC III; BHI + Tween; MRS + Tween
Klebsiella pneumoniae	306	LB
Klebsiella pneumoniae	308	LB; NYC III; BHI + Tween
Klebsiella pneumoniae	310	NYC III
Klebsiella pneumoniae	311	NYC III; BHI + Tween
Klebsiella pneumoniae	312	LB; Actinomyces
Klebsiella pneumoniae	333	Actinomyces; TSB
Klebsiella pneumoniae	337	LB
Klebsiella pneumoniae	342	MRS + Tween
Klebsiella pneumoniae	343	LB; BHI + Tween
Klebsiella pneumoniae	344	LB; Actinomyces; TSB; NYC III
Klebsiella pneumoniae	349	BHI + Tween
Klebsiella pneumoniae	359	Actinomyces
Klebsiella pneumoniae	368	LB; Actinomyces; NYC III
Klebsiella pneumoniae	390	LB; Actinomyces; TSB; NYC III; MRS + Tween
Klebsiella pneumoniae	420	Actinomyces; MRS + Tween
Klebsiella pneumoniae	441	LB; Actinomyces; TSB; NYC III; BHI + Tween
Klebsiella pneumoniae	480	TSB
Klebsiella pneumoniae	510	LB; Actinomyces; TSB; NYC III; BHI + Tween; MRS + Tween
Klebsiella pneumoniae	511	LB; Actinomyces; TSB; NYC III; BHI + Tween; MRS + Tween
Klebsiella pneumoniae	539	LB; Actinomyces; TSB; NYC III; BHI + Tween; MRS + Tween
Klebsiella pneumoniae	541	NYC III
Klebsiella pneumoniae	542	BHI + Tween
Klebsiella pneumoniae	543	LB; Actinomyces; TSB; NYC III; MRS + Tween
Klebsiella pneumoniae	564	TSB; NYC III; MRS + Tween
Klebsiella pneumoniae	610	BHI + Tween
Klebsiella pneumoniae	610	LB; Actinomyces; TSB; BYC III; BHI + Tween

Klebsiella pneumoniae	611	LB; Actinomyces; TSB; NYC III; BHI + Tween; MRS + Tween
Klebsiella pneumoniae	613	TSB
Klebsiella pneumoniae	795	LB
Klebsiella pneumoniae	853	LB; Actinomyces; TSB; NYC III; BHI + Tween; MRS + Tween
Kocuria marina	327	TSB
Kocuria palustris	513	BHI + Tween
Lactobacillus crispatus	346	NYC III; BHI + Tween; MRS + Tween
Lactobacillus crispatus	452	BHI + Tween
Lactobacillus delbrueckii	521	TSB
Lactobacillus delbrueckii	613	LB; Actinomyces; NYC III; BHI + Tween; MRS + Tween
Lactobacillus delbrueckii	614	MRS + Tween
Lactobacillus gasseri	276	BHI + Tween; MRS + Tween
Lactobacillus gasseri	286	LB; Actinomyces; TSB; NYC III; BHI + Tween; MRS + Tween
Lactobacillus gasseri	296	NYC III
Lactobacillus gasseri	323	LB; Actinomyces; NYC III; BHI + Tween; MRS + Tween
Lactobacillus gasseri	351	LB; Actinomyces; TSB; NYC III; MRS + Tween
Lactobacillus gasseri	378	Actinomyces; TSB; NYC III; BHI + Tween; MRS + Tween
Lactobacillus gasseri	412	Actinomyces
Lactobacillus gasseri	421	LB; Actinomyces; TSB; BHI + Tween
Lactobacillus gasseri	483	BHI + Tween
Lactobacillus gasseri	516	MRS + Tween
Lactobacillus gasseri	519	BHI + Tween; MRS + Tween
Lactobacillus gasseri	521	MRS + Tween
Lactobacillus gasseri	528	LB; Actinomyces; TSB; BHI + Tween; MRS + Tween
Lactobacillus jensenii	446	BHI + Tween
Lactobacillus jensenii	482	BHI + Tween
Lactobacillus reuteri	343	Actinomyces; TSB; MRS + Tween
Lactobacillus rhamnosus	285	LB; Actinomyces; TSB; NYC III; BHI + Tween; MRS + Tween
Lactobacillus rhamnosus	322	LB; Actinomyces; TSB; NYC III; BHI + Tween; MRS + Tween
Lactobacillus rhamnosus	390	BHI + Tween
Lactobacillus rhamnosus	448	BHI + Tween; MRS + Tween

Lactobacillus rhamnosus	520	LB; TSB; NYC III; BHI + Tween
Micrococcus aloeverae	330	NYC III
Micrococcus yunnanensis	331	LB; NYC III
Micrococcus yunnanensis	435	NYC III
Micrococcus yunnanensis	462	NYC III
Micrococcus yunnanensis	505	NYC III
Micrococcus yunnanensis	535	TSB; NYC III
Morganella morganii	629	LB; Actinomyces; TSB; NYC III; BHI + Tween
Paenibacillus pabuli	446	LB
Paenibacillus taichungensis	273	Actinomyces
Paenibacillus taiwanensis	434	NYC III
Pantoea eucrina	535	NYC III
Proteus mirabilis	267	LB; Actinomyces; TSB; NYC III; BHI + Tween; MRS + Tween
Proteus mirabilis	269	TSB
Proteus mirabilis	420	BHI + Tween
Proteus mirabilis	514	LB; Actinomyces; TSB; BHI + Tween
Proteus mirabilis	544	LB; Actinomyces; TSB; NYC III; BHI + Tween
Proteus mirabilis	545	LB; Actinomyces; TSB; NYC III; BHI + Tween
Proteus mirabilis	564	LB
Proteus mirabilis	577	LB; Actinomyces; NYC III; BHI + Tween
Proteus mirabilis	593	LB; Actinomyces; TSB; NYC III; BHI + Tween; MRS + Tween
Proteus mirabilis	602	LB; Actinomyces; TSB; NYC III; BHI + Tween
Proteus mirabilis	624	LB; Actinomyces; TSB; NYC III; BHI + Tween
Proteus mirabilis	630	BHI + Tween
Proteus mirabilis	815	LB; TSB; NYC III; MRS + Tween
Pseudoclavibacter alba	430	LB; TSB; NYC III; BHI + Tween
Pseudomonas aeruginosa	537	TSB; NYC III; BHI + Tween; MRS + Tween
Pseudomonas oryzihabitans	331	TSB
Rothia mucilaginosa	336	Actinomyces; TSB; NYC III; BHI + Tween
Shigella boydii	537	LB
Shigella boydii	604	BHI + Tween; MRS + Tween
Shigella boydii	617	LB; Actinomyces
Shigella flexneri	377	LB; Actinomyces; MRS + Tween
Sphingomonas aeria	451	LB

Sphingomonas aeria	452	LB
Staphylococcus aureus	268	LB; Actinomyces; TSB; NYC III; BHI + Tween; MRS + Tween
Staphylococcus aureus	270	BHI + Tween
Staphylococcus aureus	273	Actinomyces; TSB; NYC III; BHI + Tween; MRS + Tween
Staphylococcus aureus	337	Actinomyces; NYC III
Staphylococcus aureus	338	BHI + Tween
Staphylococcus aureus	345	Actinomyces; TSB; NYC III; BHI + Tween
Staphylococcus aureus	346	LB; Actinomyces; TSB; NYC III
Staphylococcus aureus	481	LB
Staphylococcus aureus	537	TSB; NYC III; BHI + Tween; MRS + Tween
Staphylococcus capitis	327	NYC III
Staphylococcus capitis	339	LB; BHI + Tween
Staphylococcus capitis	371	LB; BHI + Tween
Staphylococcus capitis	373	BHI + Tween
Staphylococcus capitis	443	LB; TSB; NYC III; MRS + Tween
Staphylococcus capitis	444	LB
Staphylococcus capitis	445	NYC III
Staphylococcus capitis	446	Actinomyces
Staphylococcus capitis	477	Actinomyces
Staphylococcus capitis	483	TSB
Staphylococcus capitis	535	TSB
Staphylococcus capitis	746	Actinomyces
Staphylococcus condimenti	298	TSB
Staphylococcus condimenti	462	TSB
Staphylococcus epidermidis	283	TSB; MRS + Tween
Staphylococcus epidermidis	287	BHI + Tween
Staphylococcus epidermidis	295	LB; Actinomyces; TSB; NYC III; BHI + Tween; MRS + Tween
Staphylococcus epidermidis	298	LB; Actinomyces
Staphylococcus epidermidis	306	Actinomyces; TSB; NYC III; BHI + Tween; MRS + Tween
Staphylococcus epidermidis	312	NYC III

Staphylococcus epidermidis	313	LB
Staphylococcus epidermidis	331	NYC III
Staphylococcus epidermidis	344	MRS + Tween
Staphylococcus epidermidis	371	TSB; NYC III; BHI + Tween; MRS + Tween
Staphylococcus epidermidis	372	MRS + Tween
Staphylococcus epidermidis	411	LB; Actinomyces; TSB; NYC III; BHI + Tween; MRS + Tween
Staphylococcus epidermidis	415	LB; Actinomyces; TSB; NYC III; BHI + Tween; MRS + Tween
Staphylococcus epidermidis	418	Actinomyces; TSB; NYC III; BHI + Tween; MRS + Tween
Staphylococcus epidermidis	421	NYC III
Staphylococcus epidermidis	434	NYC III
Staphylococcus epidermidis	434	LB; Actinomyces; TSB; NYC III; BHI + Tween; MRS + Tween
Staphylococcus epidermidis	437	LB; Actinomyces; TSB; NYC III; BHI + Tween; MRS + Tween
Staphylococcus epidermidis	443	Actinomyces; BHI + Tween
Staphylococcus epidermidis	452	TSB; NYC III
Staphylococcus epidermidis	476	TSB; NYC III
Staphylococcus epidermidis	478	TSB; NYC III
Staphylococcus epidermidis	502	TSB
Staphylococcus epidermidis	512	LB; Actinomyces; TSB; NYC III; BHI + Tween; MRS + Tween
Staphylococcus epidermidis	516	Actinomyces; TSB; NYC III; BHI + Tween; MRS + Tween
Staphylococcus epidermidis	528	NYC III
Staphylococcus epidermidis	545	Actinomyces; TSB; NYC III
Staphylococcus epidermidis	614	LB; Actinomyces; TSB; NYC III; BHI + Tween; MRS + Tween

Staphylococcus epidermidis	614	MRS + Tween
Staphylococcus haemolyticus	298	NYC III
Staphylococcus haemolyticus	309	LB
Staphylococcus haemolyticus	330	LB; NYC III
Staphylococcus haemolyticus	521	LB; Actinomyces; TSB; NYC III; BHI + Tween
Staphylococcus haemolyticus	543	NYC III
Staphylococcus hominis	298	BHI + Tween
Staphylococcus hominis	313	TSB
Staphylococcus hominis	327	TSB; BHI + Tween
Staphylococcus hominis	435	NYC III
Staphylococcus hominis	462	NYC III
Staphylococcus hominis	504	MRS + Tween
Staphylococcus hominis	513	TSB
Staphylococcus hominis	516	TSB
Staphylococcus hominis	535	NYC III
Staphylococcus hominis	563	TSB
Staphylococcus hominis	854	LB; Actinomyces; TSB; BHI + Tween
Staphylococcus pasteuri	378	TSB
Staphylococcus pasteuri	531	BHI + Tween
Staphylococcus saccharolyticus	378	TSB
Staphylococcus warneri	435	Actinomyces; TSB; BHI + Tween
Staphylococcus warneri	436	LB; Actinomyces; TSB; NYC III; BHI + Tween; MRS + Tween
Staphylococcus warneri	437	TSB
Staphylococcus warneri	451	BHI + Tween
Streptococcus agalactiae	270	LB; Actinomyces; TSB; NYC III; BHI + Tween; MRS + Tween
Streptococcus agalactiae	329	LB; TSB; BHI + Tween
Streptococcus agalactiae	332	LB; TSB; BHI + Tween
Streptococcus agalactiae	444	TSB; NYC III; BHI + Tween
Streptococcus anginosus	269	LB; TSB; BHI + Tween; MRS + Tween
Streptococcus anginosus	368	MRS + Tween
Streptococcus anginosus	437	Actinomyces; BHI + Tween

Streptococcus anginosus	502	LB
Streptococcus anginosus	532	Actinomyces; NYC III; BHI + Tween; MRS + Tween
Streptococcus oralis	330	Actinomyces
Streptococcus pasteurianus	298	NYC III; BHI + Tween

APPENDIX C

RELATIVE CFU COUNTS

Sample	CFU (x10^7)
EC283	9.15
EC283	44.6
EC283	429.58
EC359	28.13
EC359	33.76
EC359	33.76
EC 527	42.11
EC 527	46.78
EC 527	0.44
EC 529	30.5
EC 529	1.53
EC 529	7.63
EC541	95.83
EC541	106.25
EC542	33.81
EC542	49.41
EC542	35.11
EC564	64.2
EC564	29.63
EC564	138.27
EC564	16.79
EC564	19.75
EC564	57.28
KP306	239.47
KP306	76.05
KP306	292.11
KP344	60
KP344	137.14
KP344	68.57
KP359	40.25
KP359	228.93
KP359	35.22
KP 510	31.38
KP 510	19.97
KP 510	57.06
KP 511	26.05
KP 511	31.84

KP 511	23.15
KP541	89.52
KP541	130.21
KP541	61.04
KP542	35.8
KP542	33.41
KP542	26.25
KP564	29.41
KP564	127.45
KP564	49.02
KP564	7.84
KP564	14.71
KP564	53.92
PM564	278.22
PM564	58.79
PM564	26.77
PM564	62.99
PM564	57.74
PM564	38.32
SE283	10.96
SE283	65.79
SE283	20.18
SE306	122.45
SE306	81.63
SE306	73.98
SE344	17.32
SE344	139.66
SE344	184.36
EC283+KP359	13.29
EC283+KP359	37.65
EC283+KP359	44.3
EC283+SE283	4.66
EC283+SE283	8.15
EC283+SE283	9.31
KP306+SE344	76.73
KP306+SE344	3175.02
KP306+SE344	1984.39
KP306+SE306	1275.92
KP306+SE306	7.18

KP306+SE306	46.78
KP344+SE306	1622.08
KP344+SE306	45.53
KP344+SE306	256.12
KP344+SE344	98.87
KP344+SE344	149.72
KP344+SE344	93.22
EC359+SE283	47.2
EC359+SE283	86.08
EC359+SE283	63.86
EC359+KP359	35.25
EC359+KP359	22.84
EC359+KP359	21.15
EC541+KP542	13.82
EC541+KP542	42.51
EC541+KP542	14.88
EC541+KP541	61.35
EC541+KP541	48.87
EC541+KP541	122.7
KP541+EC542	22.19
KP541+EC542	54.47
KP541+EC542	87.76
EC542+KP542	7.79
EC542+KP542	19.48
EC542+KP542	18.18
EC564+KP564	10.33
EC564+KP564	9.35
EC564+KP564	8.86
EC564+PM564	7.28
EC564+PM564	50.96
EC564+PM564	12.86
EC564+PM564	25.35
EC564+PM564	16.13
EC564+PM564	23.04
KP510+EC527	55.63
KP510+EC527	45.65
KP510+EC527	41.37
KP510+EC529	51.48
KP510+EC529	55.77

KP510+EC529	72.93
KP511+EC527	27.47
KP511+EC527	54.93
KP511+EC527	21.68
KP511+EC529	56.54
KP511+EC529	102.94
KP511+EC529	97.14

APPENDIX D

LINEAR REGRESSION MODELS

Coefficients	Estimate	Std. Error	t value	Pr(>|t|)	Significance
(Intercept)	$5.04*10^{8}$	$1.74*10^{8}$	2.903	0.00442	**
KP306	$1.08*10^{9}$	$7.54*10^{8}$	1.429	0.15566	
SE306	$8.22*10^{9}$	$7.54*10^{8}$	10.905	$< 2*10^{-16}$	***
KP306:SE306	$2.96*10^{9}$	$1.48*10^{9}$	2.004	0.04738	*

Coefficients	Estimate	Std. Error	t value	Pr(>|t|)	Significance
(Intercept)	$9.14*10^{8}$	$2.59*10^{8}$	3.523	0.000609	***
KP344	$7.50*10^{9}$	$1.13*10^{9}$	6.658	$9.22*10^{-10}$	***
SE344	$-4.44*10^{8}$	$1.13*10^{9}$	-0.394	0.694247	
KP344:SE344	$-6.98*10^{9}$	$2.21*10^{9}$	-3.163	0.001989	**

Coefficients	Estimate	Std. Error	t value	Pr(>|t|)	Significance
(Intercept)	$9.61*10^{8}$	$2.70*10^{8}$	3.557	0.000541	***
KP306	$6.62*10^{9}$	$1.17*10^{9}$	5.645	$1.16*10^{-7}$	***
SE344	$-3.80*10^{8}$	$1.17*10^{9}$	-0.324	0.746528	
KP306:SE344	$-6.43*10^{9}$	$2.30*10^{9}$	-2.799	0.005982	**

Coefficients	Estimate	Std. Error	t value	Pr(>|t|)	Significance
(Intercept)	$5.48*10^{8}$	$1.41*10^{8}$	3.902	0.000159	***
KP344	$2.46*10^{8}$	$6.10*10^{8}$	0.404	0.687194	
SE306	$6.44*10^{9}$	$6.10*10^{8}$	10.567	$< 2*10^{-16}$	***
KP344:SE306	$8.98*10^{9}$	$1.20*10^{9}$	7.517	1.19E-11	***

Coefficients	Estimate	Std. Error	t value	Pr(>|t|)	Significance
(Intercept)	1356623847	302351902	4.487	$1.69*10^{-5}$	***
EC541	-890391162	1437364533	-0.619	0.537	
KP541	-798054685	1317921386	-0.606	0.546	
EC541:KP541	945338071	2646226959	0.357	0.722	

Coefficients	Estimate	Std. Error	t value	Pr(>|t|)	Significance
(Intercept)	$1.41*10^{9}$	$3.02*10^{8}$	4.655	$8.56*10^{-6}$	***
EC542	$-1.13*10^{9}$	$1.31*10^{9}$	-0.859	0.392	
KP542	$-1.16*10^{9}$	$1.31*10^{9}$	-0.884	0.379	
EC542:KP542	$9.58*10^{8}$	$2.57*10^{9}$	0.372	0.71	

Coefficients	Estimate	Std. Error	t value	Pr(>|t|)	Significance
(Intercept)	$1.38*10^{9}$	$3.02*10^{8}$	4.559	$1.27*10^{-5}$	***
EC541	$-6.24*10^{8}$	$1.43*10^{9}$	-0.435	0.664	
KP542	$-1.16*10^{9}$	$1.32*10^{9}$	-0.88	0.381	
EC541:KP542	$5.44*10^{8}$	$2.64*10^{9}$	0.206	0.837	

Coefficients	Estimate	Std. Error	t value	Pr(>|t|)	Significance
(Intercept)	$1.38*10^{9}$	$3.03*10^{8}$	4.55	$1.31*10^{-5}$	***
KP541	$-6.25*10^{8}$	$1.32*10^{9}$	-0.475	0.636	
EC542	$-1.17*10^{9}$	$1.32*10^{9}$	-0.89	0.375	
KP541:EC542	$6.39*10^{8}$	$2.58*10^{9}$	0.248	0.805	

Coefficients	Estimate	Std. Error	t value	Pr(>|t|)	Significance
(Intercept)	$1.39*10^{9}$	$3.03*10^{8}$	4.582	$1.15*10^{-5}$	***
EC359	$-9.19*10^{8}$	$1.32*10^{9}$	-0.699	0.486	
KP359	$-1.12*10^{9}$	$1.32*10^{9}$	-0.852	0.396	
EC359:KP359	$1.00*10^{9}$	$2.58*10^{9}$	0.39	0.698	

Coefficients	Estimate	Std. Error	t value	Pr(>|t|)	Significance
(Intercept)	$1.41*10^{9}$	$3.02*10^{8}$	4.666	$8.19*10^{-6}$	***
EC283	$-1.30*10^{9}$	$1.31*10^{9}$	-0.99	0.324	
SE283	$-1.03*10^{9}$	$1.31*10^{9}$	-0.783	0.435	
EC283:SE283	$9.62*10^{8}$	$2.57*10^{9}$	0.374	0.709	

Coefficients	Estimate	Std. Error	t value	Pr(>|t|)	Significance
(Intercept)	$1.40*10^{9}$	$3.03*10^{8}$	4.623	$9.76*10^{-6}$	***
EC359	$-1.08*10^{9}$	$1.31*10^{9}$	-0.824	0.412	
SE283	$-1.32*10^{9}$	$1.31*10^{9}$	-1.006	0.317	
EC359:SE283	$1.66*10^{9}$	$2.57*10^{9}$	0.645	0.52	

Coefficients	Estimate	Std. Error	t value	Pr(>|t|)	Significance
(Intercept)	$1.41*10^{9}$	$3.02*10^{8}$	4.667	$8.15*10^{-6}$	***
KP359	$-1.03*10^{9}$	$1.31*10^{9}$	-0.788	0.432	
EC283	$-1.34*10^{9}$	$1.31*10^{9}$	-1.023	0.309	
KP359:EC283	$1.10*10^{9}$	$2.57*10^{9}$	0.427	0.67	

Coefficients	Estimate	Std. Error	t value	Pr(>|t|)	Significance
(Intercept)	$1.46*10^{9}$	$3.11*10^{8}$	4.712	$6.77*10^{-6}$	***
EC564	$-1.01*10^{9}$	$1.09*10^{9}$	-0.931	0.354	
KP564	$-1.25*10^{9}$	$1.09*10^{9}$	-1.155	0.25	
EC564:KP564	$9.06*10^{8}$	$2.35*10^{9}$	0.386	0.7	

Coefficients	Estimate	Std. Error	t value	Pr(>|t|)	Significance
(Intercept)	$1.31*10^{9}$	$3.12*10^{8}$	4.197	$5.26*10^{-5}$	***
EC564	$-8.47*10^{8}$	$1.09*10^{9}$	-0.776	0.439	
PM564	$6.30*10^{8}$	$1.09*10^{9}$	0.578	0.564	
EC564:PM564	$-1.02*10^{9}$	$2.36*10^{9}$	-0.433	0.666	

Coefficients	Estimate	Std. Error	t value	Pr(>|t|)	Significance
(Intercept)	$1.34*10^{9}$	$3.11*10^{8}$	4.291	$3.66*10^{-5}$	***
KP564	$-1.18*10^{9}$	$1.09*10^{9}$	-1.082	0.282	
PM564	$5.43*10^{8}$	$1.09*10^{9}$	0.499	0.619	
KP564:PM564	$-4.48*10^{8}$	$2.35*10^{9}$	-0.19	0.849	

Coefficients	Estimate	Std. Error	t value	Pr(>|t|)	Significance
(Intercept)	$1.38*10^{9}$	$3.03*10^{8}$	4.555	$1.28*10^{-5}$	***
KP510	$-9.67*10^{8}$	$1.32*10^{9}$	-0.735	0.464	
EC527	$-1.03*10^{9}$	$1.32*10^{9}$	-0.785	0.434	
KP510:EC527	$1.18*10^{9}$	$2.58*10^{9}$	0.456	0.649	

Coefficients	Estimate	Std. Error	t value	Pr(>|t|)	Significance
(Intercept)	$1.39*10^{9}$	$3.03*10^{8}$	4.572	$1.20*10^{-5}$	***
KP511	$-1.12*10^{9}$	$1.32*10^{9}$	-0.85	0.397	
EC529	$-9.76*10^{8}$	$1.32*10^{9}$	-0.742	0.46	
KP511:EC529	$1.27*10^{9}$	$2.58*10^{9}$	0.494	0.622	

Coefficients	Estimate	Std. Error	t value	Pr(>|t|)	Significance
(Intercept)	1376378057	303381158	4.537	$1.38*10^{-5}$	***
KP510	-941305172	1316595000	-0.715	0.476	
EC529	-941161483	1316595000	-0.715	0.476	
KP510:EC529	1020859668	2580226578	0.396	0.693	

Coefficients	Estimate	Std. Error	t value	Pr(>|t|)	Significance
(Intercept)	1381352418	303239759	4.555	$1.28*10^{-5}$	***
EC527	-892671905	1315981365	-0.678	0.499	
KP511	-968395271	1315981365	-0.736	0.463	
EC527:KP511	754370535	2579023994	0.293	0.77	

REFERENCE LIST

1. Agany, D. D. M., Potts, R., Gonzalez Hernandez, J. L., Gnimpieba, E. Z., & Pietri, J. E. (2020). Microbiome Differences between Human Head and Body Lice Ecotypes Revealed by 16S rRNA Gene Amplicon Sequencing. *Journal of Parasitology*, *106*(1), 14–24. https://doi.org/10.1645/19-132

2. Armbruster, C. E., & Mobley, H. L. T. (2012). Merging mythology and morphology: The multifaceted lifestyle of Proteus mirabilis. *Nature Reviews. Microbiology*, *10*(11), 743–754. https://doi.org/10.1038/nrmicro2890

3. Bankevich, A., Nurk, S., Antipov, D., Gurevich, A. A., Dvorkin, M., Kulikov, A. S., Lesin, V. M., Nikolenko, S. I., Pham, S., Prjibelski, A. D., Pyshkin, A. V., Sirotkin, A. V., Vyahhi, N., Tesler, G., Alekseyev, M. A., & Pevzner, P. A. (2012). SPAdes: A New Genome Assembly Algorithm and Its Applications to Single-Cell Sequencing. *Journal of Computational Biology*, *19*(5), 455–477. https://doi.org/10.1089/cmb.2012.0021

4. Benson, D. A., Karsch-Mizrachi, I., Lipman, D. J., Ostell, J., & Sayers, E. W. (2010). GenBank. *Nucleic Acids Research*, *38*(Database issue), D46–D51. https://doi.org/10.1093/nar/gkp1024

5. Bokulich, N. A., Chung, J., Battaglia, T., Henderson, N., Jay, M., Li, H., Lieber, A., Wu, F., Perez-Perez, G. I., Chen, Y., Schweizer, W., Zheng, X., Contreras, M., Dominguez-Bello, M. G., & Blaser, M. J. (2016). Antibiotics, birth mode, and diet shape microbiome maturation during early life. *Science Translational Medicine*, *8*(343), 343ra82. https://doi.org/10.1126/scitranslmed.aad7121

6. Boyt, M. A. (2005). Teachers' Knowledge of Normal and Abnormal Elimination Patterns in Elementary School Children. *The Journal of School Nursing*, *21*(6), 346–349. https://doi.org/10.1177/10598405050210060801

7. Brubaker, L., Gourdine, J.-P. F., Siddiqui, N. Y., Holland, A., Halverson, T., Limeria, R., Pride, D., Ackerman, L., Forster, C. S., Jacobs, K. M., Thomas-White, K. J., Putonti, C., Dong, Q., Weinstein, M., Lukacz, E. S., Karstens, L., & Wolfe, A. J. (2021). Forming Consensus To Advance Urobiome Research. *MSystems*, *6*(4), e0137120. https://doi.org/10.1128/mSystems.01371-20

8. Camenzind, T., Hättenschwiler, S., Treseder, K. K., Lehmann, A., & Rillig, M. C. (2018). Nutrient limitation of soil microbial processes in tropical forests. *Ecological Monographs*, *88*(1), 4–21. https://doi.org/10.1002/ecm.1279

9. Chakravorty, S., Helb, D., Burday, M., Connell, N., & Alland, D. (2007). A detailed analysis of 16S ribosomal RNA gene segments for the diagnosis of pathogenic bacteria. *Journal of Microbiological Methods*, *69*(2), 330–339. https://doi.org/10.1016/j.mimet.2007.02.005

10. Chen, S. L., Hung, C.-S., Xu, J., Reigstad, C. S., Magrini, V., Sabo, A., Blasiar, D., Bieri, T., Meyer, R. R., Ozersky, P., Armstrong, J. R., Fulton, R. S., Latreille, J. P., Spieth, J., Hooton, T. M., Mardis, E. R., Hultgren, S. J., & Gordon, J. I. (2006). Identification of genes subject to positive selection in uropathogenic strains of Escherichia coli: A comparative genomics approach. *Proceedings of the National Academy of Sciences of the United States of America*, *103*(15), 5977–5982. https://doi.org/10.1073/pnas.0600938103

11. Chen, Y.-T., Liao, T.-L., Wu, K.-M., Lauderdale, T.-L., Yan, J.-J., Huang, I.-W., Lu, M.-C., Lai, Y.-C., Liu, Y.-M., Shu, H.-Y., Wang, J.-T., Su, I.-J., & Tsai, S.-F. (2009). Genomic diversity of citrate fermentation in Klebsiella pneumoniae. *BMC Microbiology*, *9*(1), 168. https://doi.org/10.1186/1471-2180-9-168

12. Conlan, S., Kong, H. H., & Segre, J. A. (2012a). Species-Level Analysis of DNA Sequence Data from the NIH Human Microbiome Project. *PLoS ONE*, *7*(10), e47075. https://doi.org/10.1371/journal.pone.0047075

13. Conlan, S., Kong, H. H., & Segre, J. A. (2012b). Species-Level Analysis of DNA Sequence Data from the NIH Human Microbiome Project. *PLOS ONE*, *7*(10), e47075. https://doi.org/10.1371/journal.pone.0047075

14. Coorevits, L., Heytens, S., Boelens, J., & Claeys, G. (2017). The resident microflora of voided midstream urine of healthy controls: Standard versus expanded urine culture protocols. *European Journal of Clinical Microbiology & Infectious Diseases*, *36*(4), 635–639. https://doi.org/10.1007/s10096-016-2839-x

15. Corbett, D., Wise, A., Langley, T., Skinner, K., Trimby, E., Birchall, S., Dorali, A., Sandiford, S., Williams, J., Warn, P., Vaara, M., & Lister, T. (2017). Potentiation of Antibiotic Activity by a Novel Cationic Peptide: Potency and Spectrum of Activity of SPR741. *Antimicrobial Agents and Chemotherapy*, *61*(8), e00200-17. https://doi.org/10.1128/AAC.00200-17

16. Cosseau, C., Devine, D. A., Dullaghan, E., Gardy, J. L., Chikatamarla, A., Gellatly, S., Yu, L. L., Pistolic, J., Falsafi, R., Tagg, J., & Hancock, R. E. W. (2008). The Commensal Streptococcus salivarius K12 Downregulates the Innate Immune Responses of Human

Epithelial Cells and Promotes Host-Microbe Homeostasis. *Infection and Immunity*, *76*(9), 4163–4175. https://doi.org/10.1128/IAI.00188-08

17. Coyne, K. S., Wein, A. J., Tubaro, A., Sexton, C. C., Thompson, C. L., Kopp, Z. S., & Aiyer, L. P. (2009). The burden of lower urinary tract symptoms: Evaluating the effect of LUTS on health-related quality of life, anxiety and depression: EpiLUTS. *BJU International*, *103*(s3), 4–11. https://doi.org/10.1111/j.1464-410X.2009.08371.x

18. Davenport, E. R., Mizrahi-Man, O., Michelini, K., Barreiro, L. B., Ober, C., & Gilad, Y. (2014). Seasonal Variation in Human Gut Microbiome Composition. *PLoS ONE*, *9*(3), e90731. https://doi.org/10.1371/journal.pone.0090731

19. Davis, J. J., Wattam, A. R., Aziz, R. K., Brettin, T., Butler, R., Butler, R. M., Chlenski, P., Conrad, N., Dickerman, A., Dietrich, E. M., Gabbard, J. L., Gerdes, S., Guard, A., Kenyon, R. W., Machi, D., Mao, C., Murphy-Olson, D., Nguyen, M., Nordberg, E. K., … Stevens, R. (2020). The PATRIC Bioinformatics Resource Center: Expanding data and analysis capabilities. *Nucleic Acids Research*, *48*(D1), D606–D612. https://doi.org/10.1093/nar/gkz943

20. De Angelis, M., Siragusa, S., Vacca, M., Di Cagno, R., Cristofori, F., Schwarm, M., Pelzer, S., Flügel, M., Speckmann, B., Francavilla, R., & Gobbetti, M. (2021). Selection of Gut-Resistant Bacteria and Construction of Microbial Consortia for Improving Gluten Digestion under Simulated Gastrointestinal Conditions. *Nutrients*, *13*(3), 992. https://doi.org/10.3390/nu13030992

21. DiGiulio, D. B., Callahan, B. J., McMurdie, P. J., Costello, E. K., Lyell, D. J., Robaczewska, A., Sun, C. L., Goltsman, D. S. A., Wong, R. J., Shaw, G., Stevenson, D. K., Holmes, S. P., & Relman, D. A. (2015). Temporal and spatial variation of the human microbiota during pregnancy. *Proceedings of the National Academy of Sciences of the United States of America*, *112*(35), 11060–11065. https://doi.org/10.1073/pnas.1502875112

22. Dixit, K., Davray, D., Chaudhari, D., Kadam, P., Kshirsagar, R., Shouche, Y., Dhotre, D., & Saroj, S. D. (2021). Benchmarking of 16S rRNA gene databases using known strain sequences. *Bioinformation*, *17*(3), 377–391. https://doi.org/10.6026/97320630017377

23. Dong, Q., Nelson, D. E., Toh, E., Diao, L., Gao, X., Fortenberry, J. D., & Van Der Pol, B. (2011). The Microbial Communities in Male First Catch Urine Are Highly Similar to Those in Paired Urethral Swab Specimens. *PLoS ONE*, *6*(5), e19709. https://doi.org/10.1371/journal.pone.0019709

24. Ezraty, B., Henry, C., Hérisse, M., Denamur, E., & Barras, F. (2014). Commercial Lysogeny Broth culture media and oxidative stress: A cautious tale. *Free Radical Biology and Medicine*, *74*, 245–251. https://doi.org/10.1016/j.freeradbiomed.2014.07.010

25. Fouts, D. E., Pieper, R., Szpakowski, S., Pohl, H., Knoblach, S., Suh, M.-J., Huang, S.-T., Ljungberg, I., Sprague, B. M., Lucas, S. K., Torralba, M., Nelson, K. E., & Groah, S. L. (2012). 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. *Journal of Translational Medicine*, *10*(1), 174. https://doi.org/10.1186/1479-5876-10-174

26. Greenblum, S., Carr, R., & Borenstein, E. (2015). Extensive Strain-Level Copy-Number Variation across Human Gut Microbiome Species. *Cell*, *160*(4), 583–594. https://doi.org/10.1016/j.cell.2014.12.038

27. Gu, W., Crawford, E. D., O'Donovan, B. D., Wilson, M. R., Chow, E. D., Retallack, H., & DeRisi, J. L. (2016). Depletion of Abundant Sequences by Hybridization (DASH): Using Cas9 to remove unwanted high-abundance species in sequencing libraries and molecular counting applications. *Genome Biology*, *17*, 41. https://doi.org/10.1186/s13059-016-0904-5

28. Gunzburg, W. H., Aung, M. M., Toa, P., Ng, S., Read, E., Tan, W. J., Brandtner, E. M., Dangerfield, J., & Salmons, B. (2020). Efficient protection of microorganisms for delivery to the intestinal tract by cellulose sulphate encapsulation. *Microbial Cell Factories*, *19*(1), 216. https://doi.org/10.1186/s12934-020-01465-3

29. Hashemi, S., Han, M., & Kim, T. (2016). Optimization of fertilization characteristics of urine by addition of Nitrosomonas europaea bio-seed. *Journal of the Science of Food and Agriculture*, *96*(13), 4416–4422. https://doi.org/10.1002/jsfa.7652

30. Hilt, E. E., McKinley, K., Pearce, M. M., Rosenfeld, A. B., Zilliox, M. J., Mueller, E. R., Brubaker, L., Gai, X., Wolfe, A. J., & Schreckenberger, P. C. (2014). Urine is not sterile: Use of enhanced urine culture techniques to detect resident bacterial flora in the adult female bladder. *Journal of Clinical Microbiology*, *52*(3), 871–876. https://doi.org/10.1128/JCM.02876-13

31. Hoffman, C., Siddiqui, N. Y., Fields, I., Gregory, W. T., Simon, H. M., Mooney, M. A., Wolfe, A. J., & Karstens, L. (2021). Species-Level Resolution of Female Bladder Microbiota from 16S rRNA Amplicon Sequencing. *MSystems*, *6*(5), e0051821. https://doi.org/10.1128/mSystems.00518-21

32. Huang, C.-H., Wang, C.-L., Liou, J.-S., Lee, A.-Y., Blom, J., Huang, L., & Watanabe, K. (2019). Reclassification of Micrococcus aloeverae and Micrococcus yunnanensis as later heterotypic synonyms of Micrococcus luteus. *International Journal of Systematic and Evolutionary Microbiology*, *69*(11), 3512–3518. https://doi.org/10.1099/ijsem.0.003654

33. Huse, S. M., Ye, Y., Zhou, Y., & Fodor, A. A. (2012). A Core Human Microbiome as Viewed through 16S rRNA Sequence Clusters. *PLOS ONE*, *7*(6), e34242. https://doi.org/10.1371/journal.pone.0034242

34. Imirzalioglu, C., Hain, T., Chakraborty, T., & Domann, E. (2008). Hidden pathogens uncovered: Metagenomic analysis of urinary tract infections. *Andrologia*, *40*(2), 66–71. https://doi.org/10.1111/j.1439-0272.2007.00830.x

35. Irwin, D. E., Kopp, Z. S., Agatep, B., Milsom, I., & Abrams, P. (2011). Worldwide prevalence estimates of lower urinary tract symptoms, overactive bladder, urinary incontinence and bladder outlet obstruction. *BJU International*, *108*(7), 1132–1138. https://doi.org/10.1111/j.1464-410X.2010.09993.x

36. Irwin, D. E., Milsom, I., Hunskaar, S., Reilly, K., Kopp, Z., Herschorn, S., Coyne, K., Kelleher, C., Hampel, C., Artibani, W., & Abrams, P. (2006). Population-Based Survey of Urinary Incontinence, Overactive Bladder, and Other Lower Urinary Tract Symptoms in Five Countries: Results of the EPIC Study. *European Urology*, *50*(6), 1306–1315. https://doi.org/10.1016/j.eururo.2006.09.019

37. Jacobsen, S. M., Stickler, D. J., Mobley, H. L. T., & Shirtliff, M. E. (2008). Complicated catheter-associated urinary tract infections due to Escherichia coli and Proteus mirabilis. *Clinical Microbiology Reviews*, *21*(1), 26–59. https://doi.org/10.1128/CMR.00019-07

38. Jay, Z. J., & Inskeep, W. P. (2015). The distribution, diversity, and importance of 16S rRNA gene introns in the order Thermoproteales. *Biology Direct*, *10*(1), 35. https://doi.org/10.1186/s13062-015-0065-6

39. Jumpstart Consortium Human Microbiome Project Data Generation Working Group. (2012). Evaluation of 16S rDNA-Based Community Profiling for Human Microbiome Research. *PLoS ONE*, *7*(6), e39315. https://doi.org/10.1371/journal.pone.0039315

40. Kammili, N., Rani, M., Styczynski, A., latha, M., Pavuluri, P. R., Reddy, V., & Alsan, M. (2020). Plasmid-mediated antibiotic resistance among uropathogens in primigravid women—Hyderabad, India. *PLOS ONE*, *15*(5), e0232710. https://doi.org/10.1371/journal.pone.0232710

41. Kanehisa, M. (2019). Toward understanding the origin and evolution of cellular organisms. *Protein Science: A Publication of the Protein Society*, *28*(11), 1947–1951. https://doi.org/10.1002/pro.3715

42. Kanehisa, M., Furumichi, M., Sato, Y., Ishiguro-Watanabe, M., & Tanabe, M. (2021). KEGG: Integrating viruses and cellular organisms. *Nucleic Acids Research*, *49*(D1), D545–D551. https://doi.org/10.1093/nar/gkaa970

43. Kanehisa, M., & Goto, S. (2000). KEGG: Kyoto encyclopedia of genes and genomes. *Nucleic Acids Research*, *28*(1), 27–30. https://doi.org/10.1093/nar/28.1.27

44. Karstens, L., Asquith, M., Davin, S., Stauffer, P., Fair, D., Gregory, W. T., Rosenbaum, J. T., McWeeney, S. K., & Nardos, R. (2016). Does the Urinary Microbiome Play a Role in Urgency Urinary Incontinence and Its Severity? *Frontiers in Cellular and Infection Microbiology*, *6*, 78. https://doi.org/10.3389/fcimb.2016.00078

45. Katoh, K., & Standley, D. M. (2013). MAFFT Multiple Sequence Alignment Software Version 7: Improvements in Performance and Usability. *Molecular Biology and Evolution*, *30*(4), 772–780. https://doi.org/10.1093/molbev/mst010

46. Khasriya, R., Sathiananthamoorthy, S., Ismail, S., Kelsey, M., Wilson, M., Rohn, J. L., & Malone-Lee, J. (2013). Spectrum of Bacterial Colonization Associated with Urothelial Cells from Patients with Chronic Lower Urinary Tract Symptoms. *Journal of Clinical Microbiology*, *51*(7), 2054–2062. https://doi.org/10.1128/JCM.03314-12

47. Koeth, R. A., Levison, B. S., Culley, M. K., Buffa, J. A., Wang, Z., Gregory, J. C., Org, E., Wu, Y., Li, L., Smith, J. D., Tang, W. H. W., DiDonato, J. A., Lusis, A. J., & Hazen, S. L. (2014). γ-Butyrobetaine Is a Proatherogenic Intermediate in Gut Microbial Metabolism of L-Carnitine to TMAO. *Cell Metabolism*, *20*(5), 799–812. https://doi.org/10.1016/j.cmet.2014.10.006

48. Kolde, R., Franzosa, E. A., Rahnavard, G., Hall, A. B., Vlamakis, H., Stevens, C., Daly, M. J., Xavier, R. J., & Huttenhower, C. (2018). Host genetic variation and its microbiome interactions within the Human Microbiome Project. *Genome Medicine*, *10*(1), 6. https://doi.org/10.1186/s13073-018-0515-8

49. Komesu, Y. M., Richter, H. E., Carper, B., Dinwiddie, D. L., Lukacz, E. S., Siddiqui, N. Y., Sung, V. W., Zyczynski, H. M., Ridgeway, B., Rogers, R. G., Arya, L. A., Mazloomdoost, D., Gantz, M. G., & Pelvic Floor Disorders Network. (2018). The urinary microbiome in women with mixed urinary incontinence compared to similarly aged controls. *International Urogynecology Journal*, *29*(12), 1785–1795. https://doi.org/10.1007/s00192-018-3683-6

50. Krych, L., Hansen, C. H. F., Hansen, A. K., van den Berg, F. W. J., & Nielsen, D. S. (2013). Quantitatively Different, yet Qualitatively Alike: A Meta-Analysis of the Mouse Core Gut Microbiome with a View towards the Human Gut Microbiome. *PLoS ONE*, *8*(5), e62578. https://doi.org/10.1371/journal.pone.0062578

51. Letunic, I., & Bork, P. (2021). Interactive Tree Of Life (iTOL) v5: An online tool for phylogenetic tree display and annotation. *Nucleic Acids Research*, *49*(W1), W293–W296. https://doi.org/10.1093/nar/gkab301

52. Liu, Q., & Bender, R. A. (2007). Complex Regulation of Urease Formation from the Two Promoters of the *ure* Operon of *Klebsiella pneumoniae*. *Journal of Bacteriology*, *189*(21), 7593–7599. https://doi.org/10.1128/JB.01096-06

53. Locey, K. J., & Lennon, J. T. (2016). Scaling laws predict global microbial diversity. *Proceedings of the National Academy of Sciences*, *113*(21), 5970–5975. https://doi.org/10.1073/pnas.1521291113

54. Lohsiriwat, S., Hirunsai, M., & Chaiyaprasithi, B. (2011). Effect of caffeine on bladder function in patients with overactive bladder symptoms. *Urology Annals*, *3*(1), 14–18. https://doi.org/10.4103/0974-7796.75862

55. Louca, S., Doebeli, M., & Parfrey, L. W. (2018). Correcting for 16S rRNA gene copy numbers in microbiome surveys remains an unsolved problem. *Microbiome*, *6*(1), 41. https://doi.org/10.1186/s40168-018-0420-9

56. Lozano, G. L., Bravo, J. I., Diago, M. F. G., Park, H. B., Hurley, A., Peterson, S. B., Stabb, E. V., Crawford, J. M., Broderick, N. A., & Handelsman, J. (2019). Introducing THOR, a Model Microbiome for Genetic Dissection of Community Behavior. *MBio*. https://doi.org/10.1128/mBio.02846-18

57. Magliano, E., Grazioli, V., Deflorio, L., Leuci, A. I., Mattina, R., Romano, P., & Cocuzza, C. E. (2012). Gender and Age-Dependent Etiology of Community-Acquired Urinary Tract Infections. *The Scientific World Journal*, *2012*, 349597. https://doi.org/10.1100/2012/349597

58. Marchesi, J. R., Sato, T., Weightman, A. J., Martin, T. A., Fry, J. C., Hiom, S. J., Dymock, D., & Wade, W. G. (1998). Design and evaluation of useful bacterium-specific PCR primers that amplify genes coding for bacterial 16S rRNA. *Applied and Environmental Microbiology*, *64*(2), 795–799. https://doi.org/10.1128/AEM.64.2.795-799.1998

59. Maserejian, N. N., Chen, S., Chiu, G. R., Wager, C. G., Kupelian, V., Araujo, A. B., & McKinlay, J. B. (2013). Incidence of Lower Urinary Tract Symptoms in a Population-Based Study of Men and Women. *Urology*, *82*(3), 560–564. https://doi.org/10.1016/j.urology.2013.05.009

60. McDonald, D., Price, M. N., Goodrich, J., Nawrocki, E. P., DeSantis, T. Z., Probst, A., Andersen, G. L., Knight, R., & Hugenholtz, P. (2012). An improved Greengenes taxonomy with explicit ranks for ecological and evolutionary analyses of bacteria and archaea. *The ISME Journal*, *6*(3), Article 3. https://doi.org/10.1038/ismej.2011.139

61. Mizrahi-Man, O., Davenport, E. R., & Gilad, Y. (2013). Taxonomic Classification of Bacterial 16S rRNA Genes Using Short Sequencing Reads: Evaluation of Effective Study Designs. *PLoS ONE*, *8*(1), e53608. https://doi.org/10.1371/journal.pone.0053608

62. Momose, Y., Hirayama, K., & Itoh, K. (2008). Competition for proline between indigenous Escherichia coli and E. coli O157:H7 in gnotobiotic mice associated with infant intestinal microbiota and its contribution to the colonization resistance against E. coli O157:H7. *Antonie van Leeuwenhoek*, *94*(2), 165–171. https://doi.org/10.1007/s10482-008-9222-6

63. Muok, A. R., & Briegel, A. (2021). Intermicrobial Hitchhiking: How Nonmotile Microbes Leverage Communal Motility. *Trends in Microbiology*, *29*(6), 542–550. https://doi.org/10.1016/j.tim.2020.10.005

64. Mushenheim, P. C., Trivedi, R. R., Weibel, D. B., & Abbott, N. L. (2014). Using Liquid Crystals to Reveal How Mechanical Anisotropy Changes Interfacial Behaviors of Motile Bacteria. *Biophysical Journal*, *107*(1), 255–265. https://doi.org/10.1016/j.bpj.2014.04.047

65. Nelson, D. E., Van Der Pol, B., Dong, Q., Revanna, K. V., Fan, B., Easwaran, S., Sodergren, E., Weinstock, G. M., Diao, L., & Fortenberry, J. D. (2010). Characteristic Male Urine Microbiomes Associate with Asymptomatic Sexually Transmitted Infection. *PLoS ONE*, *5*(11), e14116. https://doi.org/10.1371/journal.pone.0014116

66. Neugent, M. L., Hulyalkar, N. V., Nguyen, V. H., Zimmern, P. E., & De Nisco, N. J. (2020). Advances in Understanding the Human Urinary Microbiome and Its Potential Role in Urinary Tract Infection. *MBio*, *11*(2), e00218-20. https://doi.org/10.1128/mBio.00218-20

67. Park, S.-C., & Won, S. (2018). Evaluation of 16S rRNA Databases for Taxonomic Assignments Using a Mock Community. *Genomics & Informatics*, *16*(4), e24. https://doi.org/10.5808/GI.2018.16.4.e24

68. Parks, D. H., Chuvochina, M., Reeves, P. R., Beatson, S. A., & Hugenholtz, P. (2021). *Reclassification of Shigella species as later heterotypic synonyms of Escherichia coli in the Genome Taxonomy Database* (p. 2021.09.22.461432). bioRxiv. https://doi.org/10.1101/2021.09.22.461432

69. Pearce, M. M., Hilt, E. E., Rosenfeld, A. B., Zilliox, M. J., Thomas-White, K., Fok, C., Kliethermes, S., Schreckenberger, P. C., Brubaker, L., Gai, X., & Wolfe, A. J. (2014). The female urinary microbiome: A comparison of women with and without urgency urinary incontinence. *MBio*, *5*(4), e01283-01214. https://doi.org/10.1128/mBio.01283-14

70. Pearce, M. M., Zilliox, M. J., Rosenfeld, A. B., Thomas-White, K. J., Richter, H. E., Nager, C. W., Visco, A. G., Nygaard, I. E., Barber, M. D., Schaffer, J., Moalli, P., Sung, V. W., Smith, A. L., Rogers, R., Nolen, T. L., Wallace, D., Meikle, S. F., Gai, X., Wolfe, A. J., … Pelvic Floor Disorders Network. (2015). The female urinary microbiome in urgency urinary incontinence. *American Journal of Obstetrics and Gynecology*, *213*(3), 347.e1-11. https://doi.org/10.1016/j.ajog.2015.07.009

71. Peterson, J., Garges, S., Giovanni, M., McInnes, P., Wang, L., Schloss, J. A., Bonazzi, V., McEwen, J. E., Wetterstrand, K. A., Deal, C., Baker, C. C., Di Francesco, V., Howcroft, T. K., Karp, R. W., Lunsford, R. D., Wellington, C. R., Belachew, T., Wright, M., Giblin, C., … Guyer, M. (2009). The NIH Human Microbiome Project. *Genome Research*, *19*(12), 2317–2323. https://doi.org/10.1101/gr.096651.109

72. Petrova, M. I., Reid, G., & ter Haar, J. A. (2021). Lacticaseibacillus rhamnosus GR-1, a.k.a. Lactobacillus rhamnosus GR-1: Past and Future Perspectives. *Trends in Microbiology*, *29*(8), 747–761. https://doi.org/10.1016/j.tim.2021.03.010

73. Price, M. N., Dehal, P. S., & Arkin, A. P. (2010). FastTree 2 – Approximately Maximum-Likelihood Trees for Large Alignments. *PLoS ONE*, *5*(3), e9490. https://doi.org/10.1371/journal.pone.0009490

74. Price, T. K., Dune, T., Hilt, E. E., Thomas-White, K. J., Kliethermes, S., Brincat, C., Brubaker, L., Wolfe, A. J., Mueller, E. R., & Schreckenberger, P. C. (2016). The Clinical Urine Culture: Enhanced Techniques Improve Detection of Clinically Relevant Microorganisms. *Journal of Clinical Microbiology*, *54*(5), 1216–1222. https://doi.org/10.1128/JCM.00044-16

75. Price, T. K., Hilt, E. E., Thomas-White, K., Mueller, E. R., Wolfe, A. J., & Brubaker, L. (2020). The urobiome of continent adult women: A cross-sectional study. *BJOG: An International Journal of Obstetrics and Gynaecology*, *127*(2), 193–201. https://doi.org/10.1111/1471-0528.15920

76. Price, T. K., Lin, H., Gao, X., Thomas-White, K. J., Hilt, E. E., Mueller, E. R., Wolfe, A. J., Dong, Q., & Brubaker, L. (2020). Bladder bacterial diversity differs in continent and incontinent women: A cross-sectional study. *American Journal of Obstetrics and Gynecology*, *223*(5), 729.e1-729.e10. https://doi.org/10.1016/j.ajog.2020.04.033

77. Price, T. K., Mehrtash, A., Kalesinskas, L., Malki, K., Hilt, E. E., Putonti, C., & Wolfe, A. J. (2016). Genome sequences and annotation of two urinary isolates of E. coli. *Standards in Genomic Sciences*, *11*, 79. https://doi.org/10.1186/s40793-016-0202-6

78. Price, T. K., Wolff, B., Halverson, T., Limeira, R., Brubaker, L., Dong, Q., Mueller, E. R., & Wolfe, A. J. (2020). Temporal Dynamics of the Adult Female Lower Urinary Tract Microbiota. *MBio*, *11*(2), e00475-20. https://doi.org/10.1128/mBio.00475-20

79. Quast, C., Pruesse, E., Yilmaz, P., Gerken, J., Schweer, T., Yarza, P., Peplies, J., & Glöckner, F. O. (2013). The SILVA ribosomal RNA gene database project: Improved data processing and web-based tools. *Nucleic Acids Research*, *41*(D1), D590–D596. https://doi.org/10.1093/nar/gks1219

80. Rauprich, O., Matsushita, M., Weijer, C. J., Siegert, F., Esipov, S. E., & Shapiro, J. A. (1996). Periodic phenomena in Proteus mirabilis swarm colony development. *Journal of Bacteriology*, *178*(22), 6525–6538. https://doi.org/10.1128/jb.178.22.6525-6538.1996

81. Riley, M., Abe, T., Arnaud, M. B., Berlyn, M. K. B., Blattner, F. R., Chaudhuri, R. R., Glasner, J. D., Horiuchi, T., Keseler, I. M., Kosuge, T., Mori, H., Perna, N. T., Plunkett, G., Rudd, K. E., Serres, M. H., Thomas, G. H., Thomson, N. R., Wishart, D., & Wanner, B. L. (2006). Escherichia coli K-12: A cooperatively developed annotation snapshot—2005. *Nucleic Acids Research*, *34*(1), 1–9. https://doi.org/10.1093/nar/gkj405

82. Ritari, J., Salojärvi, J., Lahti, L., & de Vos, W. M. (2015). Improved taxonomic assignment of human intestinal 16S rRNA sequences by a dedicated reference database. *BMC Genomics*, *16*, 1056. https://doi.org/10.1186/s12864-015-2265-y

83. Samad, T., Billings, N., Birjiniuk, A., Crouzier, T., Doyle, P. S., & Ribbeck, K. (2017). Swimming bacteria promote dispersal of non-motile staphylococcal species. *The ISME Journal*, *11*(8), 1933–1937. https://doi.org/10.1038/ismej.2017.23

84. Sathiananthamoorthy, S., Malone-Lee, J., Gill, K., Tymon, A., Nguyen, T. K., Gurung, S., Collins, L., Kupelian, A. S., Swamy, S., Khasriya, R., Spratt, D. A., & Rohn, J. L. (2019). Reassessment of Routine Midstream Culture in Diagnosis of Urinary Tract Infection. *Journal of Clinical Microbiology*, *57*(3), e01452-18. https://doi.org/10.1128/JCM.01452-18

85. Sayers, E. W., Cavanaugh, M., Clark, K., Pruitt, K. D., Schoch, C. L., Sherry, S. T., & Karsch-Mizrachi, I. (2021). GenBank. *Nucleic Acids Research*, *50*(D1), D161–D164. https://doi.org/10.1093/nar/gkab1135

86. Schiffer, L., Azhar, R., Shepherd, L., Ramos, M., Geistlinger, L., Huttenhower, C., Dowd, J. B., Segata, N., & Waldron, L. (2019). HMP16SData: Efficient Access to the Human Microbiome Project Through Bioconductor. *American Journal of Epidemiology*, *188*(6), 1023–1026. https://doi.org/10.1093/aje/kwz006

87. Shannon, M. B., Limeira, R., Johansen, D., Gao, X., Lin, H., Dong, Q., Wolfe, A. J., & Mueller, E. R. (2019). Bladder urinary oxygen tension is correlated with urinary microbiota composition. *International Urogynecology Journal*, *30*(8), 1261–1267. https://doi.org/10.1007/s00192-019-03931-y

88. Shrivastava, A., Patel, V. K., Tang, Y., Yost, S. C., Dewhirst, F. E., & Berg, H. C. (2018). Cargo transport shapes the spatial organization of a microbial community. *Proceedings of the National Academy of Sciences*, *115*(34), 8633–8638. https://doi.org/10.1073/pnas.1808966115

89. Siddiqui, H., Nederbragt, A. J., Lagesen, K., Jeansson, S. L., & Jakobsen, K. S. (2011). Assessing diversity of the female urine microbiota by high throughput sequencing of 16S rDNA amplicons. *BMC Microbiology*, *11*(1), 244. https://doi.org/10.1186/1471-2180-11-244

90. Thomas-White, K., Brady, M., Wolfe, A. J., & Mueller, E. R. (2016). The bladder is not sterile: History and current discoveries on the urinary microbiome. *Current Bladder Dysfunction Reports*, *11*(1), 18–24. https://doi.org/10.1007/s11884-016-0345-8

91. Thomas-White, K., Forster, S. C., Kumar, N., Van Kuiken, M., Putonti, C., Stares, M. D., Hilt, E. E., Price, T. K., Wolfe, A. J., & Lawley, T. D. (2018a). Culturing of female bladder bacteria reveals an interconnected urogenital microbiota. *Nature Communications*, *9*, 1557. https://doi.org/10.1038/s41467-018-03968-5

92. Thomas-White, K., Forster, S. C., Kumar, N., Van Kuiken, M., Putonti, C., Stares, M. D., Hilt, E. E., Price, T. K., Wolfe, A. J., & Lawley, T. D. (2018b). Culturing of female bladder bacteria reveals an interconnected urogenital microbiota. *Nature Communications*, *9*, 1557. https://doi.org/10.1038/s41467-018-03968-5

93. Thomas-White, K. J., Gao, X., Lin, H., Fok, C. S., Ghanayem, K., Mueller, E. R., Dong, Q., Brubaker, L., & Wolfe, A. J. (2018). Urinary Microbes and Post-Operative Urinary Tract Infection Risk In Urogynecologic Surgical Patients. *International Urogynecology Journal*, *29*(12), 1797–1805. https://doi.org/10.1007/s00192-018-3767-3

94. Thomas-White, K. J., Hilt, E. E., Fok, C., Pearce, M. M., Mueller, E. R., Kliethermes, S., Jacobs, K., Zilliox, M. J., Brincat, C., Price, T. K., Kuffel, G., Schreckenberger, P., Gai, X., Brubaker, L., & Wolfe, A. J. (2016). Incontinence medication response relates to the female urinary microbiota. *International Urogynecology Journal*, *27*(5), 723–733. https://doi.org/10.1007/s00192-015-2847-x

95. Tillisch, K., LABUS, J., KILPATRICK, L., JIANG, Z., STAINS, J., EBRAT, B., GUYONNET, D., LEGRAIN–RASPAUD, S., TROTIN, B., NALIBOFF, B., & MAYER, E. A. (2013). Consumption of Fermented Milk Product With Probiotic Modulates Brain Activity. *Gastroenterology*, *144*(7), 10.1053/j.gastro.2013.02.043. https://doi.org/10.1053/j.gastro.2013.02.043

96. Tran, Q., Pham, D.-T., & Phan, V. (2017). Using 16S rRNA gene as marker to detect unknown bacteria in microbial communities. *BMC Bioinformatics*, *18*(S14), 499. https://doi.org/10.1186/s12859-017-1901-8

97. Tuson, H. H., Copeland, M. F., Carey, S., Sacotte, R., & Weibel, D. B. (2013). Flagellum Density Regulates Proteus mirabilis Swarmer Cell Motility in Viscous Environments. *Journal of Bacteriology*, *195*(2), 368–377. https://doi.org/10.1128/JB.01537-12

98. Větrovský, T., & Baldrian, P. (2013). The Variability of the 16S rRNA Gene in Bacterial Genomes and Its Consequences for Bacterial Community Analyses. *PLoS ONE*, *8*(2), e57923. https://doi.org/10.1371/journal.pone.0057923

99. Wampach, L., Heintz-Buschart, A., Hogan, A., Muller, E. E. L., Narayanasamy, S., Laczny, C. C., Hugerth, L. W., Bindl, L., Bottu, J., Andersson, A. F., de Beaufort, C., & Wilmes, P. (2017). Colonization and Succession within the Human Gut Microbiome by Archaea, Bacteria, and Microeukaryotes during the First Year of Life. *Frontiers in Microbiology*, *8*, 738. https://doi.org/10.3389/fmicb.2017.00738

100. Wolfe, A. J., Toh, E., Shibata, N., Rong, R., Kenton, K., FitzGerald, M., Mueller, E. R., Schreckenberger, P., Dong, Q., Nelson, D. E., & Brubaker, L. (2012). Evidence of Uncultivated Bacteria in the Adult Female Bladder. *Journal of Clinical Microbiology*, *50*(4), 1376–1383. https://doi.org/10.1128/JCM.05852-11

101. Wu, J. M., Matthews, C. A., Vaughan, C. P., & Markland, A. D. (2015). Urinary, fecal, and dual incontinence in older U.S. Adults. *Journal of the American Geriatrics Society*, *63*(5), 947–953. https://doi.org/10.1111/jgs.13385

102. Yang, Y.-T., Wang, J.-C., & Chuang, H.-S. (2020). Developing Rapid Antimicrobial Susceptibility Testing for Motile/Non-Motile Bacteria Treated with Antibiotics Covering Five Bactericidal Mechanisms on the Basis of Bead-Based Optical Diffusometry. *Biosensors*, *10*(11), 181. https://doi.org/10.3390/bios10110181

103. Zandbergen, L. E., Halverson, T., Brons, J. K., Wolfe, A. J., & de Vos, M. G. J. (2021). The Good and the Bad: Ecological Interaction Measurements Between the Urinary Microbiota and Uropathogens. *Frontiers in Microbiology*, *12*, 659450. https://doi.org/10.3389/fmicb.2021.659450

104. Zhang, C., Jiao, S., Shu, D., & Wei, G. (2021). Inter-phylum negative interactions affect soil bacterial community dynamics and functions during soybean development under long-term nitrogen fertilization. *Stress Biology*, *1*(1), 15. https://doi.org/10.1007/s44154-021-00015-0

105. Zingue, D., Panda, A., & Drancourt, M. (2018). A protocol for culturing environmental strains of the Buruli ulcer agent, Mycobacterium ulcerans. *Scientific Reports*, *8*(1), 6778. https://doi.org/10.1038/s41598-018-25278-y

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