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Development of a Synthetic Human Urine formulation for the study of UPEC physiology and the discovery of antibiotics

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Approximately 150 million people develop a urinary tract infection (UTI) every year and uropathogenic *Escherichia coli* (UPEC) are responsible for ~75% of these infections. Nevertheless, the majority of our knowledge about how antibiotics exhibit their activities is generated from non-pathogenic lab-adapted *E. coli* grown under optimal, nutrient-rich conditions. However, UPEC strains are metabolically and physiologically adapting to grow in the harsh environment of human urine, which can have dramatic impacts on the susceptibility towards certain antibiotics. Real urine varies a lot in its composition, therefore we aimed to create a synthetic human urine formulation in which the metabolic behavior, the nutritional limitations and the stresses that UPECs face in real human urine is reflected in an axenic, highly reproducible growth medium.
We apply nuclear magnetic resonance (NMR) and mass spectrometry (MS) to detect the compounds consumed by UPECs during growth in human urine and supplement these metabolites to an axenic media with human urine characteristics.
The growth dynamics of 141 environmental and uropathogenic *E. coli* in the newly developed synthetic urine closely reflect the growth dynamics in real urine. Additionally, we applied proteomics to benchmark the cellular program of UPECs grown in the synthetic urine versus in real urine, where we see a distinct overlap of the proteome of UPECs grown in real and synthetic urine, which differs significantly from the proteomes of the sameUPEC grown in MHB or previously published synthetic urine. We compared the MIC of UPECs in rich growth media to the MIC in pooled and synthetic urine. Our results demonstrate a 250-fold lower activity in MHB (and 10’000-fold lower in LB) compared to pooled urine for the clinically relevant antibiotic Sulfamethoxazole but the same activity in synthetic urine compared to real urine. We show that this difference is due to methionine limitation in urine and it indicates that other limitations that uropathogenic bacteria experience in urine can be exploited to discover novel antibiotics in synthetic urine that would be overlooked in rich growth conditions.