Engineering *E. coli* Nissle 1917 to Consume Uric Acid in the GI Tract

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Introduction

Uric acid (UA), the end product of purine metabolism in humans, has relatively low solubility in biological fluids and can lead to the most common form of inflammatory arthritis (gout) when crystals form and accumulate in joint tissues. In healthy individuals, ~70% of the circulating UA pool is excreted by the kidneys, while the remaining 30% is processed through the GI tract. A portion of the UA excreted into the intestines can be reabsorbed and re-enter systemic circulation. This represents a pool of UA that, if eliminated, could reduce systemic levels and provide a new method of treatment for disorders involving elevated UA.

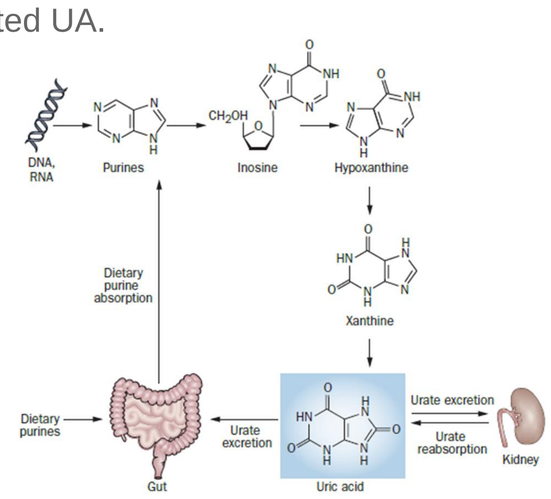

Figure 1. Purine breakdown and excretion pathways in humans. From Rock et al. *Nat Rev Rheum* 2013.

We set out to develop a new Synthetic Biotic that is capable of accessing and degrading UA from within the GI tract. We have previously shown that the probiotic bacterium *E. coli* Nissle 1917 is amenable to synthetic biology approaches, and we have engineered strains to import and degrade the amino acid phenylalanine for the treatment of phenylketonuria (PKU), as well as dietary oxalate for the treatment of hyperoxaluria. Both strains are currently being developed in human clinical trials.

Methods

The degradation of UA into allantoin by uricase enzymes enables the metabolism of UA by EcN

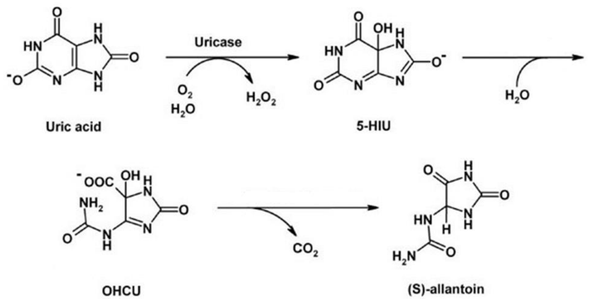

Figure 2. Enzymatic breakdown pathway of uric acid to allantoin.

A screen of >1000 uricase and urate transporter enzymes led to the final engineered components

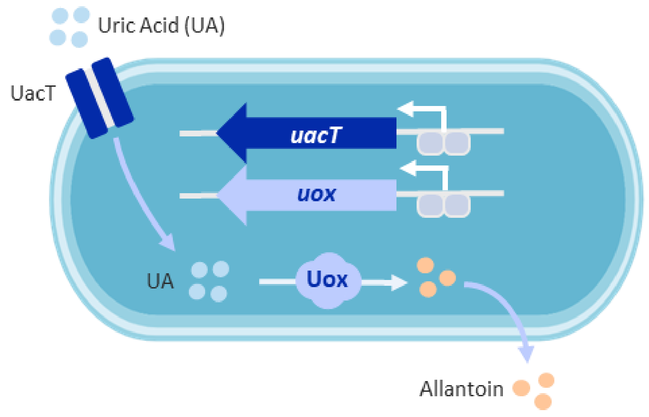

Figure 3. Schematic showing engineered components of SYN-GOUT strain of EcN capable of importing and degrading uric acid to allantoin. The *uox* gene originates from *Chimaeribacter californicus* and the *uacT* gene from *Edwardsiella tarda*.

Results

SYN-GOUT consumes UA and produces allantoin

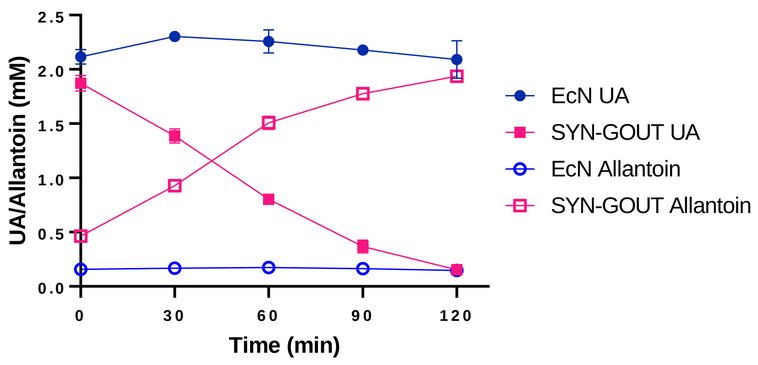

Figure 4. Consumption of uric acid and production of allantoin in vitro. 1E9 cells were added to minimal media containing 1mM UA and 0.5% glucose and incubated at 37˚C for 2hrs.

SYN-GOUT is active under low O_2 conditions

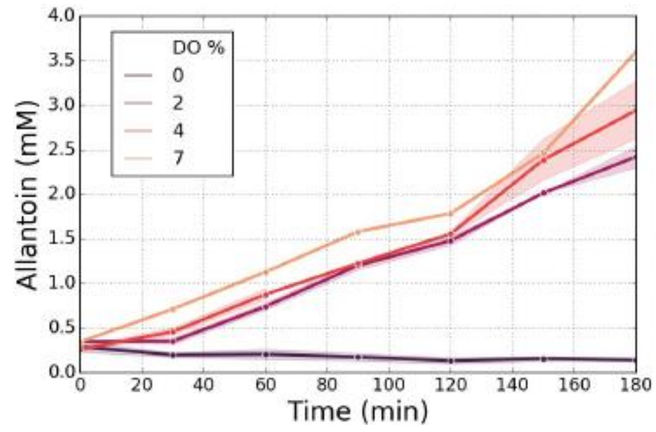

Figure 7. Strain activity as measured by production of allantoin under various dissolved oxygen (DO) conditions. 1E9 cells were added to minimal media containing 5mM UA and 0.5% glucose and incubated at 37˚C over 3hrs.

Chromosomal integration of 1 copy of uricase + transporter is less active than the plasmid prototype

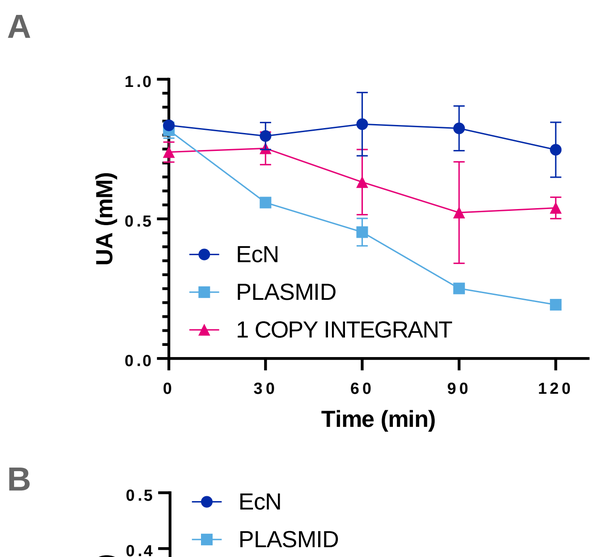

Figure 5. Consumption of uric acid (A) and production of allantoin (B) in vitro. 1E9 cells were added to minimal media containing 1mM UA (no carbon source) and incubated at 37˚C for 2hrs.

Additional rounds of optimization recovers and increases strain activity over prototype activity

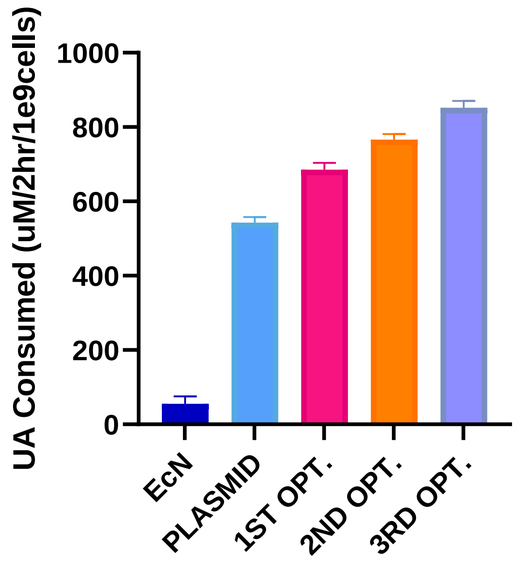

Figure 6. Consumption of uric acid in vitro. 1E9 cells were added to minimal media containing 1mM UA (no carbon source) and incubated at 37˚C for 2hrs. Optimization involved switching from a chemically-induced to an environmentally-sensitive promoter and altering the ribosomal binding site (RBS) of the uricase gene by increasing the predicted translation initiation rate.

SYN-GOUT reduces levels of endogenous UA in urinary output in nonhuman primates

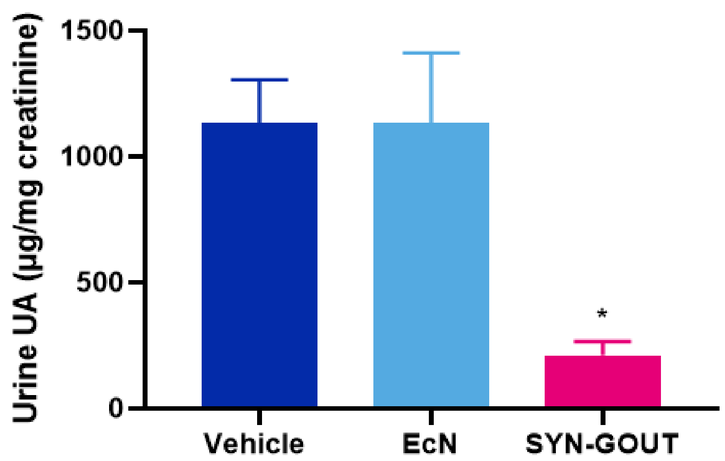

Figure 8. Demonstration of urinary uric acid lowering in nonhuman primates dosed with 15N-labeled uric acid. Endogenous (and labeled, data not shown) urinary uric acid output is significantly reduced when NHPs were given an oral gavage of 1E11 cells of SYN-GOUT compared to both vehicle treated and EcN treated NHP. *p<0.05, n=12 animals/group.

Conclusions

Synthetic biology approaches have allowed us to:

- Screen and synthesize 100s of enzymes on a rapid timescale
- Evaluate selected candidate uricase and urate transporter enzymes for their combined, optimal activities in a bacterial cell
- Iterate through multiple promoter systems and tune RBS strength to find maximal protein output without compromising strain viability
- Integrate final engineered components into the chromosome of EcN in order to satisfy one of the major FDA requirements of any potential live biotherapeutic product

We have successfully engineered a strain of *E. coli* Nissle1917 to consume and degrade UA from within the intestinal tract. SYN-GOUT shows efficacy in reducing urinary output of UA in nonhuman primates and serves as the first example of an oxygen-dependent enzyme functioning in one of our Synthetic Biotic strains even though its intended environment is substantially limited in this required cofactor.