SYNTHETIC BIOTICS AS A VERSATILE PLATFORM TO TREAT INFLAMMATORY BOWEL DISEASE

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Summary

- Synthetic Biotics are live, non-pathogenic *E. coli* Nissle (EcN) bacteria designed with drug-like properties.
- Our synthetic biology platform allows the engineering of probiotic strains with versatile and disparate modalities that can influence inflammatory pathways in vivo including:
  - Short chain fatty acid and lactate production that can alter inflammatory signaling
  - Aryl hydrocarbon receptor (AHR) metabolites to reduce inflammation and enhance mucosal healing in preclinical IBD models
  - Production and secretion of human proteins, including cytokines
- These innovations are key to the development of gut-targeted therapeutics for inflammation-based pathologies such as Inflammatory Bowel Disease (IBD).

Engineering EcN to produce Short Chain Fatty Acids and Lactate

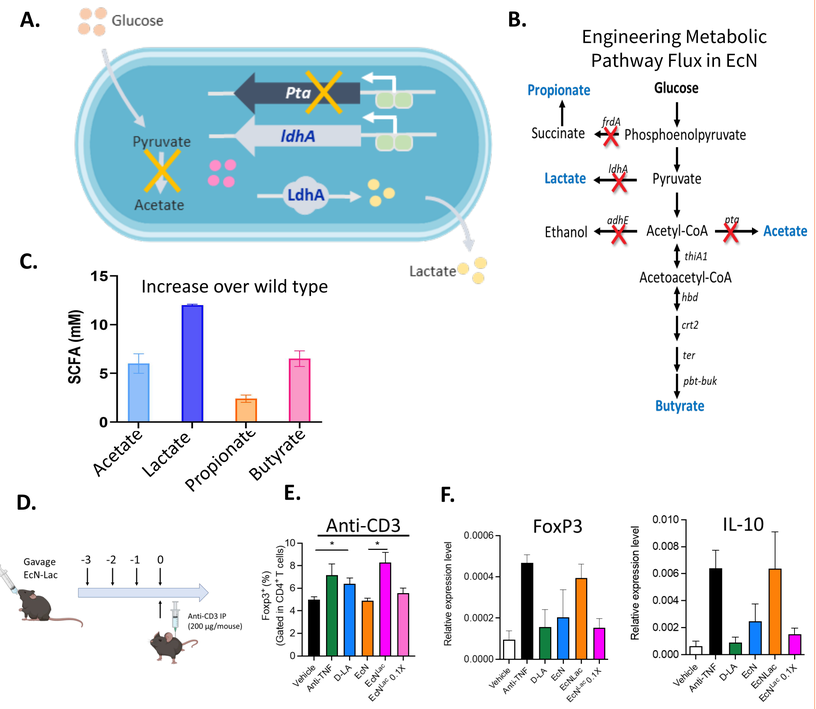

Fig. 1. A. Schematic of EcN-Lac. The lactate producing strain was engineered by removing the *pta* gene from the acetate producing pathway and introducing a plasmid containing the *E. coli ldhA* gene under the control of an inducible promoter. **B.** Schematic of SFCA metabolic pathways that can be altered for SCFA production. Red Xs denote possible genes modified for a butyrate producing strain. **C.** In vitro measurement of excess SCFAs produced from engineered EcN strains compared to wild type EcN. Dark blue var denotes EcN-Lac. **D.** Mouse study design. 1e10 CFU EcN-Lac were gavaged into wild type mice daily for 3 days prior to anti-CD3 treatment. After 24 hrs, mice were sacrificed and colons were harvested. **E.** % of FoxP3+ Tregs present in the colon. **F.** Gene expression in the colon measured by qPCR and normalized to *Gapdh.*

EcN engineered pathway for IAA production

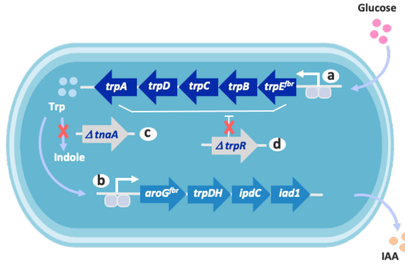

Fig 2. EcN-IAA strain contains two plasmids: **(a)** plasmid encoding the enzymatic pathway for tryptophan (Trp) production, *trpE^fbr^BCDA* **(b)** plasmid encoding the enzymatic pathway which converts Trp to indole-3-acetic acid (IAA), *trpDH-ipdC-iad1C*. A feedback inhibition resistant DAHP synthase, encoded by *aroG^fbr^*. Regulation of Trp and IAA production is carried out by P_{fnrS} anaerobic-inducible promoter. The *E. coli* Nissle (EcN) chassis contains two key chromosomal deletions: **(c)** *tnaA*, encoding tryptophanase, which converts Trp to indole, and **(d)** *trpR*, encoding Trp operon repressor, which inhibits production of Trp pathway enzymes.

Engineered EcN-IAA therapy can ameliorate IBD in DSS mouse model

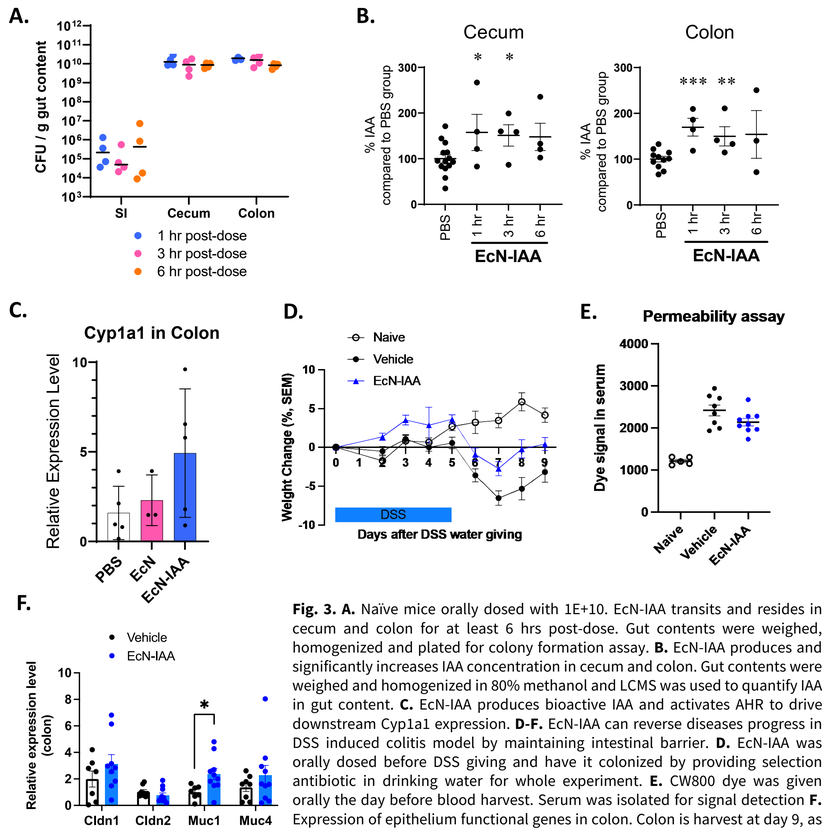

Fig. 3. A. Naïve mice orally dosed with 1E+10. EcN-IAA transits and resides in cecum and colon for at least 6 hrs post-dose. Gut contents were weighed, homogenized and plated for colony formation assay. **B.** EcN-IAA produces and significantly increases IAA concentration in cecum and colon. Gut contents were weighed and homogenized in 80% methanol and LCMS was used to quantify IAA in gut content. **C.** EcN-IAA produces bioactive IAA and activates AHR to drive downstream Cyp1a1 expression. **D-F.** EcN-IAA can reverse diseases progress in DSS induced colitis model by maintaining intestinal barrier. **D.** EcN-IAA was orally dosed before DSS giving and have it colonized by providing selection antibiotic in drinking water for whole experiment. **E.** CW800 dye was given orally the day before blood harvest. Serum was isolated for signal detection **F.** Expression of epithelium functional genes in colon. Colon is harvest at day 9, as shown in Fig. 5D. (ANOVA multiple comparison or t test is applied, *, p<0.05; **, p<0.01; ***, p<0.001)

Development and characterization of EcN-IL-22, an integrated hIL22 Secreting Strain

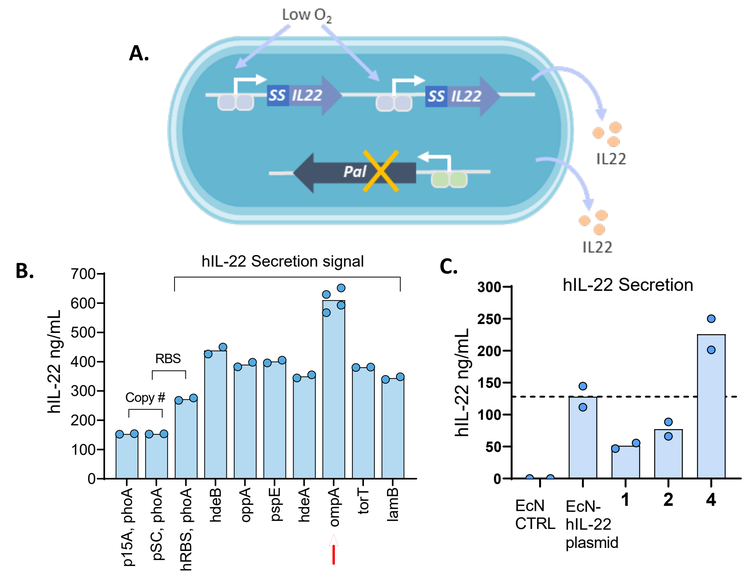

Fig. 4. A. Schematic of EcN-IL-22. Multiple copies of IL-22 fused to a secretion signal (SS) are integrated into the EcN chromosome. Removal of the *pal* gene results in a Diffusible Outer Membrane (DOM) phenotype for enhanced secretion from EcN. **B.** ELISA assay comparing the affect of plasmid copy number, ribosome-binding site (RBS) and signaling peptide on hIL-22 secretion normalized to 1e8 CFU/5hrs. hRBS, high affinity RBS **C.** IL-22 production from integrated strains containing 1, 2, or 4 copies of integrated hIL-22 with the ompA signaling peptide. The 4-copy integrant is designated EcN-IL-22.

hIL22 secreted by EcN induces IL22 dependent gene expression in vivo

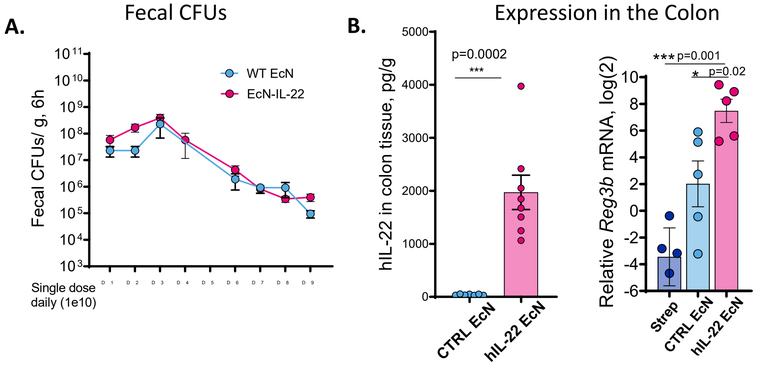

Fig. 5. A. EcN-IL-22 is viable and biologically active *in vivo.* EcN-IL-22 numbers decrease with time in naïve mice with Strep in drinking water. **B.** At day 4, high levels of secreted hIL-22 are detected in the colon by ELISA. In colon tissue, *in vivo* target engagement of bacterially-secreted IL-22 is detected as upregulation of the IL-22-dependent biomarker, Reg3b.

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