Local Delivery of Synthetic Biotics to Treat Inflammatory Bowel Disease

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Abstract

Therapeutic options available for Inflammatory Bowel Disease (IBD) are often limited by adverse events associated with systemic suppression of the immune system. A useful alternative option to locally deliver therapies in the gut are Synthetic Biotics, which are nan-pathogenic, non-colonizing engineered probiotic Escherichia coli Nissle (EcN).

In this study we aimed to characterize the viability, transit kinetics, and gene expression profiles of our Synthetic Biotics in the gut of both murine and nonhuman primate models. All EcN cells were cleared from fecal pellets by 72 hours in mice and by 120 hours in healthy nonhuman primates. To explore the spatial localization *in vivo* within the mouse gut, EcN was engineered to express mCherry under the control of a constitutive promoter. Fluorescence microscopy demonstrated that EcN was located both in the lumen and mucus layers of the colon in naïve and DSS colitis mice during transit.

To confirm *in vivo* gene induction and *de novo* protein expression is occurring in mice, EcN was engineered to express GFP or NanoLuciferase (NLuc) under an inducible promoter that responds to environmental cues *in vivo*. *In vivo* bioluminescence imaging of DSS-treated mice orally dosed with EcN expressing NLuc showed luciferase activity in the lower GI tract 3 hours post-dose.

Overall, our data demonstrate that engineered EcN are viable and active in the GI tract of naïve and DSS-treated mice and can produce a payload in the gut at both temporal and spatial sites predicted to be required to reduce inflammation and enhance mucosal healing in IBD. Synthetic Biotics offer a novel approach for delivering localized payloads directly to the site of disease.

Results

EcN engineered to express GFP, mCherry & Nanoluciferase

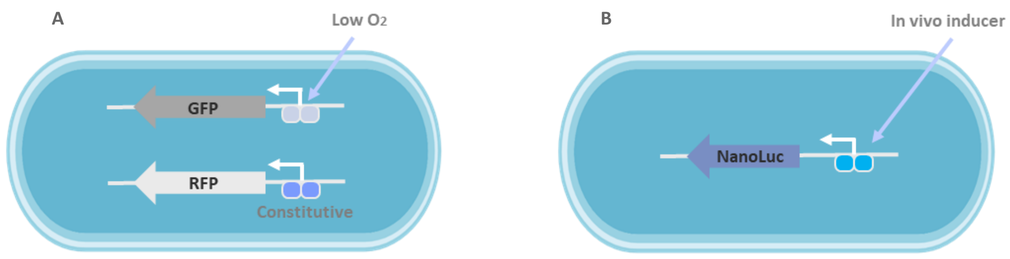

Figure 1. Schematic design of engineered EcN strains to (A) Express GFP when oxygen level is low and express mCherry under control of a constitutive promoter, **(B)** Express Nanoluciferase under control of an in vivo inducible promoter.

EcN has slower transit time in DSS colitis mice in comparison to naïve mice

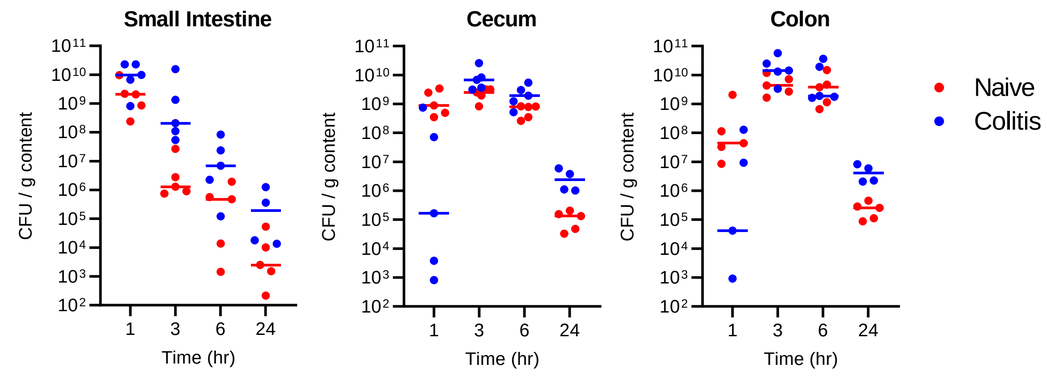

Figure 2. Transit time course in mice gut. Mice were given DSS in drinking water from day 0 to day 7 to induce colitis disease. On day 7 DSS, 1e10 EcN was orally administered in colitis or naïve mice (n=5 per group). Mice were sacrificed at 1h, 3h, 6h and 24h post-dose. Segments of the lower GI tract (small intestine, cecum and colon) were flushed with PBS. Homogenates was plated on streptomycin plates for CFU counting.

EcN transits the GI tract and does not colonize

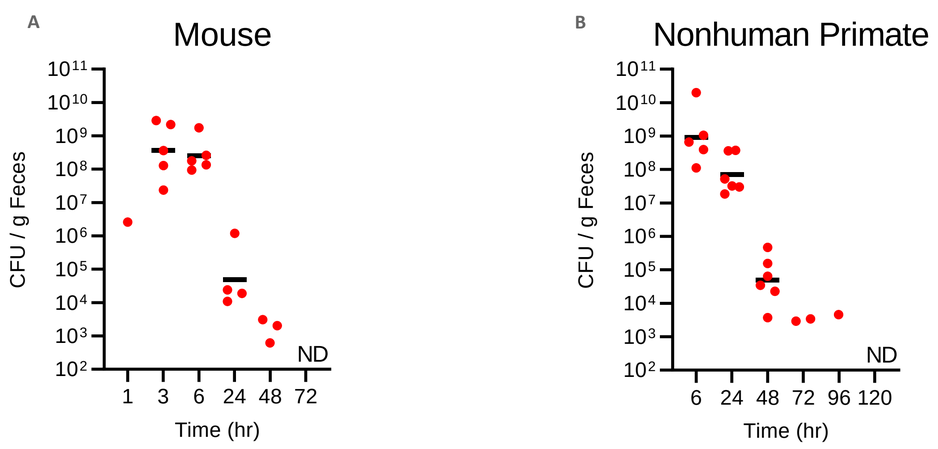

Figure 3. Clearance time course in (A) mice, **(B)** NHPs. 1e10 EcN, and 1e12 EcN was orally administered in naïve mice and NHPs respectively (n=5 per group). Feces were collected at 1h, 3h, 6h, 24h, 48h and 72h post-dose in naïve mice, while NHP feces were collected at 6h, 24h, 48h, 72h, 96h and 120h post-dose. Homogenates was plated on streptomycin plates for CFU counting.

Engineered EcN have robust gene expression in the colon

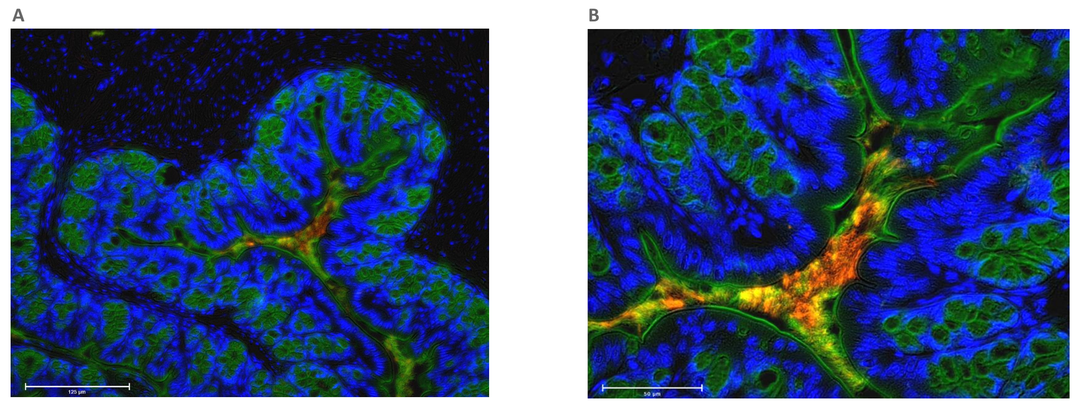

Figure 4. EcN colocalizing in mucosal layer of mouse colon. Naïve C57BL/6 mouse was orally gavaged with 1E10 CFU EcN strain that constitutively expresses mCherry. Colon tissue was collected 6hr post dose and embedded in paraffin wax. Mouse colon tissue was then stained for mCherry expression (red) and for Muc2 mucin expression (green) via fluorescence imaging. (**A)** 20X image of mouse colon tissue stained for mCherry and Muc2. (**B)** 40X image of mouse colon tissue stained for mCherry and Muc2.

Engineered EcN expresses fluorescent reporter protein under in vivo inducible promoter

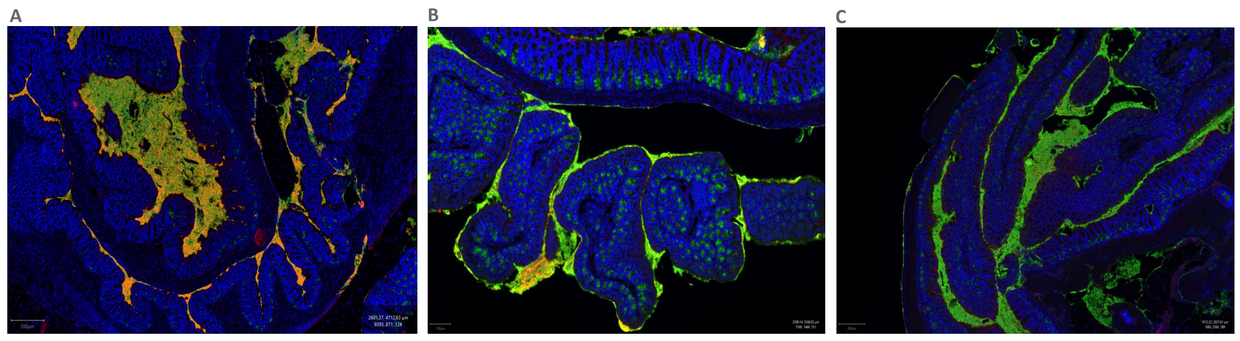

Figure 5. EcN expresses fluorescent reporter protein under environmentally inducible promoter. Naïve C57BL/6 mice were orally gavaged with different EcN strains at 1E10 that constitutively express mCherry and express GFP under an environmentally inducible promoter. Colon tissue was then collected 6hr post dose and embedded in paraffin wax. Mouse colon tissue was then stained for mCherry expression(red) and for GFP expression (green) via fluorescence imaging. (**A)** Fluorescent image of mouse colon tissue stained for mCherry and GFP expression controlled by low oxygen inducible promoter. **(B)** Fluorescent image of mouse colon tissue stained for mCherry and GFP expression controlled by temperature inducible promoter. **(C)** Fluorescent image of mouse colon tissue stained for mCherry and GFP expression controlled by nitrate inducible promoter.

EcN transit time increases through the GI tract in DSS colitis compared to healthy mice

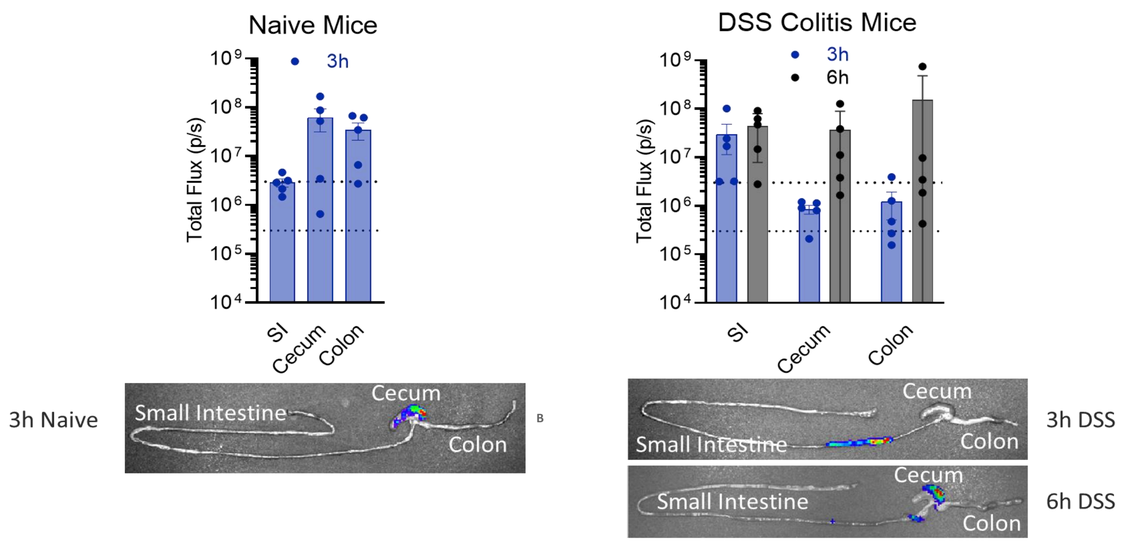

Figure 6. EcN-nLuc biodistribution in healthy vs DSS colitis mice. Mice were given DSS in drinking water from day 0 to day 7 to induce colitis disease. On day 7 DSS time 0 (T0), 1e10 EcN-temperature inducible promoter driving nanoluciferase (nLuc) were orally administered in colitis or naïve mice (n=5 per group). Two hours prior to sacrifice mice were orally dosed with flurofurimazine nanoluciferase substrate. At T3 or T6 mice were sacrificed, and the lower GI tract was imaged using IVIS bioluminescence imaging. Total flux (photons/sec) were quantitated and plotted.

Ex vivo analysis of EcN-nLuc biodistribution with CFU and luciferase activity correlates with bioluminescence imaging

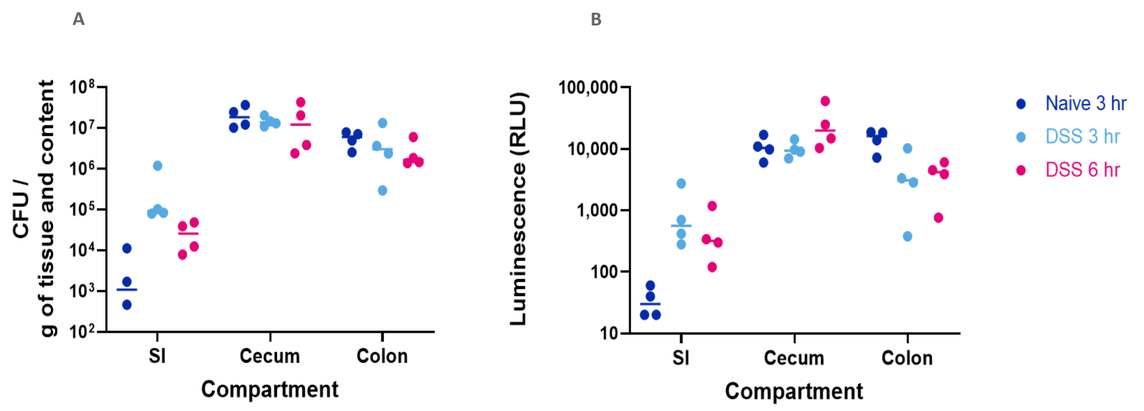

Figure 7. Ex vivo analysis of EcN-nLuc biodistribution in mice. Mice were given DSS in drinking water from day 0 to day 7 to induce colitis disease. On day 7 DSS , 1e10 EcN-temperature inducible promoter driving nanoluciferase (nLuc) were orally administered in colitis or naïve mice (n=5 per group). At T3 or T6 mice were sacrificed, and segments of the lower GI tract (small intestine, cecum and colon) were collected. Each segment were flushed, and luminal content collected; tissue was also collected and weighed. **(A)** Flushed effluent/ content and tissue homogenates were plated on streptomycin plates for CFU counting. **(B)** Luciferase substrate was added to flushed luminal content, and luminescence (RLU) was readout in a luminometer.