

## SUPPLEMENTARY INFORMATION

### ***In vitro* fermentation test bed for evaluation of engineered probiotics in polymicrobial communities**

#### **Authors**

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#### **Abbreviations**

*E. coli* Nissle (EcN); *E. coli* Nissle wild type (EcNWT); *E. coli* Nissle engineered for GFPa1 synthesis (EcN(cGFP))

#### **Table of content**

**Figure S1.** Plasmid construction and strain engineering of *E. coli* Nissle 1917 for constitutive production of the fluorescent protein GFPa1.

**Table S1.** Complex Colonic Medium (CCM) recipe.

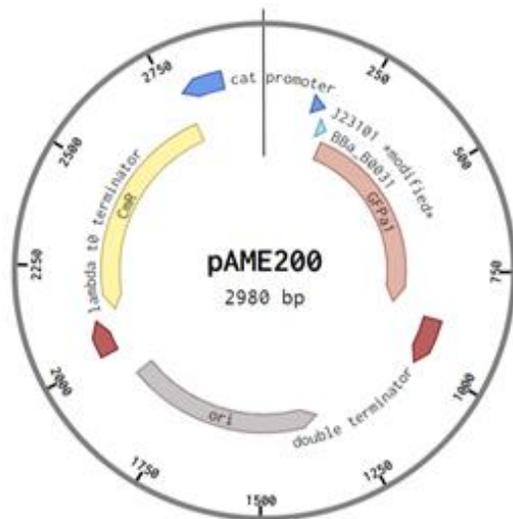
**Table S2.** Primers sequences used for qPCR.

**Figure S2:** qPCR workflow to calculate log CFU/ml culture organism persistence from sample Ct values.

**Figure S3.** Immunodetection of GFPa1 protein expression.

**Figure S4.** PCA of overall community dynamics comparing EcN(cGFP) to EcNWT.

**Figure S1. Plasmid construction and strain engineering of *E. coli* Nissle 1917 for constitutive production of the fluorescent protein GFPa1.**



DNA encoding the green fluorescent protein reporter GFPa1 was cloned downstream of a constitutive promoter and expressed from a plasmid containing chloramphenicol resistance. GFPa1 (Bomati (2009) BMC Evol Biol), along with ribosome binding site and terminator regions, was amplified from pAMP (Goodson et al (2017) ACS Synth Biol) using primers AME1000 (TCTAGAGTCACACAGGAAAC) and AME1001 (TATAAACGCAGAAAGGCC). The PCR product was reamplified with primers AME1007 (GCTTTCGCTAAGGATGATTTCTGGAATTCGGATCCTTTACATTTAGCTCAGTCCTAG GTATTATGCTAGCTCTAGAGTCACACAGGAAAC) and AME1002 (TTGCCCGTTTTTTTGCCGGACTGCAGCGGCCGCTATAAACGCAGAAAGGCC) to add promoter (underlined; two-point mutation version of BioBrick promoter BBa\_J23101 from the Anderson Promoter Collection; Registry of Standard Biological Parts <http://partsregistry.org>) and overlap regions and was cloned into plasmid J64100 at *EcoRI/NotI* using Gibson Assembly to create pAME200. The plasmid was verified via sequencing using primers AME1012 (TGCCACCTGACGTCTAAG) and AME1013 (GACCGAGCGCAGCGAG). pAME200 was transformed into *E. coli* Nissle 1917 using standard heat shock technique and designated EcN(cGFP). J64100 was a gift from Prof. Chris Voigt (MIT). *E. coli* Nissle 1917 was a gift from Dr. Scott Walper (Naval Research Laboratory).

**Table S1. Complex Colonic Medium (CCM) recipe (based on Macfarlane (1998) Microb Ecol).** CCM contains soluble and insoluble components and designed to simulate the gut nutritional environment to completely support microbe growth.

	<b>Ingredient</b>	<b>per liter</b>
<b>Soluble components</b>	Tween 80	1 ml
	Peptone	5 g
	Yeast extract	4.5 g
	Tryptone	5 g
	NaCl	4.5 g
	KCl	4.5 g
	NaHCO <sub>3</sub>	1.5 g
	MgSO <sub>4</sub> -7H <sub>2</sub> O	1.25 g
	KH <sub>2</sub> PO <sub>4</sub>	0.5 g
	FeSO <sub>4</sub> -7H <sub>2</sub> O	0.005 g
	CaCl <sub>2</sub> -2H <sub>2</sub> O	0.02 g
	Cysteine-HCl	0.8 g
	Hemin (98%)	0.05 g
	Resazurin	0.01 g
<b>Insoluble components</b>	Potato starch	5 g
	Pectin	3 g
	Guar gum	2 g
	Xylan (oat spelt)	2 g
	Arabinogalactan	2 g
	Inulin (dahlia)	1 g
	Casein (bovine milk)	3 g
	Bile salts #3	0.4 g
	Mucin (porcine type III)*	4 g
	pH to 7.0 with 1M NaOH	

\* Note: Add mucin last and stir for 2-3 hours to ensure a uniform resuspension.

**Table S2. Primers sequences used for qPCR.**

<b>Primer designation</b>	<b>Primer sequence (5' to 3')</b>	<b>uM primer</b>	<b>Target</b>
<b>Mut7f*</b>	GACCAAGCGATAACCGGATG	5 uM	MUT2 plasmid
<b>Mut8r*</b>	GTGAGATGATGGCCACGATT	5 uM	
<b>GFPa1-f</b>	GCAAGCTGTCTACCGAGTTTA	5 uM	pAME200 GFPa1 plasmid
<b>GFPa1-r</b>	AAGGGCTTGTCGAAGATGTAG	5 uM	

Primers targeting the MUT2 plasmid that is naturally occurring in *E. coli* Nissle were used to determine EcN(cGFP) and EcNWT persistence. Target efficiency; E = 80 to 100%. qPCR amplification of the off-target commensal organisms was  $\leq 0.1\%$ .

\* Blum-Oehler (2003) Res Microbiol.

**Figure S2: Bacteria concentration calculation determining log CFU/ml culture organism persistence using sample Ct values from qPCR.**

***Calculating sample log CFU/ml culture***

After determining the sample log CFU value from the standard curve, convert to log CFU/ml culture

For samples, assume:

- Volume culture = 5 ml
- Volume DNA solution after extraction = 100 ul
- Volume DNA solution in qPCR reaction = 2 ul

Calculations:

1. Use sample Ct to determine log CFU from standard curve
2. Convert log CFU to linear number (Excel POWER function) = CFU in qPCR reaction
3. Convert CFU in qPCR reaction to log CFU/ml culture
  - *Example:* If a 2 ul qPCR reaction = 7500 CFU:

$$\frac{7500 \text{ CFU}}{2 \text{ ul reaction}} \times \frac{100 \text{ ul extract}}{5 \text{ ml culture}} = 75000 \text{ CFU/ml culture}$$

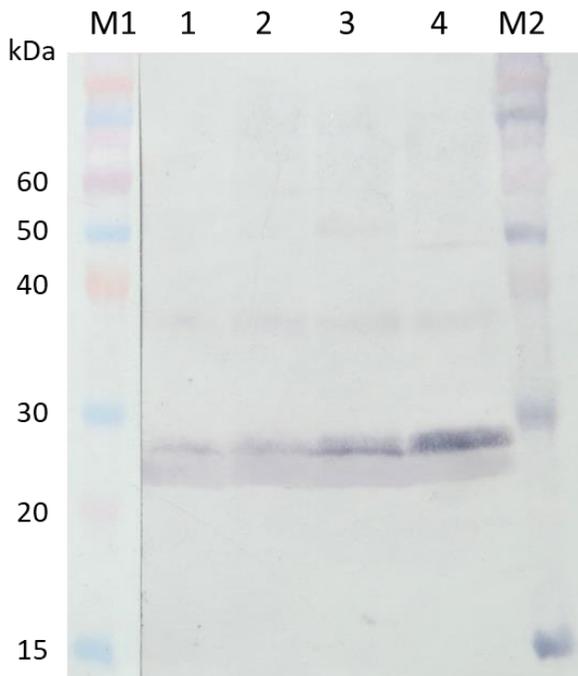
$$\text{LOG}(75000 \text{ CFU/ml culture}) = 3.8 \text{ log CFU/ml culture}$$

*NOTE:* if extraction solution needs to be diluted (e.g., due to high DNA concentration, presence of inhibitors), account for dilution factor in calculation

**Figure S3. Immunodetection of GFPa1 protein expression.**

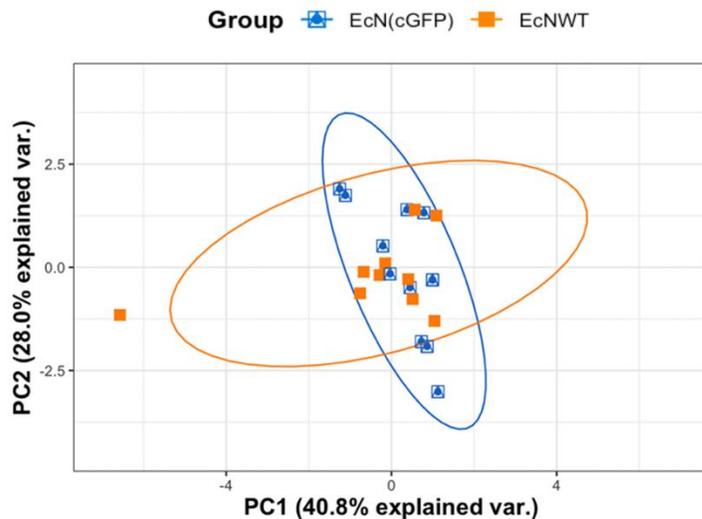
Cell lysates were run on NuPAGE 12% Bis-Tris gel (Invitrogen, Carlsbad, CA) gels in MOPS buffer according to the manufacturer's protocol. Protein was transferred onto NitroPlus nitrocellulose (Micron Separations, Inc., Westborough, MA) with carbonate transfer buffer (g/L: 0.84g  $\text{NaHCO}_3$ , 0.318g  $\text{Na}_2\text{CO}_3$ , 100 ml methanol) for 2 h at 80 mA using IMM-1 Semi-dry blotting system (WEP Co, Seattle, WA). All washes, antibody incubations and detection were done with gentle mixing. Nitrocellulose membrane was blocked for 1 h with 5% Bovine Serum Albumin in Tris Buffered Saline Tween (TBST), g/L: Tris base, 3.3; NaCl, 8.8; KCl, 0.2, pH to 7.4 with concentrated HCl, Tween 20 added to 0.1% (v/v), then washed with TBST for 5 min. Immunodetection of GFPa1 was achieved with a 1:1000 dilution (in blocking buffer) of rabbit anti-mNeonGreen antibody (Cell Signaling Technologies, Danvers, MA) incubated 4°C overnight. After washing 3x with TBST for 5 min, a 1:1000 dilution (in blocking buffer) of anti-rabbit antibody conjugated to alkaline phosphatase, washing 2x with TBST for 5 min and 1x TBS for 5 min. Detection was done using an Alkaline Phosphatase (AP) Conjugate Substrate Kit (BioRad, Hercules, CA) in 100 mM Tris-Cl, pH 9.5.

**Figure S3A. Western blot of EcN(cGFP) 24 h culture lysates.** M1. Novex Sharp marker, pre-Western; 1. EcN(cGFP) mono; 2. EcN(cGFP) 4-member; 3. EcN(cGFP) 6-member; 4. EcN(cGFP) 8-member; M2. Novex Sharp marker, post-Western. M1 Novex Sharp lane cut from NC membrane post-blot and dried. Remaining NC brought through Western protocol. Western blot analysis using mNeonGreen antibody showed increased production of GFPa1 in the 8-way (and to a lesser extent the 6-way) fermentations, confirming the fluorescent reporter function results. mNeonGreen antibody was used since there is no commercially available GFPa1 antibody.





**Fig. S4. PCA of overall community dynamics comparing EcN(cGFP) to EcNWT.** No distinctive clustering is observed, revealing no significant difference and that engineering EcN does not affect community dynamics.



## References

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