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Letter

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Engineered Probiotic for the Inhibition of *Salmonella* via Tetrathionate-Induced Production of Microcin H47

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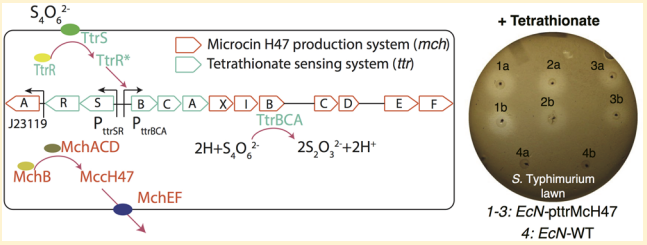
Supporting Information

ABSTRACT: Complications arising from antibiotic-resistant bacteria are becoming one of the key issues in modern medicine. Members of drug-resistant *Enterobacteriaceae* spp. include opportunistic pathogens (e.g., *Salmonella* spp.) that are among the leading causes of morbidity and mortality worldwide. Overgrowth of these bacteria is considered a hallmark of intestinal dysbiosis. Microcins (small antimicrobial peptides) produced by some gut commensals can potentially cure these conditions by inhibiting these pathogens and have been proposed as a viable alternative to antibiotic treatment. In this proof-of-concept work, we leverage this idea to develop a genetically engineered prototype probiotic to inhibit *Salmonella* spp. upon exposure to tetrathionate, a molecule produced in the inflamed gut during the course of *Salmonella* infection. We developed a plasmid-based system capable of conferring the ability to detect and utilize tetrathionate, while at the same time producing microcin H47. We transferred this plasmid-based system to *Escherichia coli* and demonstrated the ability of the engineered strain to inhibit growth of *Salmonella* in anaerobic conditions while in the presence of tetrathionate, with no detectable inhibition in the absence of tetrathionate. In direct competition assays between the engineered *E. coli* and *Salmonella*, the engineered *E. coli* had a considerable increase in fitness advantage in the presence of 1 mM tetrathionate as compared to the absence of tetrathionate. In this work, we have demonstrated the ability to engineer a strain of *E. coli* capable of using an environmental signal indicative of intestinal inflammation as an inducing molecule, resulting in production of a microcin capable of inhibiting the organism responsible for the inflammation.



KEYWORDS: *microbiome, engineering, probiotics, Salmonella, tetrathionate, microcins, Escherichia coli Nissle*

Medical complications related to the emergence of antibiotic-resistant bacteria are a major issue in modern healthcare due to the resulting increase in morbidity, mortality, length of hospitalization, and related healthcare costs.[1] The CDC estimates that, every year, more than 2 million people acquire multidrug-resistant infections, resulting in over 23 000 related deaths.[2] *Enterobacteriaceae* spp. include opportunistic pathogens (Carbapenem-resistant *Klebsiella* spp., Fluoroquinolone-resistant *Salmonella* spp., adherent−invasive *Escherichia coli*) that are among the leading causes of morbidity and mortality worldwide.[3,4] According to the CDC, drug-resistant *Salmonella* spp. are responsible for more than 100 000 infections, while their nonresistant counterparts already account for 1.2 million infections and 450 deaths in the US every year.[2] It was recently demonstrated that microcin production by specific gut bacteria can ameliorate these conditions by inhibiting certain pathogens.[5] However, in these organisms, microcin production is dependent upon a narrow range of environmental conditions[6,7] and regulatory mechanisms which are, in many cases, not fully understood.[5]

Engineered microbes as diagnostic tools or therapeutics have recently been proposed as "smart" alternatives to traditional drugs. For the former, recent remarkable examples have included engineering microbes to sense cancer or inflammation deriving from the production of reactive oxygen species by the host.[8,9] For the latter, major examples have included the construction of nonpathogenic *E. coli* that kill *Pseudomonas*

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aeruginosa by means of microcins or pyocins, elicited through this pathogen's quorum-sensing signaling system.[10−12]

A recent report from Sassone-Corsi et al. demonstrated that the production of microcins from *E. coli* strain Nissle 1917 (*EcN*) can therapeutically displace pathogenic *Enterobacteriaceae* species in specific environmental conditions (e.g., iron limitation).[5] Our group independently verified that the class IIb sideromycin, microcin H47 (MccH47),[13] a high molecular mass, post-translationally modified peptide, originally isolated from *E. coli* strain H47, can inhibit *Salmonella* growth *in vitro*. Therefore, rather than relying on the wild-type regulatory mechanisms, we sought to develop a probiotic with a specifically designed induction system for MccH47.

During gut inflammation, reactive oxygen species produced by the host react with luminal thiosulfate, resulting in production of tetrathionate.[14] *Salmonella* species utilize the gene products of the *ttr* operon (*ttrRSBCA*), which provide this pathogen with the ability to utilize tetrathionate as a terminal electron acceptor, conferring a growth advantage over the competing microbiota during inflammation conditions.[14] The *ttrBCA* genes of *Salmonella*, encoding the three subunits of tetrathionate reductase, have previously been transferred to *E. coli*, resulting in functional tetrathionate reductase activity.[14] The *ttrBCA* promoter is positively regulated by TtrR in the presence of tetrathionate and by Fnr under anoxic conditions.[14,15] Very recently, the *ttrRS* genes of *Salmonella*, encoding the tetrathionate sensor and response regulator, were chromosomally integrated into *E. coli* strain NGF-1 and served as a functional tetrathionate detection probiotic in a mouse model of inflammation for over 6 months.[9] In light of these advances, we developed an engineered strain of *EcN* harboring a plasmid-based system carrying *mchAXIBCDEF* and *ttrRSBCA*, capable of producing MccH47 in response to environmental tetrathionate, resulting in the ability to inhibit and out-compete *Salmonella* during *in vitro* experiments.

RESULTS AND DISCUSSION

Construction and Analysis of a Plasmid-Based System for the Inducible Production of Microcin H47. Plasmid pJPMcH47 was developed for the L-rhamnose dependent production of MccH47 and constructed by Gibson Assembly (see Methods). pJPMcH47 contains all *mch* genes of *E. coli* H47 (*mchAXIBCDEF*), with the *mchXIB* genes immediately downstream of the $rhaP_{BAD}$ promoter (Figure 1A). The aim of this design is to specifically regulate production of the MccH47-precursor (MchB), on the basis of L-rhamnose concentration. For the purpose of *in vitro* assays, we developed *E. coli* strain NEB10β pJPMcH47 and *EcN* pJPMcH47 and then assessed the ability of each strain to inhibit *Salmonella enterica* subsp. *Enterica* serovar Typhimurium (hereafter referred to as *S.* Typhimurium) and *E. coli* strain DH5α.

Inhibition assays were based on previous work with microcin J25 by Delgado et al.,[16] where inhibition was evaluated visually by measuring a zone of inhibition in susceptible lawns grown on agar plates previously stabbed with a strain carrying pJPMcH47 and inactivated with chloroform and UV. Both *EcN* pJPMcH47 (Figure 2A) and *E. coli* NEB10β pJPMcH47 (Figure S1) were capable of inhibiting both *S.* Typhimurium and *E. coli* DH5α. As expected, on the basis of the nature of the L-rhamnose dependent induction system utilized,[17] inhibition increases with increasing L-rhamnose concentration, while *EcN* WT showed no inhibition (Figure 2B). Taken together, these results demonstrate that the MccH47 produced by our

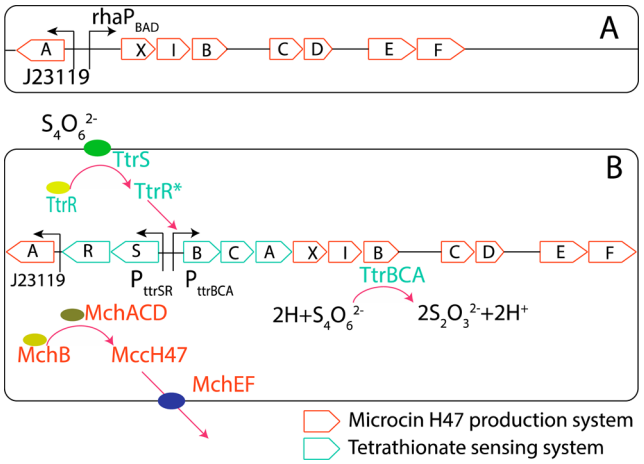

Figure 1. (A) Plasmid pJPMcH47 for the L-rhamnose-induced production of MccH47. (B) Plasmid pttrMcH47 for the tetrathionate-induced production of MccH47. Orange indicates genes and reactions needed for MccH47 production; cyan indicates genes and reactions related to tetrathionate sensing and utilization.

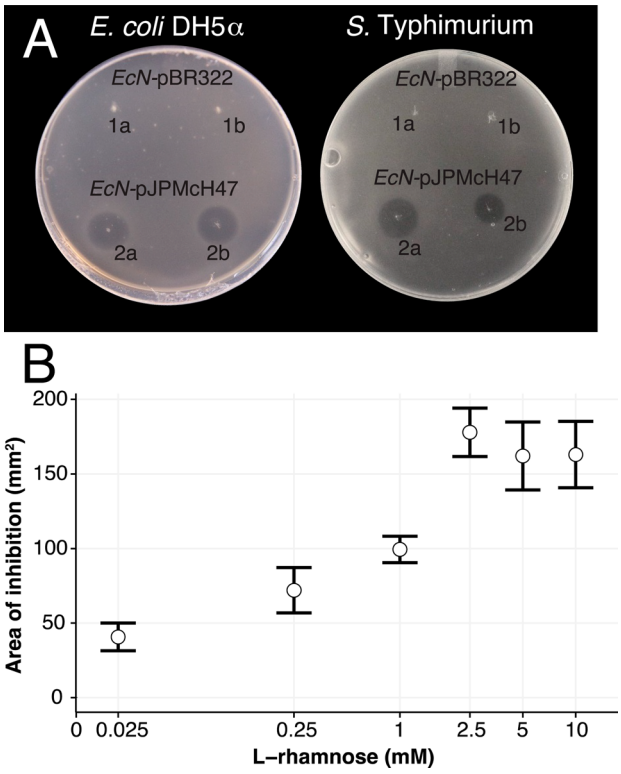

Figure 2. (A) Inhibition assays comparing the effect of *EcN* WT and *EcN* pJPMcH47 against lawns of pathogenic *S.* Typhimurium (left) and *E. coli* DH5α (right) on minimal media, supplemented with 22 mM L-rhamnose and 0.2 mM 2,2′-dipyridyl (see Methods). (B) Measurement of inhibition area in the *S.* Typhimurium lawn as a function of L-rhamnose concentration on LB agar plates supplemented with variable L-rhamnose (0.025−10 mM) and 0.2 mM 2,2′-dipyridyl. 2,2′-Dipyridyl addition was necessary for maximum *S.* Typhimurium inhibition by MccH47 (see Methods and Figure S2).

engineered strains is the causative agent in the inhibition of *S.* Typhimurium. This is in line with reported literature, showing that MccH47 produced from *EcN* WT is unable to inhibit *S.* Typhimurium even during iron starvation,[5,6] while MccH47 produced from our donor *E. coli* strain H47 is able to

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inhibit *S.* Typhimurium.[6,13] One reason could be the lack of post-translational modifications in MccH47 derived from *EcN* WT,[18] potentially due to the lack of *mchA*, a necessary gene for mature MccH47 antibacterial activity. Another possibility could be the observed divergence in amino acid sequence for genes *mchE* and *mchF* between *EcN* and *E. coli* H47.[6]

Construction and Analysis of a Plasmid-Based Tetrathionate-Detection System. Plasmid pttrMcH47 (Figure 1B) was developed to confer utilization of tetrathionate reductase activity and tetrathionate dependent production of MccH47 and was constructed by Gibson Assembly (see Methods). Plasmid pttrMcH47 contains all genes of the *ttr* operon from *S.* Typhimurium (*ttrRSBCA*) and all genes necessary for mature MccH47 production, immunity, and secretion (*mchAXIBCDEF*). *mchXIB* is encoded immediately downstream of *ttrA* resulting in cotranscription along with *ttrBCA* from the *ttrBCA* promoter. To assess successful tetrathionate-induced MccH47 production, we compared the inhibitory effect of *EcN* pttrMcH47 against *E. coli* DH5*α* in variable conditions (Figure 3). We observed that MccH47

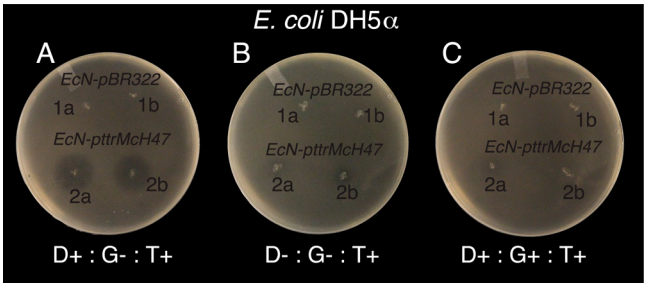

Figure 3. Characterization of MccH47-based inhibition of *E. coli* DH5*α* by EcN pttrMcH47, with EcN pBR322 control, as a function of 0.2 mM 2,2′-dipyridyl (D), 0.1% D-Glucose (G), and 1 mM of potassium tetrathionate (T). + stands for compound present; − stands for compound absent.

dependent inhibition increases in iron limited conditions (Figure 3A,B). This is consistent with previous studies that have shown that species susceptible to inhibition by MccH47 express catecholate siderophore receptors *iroN*/*fui*, *cir*, and *fepA*,[6] which serve as the mode of entry into susceptible cells. Importantly, this should not constitute an issue with respect to the functioning of our construct *in vivo,* as it is known that the iron limitation is a typical feature of a host's inflamed gut.[18] When testing for tetrathionate addition in iron limitation, we observe the expected emergence of inhibition only in the presence of tetrathionate (Figure 4A). Supplementation to the media with 0.1% D-glucose reduced inhibition across all experimental conditions (Figure 3A,C), which is noteworthy in light of published reports which utilize glucose supplementation alongside P_{ttrRS}-based induction and MccH47 production.[9,19]

Analysis of Tetrathionate-Induced Inhibition. We proceeded to test the tetrathionate-induced inhibition *in vitro* against *S.* Typhimurium. First, solid media inhibition assays were carried out anaerobically in LB agar, again supplemented with 0.2 mM 2,2′-dipyridyl, with and without 1.5 mM potassium tetrathionate. In media containing potassium tetrathionate, *EcN* pttrMcH47 was capable of inhibiting *S.* Typhimurium while *EcN* WT did not (inhibition zone not detected) (Figure 4A) confirming the ability of our construct to

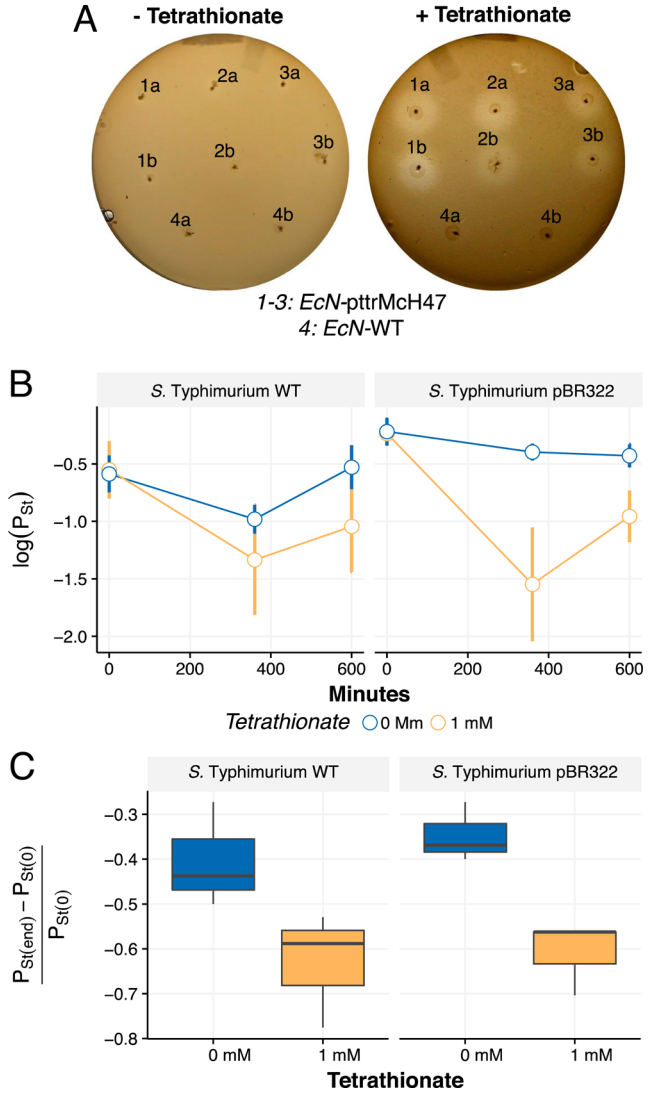

Figure 4. Tetrathionate-induced inhibition by *EcN* pttrMcH47 MccH47 production. (A) Inhibition assays comparing the ability of three separate clones of *EcN* pttrMcH47 and *EcN* WT to inhibit growth of *S.* Typhimurium in the absence (left) and presence (right) of 1 mM potassium tetrathionate (a,b indicate duplicate stabs).·(B) Results from *in vitro* ecological competition experiments, showing the proportion of *S.* Typhimurium WT (left) or *S.* Typhimurium pBR322 (right) over time. (C) *S.* Typhimurium fitness estimation obtained from competition experiments (see ref 21). Supplementation of 1 mM potassium tetrathionate leads to production of MccH47 and a statistically significant reduction ($p < 0.05$) in *S.* Typhimurium fitness.

inhibit *S.* Typhimurium at a physiologically relevant disease state.[14]

While static plate assays demonstrate the functional capability of the constructs, these experiments do not account for the effect of competition for growth nutrients. Therefore, we performed competition experiments between *EcN* pttrMcH47 and either *S.* Typhimurium WT or *S.* Typhimurium pBR322. Assays were conducted anaerobically, in LB broth supplemented with 0.2 mM 2,2′-dipyridyl, in the presence or absence of 1 mM potassium tetrathionate, and supplemented with 100 *μ*g/mL carbenicillin when both strains contained resistance. In experiments to analyze growth rate, we determined 1 mM of potassium tetrathionate to be a plausible concentration resulting in no significant fitness cost due to

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Table 1. Strains, Plasmids, and Oligonucleotides

	relevant characteristics/sequence (5′−3′)	source or reference
strains		
Escherichia coli Nissle 1917		27
Salmonella Typhimurium	*Salmonella enterica* subsp. enterica serovar Typhimurium ATCC 29630	ATCC
Escherichia coli NEB10β	Δ(ara-leu) 7697 araD139 fhuA ΔlacX74 galK16 galE15 e14- ϕ80dlacZΔM15 recA1 relA1 endA1 nupG rpsL (StrR) rph spoT1 Δ(mrr-hsdRMS-mcrBC)	New England Biolabs
Escherichia coli DH5α	F− Φ80lacZΔM15 Δ(lacZYA-argF) U169 recA1 endA1 hsdR17 (rK−, mK+) phoA supE44 λ− thi-1 gyrA96 relA1	New England Biolabs
plasmids		
pBR322	pMB1, ApR, TcR	New England Biolabs
pLJV3	pUC57, CFP, mchA, rhaPBAD	this study; synthesized by General Biosystems
pJPMcH47	rhaPBAD, mchAXIBCDEF	this study
pttrMcH47	ttrRSBCA, mchAXIBCDEF	this study
pEX2000	pBR322, mchAXIBCDEF	29
oligonucleotides		
pBR322FWD	GGATTATTTCTATTTAAAATGAGGCCCTTTCGTCTTCAAGAATTCT	
pBR322REV	GAGATAGCGGTAGCTAACTAGACGTCAGGTGGCACTTTTCG	
pLJV3FWD	TTACCAGACCTCACCCAGACTTCATTACGACCAGTCTAAAAAGCGCCTG	
pLJV3REV	GAAAAGTGCCACCTGACGTCTAGTTAGCTACCGCTATCTCCAACGTGC	
pEX2000FWD	TTTAGACTGGTCGTAATGAAGTCTGGGTGAGGTCTGGTAAGA	
pEX2000REV	CTTGAAGACGAAAGGGCCTCATTTTAAATAGAAATAATCCTGTCAACAGTTCTCAACG	
pJPMcH47FWD	AAAAATCGAGCGTATATAACGTCTGGGTGAGGTCTGGTAAGA	
pJPMcH47REV	TCTGTTGGTTTGATCTGGCGGGATGTGACGATCGTTGACAGC	
SentFWD	TTACCAGACCTCACCCAGACGTTATATACGCTCGATTTTTGCCGGC	
SentREV	TGTCAACGATCGTCACATCCCGCCAGATCAAACCAACAGAA	

tetrathionate addition (Figure S3). Competition experiments were initiated with a *S.* Typhimurium (pBR322)−*EcN* pttrMcH47 ratio of approximately 1:1 (Figure 4B). Bacteria were grown for a total of 10 h, and 10-fold dilutions were plated onto MacConkey agar for colony enumeration. For both strains, we followed Lenski et al.[20] and our previous work[21,22] to determine relative fitness as

$$W_i = \frac{\Delta P_i}{P_i(0)} = \frac{P_i(end) - P_i(0)}{P_i(0)} \quad (1)$$

where $P_i(t)$ is the proportion of strain i at time t. We performed linear regression analysis and fitted the model $W_{SE} \sim 1 + Tet + Tag$ where *Tet* and *Tag* are two "dummy" variables, with *Tet* indicating absence/presence of tetrathionate and *Tag* indicating use of *S.* Typhimurium WT or *S.* Typhimurium pBR322. Results of linear regression show a significant decrease in *S.* Typhimurium fitness at 1 mM potassium tetrathionate irrespective of *S.* Typhimurium WT or *S.* Typhimurium pBR322 ($p < 0.02$). We also observe that the inhibitory effect is reduced when competition is performed against *S.* Typhimurium WT in a media lacking antibiotic addition ($p < 0.05$). However, because we do not detect significant differences in growth curve dynamics in isolation for any of the strains used in this experiment, neither in the presence nor in the absence of tetrathionate (Figure S3), we speculate that this reduced competitiveness against *S.* Typhimurium WT may be due to plasmid loss in the absence of antibiotic. When testing for an increase in *S.* Typhimurium proportion observed at 600 min compared to 360 min for the *EcN* pttrMch47 vs *S.* Typhimurium WT, we do not observe statistical significance ($p > 0.05$) between the two data points. We acknowledge but cannot explain the recovery by *S.* Typhimurium pBR322 from 360 to 600 min. Our results confirm the ability of *EcN* pttrMcH47 to suppress *S.* Typhimurium growth beyond what is obtained in direct competition experiments in an environment without the supplementation of tetrathionate (Figure 4C). The increased competitive advantage of *EcN* pttrMcH47 over *S.* Typhimurium in an environment supplemented with tetrathionate is particularly important considering recent work which has indicated that tetrathionate in the lumen of the inflamed gut provides a growth advantage for *S.* Typhimurium over the rest of the competing microbiota.[4]

Outlook. The use of microcins for the treatment of enterobacterial colitis can be regarded as a novel and potent alternative to antibiotics.[23] Here, we have developed a genetically engineered *E. coli* that produces a *S.* Typhimurium-inhibiting microcin in response to tetrathionate, a molecule resulting from the inflammation of the intestine, a hallmark of a *Salmonella* infection.[4] Using *in vitro* assays, we have shown that *EcN* pttrMcH47 not only prevents *S.* Typhimurium growth in static agar inhibition assays but also significantly reduces *S.* Typhimurium fitness in pairwise ecological competition experiments.

While current focus on engineered probiotics (with respect to gut pathogen infections) has been mostly directed toward disease sensing and reporting,[8,9] our work provides a first proof-of-concept toward using a genetically engineered living therapeutic to induce a specific microbiome correction during the course, or at the onset of, a particular disease state.

The realization that microcins play a crucial role in gut microbiome ecology[5] opens a new door for the treatment and prevention of a range of gut pathogens. As new microcins or related antimicrobial peptides with narrow target specificity are further discovered and characterized,[24] our work provides a demonstration of a plausible delivery method for timely gut pathogen eradication with minimal off-target effects, as compared to broad spectrum antibiotics.

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To obtain a proof-of-concept system that would ensure a fully functional inflammation sensing−pathogen killing phenotype, without the need of any extra strain-specific chromosomal features, we included all the necessary genes for tetrathionate sensing and MccH47 production into a single DNA construct. We then transferred this system to *EcN*, an FDA-approved probiotic that has been shown to be amenable toward engineered probiotic applications.[8,11] For illustrative purposes and in line with recent studies on the construction and preliminary testing of engineered bacteria,[8,10,11,25] we opted for a plasmid-based approach. However, for translation to clinics, as well as for trials using *in vivo* models, future work will be performed to achieve stable chromosomal integration. This may involve using a background genome with promising characteristics regarding host colonization and retention of recombinant genes, such as *E. coli* NGF-1.[9]

A possible issue that is common to biocontrol through supplementation of native and engineered bacteria comes from the emergence of mutations that may alter the regulation or function of the system. However, in light of recent work that demonstrated wild-type stability and mutational rate of the *ttr* operon in an engineered *E. coli*,[9] we expect our probiotic to properly work over the time frame of a *Salmonella* infection. Moreover, future efforts to properly control for this possible issue could lead to the development of a probiotic that autolyzes within a certain time frame after supplementation.[26] As such, utilizing this method, it is possible to envision a single probiotic bacterium capable of colonizing a host, sensing a disease state or particular pathogen, and eradicating the disease-causing organism without the need for physician intervention or use of broad-spectrum antibiotics.

METHODS

Microbial Strains, Media, and Growth Conditions. Strains used in this study include *Escherichia coli* strain NEB10β (New England Biolabs. Ipswich, MA), *E. coli* strain DH5α (New England Biolabs. Ipswich, MA), *E. coli* strain Nissle 1917 (gift from),[21] and/or *Salmonella enterica* subsp. enterica; serovar Typhimurium ATCC 29630 (purchased from ATCC, Manassas, VA). Plasmid constructs developed in this work were first transformed by electroporation into *E. coli* NEB10β cells and then to *E. coli* Nissle (*EcN*). Oligonucleotides used in this study are listed in Table 1. All media and additional reagents listed in this study were purchased from Sigma-Aldrich, St. Louis, MO, unless otherwise indicated.

pJPMcH47 and pttrMcH47 were constructed using standard methods for Gibson Assembly[28] and the Gibson Assembly Master Mix (New England Biolabs. Ipswich, MA). To construct pJPMcH47, a linear version of pBR322 was produced by polymerase chain reaction using primers pBR322FWD and pBR322REV, and select regions of pLJV3 (encoding rhaP$_{BAD}$, CFP, *mccF*, and *mchA*) and pEX2000 (encoding *mchXIBCDEF*) were amplified using primer sets pLJV3FWD/pLJV3REV and pEX2000FWD/pEX2000REV, respectively. pttrMcH47 was constructed by amplification of pJPMcH47 using primers pJPMcH47FWD/pJPMcH47REV and amplification of the *ttrRSBCA* operon of *S.* Typhimurium using primers SentFWD/SentREV. *ttrRSBCA* was included in the plasmid as opposed to just *ttrRS* because in Riglar et al.[9] the constructed sensor strain, when in direct competition with *Salmonella*, only detected tetrathionate if the *Salmonella* was incapable of tetrathionate utilization (ΔttrR).

Solid Media Inhibition Assays. Inhibition assays in solid media were designed and carried out on the basis of previous work by Delgado et al.[16] Briefly, *E. coli* strains were grown overnight on LB agar plates, supplemented with carbenicillin (100 μg/mL) when relevant. Individual colonies were then used to inoculate 3 mL of LB broth, supplemented with carbenicillin (100 μg/mL) when relevant. 1 μL of liquid culture was then used to create an agar stab in solid media and incubated at 37 °C, either aerobically or anaerobically, for 24 h. Postincubation, cells were inactivated with chloroform and UV. Molten 3% agar was then added to an overnight culture of susceptible cells to a final concentration of 0.75%, and then, 3 mL of the mixture was overlaid on top of the inactivated agar stab plates and allowed to solidify. Plates were then incubated aerobically overnight at 37 °C, and inhibition halos were observed. When inhibition halo size was a relevant characteristic, ImageJ software (https://imagej.nih.gov/ij/) was utilized. For solid medium inhibition assays of *S.* Typhimurium and *E. coli* DH5α by *EcN* pJPMcH47, agar stabs were made in M9 minimal salts supplemented with 0.1 mM $CaCl_2$, 2 mM $MgSO_4$, 0.2 mM 2,2′-dipyridyl, and 0.4% L-rhamnose. For variable L-rhamnose concentration inhibition experiments, culture stabs of *EcN* pJPMcH47 were made in LB agar supplemented with 0.2 mM 2,2′-dipyridyl and L-rhamnose, ranging from 0.25 μM to 10 mM. All aspects of inhibition assays utilizing plasmid pJPMcH47 were performed aerobically.

For solid medium inhibition assays of *S.* Typhimurium by *EcN* pttrMcH47, culture stabs in LB agar supplemented with 0.2 mM 2,2′-dipyridyl and 1.0 or 1.5 mM (see main text) potassium tetrathionate were incubated anaerobically at 37 °C for 24 h in Oxoid anaerobic jars with anaerobic atmosphere generation bags. Notably, potassium tetrathionate was not added to the LB agar medium until the temperature had reached 50 °C and was prepared immediately before introduction to the media and sterilized by filtration using a 0.22 μm filter membrane. Upon removal from the jars, cells were immediately inactivated, overlaid with *S.* Typhimurium culture in 0.75% agar, and incubated aerobically overnight.

Because microcin H47 mode of action relies on functional catecholate siderophore receptors *iroN*, *cir*, and *fepA*,[6] 2,2′-dipyridyl addition was essential to render *S.* Typhimurium maximally susceptible to inhibition by our probiotic. Control experiments using the laboratory strain *E. coli* DH5a allowed us to rule out necessity of iron limitation for the MccH47 production by our constructs (Figure 3A,B).

Liquid Media Competition Assays. Competition assays were carried out in triplicate for each experimental condition in a Forma Scientific Model 1025 anaerobic chamber. LB was allowed to equilibrate in anaerobic conditions overnight, prior to initiation of any competition assays. 3 mL of LB was distributed to individual test tubes, with a final concentration of 0.2 mM 2,2′-dipyridyl and both 1 mM potassium tetrathionate and 100 μg/mL carbenicillin, as indicated. Potassium tetrathionate was prepared immediately before inoculation and sterilized by filtration using a 0.22 μm filter membrane. Individual colonies of *EcN* pttrMcH47 and *S.* Typhimurium and *S.* Typhimurium pBR322 were taken from LB agar plates and incubated aerobically overnight at 37 °C in 3 mL of LB, supplemented with 100 μg/mL carbenicillin for plasmid retention when relevant. Liquid cultures were then transferred to the anaerobic chamber, and ∼10^5 cells of each culture were transferred into media for experimental conditions for analysis of relative fitness in competition, on the basis of the presence of

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tetrathionate. Competition assays were set up to compete *EcN* pttrMcH47 with *S.* Typhimurium and *EcN* pttrMcH47 with *S.* Typhimurium pBR322, in which case 100 μg/mL carbenicillin was supplemented to the media. Cultures were serially diluted and plated onto MacConkey Agar for colony enumeration.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acsinfecdis.7b00114.

Figure S1, *E. coli* Nissle 1917 pBR322 and *E. coli* NEB10β pJPMcH47 inhibition of *E. coli* DH5α and *S.* Typhimurium; Figure S2, *E. coli* Nissle 1917 pBR322 and *E. coli* Nissle 1917 pJPMcH47 inhibition of *E. coli* DH5α and *S.* Typhimurium in the presence and absence of 0.2 mM 2,2′-dipyridyl; Figure S3, growth curves in LB media under anaerobic conditions with 0.2 mM 2,2′-dipyridyl (PDF)

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Author Contributions

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

Notes

The authors declare no competing financial interest.

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