**INTRODUCTION**

Cholera is a life-threatening gastrointestinal infection that principally affects populations where basic sanitation and healthcare are insufficient (1). Severe dehydration from cholera can lead to death within hours if treatment is not provided promptly. Strengthening surveillance and early identification of cholera cases are top priorities of the World Health Organization for the prevention of cholera outbreaks. Therefore, implementation of cost-effective measures that prevent disease progression—from initial infection to active disease and dissemination—is urgently needed. Microbiota interventions have been used to treat or alleviate a variety of gastrointestinal disorders (2, 3). In the case of cholera, it has been demonstrated that natural members of the human microbiome can reduce colonization of *Vibrio cholerae* in gnotobiotic mice through interference of the pathogen’s quorum-sensing signaling (4). Furthermore, engineered probiotics have been used to interfere with bacterial communication to suppress virulence (5), or to compete with intestinal receptor binding to the cholera toxins (6). Laboratory strains of *Escherichia coli* have also been engineered for the detection of *V. cholerae* signals under culture conditions (7). However, the demonstration of the in vivo functionality of engineered *V. cholerae* detection systems in safely consumed, gut-relevant bacteria is lacking.

Food-associated bacteria play a central role in the function and health of the gastrointestinal tract (8). High doses of ingested, live bacteria can create a microbiota shift that allows transient modulation of the human intestinal microbiome (8), a phenomenon that can be leveraged to reduce pathogen colonization (9) and enable in situ diagnosis at an early stage of infection. We hypothesized that dietary-associated bacteria with natural infection-antagonizing properties could be identified, applied, and further enabled with engineered diagnostic functions to ameliorate the impact of cholera and facilitate disease surveillance in populations at risk of cholera outbreaks.

**RESULTS**

Promoting colonization resistance against *V. cholerae* with *Lactococcus lactis*

*L. lactis* is a food-associated lactic acid bacterium that has been safely consumed for millennia as part of fermented milk products (10). This bacterium displays strong acidification capabilities that stem from its high carbon flux metabolism, which is optimized for the conversion of simple carbohydrates into lactic acid (11, 12). Given that *V. cholerae* is known to be particularly sensitive to acidic conditions (13) and that both *Lactis* and *V. cholerae* find temporary residence in the small intestine (14, 15), we sought to determine whether *L. lactis* could be used to promote intestinal colonization resistance against *V. cholerae* through the production of lactic acid. Initial in vitro agar diffusion and liquid coculture assays showed that *L. lactis* could effectively antagonize *V. cholerae* growth (Fig. 1, A and B, and figs. S1 and S2). The *L. lactis* antibacterial effect on *V. cholerae* was abolished when lactic acid production was impaired by mutation of the lactate dehydrogenase (*ldh*) gene or when the pH of the coculture was strongly buffered (Fig. 1, A and B). These results indicate that lactic acid, a major product of *L. lactis* metabolism, is a *V. cholerae*–antagonizing agent (figs. S2 and S3).

We next used an infant mouse model of cholera infection to test the ability of *L. lactis* to enhance colonization resistance against *V. cholerae* (5, 16). We evaluated the intestinal transit dynamics of *L. lactis* in the infant mouse model and determined that dosing 10⁹ colony-forming units (CFU) of *L. lactis* cells every 10 hours ensures its sustained presence in the mouse small intestine (fig. S4). Four-day-old mice were inoculated with 10⁷ CFU of *V. cholerae* either at the same time (coadministration regimen) or 5 hours after the first *L. lactis* dose (preadministration regimen), and host survival was monitored for the next 42 hours (Fig. 2A). We found that *V. cholerae*–infected mice were substantially more likely to survive when they were coinoculated with *L. lactis* (84.6%) compared to when they were mock-fed (45.7%) (Fig. 2B). Three independent trials showed that the improved survival is not affected by litter variation (fig. S5). Mouse survival was comparable for the coinoculation and preadministration regimens, indicating that the *L. lactis* dietary intervention provides protection against *V. cholerae* infection within the 10-hour window between the probiotic doses (Fig. 2B). Furthermore, in the coinoculation regimen, the *V. cholerae* burden in *L. lactis*–fed mice was reduced by about 100-fold compared to that in
mock-treated mice at 26 hours after infection (Fig. 2C). We found that the *V. cholerae* load correlates with the infection outcome (Fig. 2C). Regardless of the probiotic intervention, mice that died from cholera have *V. cholerae* developed to 10⁷ CFU in the gut; the ones that survived until the end of study had lower than 10⁶ CFU. This correlation between reduced *V. cholerae* burden and mouse survival includes mice that were not fed *L. lactis* (Fig. 2C), further suggesting that *L. lactis* assists in mouse survival through reduced *V. cholerae* colonization. These findings indicate that a regular feeding regimen of *L. lactis* can prevent the aggressive development of cholera by limiting the bacterial burden to nonlethal concentrations in infant mice.

To further investigate the involvement of *L. lactis* lactic acid production in the reduction of *V. cholerae* burden in vivo, we measured the intestinal lactate levels during *V. cholerae* mouse infections. At 26 hours after infection, mice that were fed *L. lactis* exhibited increased levels of lactate in the gut compared to mice that had been mock inoculated (Fig. 2D). When the lactate dehydrogenase mutant strain of *L. lactis* was used, the protective effect against *V. cholerae* infection disappeared (Fig. 2E). Abolishing lactate dehydrogenase function reroutes carbon flux toward mixed acid fermentation pathways and results in a slower growth rate (Fig. S6), an effect that might also contribute to its reduced protective effect in vivo. Genetic complementation with plasmid-encoded *ldh* restored both the strain’s growth rate and acidification capabilities, and consequently its protective function in vivo (Fig. 2E and Fig. S6). As expected, when heat-inactivated cells were used instead of live *L. lactis*, the survival rate of the treated mice was similar to the mock-fed group, indicating that metabolically active *L. lactis* are needed for effective protection (Fig. 2F). These findings suggest that in situ production of lactic acid by metabolically active bacteria inhibits *V. cholerae* colonization and is essential for *L. lactis* to provide the observed protective effect.

To investigate the effect of direct intake of lactic acid on *V. cholerae* intestinal colonization, we performed oral gavage of lactic acid into *V. cholerae*-infected infant mice and observed a reduction of pathogen load similar to the treatment with *L. lactis* in 62.5% of the mice, whereas the rest of the litter showed a colonization akin to the mock-treated control (Fig. S7). These results indicate that although direct administration of lactic acid to the gastrointestinal tract can be sufficient to recapitulate the effect of *L. lactis* in the reduction of *V. cholerae* colonization, it is not as effective as the dietary intervention with live bacteria. Orally administered lactic acid is susceptible to neutralization during translocation from the stomach into the small intestine. An in vitro study showed that intestinal lactate concentration in *L. lactis*–treated and mock-treated infant mice at 26 hours after infection disappeared (Fig. 2E). Abolishing lactate dehydrogenase function might also contribute to its reduced protective effect in vivo. Genetic knockout of lactate dehydrogenase 

Fig. 2. *L. lactis*–derived lactic acid antagonizes *V. cholerae* infection in mice. (A) Intragastric feeding regimens of *L. lactis* and inoculation time of *V. cholerae* to infant mice. Mock-fed mice were administered GM17 medium instead of *L. lactis*. (B) Effects of *L. lactis* intervention on infant mouse survival to cholera infection under both feeding regimens. Coadministration with *V. cholerae*, ***P = 0.0005; pre-administration, **P = 0.0187, log-rank test against mock-fed (*). V: n = 39, N = 6; V + L, coadmin: n = 39, N = 6; V + L, preadmin: n = 19, N = 6. (C) Effect of *L. lactis* intervention on *V. cholerae* colonization in the infant mouse gut. (C) Left: *V. cholerae* intestinal colonization at 26 hours after infection. Right: *V. cholerae* burden in mice that died from cholera and in mice that survived the infection by 42 hours after infection. Each data point represents an individual mouse. Horizontal lines are medians. P values from Mann-Whitney test (n = 7, N = 7 for nontreated/treated; n = 11, N = 7 for dead versus survived, respectively). Data correspond to coadминистration regimen. (D) Intestinal lactate concentration in *L. lactis*–treated and mock-treated infant mice at 26 hours after infection. Mann-Whitney test (n = 7, N = 7). Data correspond to coadministration regimen. (E) Effects of lactate dehydrogenase mutants (*Δldh*) and plasmid-complemented lactate dehydrogenase *Δldh* plDH in *L. lactis* strains on infant mouse survival to cholera infection. Functional knockout of lactate dehydrogenase compromises the protective effect against cholera (n = 20, N = 6), ns, not significant by log-rank test compared to mock treatment (*). Complementation with a plasmid-based *ldh* restores the protective effect (**P = 0.0349; log-rank test; n = 9, N = 3). (F) Effect of treatment with heat-inactivated *L. lactis* on infant mice survival to cholera infection, n = 10, N = 3, n, number of individual mice; N, number of litters covered in each group.

To understand the protective effect of *L. lactis*, we performed agar diffusion assays with wild-type (WT), lactate dehydrogenase mutant (*Δldh*), and plasmid-complemented lactate dehydrogenase mutant *Δldh* (*pLDH*) strains of *L. lactis* grown on a lawn of *V. cholerae*. Lactate dehydrogenase activity is required for the growth of *L. lactis* on agar containing *V. cholerae* lawn. Inhibition zones correlate with the acidification range of *L. lactis* colonies. *L. lactis* cell density is in coculture with *V. cholerae* in minimally buffered media and strongly buffered media (+buffer). For detailed media conditions, see Materials and Methods and fig. S2. Bars represent range of technical duplicates.

Fig. 1. Lactic acid–dependent antibacterial effect of *L. lactis* against *V. cholerae* in vitro. (A) Top: Agar diffusion assay of wild-type (WT), lactate dehydrogenase mutant (*Δldh*), and plasmid-complemented lactate dehydrogenase mutant *Δldh* (*pLDH*) strains of *L. lactis* grown on a lawn of *V. cholerae*. Bottom: Agar diffusion assay of *L. lactis* strains in minimally buffered GM17 agar plates containing pH indicator bromocresol purple, which turns yellow when pH drops below 5.2. Inhibition zones correlate with the acidification range of *L. lactis* colonies. (B) Acidification dynamics and *V. cholerae* cell density in coculture with *L. lactis* in minimally buffered media and strongly buffered media (+buffer). For detailed media conditions, see Materials and Methods and fig. S2. Bars represent range of technical duplicates.

upper small intestine; in contrast, in situ production of lactic acid by transit-
ing \textit{L. lactis} cells allows a direct supply of acid that is independent of upstream gastrointestinal pH changes.

Higher gastrointestinal pH increases the susceptibility to \textit{V. cholerae} infection in humans, especially due to the use of acid-suppressing drugs such as proton pump inhibitors (17). We evaluated the effect of the \textit{L. lactis} intervention on \textit{V. cholerae}-infected infant mice when oral gavage is performed using media buffered to pH 7.4 (fig. S8). We found that under acid-neutralizing conditions, the survival of mock-treated mice was reduced to 11%, whereas the survival of \textit{L. lactis}-treated mice was 50.8%, which suggests that probiotic intervention might ameliorate the impact of the disease even under conditions of increased susceptibility of infection.

**Engineering cholera-sensing functions into \textit{L. lactis}**

Next, we sought to engineer \textit{L. lactis} to specifically detect and report on \textit{V. cholerae} presence in the intestinal environment. \textit{V. cholerae} produces the quorum-sensing molecules autoinducer-2 (AI-2) and cholera autoinducer 1 (CAI-1), and uses the two-component histidine kinase receptors LuxQ and CqsS to specifically detect each molecule, respectively (18). Unlike AI-2, CAI-1 is unique to the genus \textit{Vibrio}; therefore, we sought to develop a receptor in \textit{L. lactis} to detect CAI-1 from \textit{V. cholerae}. We took advantage of CqsS’s specificity to CAI-1 and the modularity of histidine kinase receptors to design an \textit{L. lactis} hybrid receptor (HR) that combines the transmembrane ligand binding domain of CqsS with the signal transduction domain of NisK, a two-component receptor in \textit{L. lactis} that regulates the production of the lantibiotic nisin (Fig. 3A) (19).

We designed 10 HR variants with different CqsS-NisK junction points (fig. S9A). Because of the known effect of altered NisK expression levels on signaling (20), we cloned a randomized ribosome binding site (RBS) sequence upstream of each HR variant and screened the resulting HR libraries for CAI-1–dependent activity (fig. S9B). To create a convenient screening platform, we cloned the fluorescent reporter gene encoding mCherry downstream of the

---

**Fig. 3. Construction of HR for \textit{V. cholerae} sensing.** (A) Creation of a CAI-1–sensing function in \textit{L. lactis} requires the fusion of two distantly related quorum-sensing signaling pathways. The proposed HR fuses the CAI-1–binding domain of the CqsS receptor with the histidine kinase domain of NisK to achieve CAI-1–dependent signaling in \textit{L. lactis}. The design of the HR cannot predict whether the regulatory outcome of CAI-1 binding will be activation (arrow-headed line) or inactivation of NisR (bar-headed line). (B) Functional screen for HR variants. CqsS-NisK fusion variants with randomized RBS strengths (fig. S9) were screened for their ability to modulate output gene expression in response to CAI-1. CAI-1–deficient \textit{V. cholerae} (\textit{VΔcqsA}) serves as a negative control. (C) mCherry fluorescence output of HR variants in response to CAI-1. Hybrid receptor 4 mutant (HR4M) is a functional variant. Hybrid receptor 2 (HR2) is an example of a nonfunctional variant. (D) Primary sequence map of HR4M. S\text{177} indicates the last residue of N-terminal part of CqsS, and A\text{221} indicates the first residue of the C-terminal part of NisK. The HR contains a spontaneous Glu-to-Gly mutation at residue 182. (E) Reporter gene expression dynamics in \textit{L. lactis} with HR4M in response to CAI-1. Error bars are SEM of three technical replicates. a.u., arbitrary units.
NisR-responsive nisA promoter and assayed each HR variant in a high-throughput coculture system with both wild-type V. cholerae and a V. cholerae cqsA deletion strain that is unable to produce CAI-1 (Fig. 3B).

The native nisRK two-component system and the majority of HR variants were not responsive to CAI-1 (fig. S9, C and D). However, a functional variant with a Glu-to-Gly mutation at residue 182 (HR4M) displayed robust repression of mCherry in the presence of CAI-1 (Fig. 3, C and D). Detailed characterization of the dynamic response of HR4M to CAI-1 showed that mCherry repression occurred within 4 hours of L. lactis–V. cholerae coculture initiation, and the transcriptional response was entirely dependent on CAI-1 production by V. cholerae (Fig. 3E). Nisin binding to NisRK is known to cause a conformational shift that activates autophosphorylation and induces transcription of the target gene (18). However, the transcriptional repression observed suggested that HR4M preserves the phosphorelay properties of CqsS, wherein constitutive autophosphorylation is inhibited upon CAI-1 binding, leading to reduced transcription of target genes.

**In situ detection of V. cholerae infection**

We next incorporated a transcriptional inverter circuit that programs HR4M signaling into an activation response upon pathogen detection. As shown in Fig. 4A, we placed the gene encoding the transcriptional repressor TetR downstream of the HR4M-controlled nisA promoter to enable constitutive repression of an engineered Bacillus subtilis xylA-tetO promoter (21) in the absence of CAI-1. Upon CAI-1 binding, HR4M repression of TetR expression enabled transcription from the xylA-tetO promoter (fig. S10A). We screened a library of tetR RBS sequences to identify an optimal TetR translation rate that would provide a suitable dynamic range of reporter gene expression upon CAI-1 induction (fig. S10B). Detection circuit characterization in coculture conditions indicates that mCherry expression increased 60-fold in response to CAI-1 from V. cholerae with minimal signal background (Fig. 4B). Although CAI-1–based quorum-sensing systems are present in most Vibrio spp., CAI-1 moieties might display different acyl chain lengths and modifications depending on the bacterial species (22). We tested the specificity of HR4M to other CAI-1–producing and nonproducing bacterial species and found that the detection circuit did not respond to most of the environmental Vibrio strains tested or to non–CAI-1–producing bacteria (fig. S11). However, Vibrio spp. producing CAI-1 moieties that are compatible with CqsS from V. cholerae such as Vibrio alginolyticus (22) were detected by HR4M (fig. S11), suggesting that this system might be used for the detection of other pathogenic Vibrio spp. that produce CqsS-compatible quorum-sensing molecules, such as Vibrio parahaemolyticus, Vibrio anguillarum, and Vibrio furnissii (22).

As a proof of concept of a living diagnostic tool with an easy, point-of-need readout, we replaced mCherry with a secreted β-lactamase reporter whose activity is readily visualized by a colorimetric shift from yellow to red after hydrolysis of the chromogenic substrate nitrocefin (23, 24). Evaluation by in vitro coculture showed that the reporter strain, termed CSL, could generate a visible color.
change within 30 min when the coculture had reached a density of $10^8$ CFU/ml of *V. cholerae* (Fig. 4C). When the reporter assay was tested in the infant mouse model (Fig. 4D), only the fecal pellets of mice dosed with a constitutive producer of β-lactamase displayed a positive signal, demonstrating both the orthogonality and stability of the β-lactamase reporter in the mouse intestinal environment (Fig. 4E). Also, fecal pellets of mice dosed only with the *L. lactis* CSL strain did not display a color change, which demonstrates a tight regulation of the engineered detection circuit under in vivo conditions (Fig. 4E). Finally, to determine whether *L. lactis* CSL could detect cholera infection in the infant mouse model, we administered two doses of the engineered strain, the first concurrent with *V. cholerae* and the second 10 hours after infection, to mice and collected stool samples for colorimetric assays (Fig. 4D). The fecal samples of cholera-infected mice treated with *L. lactis* CSL showed positive signals after overnight incubation with nitrocefin, indicating circuit activation and β-lactamase secretion in the gut (Fig. 4E). In contrast, stool samples from uninfected mice and mice infected with the *csaA* mutant of *V. cholerae* did not show colorimetric change, demonstrating that the circuit is CAI-1–specific.

**Probiotic intervention with natural and engineered *L. lactis***

We found that, compared to wild-type *L. lactis*, the *L. lactis* CSL strain exhibited a reduced protective effect in our infant mouse model for cholera infection (Fig. 5A). To understand this phenomenon, we studied the strain’s growth and acidification dynamics in monoculture and coculture under anaerobic conditions (figs. S12 and S13). When assayed in the infant mouse model, the engineered *L. lactis* CSL strain had a lower median of recovered cells from the gut than the wild-type strain; however, no significant difference was detected between strains (fig. S12A). Under liquid culture conditions, *L. lactis* CSL acidifies medium slower than the wild-type strain (fig. S12B). Furthermore, the viability of the *L. lactis* CSL strain decreases under extended coculture condition, and the steady-state pH is higher than the coculture with wild-type *L. lactis* (fig. S12B). Also, given the observed defect in acidification, the *V. cholerae* killing by *L. lactis* CSL is delayed by 4 hours compared to wild-type *L. lactis* (fig. S12C). The results we obtained indicate that circuit activation and β-lactamase secretion in the engineered *L. lactis* strain create a metabolic burden that reduces the bacteria’s natural acidification capacities and its antagonistic effect on *V. cholerae*, further supporting the hypothesis that lactic acid production in the gut environment plays a key role in the protective effect. To achieve a combined protective and diagnostic function, we introduced a mixed population of wild-type and CSL *L. lactis* into the feeding regimen (Fig. 5A). The combined dose of natural and engineered *L. lactis* successfully restored the protective effect and provided an effective diagnostic readout in stools (Fig. 5, A and B).

**DISCUSSION**

Here, we present evidence indicating that the common lactic acid bacterium *L. lactis*, through its natural acidification function, can be used to inhibit *V. cholerae* infection in a mouse model. Our experiments demonstrate that dietary interventions with metabolically active probiotic bacteria can be used as an effective strategy to induce modifications of the intestinal microenvironment that in turn lead to the enhancement of colonization resistance against a major intestinal pathogen. The infection model used in this work allows infant mice to feed ad libitum from the mother, a situation that provides them with a constant source of lactose, favoring the *L. lactis*–mediated production of lactic acid in the intestinal environment. The beneficial effects of ingesting metabolically active *L. lactis* have been observed in other animal models of cholera infection—Zamri *et al.* (25) noted that oral vaccination of adult rabbits with wild-type and recombinant *L. lactis* expressing *V. cholerae* membrane proteins abolished the diarrheal symptoms of the cholera infection. Although antimicrobial properties of lactic acid have been widely applied as a food preservation method (26), our findings uncover that regular ingestion of metabolically active *L. lactis* represents an effective means to deliver lactic acid to the intestinal environment and create inhospitable conditions for *V. cholerae* proliferation. Future implementation of probiotic interventions with *L. lactis* in humans will require a formulation system that ensures the delivery of sufficient quantities of probiotic bacteria and key metabolic substrates so that lactic acid production occurs regardless
of the individual’s diet or intestinal conditions. The use of microbiota-
modulating interventions represents an emerging strategy that
might confer rapid protection against fast-developing infections
like cholera. Notably, Hubbard et al. (27) created a live cholera vac-
cine candidate that provides colonization resistance in a probiotic-
like fashion, conferring cholera protection within 1 day in an infant
rabbit model.

Here, we have also demonstrated the rational design and devel-
opment of a synthetic gene circuit that enables L. lactis to detect
and respond to V. cholerae both in vitro and in the intestinal envi-
ronment. We further coupled the circuit to a proof-of-concept
enzyme-based assay that reports the in situ detection of the path-
ogen through a simple colorimetric assay on stools, demonstrating
the potential of engineered probiotics as point-of-care diagnostic
tools. Our results indicate that in situ biosensing and reporting of
the pathogen presence is feasible through the use of synthetic gene
networks and stable, orthogonal reporter enzymes that can survive
the intestinal environment. Future application of such living diag-
nostics in the field will entail optimization of the response time to
readable output to obtain diagnostic results within clinically rele-
vant time scales. Furthermore, there are additional technological
and regulatory limitations that need to be addressed before in vivo
diagnostic systems are suitable for deployment in cholera endemic
areas. The β-lactamase–based enzymatic reporter used here as sig-
nal output is suitable for laboratory experiments; however, its use in
the field is restricted due to potential horizontal gene transfer of
the β-lactamase gene to pathogenic bacteria and cost-effectiveness
of the use of its substrate, nitrocefin. Therefore, there is a need for
the development of a food-grade enzymatic reporter that is orthog-
onal to the human intestine and whose cognate substrate can be
produced cost-effectively and distributed en masse in low-resource
settings.

Our work sets the stage for dietary interventions with natural
and engineered probiotics as an alternative strategy to combat the
spread of cholera in vulnerable populations. Household contact
with infected individuals is among the highest known risk factors
for subsequent infection and dehydration (28). Regular consump-
tion of affordable, home-fermented food products containing live
L. lactis may not only diminish the severity of the disease through
colonization resistance, but if used in combination with a living di-
agnostic L. lactis strain, it may also provide an opportune alert for
patients to seek further medical intervention and a timely warning
for close contacts to apply sanitary measures to prevent transmis-
sion. The use of biologics–based prophylaxis, such as the probiotic
intervention presented in this study or the recently proposed phage-
based prophylaxis (29), could serve as an alternative to convention-
al chemoprophylaxis, limiting the emergence of antibiotic resistance
in the population (28). The implementation of engineered probiot-
ics, such as the L. lactis CSL strain, has the potential to provide
community-level surveillance of cholera cases and could help to
elucidate the contribution of asymptomatic carriage of V. cholerae
to disease transmission (30). The development of living diagnostic
bacteria (31–35) represents a promising approach to achieve near
real-time surveillance of multiple pathological conditions. Our work
advances this endeavor by demonstrating the design and construc-
tion of a hybrid sensing and reporting system, which provides a mo-
olecular architecture strategy for generalizable “sense-and-respond”
bioengineering, expanding the possible targets of detection and the
variety of engineerable sensor bacteria.

MATERIALS AND METHODS
Study design
The overall objective of this work was to demonstrate that microbiota-
modulating interventions with natural and engineered probiotics
might represent a plausible strategy to achieve two high-priority
goals in the fight against cholera, namely, enhancement of coloniza-
tion resistance and point-of-need diagnostics for disease surveil-
ance. We hypothesized that the transient colocalization of ingested
L. lactis and V. cholerae could be leveraged to promote colonization
resistance through the in situ production of lactic acid and to detect
the presence of the pathogen at an early stage of the infection. We
used a synthetic biology approach to construct a hybrid-signaling
pathway in L. lactis that enables the detection of quorum-sensing
signals that are indicative of V. cholerae infection. After in vitro val-
idation of these hypotheses, we used the infant mouse model of
cholera infection to evaluate the efficacy of the proposed dietary
intervention using survival, intestinal colonization, and production
of diagnostic enzymatic reporters as the principal outcomes. To op-
timize the number of mice needed in each trial without compromis-
ing the power of the study, we performed the survival studies in a
randomized cross-litter manner (except for the three whole-litter
studies in fig. S5). Each trial was performed with at least three litters.
Each litter was randomly divided into two to three groups with a
random number generator. Each group was assigned to a different
treatment. Overall, each treatment covered individual mice from at
least three litters, and data were collected over two or more inde-
hendent trials [apart from the heat-inactivated (Fig. 2F) and
ldh-complemented (Fig. 2E) L. lactis tests, each from one independent
trial]. No blinding was performed in either experiments or data
analysis. Sample sizes were determined by power analysis to reach a
statistical power of 0.8 or higher using the pwr package in R.

All mice in this study were treated in accordance with protocol
number IS00000852, approved by Harvard Medical School Institu-
tional Animal Care and Use Committee and the Committee on
Microbiological Safety. Primary data are reported in table S1. A de-
tailed description of all experimental procedures performed in this
study is included in the Supplementary Materials and Methods.

Statistical analysis
Statistics were performed with GraphPad Prism 5.0c. Log-rank tests
were performed for all survival studies, assuming the mice in each
group were independent and the hazard ratio was consistent over the
study period. Each treatment group was predefined randomly cross
litters at the beginning of the study (details in the Supplementary
Materials and Methods). Mann-Whitney test was used where each
data point was independent from each other, and no Gaussian dis-
tribution was assumed. P values reported in the figure legends are
two-tailed. Specific sample size, P value, and other details for each test
are described in the figure legends.

SUPPLEMENTARY MATERIALS
www.sciencetranslationalmedicine.org/cgi/content/full/10/445/eaao2586/DC1
Materials and Methods
Fig. S1. Homolactic fermentation of glucose by L. lactis leads to growth inhibition of V. cholerae.
Fig. S2. L. lactis–V. cholerae coculture system.
Fig. S3. Lactic acid displays a bactericidal effect on V. cholerae.
Fig. S4. L. lactis transit dynamics in mouse gut.
Fig. S5. Litter variation in infant mouse survival study.
Fig. S6. Growth and acidification capability of L. lactis strains used in this study.
Fig. S7. V. cholerae colonization in infant mice treated with lactic acid.
REFERENCES AND NOTES

21. R Development Core Team, “R: A Language and Environment for Statistical Computing” (Vienna, 2016); www.r-project.org/.
22. EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP), Safety and efficacy of lactic acid and calcium lactate when used as technological additives for all animal species. EFSA J. 13, 4198 (2015).
Acknowledgments: We are grateful to W. Zhao (Mekalanos Lab, Harvard Medical School) and A. Graveline (Wyss Institute) for help on the animal protocol setup. We thank F. Hussain and D. VanInsberghe (Polz Lab, Massachusetts Institute of Technology) for providing us with environmental Vibrio spp. strains. We also thank X. Tan, M. Takahashi, and S. Slomovic for the helpful discussions. Funding: This work was supported by funding from the Defense Threat Reduction Agency grant HDTRA1-14-1-0006 (to J.J.C.), the Gates Foundation OPP1058951 (to D.E.C.), and the Paul G. Allen Frontiers Group (to J.J.C.).

Author contributions: J.J.C., D.E.C., A.C.-R., and N.M. conceived the project. N.M. and A.C.-R. designed and performed the colonization resistance studies. N.M. and D.E.C. designed and performed the diagnostic circuit experiments. N.M., D.E.C., and A.C.-R. designed the animal experiments. N.M. performed the animal experiments and analyzed the data. All the authors wrote and edited the manuscript. Competing interests: J.J.C. is the scientific co-founder of Synlogic, a biotech startup focused on developing engineered probiotics to treat disease. Patent disclosure: Synthetic hybrid receptor and genetic circuit in probiotic bacteria to detect enteric pathogenic microorganisms. Inventors: D.E.C., N.M., and J.J.C. (U.S. provisional application no. 62/172,971; filed on 9 June 2015). Data and materials availability: Primary data for experiments presented here are in the Supplementary Materials. Correspondence and requests for materials should be addressed to J.J.C.

Submitted 30 June 2017
Resubmitted 22 January 2018
Accepted 8 May 2018
Published 13 June 2018
10.1126/scitranslmed.aao2586

Probiotic strains detect and suppress cholera in mice
Ning Mao, Andres Cubillos-Ruiz, D. Ewen Cameron and James J. Collins

Sci Transl Med 10, eaao2586.
DOI: 10.1126/scitranslmed.aao2586

Designer bugs as drugs
The endemic persistence and outbreaks of Vibrio cholerae indicate a need for new methods of control; in this issue, two groups investigated the potential of engineered bacteria to mediate cholera resistance in animal models. Mao et al. discovered that lactic acid production by the probiotic Lactococcus lactis rendered the infant mouse gut hostile to V. cholerae and engineered L. lactis to detect breakthrough infection. Hubbard et al. extensively modified a contemporary V. cholerae strain for a live oral vaccine, which resulted in an attenuated strain that could protect infant rabbits from V. cholerae challenge within 24 hours of vaccine administration, indicating the protective effects were not dependent on adaptive immunity. These papers showcase innovative approaches to tackling cholera.