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Bacteriocin production augments niche competition by enterococci in the mammalian GI tract

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Abstract

Enterococcus faecalis (EF) is both a common commensal of the human gastrointestinal tract (GI) and a leading cause of hospital acquired infections[1]. Systemic infections with multi-drug resistant enterococci occur subsequent to GI colonization[2]. Preventing colonization by multi-drug resistant EF could therefore be a valuable approach to limiting infection. However, little is known about mechanisms EF uses to colonize and compete for stable gastrointestinal niches. Pheromone-responsive, conjugative plasmids encoding bacteriocins are common among enterococcal strains[3], and could modulate niche competition among enterococci or between enterococci and the intestinal microbiota. We developed a model of mouse gut colonization with EF without disrupting the microbiota, to evaluate the role of the conjugative plasmid pPD1 expressing bacteriocin 21[4] on enterococcal colonization. Here we show that EF harboring pPD1 replaces indigenous enterococci and outcompetes EF lacking pPD1. Furthermore, in the intestine, pPD1 is transferred to other EF strains by conjugation, enhancing their survival. Moreover, colonization with an EF strain carrying a conjugation-defective pPD1 mutant resulted in clearance of vancomycin-resistant enterococci, without plasmid transfer. Therefore bacteriocin expression by commensal bacteria can influence niche-competition in the GI tract, and bacteriocins, delivered by commensals that occupy a precise intestinal bacterial niche, may be an effective therapeutic approach to specifically eliminate

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Supplementary Information is available in the online version of the paper.

Author Contributions S.K., C.J.K. and N.H.S. designed and conceived the study. S.K. performed most of the experiments and the analysis; D.B. and M.H. assisted in the development of the colonization model. V.L. performed bioinformatics analysis. R.C. and P.B. contributed to the sequential colonization experiment. P.S. and Y.C. performed statistical analysis; S.K., C.J.K. and N.H.S. interpreted the data and wrote the manuscript. C.J.K. and N.H.S. secured funding.

Author Information The complete sequence of pPD1 was deposited in GenBank under accession number KT290268. 16S rDNA sequences generated for microbiome analyses are deposited in NCBI-SRA archive under study accession number SRP061808 and BioProject accession number PRJNA290480.

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intestinal colonization by multi-drug resistant bacteria, without profound disruption of the indigenous microbiota.

The mammalian host is colonized by trillions of microbes that live in a predominantly symbiotic relationship with their host[5, 6]. Some of these symbionts can invade and cause serious disease when homeostasis is disrupted[7]. One such symbiont is *Enterococcus faecalis* (EF), a Gram-positive member of the gut microbiome of a wide range of mammals, including humans[8]. While enterococci are not pathogenic in most normal hosts, they can cause significant disease in immune compromised individuals. Treating enterococcal infections is challenging due to their intrinsic and acquired resistance to a wide range of antibiotics[9, 10]. Prior antibiotic therapy is a well-known risk factor for enterococcal infection[9, 11]. Antibiotic disruption of the intestinal biota enables resistant enterococci to proliferate profusely in the GI tract[12] and invade the host[11]. Reducing colonization of the GI tract by antibiotic-resistant enterococci could therefore represent a promising approach for preventing enterococcal infections. Unfortunately, our mechanistic understanding of enterococcal GI colonization is limited. Most previous studies employed antibiotic disruption of the intestinal microbiota prior to enterococcal challenge, resulting in invasion rather than colonization. Our model establishes long-term colonization of mice with a marked strain of EF without antibiotic pretreatment, allowing us to study EF dynamics in an unperturbed intestinal environment.

We established long-term colonization of FVB mice by prolonged feeding with a rifampicin-resistant EF strain, CK135 (EF_r). After withdrawal of EF_r from the drinking water, mice maintained persistent colonization of the GI tract and shedding of EF_r in feces, for more than eleven weeks (data shown through 4 weeks, Fig. 1a, b). In contrast, mice gavaged with ~10^9 CFU of EF_r, demonstrated short-lived colonization (2–3 days) with significant mouse-to-mouse variability. While the specific factors that determine bacterial GI colonization fitness are unclear, the success of our model depends on both the nature and route of delivery, and offers a means of investigating the underlying mechanisms. Similar levels and patterns of EF_r colonization were observed in C57Bl6 mice and with multiple lineages of EF (Extended Data Fig. 1a, b), suggesting that this approach for mouse GI colonization by EF is generalizable.

We sought to understand the role of bacteriocins, antimicrobial peptides often encoded on pheromone responsive plasmids, on enterococcal colonization dynamics of the GI tract. Bacteriocin production is a tool bacteria can use to enhance the stability of their communities[13], by competing out closely related bacterial species and establishing a stable niche for the producing strain[14, 15]. Many lactic acid bacteria produce bacteriocins[15, 16, 17, 18]. The enterococcal bacteriocin, Bacteriocin-21 (Bac-21), is encoded on the sex-pheromone-responsive conjugative plasmid pPD1[19] and is identical in nucleotide sequence to another well-characterized enterococcal bacteriocin, AS-48[20] (Supplementary Table 1a, b). First isolated from an enterococcal strain of human gingival origin, the prevalence of pPD1 has not been described. Only the pheromone responsive and bacteriocin (*bac*) operons of pPD1 have previously been sequenced[4, 19]. Complete sequencing and annotation of pPD1 revealed a 57,732 bp plasmid with a total of 59 ORFs (Extended Data

Fig. 2 and Supplementary Table 2). The pPD1 *bac* operon contains nine genes designated from *bacA* to *bacI*[19]. The first gene in the *bac* operon consists of the bacteriocin gene (*bacA)*, which encodes a 105-amino acid precursor of Bac-21[19]. The genes *bacB* to *bacI* are thought to be involved in processing, modification and secretion of the 70-aa mature bacteriocin, and providing immunity to the bacteriocin producer[19].

Colonization experiments with an EF strain harboring pPD1 (EF_r +pPD1) revealed that EF_r +pPD1 was significantly more abundant than EF_r in feces and throughout the GI tract, suggesting more effective colonization (Fig.1a, b). To test if pPD1-encoded Bac-21 drives enhanced enterococcal colonization, we introduced an in-frame deletion of *bacAB* into pPD1 (EF_r+pPD1::*ΔbacAB*). EF_r+pPD1::*ΔbacAB* did not exhibit a growth defect *in vitro*, and lacked bacteriocin activity (Extended Data Fig. 3a). Mice were colonized with either EF_r+pPD1, EF_r+pPD1::*ΔbacAB*, or EF_r. EF_r+pPD1::*ΔbacAB* showed no colonization advantage over EF_r, and demonstrated impaired long-term persistence in the GI tract compared to EF_r+pPD1 or EF_r (Fig. 1c, d), possibly due to the excessive burden of maintaining a large non-functional plasmid. Alternatively, it is possible that unknown colonization factors expressed in the presence of an intact pPD1 are affected by *bacAB* deletion resulting in loss of colonization.

Mice obtained from commercial vendors possess a variable indigenous intestinal enterococcal (IE) population (Fig. 1e, Extended Data Fig. 3b–e). To determine whether exogenous colonization with EF_r strains replaced the IE to establish a stable niche in the gut, IE was monitored after colonization with EF_r or EF_r+pPD1 (Extended Data Fig. 3b and c). EF_r comprised approximately 5%–10% of the total enterococci in the feces and declined over time to 0.1%–1% (Fig.1e and Extended Data Fig. 3b–e). However, EF_r+pPD1 rapidly dominated the niche within one week and maintained this dominance over time (Extended Data Fig. 3b, c), suggesting that it was displacing or outcompeting the majority of IE in the mouse gut. EF_r+pPD1::*ΔbacAB* could not compete in the gut as it failed to displace the IE (Fig. 1e and Extended Data Fig. 3b–e). Moreover, colonization of the gut with EF_r carrying another pheromone-inducible conjugative plasmid that lacks bacteriocins (pCF10) did not result in domination of the intestinal niche (Extended Data Fig 4). Hence, Bac-21 is responsible for the colonization advantage conferred by pPD1.

While this evidence suggests that EF_r+pPD1 directly kills competing enterococci in the gut, Bac-21 might also alter physical or nutritional niches by killing other competing bacterial species[4, 19]. To investigate this, 16S rDNA high throughput pyrosequencing was used to assess the gut community composition of colonized mice. We found no significant differences in composition between the ceca of control mice and those colonized by EF_r indicating that colonization of mice with laboratory strains of EF does not substantially alter intestinal microbial ecology (Extended Data Fig. 5a). The cecal composition of EF_r and EF_r+pPD1 were significantly different from each other ($P = 0.007$) and clustered separately (Fig. 1f). However only the Gram-negative Deferribacteraceae; Mucispirillum were significantly different between groups (Extended Data Fig. 5b). We speculate that this is likely a secondary effect as Gram-positive bacteriocins such as Bac-21 have low efficacy against Gram-negative bacteria. We conclude the primary mechanism by which EFr+pPD1 enhances its colonization is through elimination of competing enterococcal strains.

To further investigate Bac21-mediated enterococcal competition in the gut, a competitive colonization experiment using differentially marked EF strains was performed. EF_r and spectinomycin-resistant EF_s+pPD1, or EF_r and EF_s+pPD1::*ΔbacAB* were given in combination to groups of mice, at indicated ratios (Fig. 2). At week 0, fecal shedding of EF_r, EF_s+pPD1 and EF_s+pPD1::*ΔbacAB* reflected the ratio of the bacteria given in the drinking water (Fig. 2). When EF_s+pPD1 was given in excess, it rapidly outcompeted EF_r (Fig. 2g). When EF_r was given in excess (Fig. 2a) or equal abundance to EF_s+pPD1 (Fig. 2d), although it was able to persist at lower levels it did not outcompete EF_s+pPD1. Subsequent experiments indicated that the ability of EF_r to persist was due to conjugative transfer of pPD1 to the EF_r host (see below), thus confirming the competitive advantage conferred by Bac-21. To control for unanticipated effects of the specific antibiotic resistance marker on EF survival advantage, the reciprocal experiment using EF_s and EF_r+pPD1 yielded similar results (Extended Data Fig. 6). EF_s+pPD1::Δ*bacAB* is outcompeted by EF_r in all circumstances (Fig. 2b, e, h), confirming its loss of colonization fitness. pPD1 also provided a competitive advantage during *in vitro* co-culturing competitions (Extended Data Fig. 7). Complementation of the Δ*bacAB* strain restored bacteriocin activity, stable gut colonization (Extended Data Figure 8), and competitive advantage against other enterococci (Fig. 2c, f, i and Extended Data Fig. 8).

During competition experiments, we noted the persistence of EF_r when given in combination with EF_s+pPD1. This suggested that either EF_s+pPD1 was able to clear a niche and enhance overall EF_r survival, or alternatively that EF_s+pPD1 was able to transfer pPD1 to EF_r by conjugation. To determine whether conjugation of pPD1 was occurring in the GI tract, we screened drinking water, GI tissue (Supplementary Table 3), and fecal isolates (Fig. 3a, b) of EF_r by colony PCR. No evidence of conjugation was observed in drinking water. The detection of transconjugants in the intestine or feces indicates that conjugation occurred in the GI tract. At week 1, approximately 25% of the EF_r fecal colonies selected had obtained the pPD1plasmid via conjugation (Fig. 3a, b). By week 4, nearly 100% of the EF_r colonies harbored pPD1 (Fig. 3a, b) and produced bacteriocin (not shown). Similar results were observed in each segment of the lower GI tract (Supplementary Table 3). EF_s+pPD1::Δ*bacAB* showed no evidence of ability to conjugate its defective plasmid (not shown). Although complementation of *bacA-E* ectopically (pSK29) restored Bac-21 production in EF_s+pPD1::*ΔbacAB*, plasmid conjugation was not restored *in vitro* or *in vivo*. The mechanism underlying this observation is currently unknown.

To determine if EF_s+pPD1 could displace an established resident enterococcal population, we performed sequential colonization experiments, in which EF_r colonization was stably established, followed by challenge with EF_s+pPD1. The levels of EF_r were not significantly altered after introducing EF_s+pPD1 (Extended Data Fig. 9); however, transconjugants of EF_r containing pPD1 started to appear as early as week 0 and neared 100% by week 4 (Fig. 3c). Although these results cannot distinguish extensive transfer of pPD1 by conjugation from a small number of transfer events followed by proliferation of pPD1-containing transconjugants, we conclude that pPD1 enhances EF competition in the GI tract by Bac-21-mediated killing, or through transfer of functional pPD1 plasmid via conjugation. To probe conjugation dynamics between non-isogeneic enterococci, we investigated IE transconjugants in mice that were colonized with EF_r+pPD1 (Fig. 1e and Extended Data Fig.

3b–e). We found that conjugation of pPD1 to IE is possible and can be observed both *in vivo* and *in vitro*, but happens at a low and variable frequency (Extended Data Fig.10). The reasons for this remain unknown, but are likely multifactorial, especially in the GI tract.

Colonization with antibiotic-resistant enterococci precedes emergence of enterococcal infections. Bac-21 producing EF exhibits bacteriocin activity *in vitro* against many multidrug-resistant clinical isolates of *E. faecium* and *E. faecalis* (Supplementary Table 4). To determine whether Bac-21 can disrupt colonization *in vivo* by vancomycin-resistant *E. faecalis* V583, mice colonized with rifampicin-resistant V583 ($V583_r$) were challenged with the complemented Bac-21^+ strain (EF_s+pPD1::*ΔbacAB bacA-E+*). The conjugation defect of this strain eliminated the risk for bacteriocin transfer to $V583_r$. EF_s+pPD1::*ΔbacAB bacA-E+* successfully eliminated $V583_r$ levels to below the detection limit in most of the mice compared to the control group, which showed steady levels of $V583_r$ in feces (Fig. 4a). As expected, EF_s+pPD1::*ΔbacAB bacA-E+* did not transfer bacteriocin to $V583_r$ during gut colonization, and the loss of pAM401::*bacA-E* resulted in persistent colonization by the bacteriocin-defective Δ*bacAB* strain (Fig. 4b). $V583_r$ clones recovered from the one mouse with persistent $V583_r$ levels in the treated group (group 2) were examined and found to be susceptible to Bac21 *in vitro*. We speculate that a stochastic, particularly rapid loss of complementing plasmid (pAM401::*bacA-E)* from the challenging strain (EF+pPD1::*bacAB*, *bacA-E+*) in that particular mouse allowed $V583_r$ to persist over time. These results demonstrate that introducing a conjugation defective Bac-21 producing strain into the GI tract can successfully reduce colonization of the GI tract by multidrug-resistant EF.

Our findings provide proof-of-concept for a novel therapeutic strategy to specifically de-colonize antibiotic-resistant enterococci from the GI tract (of patients) and thereby prevent the emergence of resistant enterococcal infections that are otherwise difficult, or impossible, to treat. Although stable colonization by our Bac-21-producing EF strain (EF +pPD1::Δ*bacAB*, *bacA-E+*) can eliminate multi-drug resistant enterococci from the gut, we imagine that the bacteriocin-producing strain may itself eventually acquire antibiotic resistance determinants, compromising its utility. Thus, therapeutic implementation of this strategy will likely require a modified approach. Nevertheless, use of a commensal bacterium to deliver Bac-21 leverages the niche specificity of the therapeutic strain to produce Bac-21 directly in the appropriate niche(s) at levels sufficient to inhibit other EF, yet not perturb the overall community. We imagine that the concept presented here, specific inhibition of particular members of the gut ecosystem, will be widely applicable to modulate gut colonization by other problematic organisms as well.

Methods

Bacterial strains, growth media and chemicals

The strains used in this study are listed in Supplementary Table 5. Brain heart infusion medium (BHI) and m-Enterococcus agar (Difco™) (Ent-agar) were prepared as described by the manufacturer (Becton Dickinson). Antibiotics were purchased from Sigma and used at the following concentrations: rifampicin, 200 μg/ml; spectinomycin, 500 μg/ml; erythromycin, 10 μg/ml; chloramphenicol, 15 μg/ml; tetracycline, 10 μg/ml. EF was cultured in BHI media at 37°C. All restriction enzymes were purchased from New England BioLabs.

Phusion High-Fidelity DNA Polymerase (Thermo Scientific) was used for all PCRs performed for strain and plasmid construction. Oligonucleotides were synthesized by Fisher Scientific (Supplementary Table 6).

Animals

The animal care and use committee at the Medical College of Wisconsin approved all animal-related procedures and experiments. Five-week-old male FVB and C57Bl6 mice were obtained from Taconic Laboratories (Oxnard and Germantown facilities). Upon arrival, mice were allowed to adapt to the new environment for a minimum period of 1 week before beginning any experiment. Animals were housed under specific-pathogen-free conditions in the Medical College of Wisconsin vivarium. Experimental sample sizes were determined by appropriate husbandry considerations as determined by the Medical College of Wisconsin vivarium, and experiments were repeated as described. No blinding was done and no scheme of randomization was applied when allocating mice for the experiments.

Generation of Δ*bacAB* deletion in pPD1

A mutant lacking *bacAB* was constructed using marker gene insertion (*ermC*) in an in-frame deletion. The design for in-frame deletion and the procedure for mutant construction was similar to that used previously with pCJK218[21]. The PCR products with first and last codons in the *bacAB* fragment and flanking the *ermC* gene were inserted between the XbaI and SphI sites of pCJK218 by Gibson Assembly[22] to generate pSK21. Deletion mutants were isolated by plating on counter-selection medium containing *p*-Cl-Phe followed by prolonged incubation at 30°C[21]. The desired mutants harboring *bacAB* deletion in pPD1 plasmid (pSK35) were identified by PCR analysis and erythromycin selection. Two independent isolates were obtained and were analyzed to exhibit identical phenotypes.

Complementation studies

The *bacABCDE* fragment was amplified using primers listed in Supplementary Table 6 and was cloned into pAM401 vector using the Gibson assembly[22] approach, resulting in pSK29. Strains with *bacAB* deletion in pPD1 (CK135 pSK35 or OG1sp pSK35) were electroporated with pSK29. PCR analysis, chloramphenicol resistance, and bacteriocin assay confirmed the complementation construct.

Bacteriocin Assays

As previously described[23], 50 μl of an overnight culture of the indicator strain (CK135 or OG1sp) that was grown in BHI broth was added to 5 ml of molten BHI soft agar and then evenly spread onto a BHI agar plate. After solidification, 2 μl of the test strain that was grown overnight was spotted on the soft agar. Zones of inhibition of the susceptible strain around the spots were monitored after overnight incubation at 37°C.

Mouse colonization model

Overnight culture of EF was washed with sterile water and added to autoclaved water to a final concentration of 5×10^8 cfu/ml. Persistence of EF in drinking water was determined daily, and remained between 10^7 and 10^8 cfu/ml over 3 days. Drinking water was changed

every 3–4 days to maintain the appropriate inoculum, and mice were allowed to drink ad libitum. After two weeks, EF-inoculated drinking water was replaced with sterile water for the duration of the experiment. For the sequential colonization experiment, mice were first colonized with a strain lacking pPD1 (initial strain) by feeding the bacteria through drinking water for two weeks. Three days after the initial strain was withdrawn from drinking water, the challenge strain was introduced in the drinking water for two weeks at which time (Week 0) animals were returned back to regular sterile water.

Bacterial culture and quantification of EF from mouse feces and intestines

Fecal pellets obtained directly from living mice were weighed and homogenized in 1 ml of PBS. Fecal homogenates were plated in serial dilution on BHI agar plates with appropriate antibiotics to enumerate levels of EF colonization. After mice were euthanized, the intestinal tract was removed and divided into three segments: distal 10 cm of small intestine (DSI), cecum, and large intestine (LI). Each segment was homogenized in 2 ml PBS and cultured on BHI media with appropriate antibiotics. To enumerate indigenous enterococci or total enterococci, fecal and tissue homogenates were plated in serial dilution on Ent-agar (Difco™). Alternative to BHI, lab strains were enumerated on Ent-agar supplemented with appropriate antibiotics.

Colony PCR for *bacA* and *bacD* gene

To determine transconjugants, colonies were selected and used as PCR templates for identification of the *bacA* or *bacD* gene using gene specific primers as described in the Supplementary Table 6. PCR products were identified using agarose gel electrophoresis.

Plasmid DNA preparation and sequencing of pPD1

pPD1 plasmid was purified as described previously[24]. Sequencing was performed at GENEWIZ, Inc. (South Plainfield, NJ) on the Illumina HiSeq Platform in a 2×150 bp paired-end (PE) configuration. The *de novo* assembly using CLC Genomics Server 6.5.1 was used to obtain assembled contigs/scaffolds. In total, 59 open reading frames (ORFs) larger than 100bp that showed sequence similarity to known sequences in NCBI database were identified by NCBI ORF- finder (http://www.ncbi.nlm.nih.gov/projects/gorf/)

Bacterial genomic DNA extraction

Cecum was isolated from experimental animals and homogenized as described[25]. Genomic DNA was extracted from tissues using the MO BIO PowerFecal DNA Isolation kit (MO BIO, Carlsbad, CA) with slight modification in the protocol. After addition of C1 solution and heating the samples at 65°C for 10 minutes, the sample was further subjected to heating at 95°C for 10 minutes followed by vigorous bead beating using PowerLyzer^{R} (MO BIO).

Amplification of bacterial 16S gene sequences and high throughput sequencing

The 16S rDNA V4 region amplicons (single index) were produced by PCR and sequenced on the MiSeq platform (Illumina) using the 2×250 bp protocol yielding paired-end reads that overlap by ~247 bps[26] (performed by Metanome™, Baylor College of Medicine, Waco, Texas). Following sequencing, raw BCL files were retrieved from the MiSeq platform and

called into fastqs by Casava v1.8.3 (Illumina). The read pairs were demultiplexed based on unique molecular barcodes, filtered for PhiX using Bowtie2 v2.2.1[27], and reconstituted into two fastq files for each read using standard BASH. Sequencing reads were merged (allowing 4 mismatches per ≥50 bases) and processed using USEARCH v7.0.1001[28]. Sequences were demultiplexed using QIIME v1.8.0[29] and then clustered using the UPARSE pipeline[28]. Operational taxonomic unit (OTU) classification was achieved by mapping the UPARSE OTU table to the SILVA database[30]. Abundances were recovered by mapping the demultiplexed reads to the UPARSE OTUs. A custom script constructed an OTU table from the output files generated in the previous two steps. The OTU table was used to calculate alpha-diversity, beta-diversity and provide taxonomic summaries[31, 32, 33].

Bioinformatic analysis

Microbiome data was analyzed using the Vegan[34] and Ecodist[35] packages in R 3.0.2[36]. Sequence counts of each sample were normalized to the average sequencing depth, and the Bray-Curtis metric was used to assess inter-sample (beta) diversity. Statistical significance for differences in microbiome diversity between groups was determined using Adonis (in Vegan). Non-parametric multi-dimensional scaling (NMDS) ordination (in Ecodist) was used to visualize group clustering and diversity distance between samples. Log transformed abundance data (Log_{10} of sequence count+1) was used to normalize the data, and statistically significant differences in OTU abundance between groups was determined by heteroscedastic, two-sided Student's t-test.

Statistical analysis of data

To compare the EF in feces/tissues under different experimental conditions, we applied either a non-parametric Mann-Whitney-Wilcoxon or Kruskal-Wallis test due to the skewness of the data. To model the response changes over time, we fitted an exponential decay in the following form:

$$EF\ in\ feces\ (\log\ CFU\ per\ gram) = k_0 * e^{(-k_1 * time)}$$

where k_0 is the EF in feces (log CFU per gram) at time 0, and k_1 is the rate of decay. We also fitted a logistic curve to model the percent of transconjugants using the following equation:

$$\%\ of\ transconjugants = \frac{k_1}{1+e^{(-k_2*(time-k_3))}}$$

where k_1 is the maximum value of the curve; k_2 is the steepness of the curve; and k_3 is the time of the midpoint of the sigmoid.

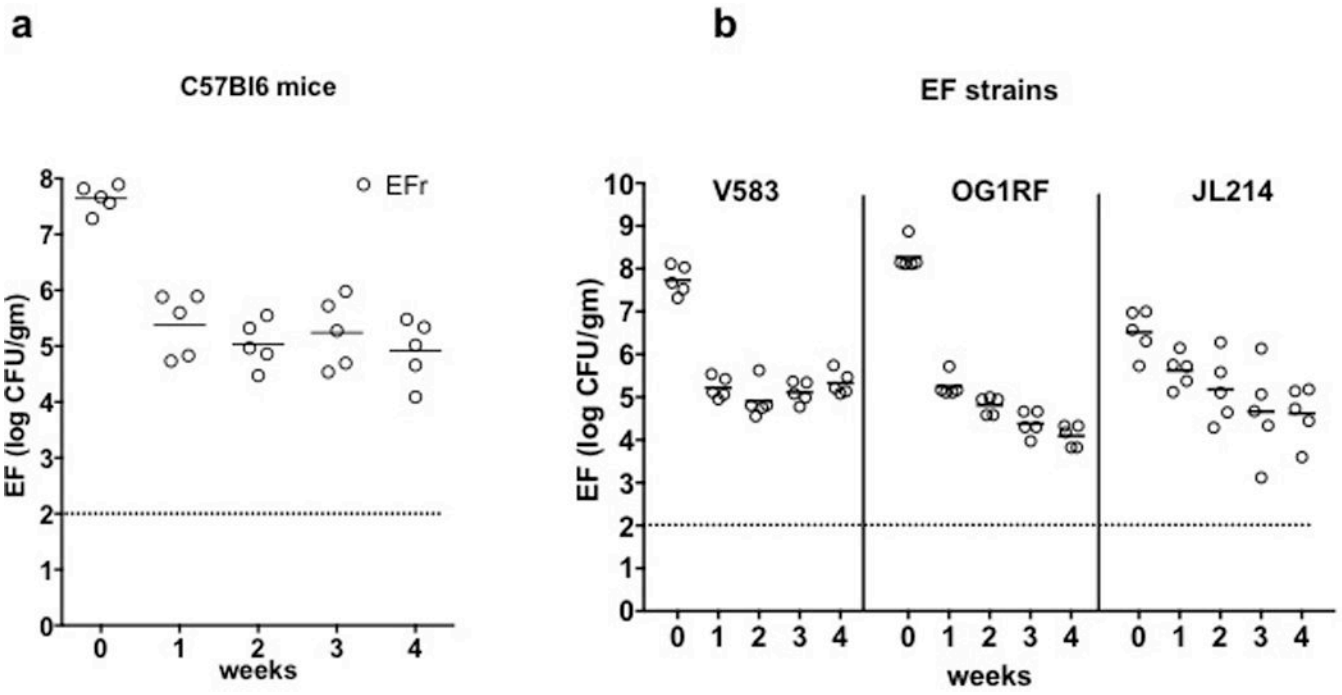
For the above nonlinear mixed effects models, we used a compound symmetry correlation structure to model the dependency among observations. If compound symmetry was not a good fit, a general correlation matrix or AR(1) (autocorrelation structure of order 1) correlation matrix was used. For those responses that have non-zero values only at one or two time points, a model cannot be fit. In these cases, we compared the responses at each

time point. A linear mixed effect model with autoregressive covariance structure was used to compare the EF in feces over time for EF and EF+pPD1. Mice were treated as random. The response was log transformed, and groups were compared using two-tailed t-test at an alpha 0.05. With 5 mice in each group, we have at least 80% power to detect a difference of at least 2.4 standard deviations (SDs). PASS 2008 was used for the calculation (www.ncss.com). SAS (SAS Institute, Cary, NC), S-PLUS (Insightful Corporation, Seattle, WA), R[36], and SPSS (IBM Corporation, Armonk, NY) were used for statistical analysis. All tests were two-tailed. A *P* value < 0.05 was considered statistically significant.

Supplementary Material

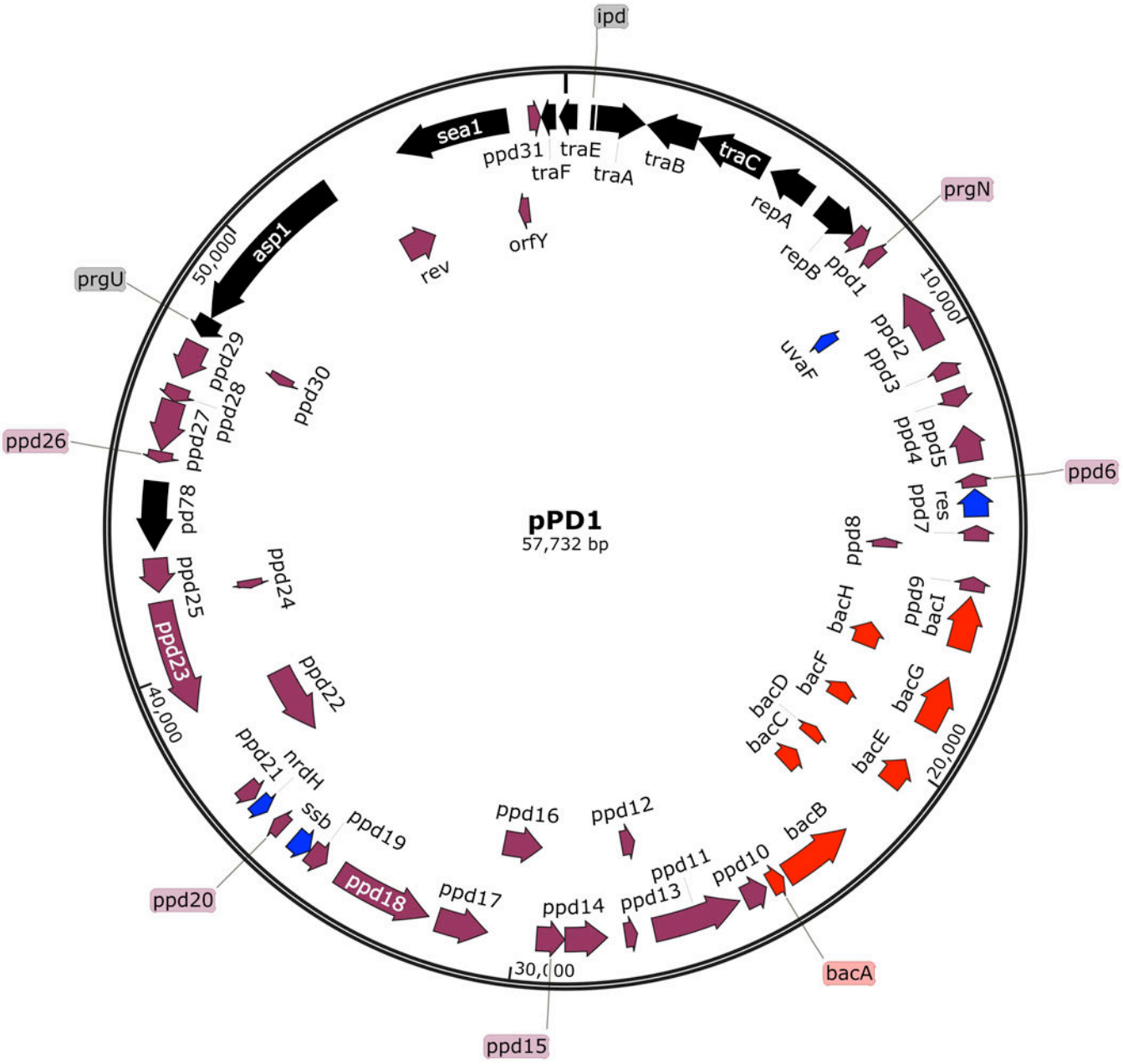
Refer to Web version on PubMed Central for supplementary material.

Extended Data



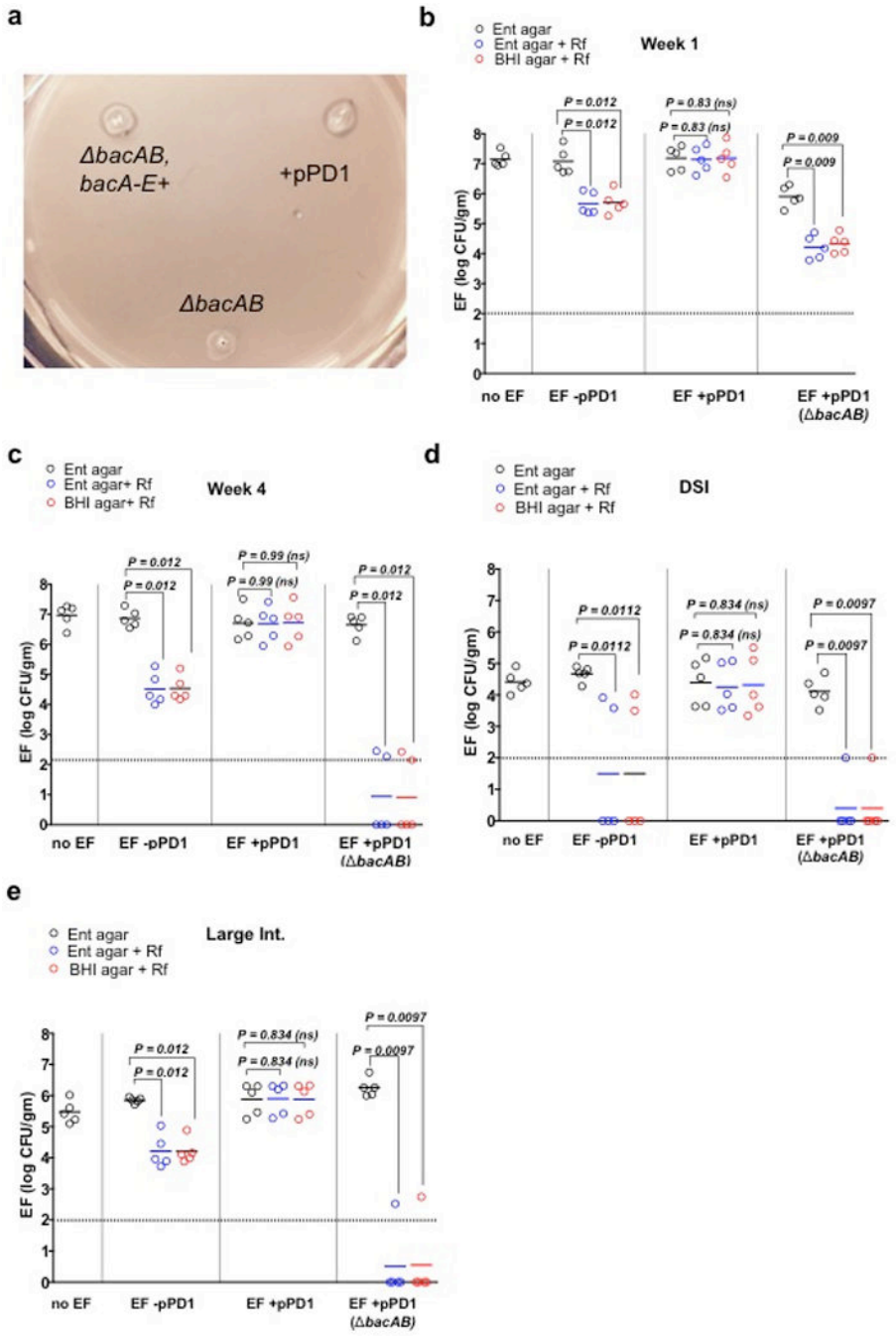
Extended Data Figure 1. EF colonization

(a) C57Bl6 mice (N=5) were given EF_r in drinking water for 14 days. Fecal samples were taken from each animal at the transition to sterile drinking water (week 0) and then weekly. EF_r abundance was determined by enumeration on BHI agar with rifampicin. (b) $V583_r$, OG1RF, JL214 strains of EF that are rifampicin resistant were fed to groups of mice (N=5/group). EF strains were enumerated weekly as described above. Horizontal lines indicate geometric mean. Each symbol represents an individual animal, data is representative of more than three experiments in (a) and (b; for $V583_r$ and OG1RF) and result of one experiment for (b; JL214).



Extended Data Figure 2. Physical map of plasmid pPD1

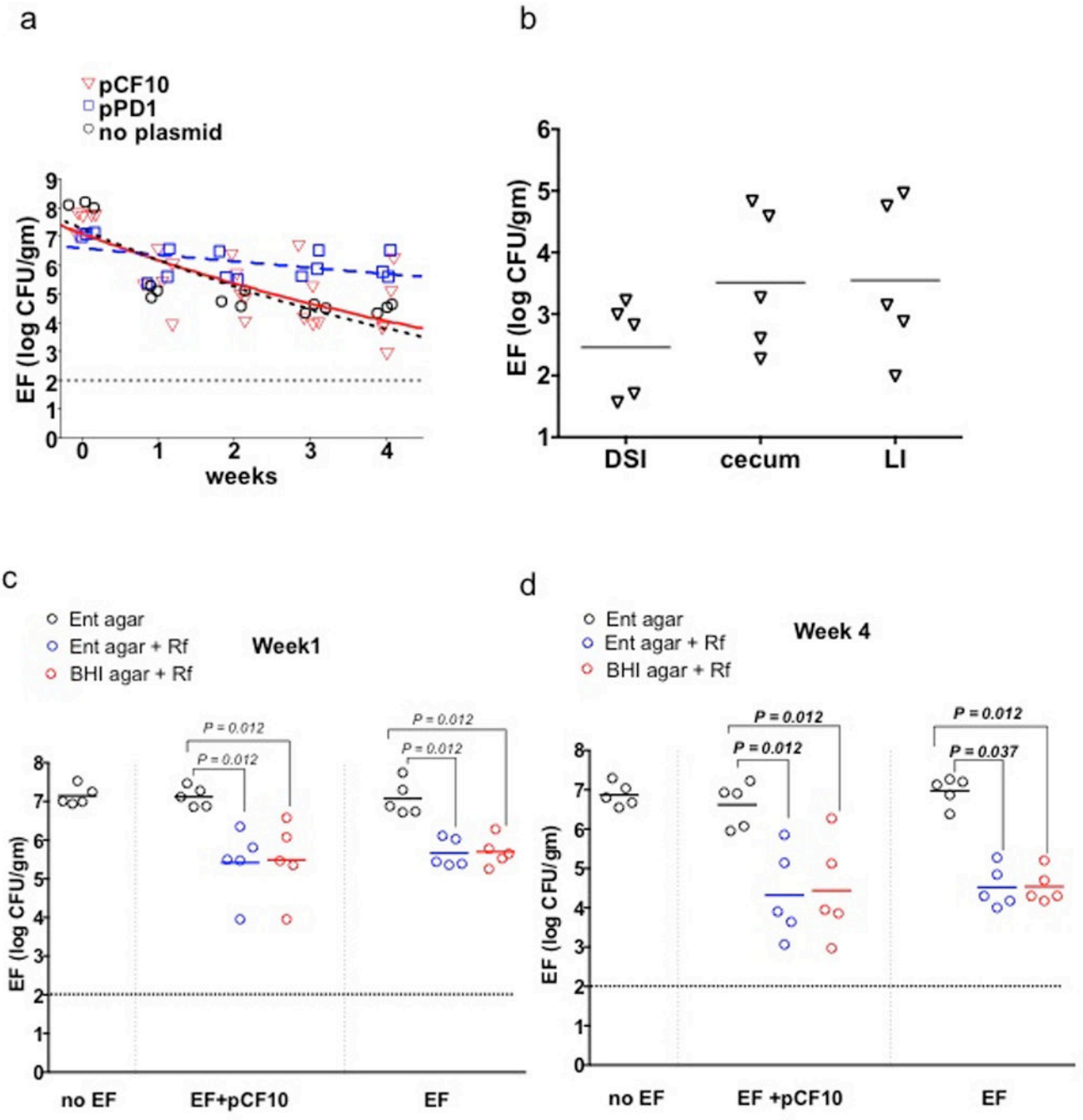
The 59 ORFs identified in the nucleotide sequence of pPD1 are located on a circular map. Arrows indicating the direction of transcription show the ORFs. Different colors indicate coding regions for conjugation (black), *bac* operon (red) and maintenance/repair (blue). Hypothetical coding regions are shown in magenta. A circular plasmid map was generated using the SnapGene software (from GSL Biotech; available at snapgene.com). Schematic diagrams of multiple alignments of plasmids were produced by manually realigning the linear plasmid maps generated by the SnapGene Viewer.



Extended Data Figure 3. pPD1 enhances EF competition for an intestinal niche

(a) Bacteriocin assay by the soft agar method with EF_r+pPD1::Δ*bacAB, bacA-E*+, EF_r+pPD1 and EF_r+pPD1::Δ*bacAB* spotted on a lawn of susceptible EF. Mice (N=5/group) were given EF_r or EF_r +pPD1 or EF_r+pPD1::Δ*bacAB* or sterile drinking water for 14 days, at which time all mice were given sterile water. (b) One week and (c) four weeks after withdrawal of EF from drinking water, fecal samples were collected and abundance of total enterococci was determined by enumeration on m-Enterococcus selective agar (Ent agar, ○). Lab strain of EF was enumerated using Ent agar with rifampicin (Rf) (○), or BHI agar with

rifampicin (○). (d and e and Fig. 1e) At the end of week four, animals were euthanized and abundance of EF was determined in distal small intestine (DSI) and large intestine (LI). The results shown are representative of two biologically independent experiments. Horizontal lines indicate geometric mean. Each symbol represents an individual animal.

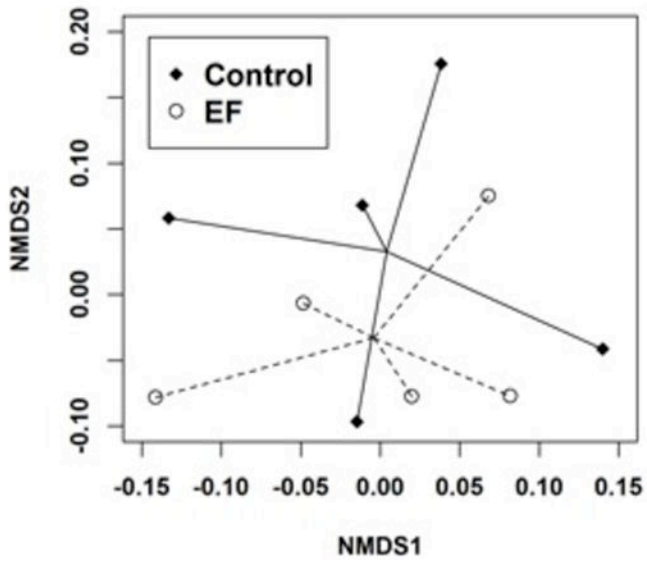


Extended Data Figure 4. EF+pPD1 but not EF $_r$+pCF10 dominates the intestinal enterococcal population

pCF10 is a well-studied pheromone-inducible conjugative plasmid of EF that encodes resistance to tetracycline but does not encode a known bacteriocin determinant. (a) EF_r: (o,

N=3 mice, no plasmid), EF_r+pPD1: (□, N=3 mice, pPD1) or EF_r+pCF10, (∇, N=5 mice, pCF10) was added to drinking water for 14 days, and then replaced by sterile drinking water. (a) Fecal samples were taken from each animal at the transition to sterile drinking water (week 0) and then weekly. EF abundance was determined by enumeration on BHI agar with rifampicin. (b) Four weeks after withdrawal of EF from drinking water, animals were euthanized and abundance of EF_r+pCF10 was determined in each segment of the GI tract (distal small intestine, DSI; cecum; and large intestine, LI). Mice colonized with EF_r+pCF10 maintained long-term fecal shedding of EF_r+pCF10 similar to EF_r, and persistent colonization throughout the GI tract. Abundance of enterococci in the feces was determined by enumeration on m-Enterococcus agar (Ent agar, o), Ent agar with rifampicin (Rf) (○), or BHI agar with rifampicin (○) at week 1 (c) and week 4 (d). Unlike EF_r+pPD1 that dominated the enterococcal niche in the GI tract (Fig. 1e), EF_r+pCF10 did not outcompete the indigenous enterococci, colonizing at levels comparable to EF_r (c and d). (a) The lines are fitted using an exponential decay model, and there are significant differences in rate of decay for the “pCF10” group vs. “pPD1” group (P=0.012) and for the “pPD1” vs. “no plasmid” group (P=0.007). Horizontal lines in (b, c, and d) indicate geometric mean. Each symbol represents an individual animal; data are obtained from one experiment.

a



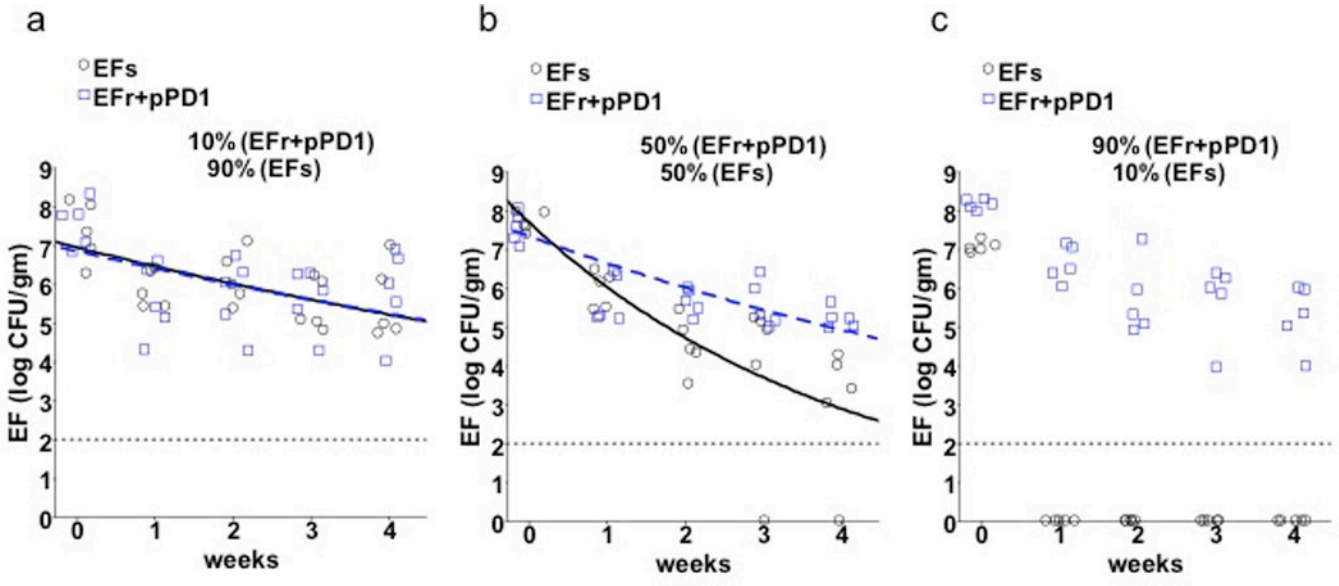
b

Taxon	EF vs EF +pPD1 t-test	Magnitude: (EF+pPD1) - (EF)
Family level		
Bacteria;__Deferribacteres;__Deferribacteres;__ Deferribacterales;__Deferribacteraceae	0.00010	-1.04280
Bacteria;__Firmicutes;__Clostridia;__Clostridiales;__ Defluviitaleaceae	0.04195	-0.21928
Genus level		
Bacteria; __Deferribacteres; __Deferribacteres; __Deferribacterales; __Deferribacteraceae; __Mucispirillum;Other	0.00010	-1.04280
Bacteria; __Firmicutes; __Clostridia; __Clostridiales; __Lachnospiraceae; __Incertae_Sedis;Other	0.00545	-0.29577
Bacteria; __Firmicutes; __Clostridia; __Clostridiales; __Lachnospiraceae; __Blautia;Other	0.03635	0.14052
Bacteria; __Firmicutes; __Clostridia; __Clostridiales; __Lachnospiraceae; __Incertae_Sedis;Other	0.03877	-0.23034

Extended Data Figure 5. Microbiome analysis

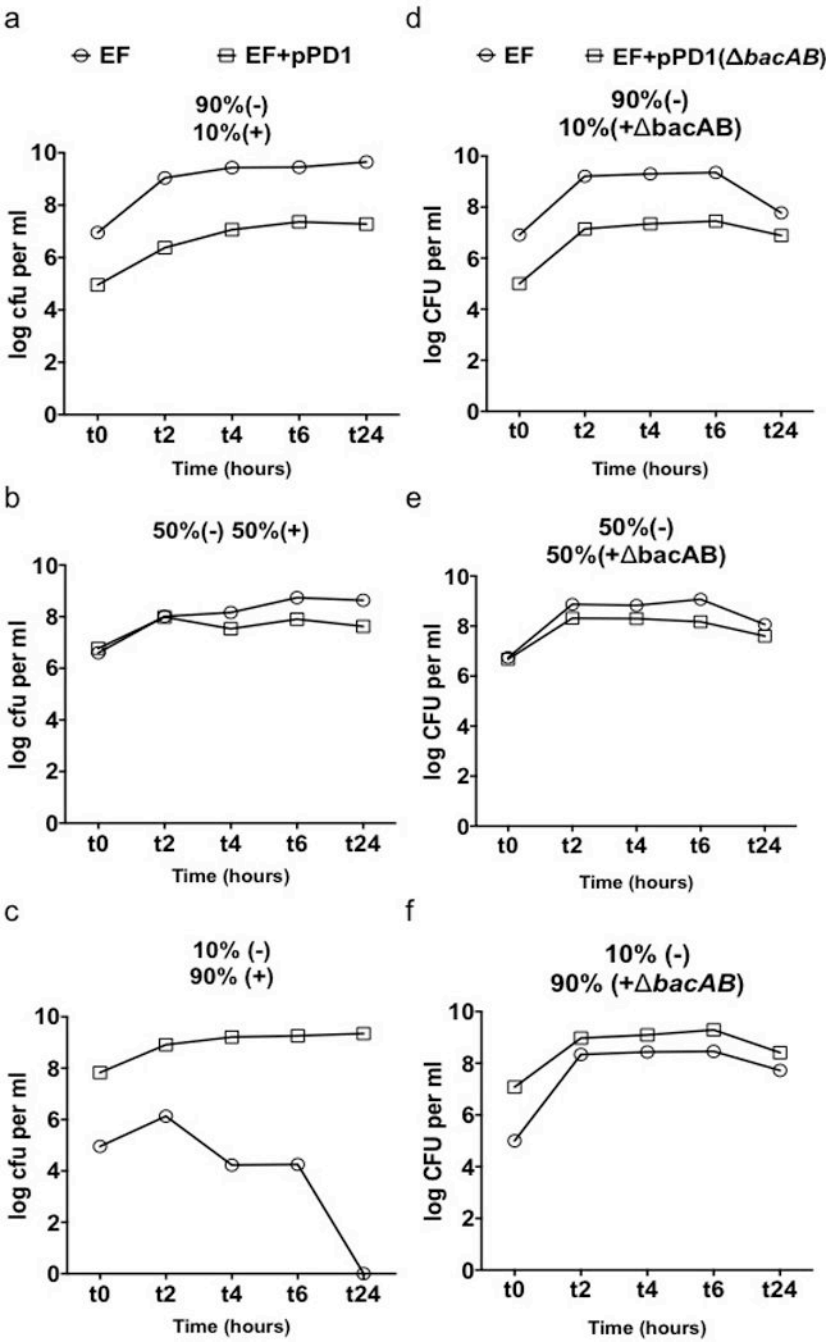
(a) NMDS Ordination of control and EF samples as separated by Bray-Curtis beta diversity metric. Control (no EF treatment, N=5 mice) and EF samples (N= 5 mice) are intermixed and showed no significant difference in beta diversity between the two groups. Adonis *P* value=0.298. Samples are connected with lines to help visualize grouping. (b) Analysis at the OTU level between EF and EF+pPD1 suggests changes in the abundance of four bacterial genera, in particular Deferribacteraceae; Mucispirillum and Lachnospiraceae; Incertae Sedis (*P* = 0.0001 and 0.005, respectively; heteroscedastic two-sided Student's t-

test). Bacteria belonging to these genuses were ten-fold and two-fold lower respectively in EF+pPD1 colonized mice (note the magnitude of change is shown in log10 scale). Changes in Defluviitaleaceae; Incertae Sedis, and Lachnospiraceae; Blautia were not as pronounced ($P = 0.03$; heteroscedastic two-sided Student's t-test). Nevertheless, analyses at the family taxonomy level suggest that the change in Deferribacteraceae was statistically significant.



Extended Data Figure 6. Reciprocal experiment for Fig. 2a, d and g

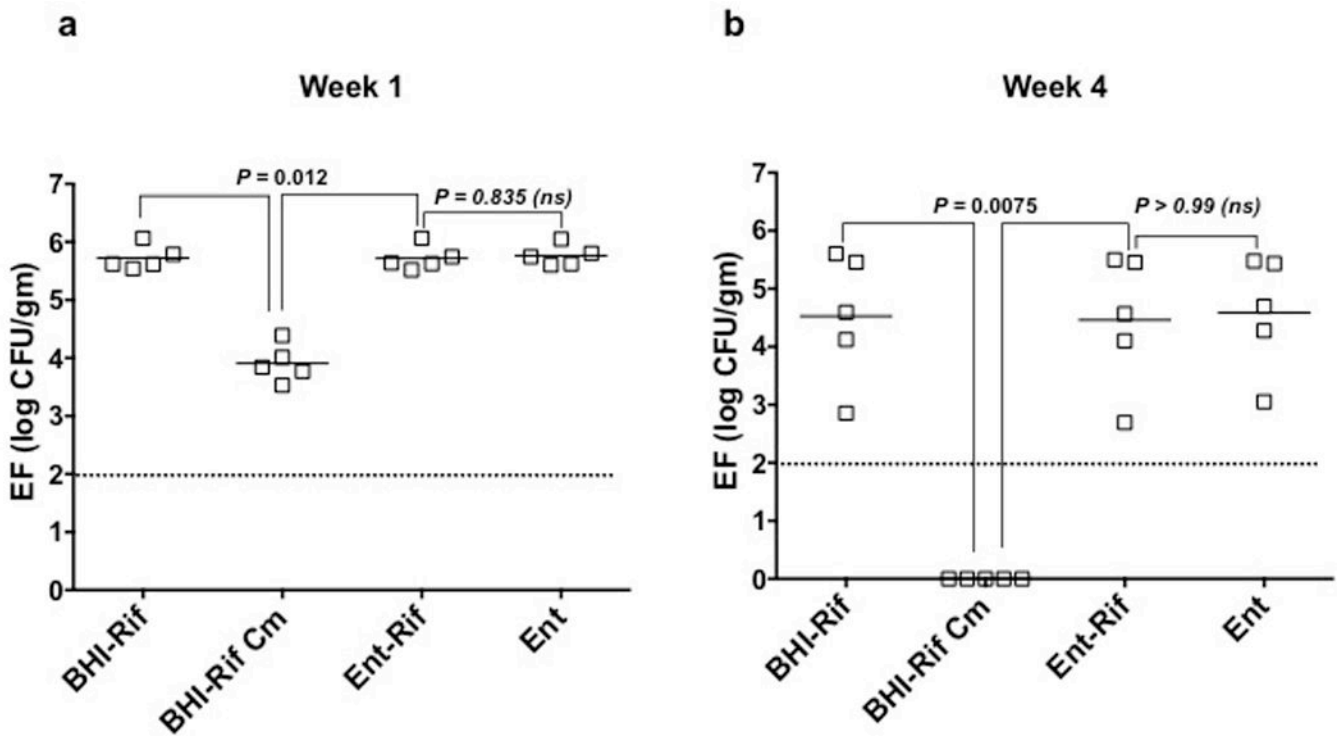
Groups of mice (N=5 mice per group) were given mixtures of EF_r+pPD1 and EF_s in drinking water at ratios of 10%/90% (a), 50%/50% (b) and 90%/10% (c) respectively. Fecal samples were obtained at the transition to sterile drinking water (week 0) and then weekly. Abundance of each EF strain in feces was determined by enumeration on BHI agar with rifampicin and BHI agar with spectinomycin. (□) represent abundance of EF_r+pPD1 and (○) represent abundance of EF_s. Each symbol represents an individual animal. The differences between the two groups at each week were compared using a nonparametric Wilcoxon test, and all *P* values were *ns* (non significant) (a); and <0.005 (b). The *P* values in (c) are 0.0122 (week 0) and 0.0075 (week 1–4). The lines are fitted using an exponential decay model in (a) and (b). The results in a, b and c are from one experiment.



Extended Data Figure 7. pPD1 associated competition *in vitro*

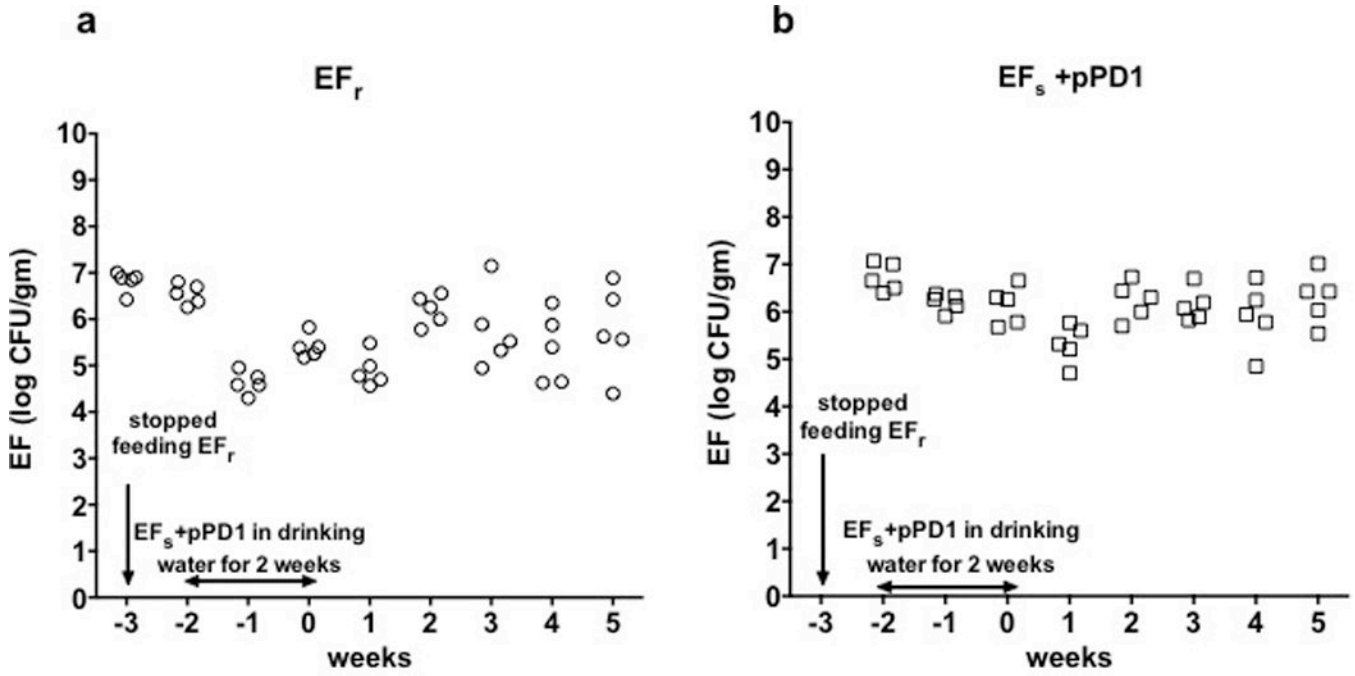
Three independent cultures were carried out with various mixed populations of EF_r (−) and EF_s+pPD1(+) or EF_r (−) and EF_s +pPD1::Δ*bacAB* (+Δ*bacAB*) in 10 ml BHI broth at ratios of 90%/10% (a and d), 50%/50% (b and e) and 10%/90% (c and f). Samples were taken for serial dilution at 0, 2, 4, 6 and 24 hours after the start of the experiment. Abundance of each EF strain in feces was determined by enumeration on BHI agar with rifampicin and BHI agar with spectinomycin. Evidence of conjugation was observed *in vitro* co-cultures of (a) and (b) only by screening for transconjugants (EF_r+pPD1) via colony PCR. (□) represent

abundance of EF_s+pPD1 (a, b and c) or EF_s+pPD1:: *ΔbacAB* (d, e and f). (○) represent abundance of EF_r (a–e). Data is representative of two biologically independent experiments.



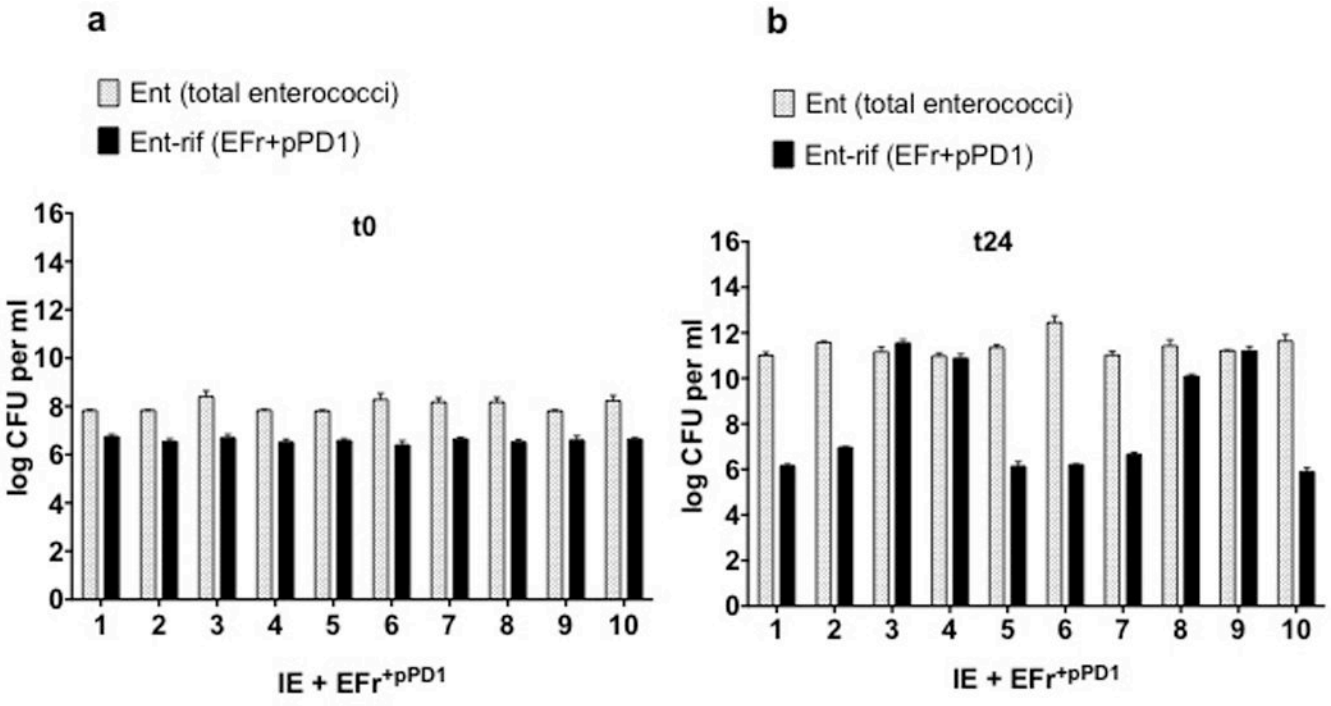
Extended Data Figure 8. Complementation of Bac-21 production restores colonization phenotype by providing competitive advantage

Bacteriocin activity was restored upon ectopic expression of *bacABCDE* (from pAM401) in EF+pPD1::Δ*bacAB* but not in EF lacking pPD1, indicating that the distal part of the *bac* operon (*bacFGHI)* is necessary for bacteriocin expression and that the *bacAB* in-frame deletion is not polar on downstream genes. Mice (N=5) were given EF_r+pPD1::Δ*bacAB, bacABCDE*+ as described in the methods and abundance was determined by enumeration on m-Enterococcus agar (Ent agar), Ent agar plus rifampicin (Rf), or BHI agar with rifampicin. The presence of pAM401A::*bacABCDE+* (complementing plasmid) was determined by enumerating CFU on BHI agar with rifampicin and chloramphenicol (Cm). Fecal samples were obtained at week 1 (a) and week 4 (b) after transition to sterile drinking water. Horizontal lines indicate geometric mean. Each symbol represents an individual animal and data is a representative of two biologically independent experiments. EF_s +pPD1::*ΔbacAB bacABCDE*+ stably colonized the GI tract (a), although in the absence of chloramphenicol selection, pAM401::*bacABCDE* was gradually lost from the population (b). Over time, loss of pAM401::*bacABCDE* resulted in the complemented strain reverting back to the bacteriocin-defective Δ*bacAB* strain, with the loss of bacteriocin activity. Nevertheless, this strain persisted in the gut, suggesting that Bac-21 was essential for clearing a niche for EF, but once cleared, EF uses other mechanisms to maintain colonization (a and b).



Extended Data Figure 9. EF$_r$ levels are not altered by sequential colonization with EF$_s$+pPD1

Groups of mice (N=5 mice per group) were given EF$_r$ in drinking water for two weeks and subsequently challenged with EF$_s$+pPD1 in drinking water for another 2 weeks (beginning at week −2) before transition to sterile water (week 0). Fecal samples were obtained weekly to enumerate the abundance of EF$_r$ (a) and EF$_s$+pPD1 (b) on BHI agar with rifampicin and spectinomycin respectively. Each symbol represents an individual animal and data is from one experiment.



Extended Data Figure 10. Conjugation frequency of pPD1 between lab strain (EF+pPD1) and indigenous enterococci (IE) *in vitro*

To understand conjugation dynamics between non-isogeneic species of enterococci, we investigated indigenous enterococcal (IE) transconjugants in mice that were colonized with EF_r+pPD1 (Fig. 1e and Extended Data Fig. 3b–e). However, we were unable to detect Rf-sensitive enterococci (IE) from the feces sample at week 4 (Extended Data Fig. 3c). At week one, only 9 clones of IE that were Rf-sensitive (out of 730 enterococci) were isolated from 3 out of 5 mice (Extended Data Fig 3b). Bacteriocin assays and probing for *bacA* gene sequence confirmed that 6 out of 9 clones were transconjugants and were Bac-21 positive. 16S rDNA gene sequencing of these 9 clones show high similarities to *E. faecalis* 16S rDNA. To understand the reason behind low conjugation frequency between IE and the lab strain of EF_r+pPD1, *in vitro* conjugation experiments were performed to assess the frequency of plasmid transfer. Ten new clones of IE were isolated from the feces of five mice (2 clones per mouse that were not colonized with any lab strain) by culturing on m-Enterococcus agar. EF_r +pPD1 was mixed with each of these ten IE clones in BHI broth at a 1:9 ratio. Samples were taken for serial dilution at the start of the culture (t0; a) and after 24 hours (t24; b) from the start of the experiment. Abundance of total enterococci was determined by using m-Enterococcus agar, and EF_r +pPD1 on BHI agar+Rf. Data is representative of 3 biologically independent experiments. *In vitro* bacteriocin assays revealed that EF_r +pPD1 is capable of killing most of the non-pPD1 containing IE strains (not shown). *In vitro* conjugation assays between individual IE clones and EF_r+pPD1 led to three observations: (i) 4 out of 10 clones were susceptible to Bac-21 and were eliminated by EF_r+pPD1 (#3, #4, #8 and #9); (ii) 6 out of 10 were immune to Bac-21 killing (#1, #2, #5, #6, #7 and #10); (iii) probing for *bacA* provided evidence for pPD1-containing IE transconjugants in 4 out of 6 above-mentioned immune IE clones. The two clones that failed to conjugate and were resistant to Bac-21 killing in mixed culture experiment were also

resistant to EF_r +pPD1 on bacteriocin assay plates. The mechanism for resistance of these two clones is not clear, however one could speculate that they might harbor cross-resistance traits.

Acknowledgments

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References

1. Richards MJ, Edwards JR, Culver DH, Gaynes RP. Nosocomial infections in combined medical-surgical intensive care units in the United States. Infect Control Hosp Epidemiol. 2000; 21(8):510–515. [PubMed: 10968716]
2. Broaders E, Gahan CG, Marchesi JR. Mobile genetic elements of the human gastrointestinal tract: potential for spread of antibiotic resistance genes. Gut Microbes. 2013; 4(4):271–280. [PubMed: 23651955]
3. Nes, Ingolf F.; D, BD.; Yasuyoshi, Ike. Enterococcal Bacteriocins and Antimicrobial Proteins that Contribute to Niche Control BTI - Enterococci: From Commensals to Leading Causes of Drug Resistant Infection. In: Gilmore E-i-c, Michael S.; Clewell, Don B.; Ike, Yasuyoshi; Shankar, Nathan, editors. Enterococci: From Commensals to Leading Causes of Drug Resistant Infection. Massachusetts Eye and Ear Infirmary; Boston: 2014.
4. Fujimoto S, Tomita H, Wakamatsu E, Tanimoto K, Ike Y. Physical mapping of the conjugative bacteriocin plasmid pPD1 of Enterococcus faecalis and identification of the determinant related to the pheromone response. Journal of Bacteriology. 1995; 177(19):5574–5581. [PubMed: 7559345]
5. Hooper LV, Midtvedt T, Gordon JI. How host-microbial interactions shape the nutrient environment of the mammalian intestine. Annu Rev Nutr. 2002; 22(0199-9885):283–307. (Print). [PubMed: 12055347]
6. Turnbaugh PJ, Ley RE, Hamady M, Fraser-Liggett CM, Knight R, Gordon JI. The human microbiome project. Nature. 2007; 449(7164):804–810. [PubMed: 17943116]
7. Round JL, Mazmanian SK. The gut microbiota shapes intestinal immune responses during health and disease. Nat Rev Immunol. 2009; 9(5):313–323. [PubMed: 19343057]
8. Hidron AI, Edwards JR, Patel J, Horan TC, Sievert DM, Pollock DA, et al. NHSN annual update: antimicrobial-resistant pathogens associated with healthcare-associated infections: annual summary of data reported to the National Healthcare Safety Network at the Centers for Disease Control and Prevention 2006–2007. Infect Control Hosp Epidemiol. 2008; 29(11):996–1011. [PubMed: 18947320]
9. Shepard BD, Gilmore MS. Antibiotic-resistant enterococci: the mechanisms and dynamics of drug introduction and resistance. Microbes and Infection/Institut Pasteur. 2002; 4(2):215–224. [PubMed: 11880055]
10. Paulsen IT, Banerjei L, Myers GS, Nelson KE, Seshadri R, Read TD, et al. Role of mobile DNA in the evolution of vancomycin-resistant Enterococcus faecalis. Science. 2003; 299(5615):2071–2074. [PubMed: 12663927]
11. Brandl K, Plitas G, Mihu CN, Ubeda C, Jia T, Fleisher M, et al. Vancomycin-resistant enterococci exploit antibiotic-induced innate immune deficits. Nature. 2008; 455(7214):804–807. [PubMed: 18724361]
12. Ubeda C, Taur Y, Jenq RR, Equinda MJ, Son T, Samstein M, et al. Vancomycin-resistant Enterococcus domination of intestinal microbiota is enabled by antibiotic treatment in mice and precedes bloodstream invasion in humans. J Clin Invest. 2010; 120(12):4332–4341. [PubMed: 21099116]

13. Dobson A, Cotter PD, Ross RP, Hill C. Bacteriocin production: a probiotic trait? Applied and Environmental Microbiology. 2012; 78(1):1–6. [PubMed: 22038602]
14. Hibbing ME, Fuqua C, Parsek MR, Peterson SB. Bacterial competition: surviving and thriving in the microbial jungle. Nat Rev Microbiol. 2010; 8(1):15–25. [PubMed: 19946288]
15. Corr SC, Li Y, Riedel CU, O'Toole PW, Hill C, Gahan CG. Bacteriocin production as a mechanism for the antiinfective activity of Lactobacillus salivarius UCC118. Proc Natl Acad Sci U S A. 2007; 104(18):7617–7621. [PubMed: 17456596]
16. Perez RH, Zendo T, Sonomoto K. Novel bacteriocins from lactic acid bacteria (LAB): various structures and applications. Microbial Cell Factories. 2014; 13(Suppl 1):S3. [PubMed: 25186038]
17. Grande Burgos MJ, Pulido RP, Del Carmen Lopez Aguayo M, Galvez A, Lucas R. The Cyclic Antibacterial Peptide Enterocin AS-48: Isolation, Mode of Action, and Possible Food Applications. International Journal of Molecular Sciences. 2014; 15(12):22706–22727. [PubMed: 25493478]
18. Cruz VL, Ramos J, Melo MN, Martinez-Salazar J. Bacteriocin AS-48 binding to model membranes and pore formation as revealed by coarse-grained simulations. Biochimica et Biophysica Acta. 2013; 1828(11):2524–2531. [PubMed: 23756777]
19. Tomita H, Fujimoto S, Tanimoto K, Ike Y. Cloning and genetic and sequence analyses of the bacteriocin 21 determinant encoded on the Enterococcus faecalis pheromone-responsive conjugative plasmid pPD1. Journal of Bacteriology. 1997; 179(24):7843–7855. [PubMed: 9401046]
20. Galvez A, Maqueda M, Martinez-Bueno M, Valdivia E. Bactericidal and bacteriolytic action of peptide antibiotic AS-48 against gram-positive and gram-negative bacteria and other organisms. Research in Microbiology. 1989; 140(1):57–68. [PubMed: 2501837]
21. Vesic D, Kristich CJ. A Rex family transcriptional repressor influences H2O2 accumulation by Enterococcus faecalis. Journal of Bacteriology. 2013; 195(8):1815–1824. [PubMed: 23417491]
22. Gibson DG, Young L, Chuang R-Y, Venter JC, Hutchison CA, Smith HO. Enzymatic assembly of DNA molecules up to several hundred kilobases. Nat Meth. 2009; 6(5):343–345.
23. Tomita H, Fujimoto S, Tanimoto K, Ike Y. Cloning and genetic and sequence analyses of the bacteriocin 21 determinant encoded on the Enterococcus faecalis pheromone-responsive conjugative plasmid pPD1. Journal of Bacteriology. 1997; 179(24):7843–7855. [PubMed: 9401046]
24. O'Sullivan DJ, Klaenhammer TR. Rapid Mini-Prep Isolation of High-Quality Plasmid DNA from Lactococcus and Lactobacillus spp. Applied and Environmental Microbiology. 1993; 59(8):2730–2733. [PubMed: 16349028]
25. Croswell A, Amir E, Teggatz P, Barman M, Salzman NH. Prolonged impact of antibiotics on intestinal microbial ecology and susceptibility to enteric Salmonella infection. Infection and Immunity. 2009; 77(7):2741–2753. [PubMed: 19380465]
26. Caporaso JG, Lauber CL, Walters WA, Berg-Lyons D, Huntley J, Fierer N, et al. Ultra-high-throughput microbial community analysis on the Illumina HiSeq and MiSeq platforms. ISME J. 2012; 6(8):1621–1624. [PubMed: 22402401]
27. Langmead B, Salzberg SL. Fast gapped-read alignment with Bowtie 2. Nat Methods. 2012; 9(4): 357–359. [PubMed: 22388286]
28. Edgar RC. UPARSE: highly accurate OTU sequences from microbial amplicon reads. Nat Methods. 2013; 10(10):996–998. [PubMed: 23955772]
29. Caporaso JG, Kuczynski J, Stombaugh J, Bittinger K, Bushman FD, Costello EK, et al. QIIME allows analysis of high-throughput community sequencing data. Nat Methods. 2010; 7(5):335–336. [PubMed: 20383131]
30. Quast C, Pruesse E, Yilmaz P, Gerken J, Schweer T, Yarza P, et al. The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. Nucleic Acids Res. 2013; 41:D590–596. [PubMed: 23193283]
31. Lozupone C, Hamady M, Knight R. UniFrac–an online tool for comparing microbial community diversity in a phylogenetic context. BMC Bioinformatics. 2006; 7:371. [PubMed: 16893466]

32. Chao A, Chazdon RL, Colwell RK, Shen TJ. Abundance-based similarity indices and their estimation when there are unseen species in samples. Biometrics. 2006; 62(2):361–371. [PubMed: 16918900]

33. Shannon CE. A mathematical theory of communication. The Bell System Technical Journal. 1948; 27:379–423.

34. Jari Oksanen FGB, Roeland Kindt, Legendre Pierre, Peter R, Minchin RB, O'Hara Gavin L, Simpson Peter, Solymos M, Henry H. Stevens and Helene Wagner vegan: Community Ecology Package. R package version. 2013:20–10.

35. Goslee SCaU DL. The ecodist package for dissimilarity-based analysis of ecological data. Journal of Statistical Software. 2007; 22(7):1–19.

36. Team, RC. R: A language and environment for statistical computing. R Foundation for Statistical Computing; Vienna, Austria: 2014. http://www.R-project.org/

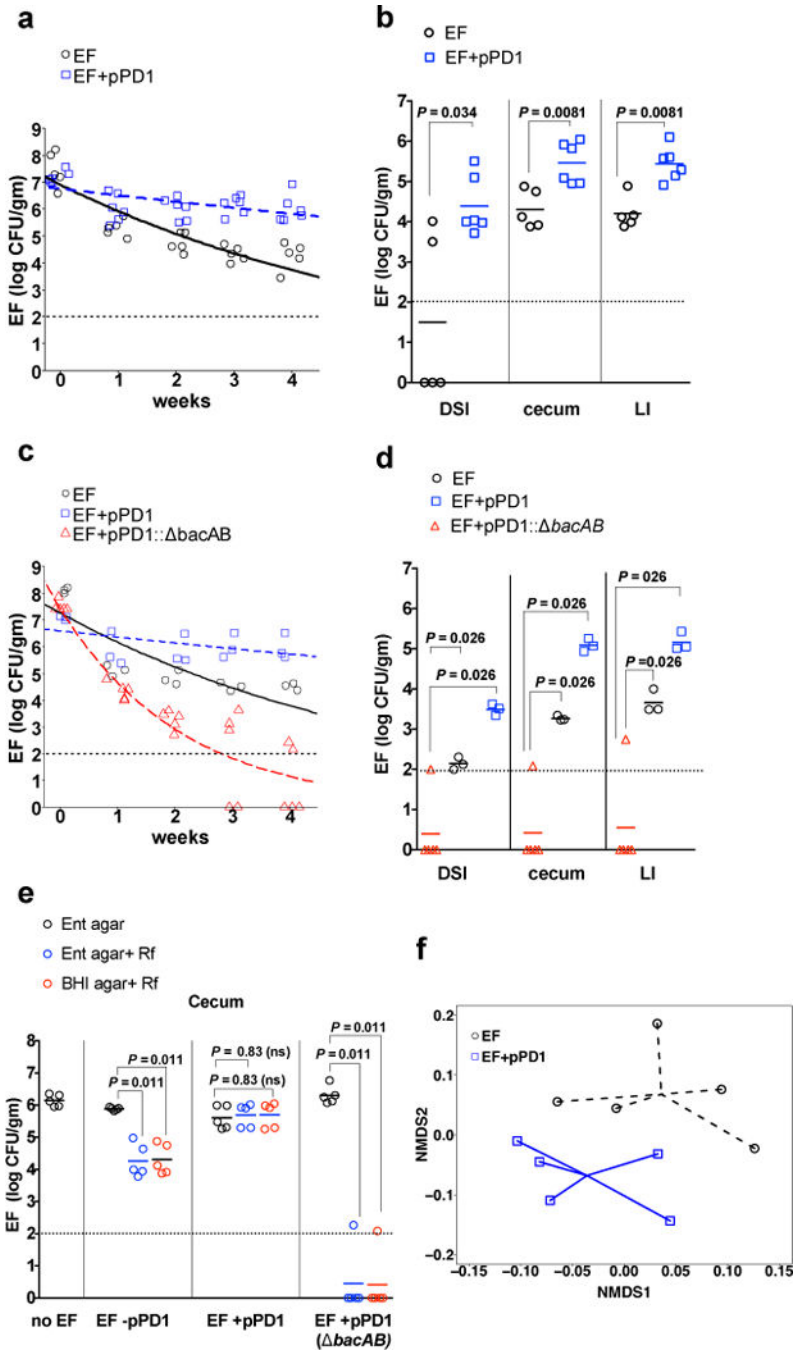

Figure 1. pPD1 enhances EF competition for an intestinal niche

Mice were colonized by EF_r (o, N=5) or EF_r+pPD1 (□, N=6) which were enumerated weekly from feces (a), and at week 4 from each segment of the GI tract (b) (distal small intestine, DSI; cecum; and large intestine, LI). (c–d) Mice were colonized with EF_r+pPD1::Δ*bacAB* (Δ, N=5), EF_r (o, N=3) or EF_r +pPD1 (□, N=3). EF fecal abundance was determined weekly (c) and in GI tract at week 4 (d). (e) Mice (N=5/group) were colonized with EF_r, EF_r+pPD1, EF_r+pPD1::Δ*bacAB* or sterile drinking water; at week four, abundance of total enterococci (indigenous + lab strains) and rifampicin–resistant lab strains

of EF were enumerated in each segment of the GI tract. (f) **Microbiome analysis:** Ordination of EF (o, N=5 mice) and EF+pPD1 (□, N=5 mice) samples were separated by Bray-Curtis beta diversity metric (Adonis P=0.007). Samples are connected to help visualize grouping. An exponential decay model was fitted to the data in (a) and (c). (a) Rate of decay is significantly different between the two groups in (a) P<0.0001) and (c) EF_r+pPD1:: Δ*bacAB* vs EF_r (P<0.0001) and EF_r+pPD1:: Δ*bacAB* vs. EF+pPD1 (P<0.0001). Horizontal lines indicate geometric mean. Each symbol represents an individual animal; data are representative of six (a–b) and two (e) biologically independent experiments. Data is from one experiment in c, d and f. All graphs: dashed line indicates the limit of detection at 100 CFU per gram feces.

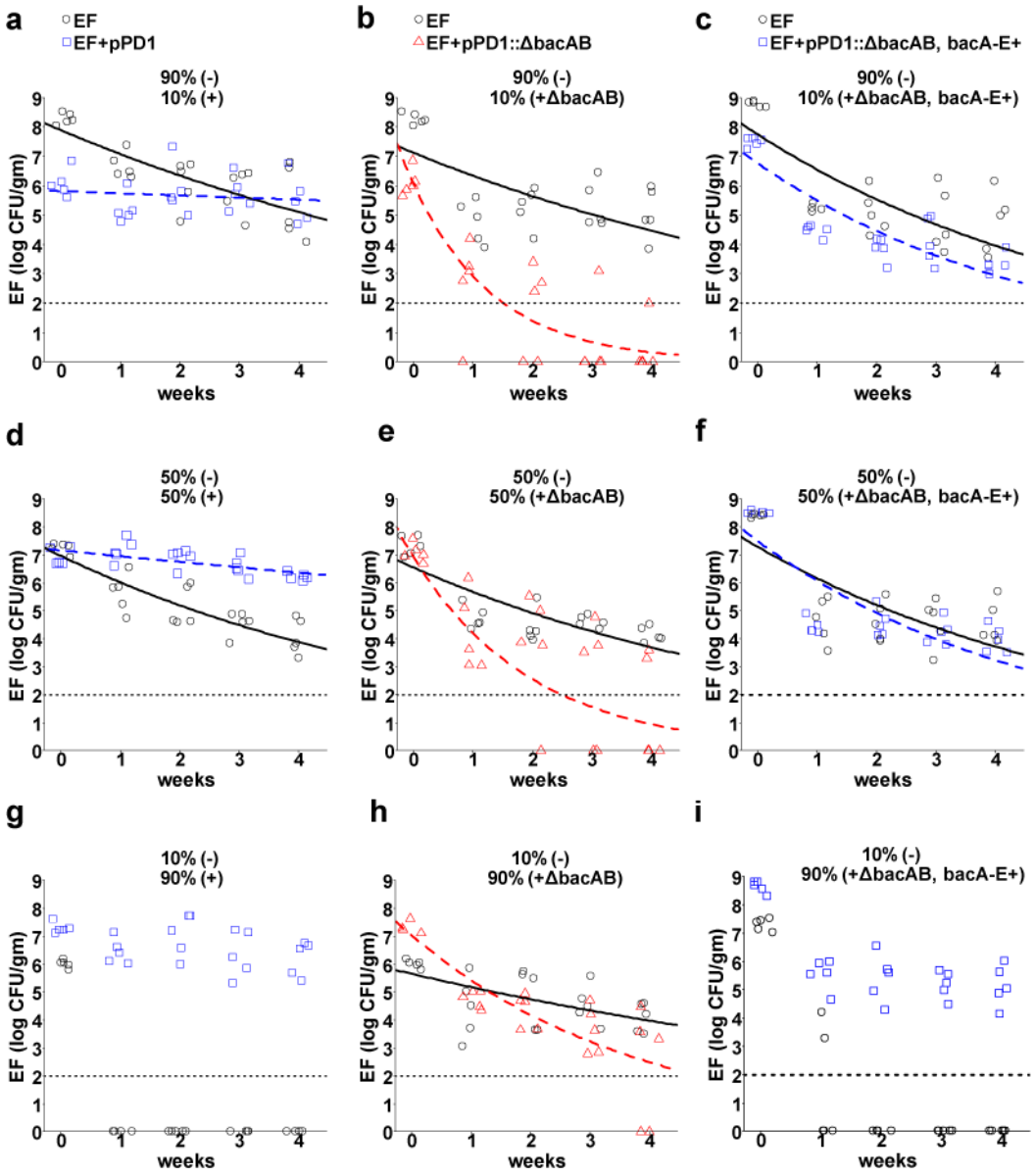

Figure 2. Bacteriocin provides a competitive survival advantage to EF in the GI tract

Mice (N=5/group) were given mixtures of [EF_r (−) and EF_s +pPD1 (+)] or [EF_r and EF_s +pPD1::Δ*bacAB*] or [EF_r and EF_s+pPD1::*ΔbacAB, bacA-E*+] in drinking water at indicated ratios, and fecal shedding was determined weekly. Each symbol represents an individual animal. Lines are fitted using an exponential decay model. Rate of decay is significantly different between the two groups (a) P=0.0004; (b) P<0.0001; (c) P=*ns* (d) P<0.0001; (e) P=0.001; (f) P=*ns*; (g) P=0.0117 (Week 0) and 0.0075 (Week 1, 2, 3, and 4); (h) P=0.008; (i) P=0.012 (Week 0 and 1) and 0.0075 (Week 2, 3, and 4). The results in a, d and g are representative of three biologically independent experiments; data in b, c, e, f, h and i are results of one experiment.

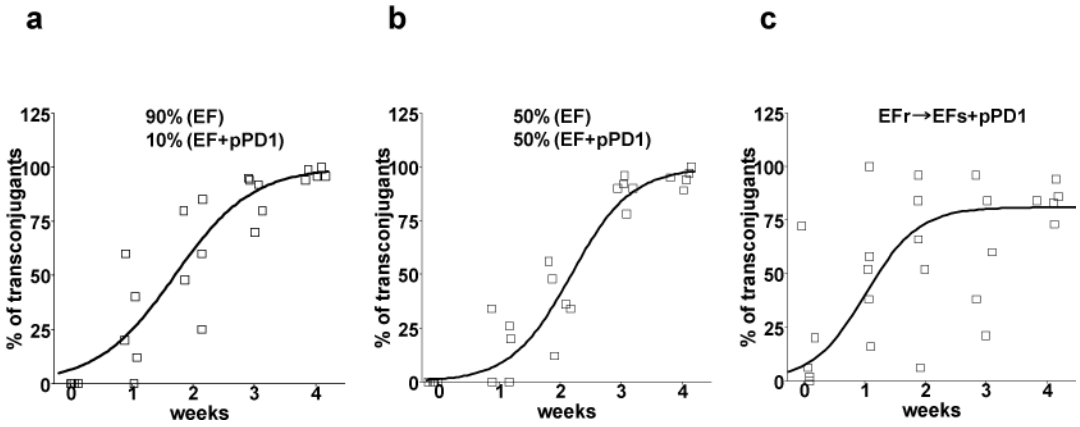

Figure 3. pPD1 is transferred via conjugation within the mouse GI tract

(a and b) Mice (N=5/group) were colonized with mixtures of EF_r and EF_s+pPD1 water at indicated ratios. One hundred EF_r colonies (from feces) per animal were screened weekly for pPD1. (c) Mice (N=5/group) were stably colonized with EF_r and then challenged with EF_s+pPD1. Fifty EF_r colonies (from feces) per animal were screened weekly for pPD1. (a–c) Each symbol represents the percentage of fecal transconjugants in an individual animal. The line is the fitted logistic curve. The results in a, b and c are from one experiment each.

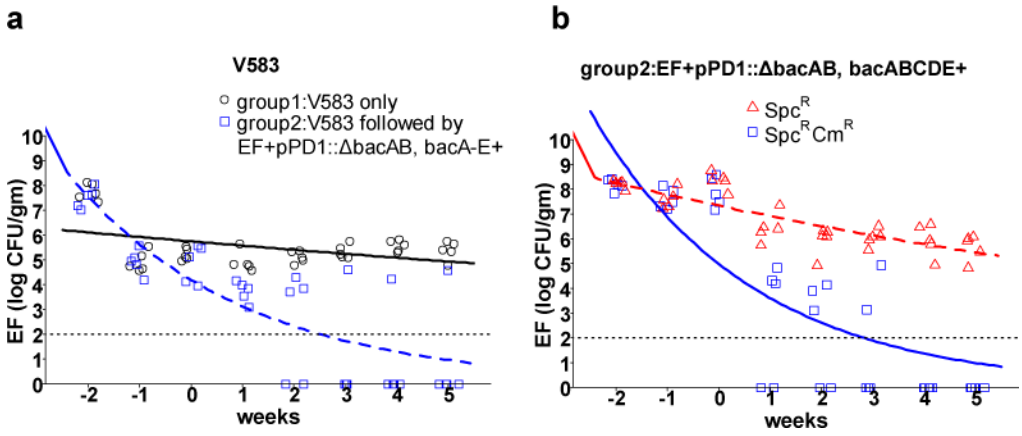

Figure 4. Bacteriocin reduces $V583_r$ colonization

Mice (N=5/group) were colonized with $V583_r$. $V583_r$ was removed from drinking water for both groups (week -2). Group 1 received sterile water and group 2 received EF_s+pPD1::*ΔbacAB, bacA-E+* in drinking water for two additional weeks, followed by sterile water at Week 0. Fecal $V583_r$ (a) and EF_s +pPD1::*ΔbacAB, bacA-E+* (b) levels were enumerated weekly. The retention of complementation plasmid pAM401A::*bacA-E+* by EF_s+pPD1::*ΔbacAB, bacA-E+* was determined weekly (b). Lines are fitted using an exponential decay model, and the rate of decay is significantly different between the two groups in (a) $P<0.0001$ and (b) $P<0.0001$. Each symbol represents an individual animal and data are representative of three biologically independent experiments. Dashed line indicates the limit of detection at 100 CFU per gram feces.