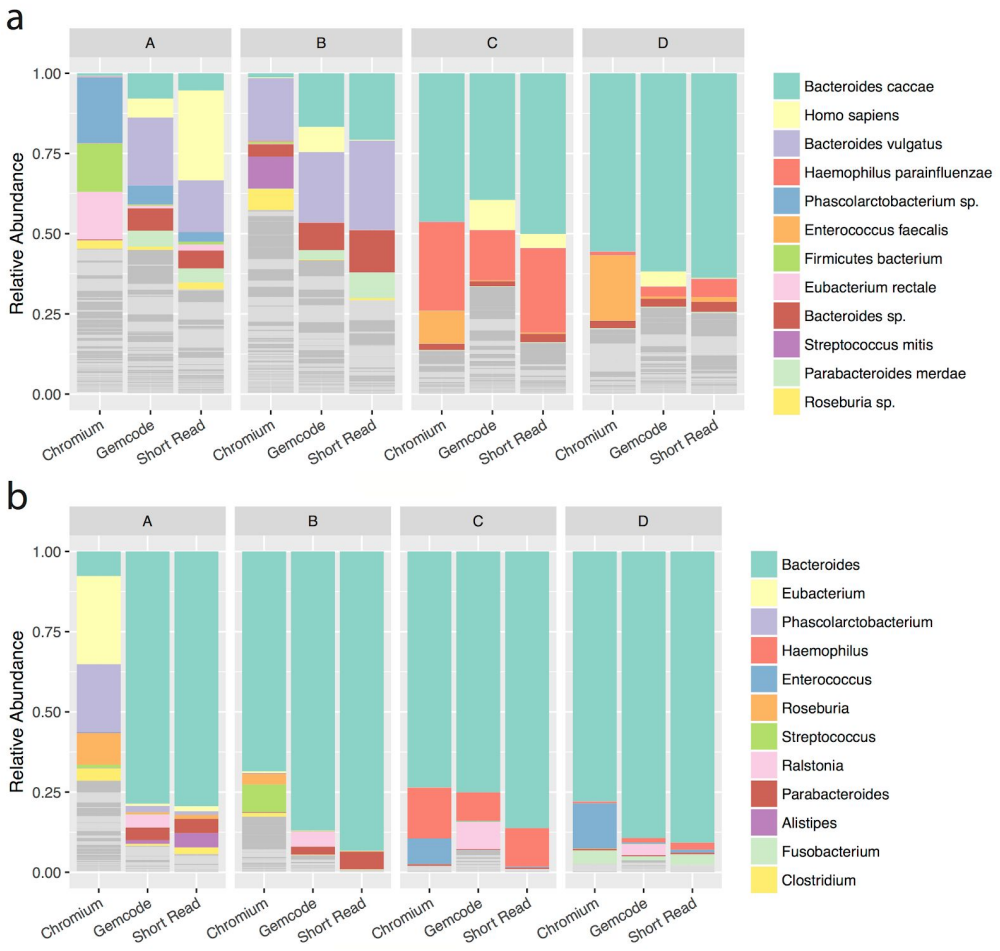
Supplementary Material

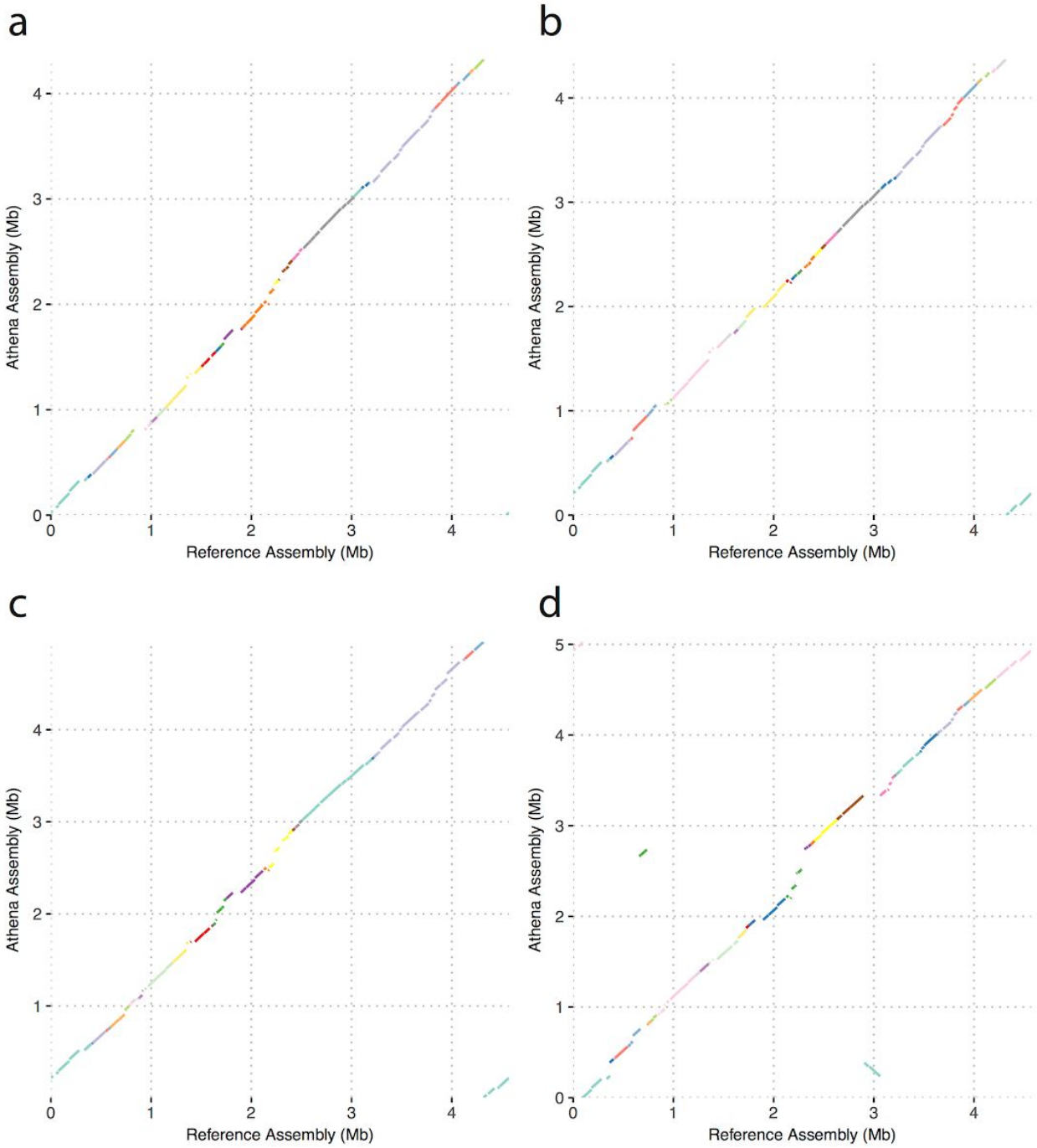
Supplementary Figures and Tables



Supplementary Figure 1

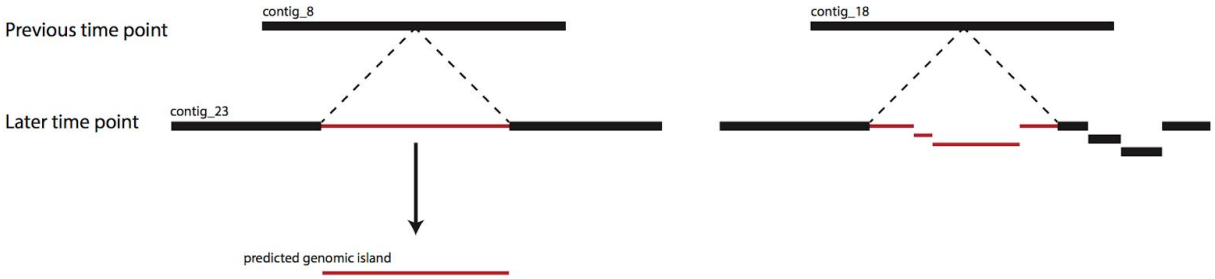
Species-level (a) and genus-level (b) taxonomic composition of the patient stool time series for Illumina Truseq short-read libraries, primary read cloud libraries prepared using the 10X Chromium platform, and previous read cloud libraries prepared using the now discontinued 10X Gemcode platform. For visual clarity, assignments for only the top 12 most abundant species and genera are identified. Reads receiving no classification, as well as reads classified at broader taxonomic levels than genus, are omitted for visual clarity.

Reads classified as *Ralstonia* were only observed in the 10X Gemcode read cloud libraries. We attributed these reads to DNA contamination of Gemcode library preparation kit reagents.



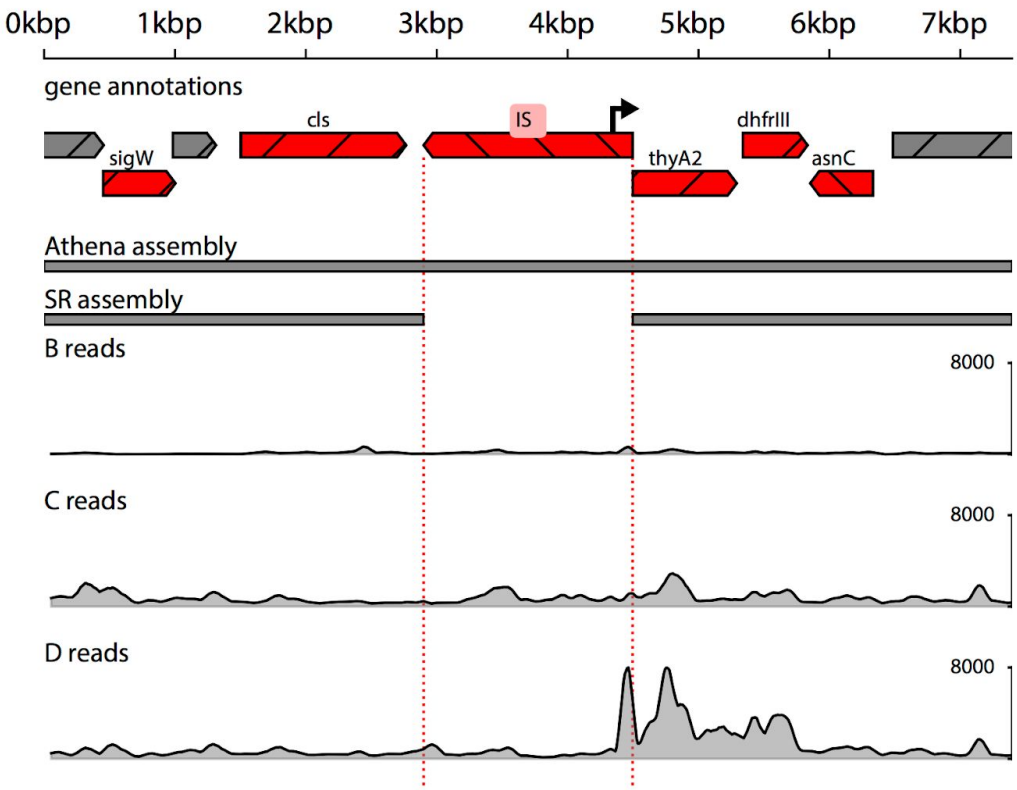
Supplementary Figure 2

Dot-plot alignments between *B. caccae* read cloud drafts from the four time points (A, B, C, and D) against the available closed reference isolate genome (Genbank ID GCF_002222615.2).



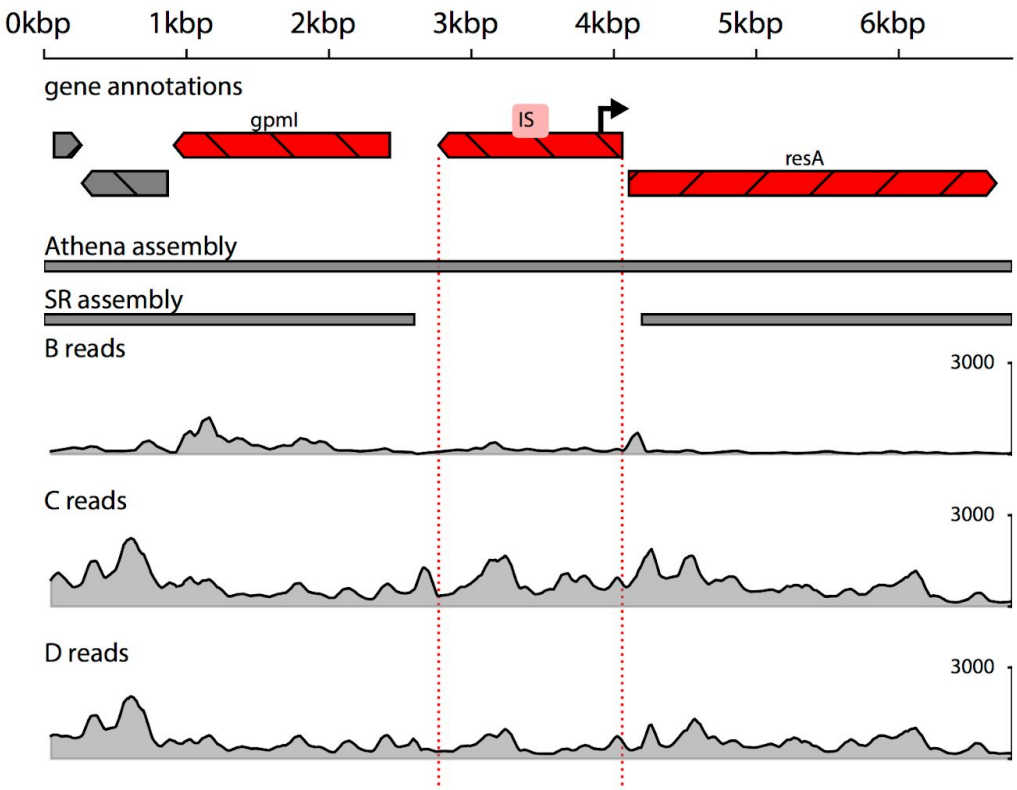
Supplementary Figure 3

Alignments between single contigs before and after genomic island integration allow prediction of genomic island sequence (left). Genomic island sequences that are not fully assembled into a single contig within their genomic contexts in the later time point cannot be accurately predicted (right).



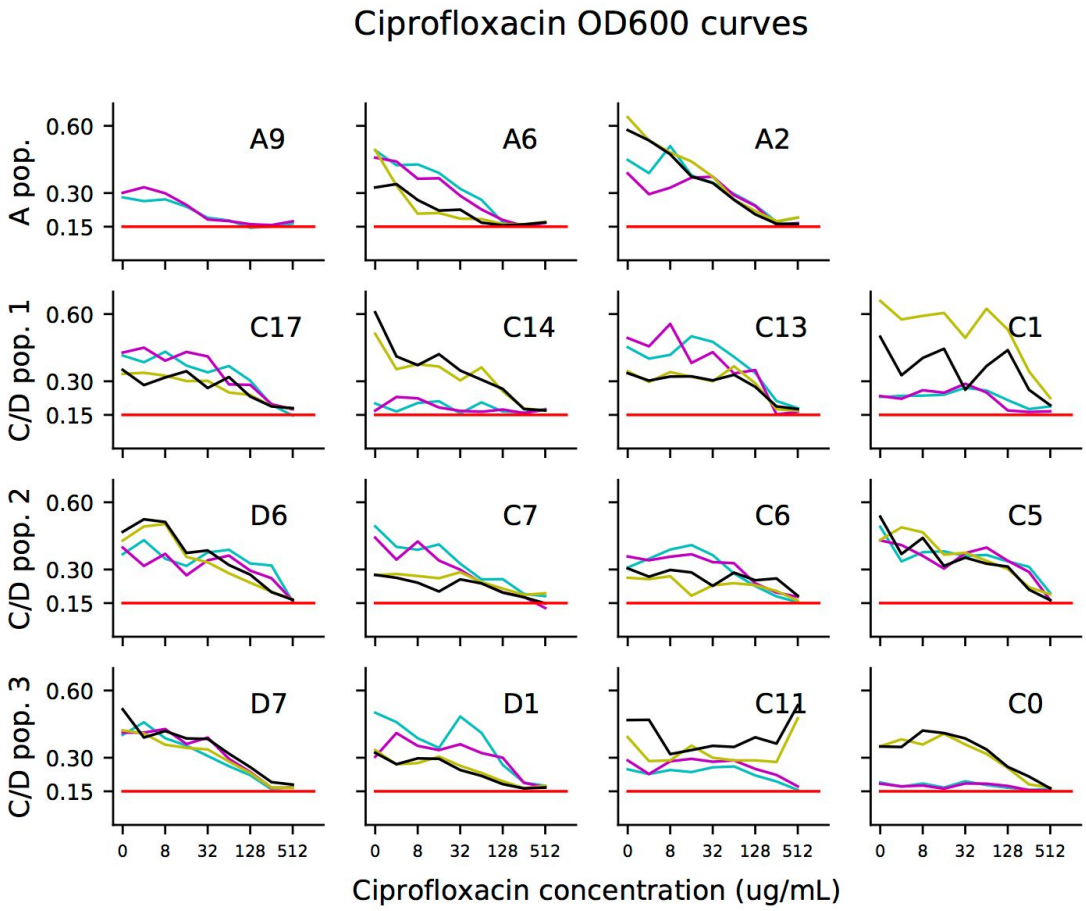
Supplementary Figure 4

Metagenomic RNA sequencing supports IS-mediated transcription within *B. caccae.* Example of IS-mediated transcription for an additional gene, *thyA2*. Dominant strains in time point D harbor an introduced promoter and in the preceding time points B and C they do not. The transcriptional contribution of the IS is supported by increased RNA sequencing read depth in downstream genes relative to upstream genes, which coincides with an increase in the proportion of strains harboring the IS.



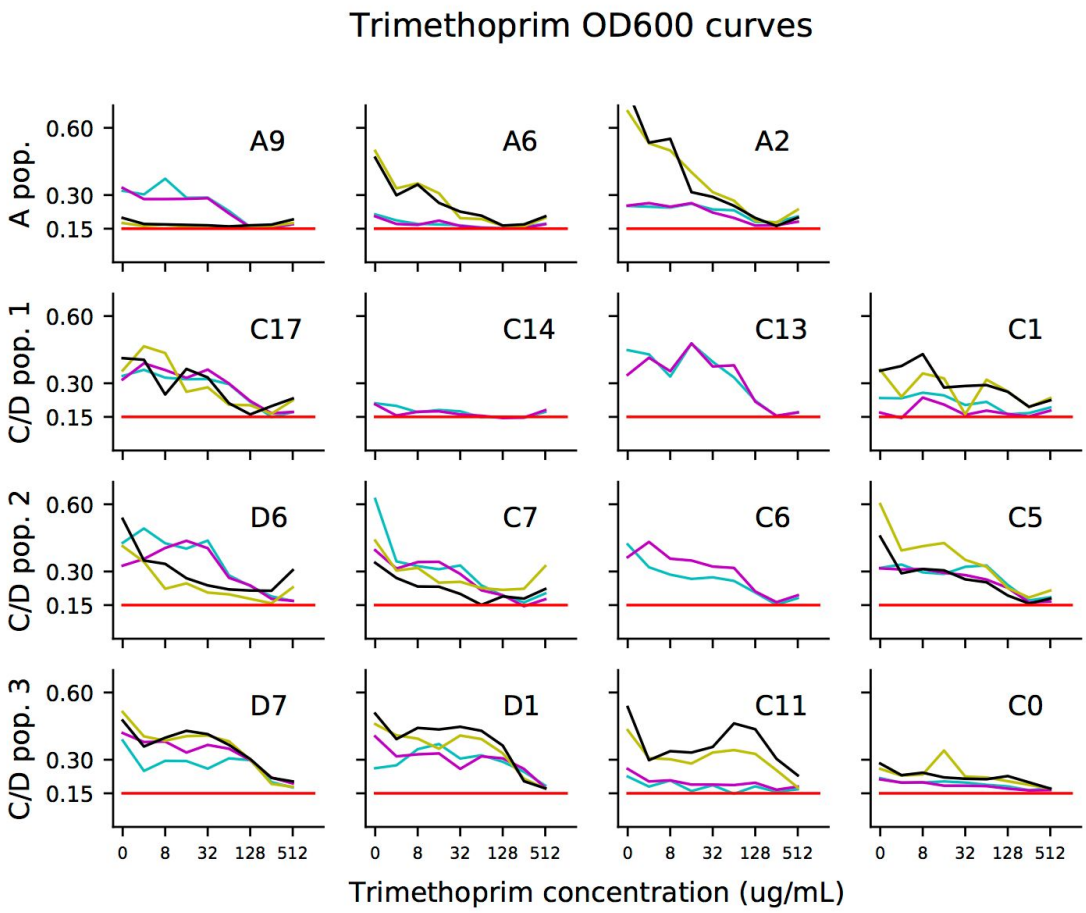
Supplementary Figure 5

Metagenomic RNA sequencing supports IS-mediated transcription within *B. caccae.* Example of IS-mediated transcription for an additional gene, *resA*. Dominant strains in time point C harbor an introduced promoter and in the preceding time point B they do not. The transcriptional contribution of the IS is supported by increased RNA sequencing read depth in downstream genes relative to upstream genes, which coincides with an increase in the proportion of strains harboring the IS.



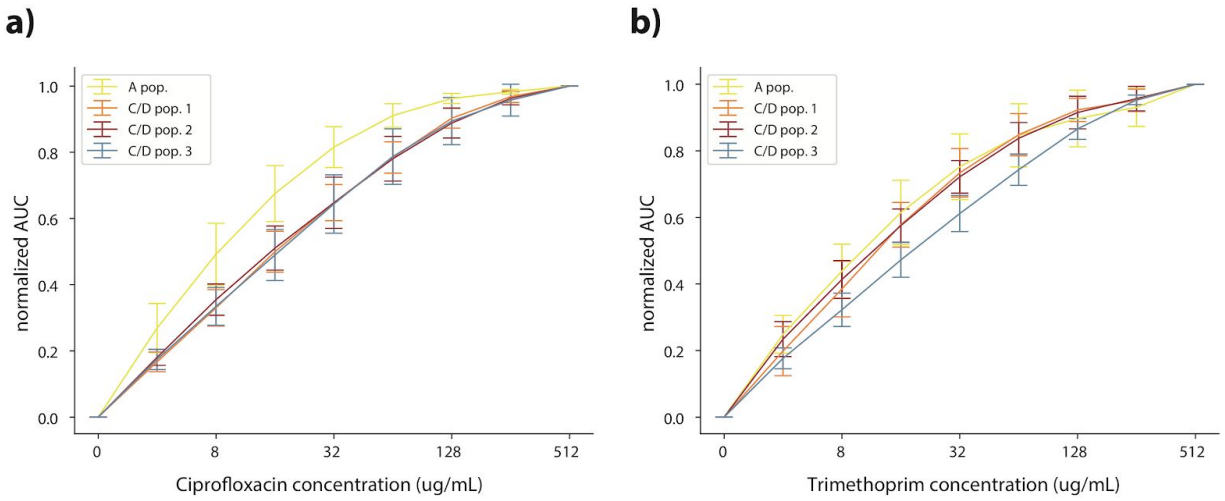
Supplementary Figures 6

Raw OD600 readings of each tested *B. caccae* isolate strain against different concentrations of ciprofloxacin. Each strain was tested using two to four replicates. Strains are organized by their subpopulation assignment as determined by whole genome sequencing. The area-under-the-curve (AUC) of each growth curve was used to determine MIC. Replicates showing minimal growth over a blank OD600 reading of sterile TYG media were excluded from AUC/MIC analysis. Although higher concentrations of the drug were tested, OD600 readings above 512ug/mL were excluded as ciprofloxacin was observed to precipitate at these higher concentrations after the 48 hour incubation period of the assay.



Supplementary Figure 7

Raw OD600 readings of each tested *B. caccae* isolate strain against different concentrations of trimethoprim. Each strain was tested using two to four replicates. Strains are organized by their subpopulation assignment as determined by whole genome sequencing. The area-under-the-curve (AUC) of each growth curve was used to determine MIC. Replicates showing minimal growth over a blank OD600 reading of sterile TYG media were excluded from AUC/MIC analysis. OD600 readings above 512ug/mL were excluded as ciprofloxacin was observed to precipitate after the 48 hour incubation period of the assay.



Supplementary Figure 8

Normalized AUC of all replicates (total area for each normalized to one) of all tested strains of each *B. caccae* strain subpopulation. Error bars show the variance of normalized AUC at each drug concentration. Isolate strains from time point A show markedly reduced overall growth at higher drug concentrations than strains from time points C and D. Isolate strains from the third subpopulation from time points C and D show a modest increase in overall growth at higher concentrations of trimethoprim as compared to the rest.

Library	Time point	Total Reads	Total Bases	Total Bases (Gb)	Total Reads w/QC	Total Bases w/QC	Total Bases w/QC (Gb)
Short read	A	366,125,362	34,549,490,342	34.55	335,984,623	31,582,554,562	31.58
Short read	B	57,939,592	5,909,838,384	5.91	53,327,906	5,187,200,671	5.19
Short read	C	66,691,350	6,802,517,700	6.80	61,961,698	6,035,573,394	6.04
Short read	D	27,814,734	2,837,102,868	2.84	25,920,034	2,521,145,129	2.52
Chromium	A	147,103,476	20,594,486,640	20.59	144,405,692	19,855,169,050	19.86
Chromium	B	140,586,910	19,682,167,400	19.68	138,156,773	19,033,140,760	19.03
Chromium	C	135,132,378	18,918,532,920	18.92	132,255,256	18,201,215,198	18.20
Chromium	D	152,224,794	21,311,471,160	21.31	149,329,401	20,575,219,967	20.58
Gemcode	A	49,121,872	7,073,549,568	7.07	23,607,499	997,034,214	1.00
Gemcode	B	39,171,402	5,640,681,888	5.64	17,394,123	749,959,878	0.75
Gemcode	C	29,894,142	4,304,756,448	4.30	11,665,434	564,141,282	0.56
Gemcode	D	40,573,138	5,842,531,872	5.84	19,771,511	944,012,183	0.94
RNA	A	-	-	-	-	-	-
RNA	B	118,775,928	12,115,144,656	12.12	-	-	-
RNA	C	83,575,948	8,524,746,696	8.52	-	-	-
RNA	D	79,094,650	8,067,654,300	8.07	-	-	-

Supplementary Table 1

Total reads and sequencing coverage for all metagenomic sequencing libraries before and after quality control.

reference Genbank ID	draft	reference length	draft length	median sequence identity (%)	unaligned reference bases	unaligned draft bases
NZ_JH724079.1	Read cloud	5493117	5476141	99.175	1078924	1076271
NZ_CP022412.2	Read cloud	4570803	5476141	99.474	513456	1431898
NZ_AAVM02000021.1	Read cloud	4564814	5476141	99.479	515847	1437337
NZ_PUEQ01000001.1	Read cloud	4577788	5476141	99.479	528503	1434141
NZ_CZBL01000001.1	Read cloud	5291863	5476141	99.484	910158	1099942
NZ_CZAI01000001.1	Read cloud	5337582	5476141	99.633	733275	899825
combined	Read cloud		5476141			638951
NZ_JH724079.1	Short read	5493117	4713495	99.263	1348485	602650
NZ_CP022412.2	Short read	4570803	4713495	99.486	654106	796989
NZ_AAVM02000021.1	Short read	4564814	4713495	99.485	658494	797371
NZ_PUEQ01000001.1	Short read	4577788	4713495	99.486	671140	796988
NZ_CZBL01000001.1	Short read	5291863	4713495	99.683	1181934	648442
NZ_CZAI01000001.1	Short read	5337582	4713495	99.692	1047949	464972
combined	Short read		4713495			317696

Supplementary Table 4

Median sequence identity and total number of unaligned bases between *B. caccae* drafts from time point C drafts and six available reference isolate genomes.

Time point	Read cloud draft location	Size	Other organisms
C	contig_3:715785-732470	16685	B. vulgatus and B. uniformis
C	contig_3:849029-906491	57462	B. vulgatus
C	scaffold_20:157993-228816	70823	
D	contig_67:129068-179225	50157	
D	contig_67:60290-102957	42667	

Supplementary Table 5

The locations of large-scale genomic islands within the assembled read cloud drafts detected by pairwise sequence alignments of *B. caccae* drafts from successive time points. Two of these islands were also found to be present within the draft genomes of other organisms present in the samples.

Source time point draft	Read cloud draft IS612 location	A	B	C	D	downstream gene (where applicable)
a	contig_133:369670-371266	0.452	0.513	0.646	0.760	resA
a	contig_3:281420-283016	0.000	0.000	0.000	0.000	
a	contig_34:3881-5477	0.000	0.000	0.000	0.000	per1
a	contig_34:30545-32141	0.688	0.573	0.709	0.685	
a	contig_366:11196-12792	0.220	0.585	0.756	0.669	
a	contig_452:35256-36852	0.051	0.015	0.022	0.672	
a	contig_70:225046-226642	0.094	0.016	0.044	0.666	
a	contig_70:315579-317175	0.033	0.008	0.014	0.288	
a	contig_87:7379-8975	0.000	0.000	0.000	0.000	
a	contig_87:36950-38546	0.021	0.025	0.027	0.554	
b	contig_171:85806-87402	0.000	0.000	0.000	0.000	
b	contig_388:58644-60240	0.034	0.013	0.022	0.556	
c	contig_2:345613-347209	0.044	0.025	0.018	0.561	
c	contig_21:136948-138544	0.583	0.854	0.136	0.583	
c	contig_3:133306-134902	0.515	0.594	0.162	0.630	
c	contig_3:713286-714882	0.305	0.834	0.164	0.743	
d	contig_18:332510-334106	0.623	0.826	0.674	0.149	thyA2
d	contig_4:129400-130996	0.491	0.723	0.000	0.000	norM

Supplementary Table 6

Estimated fractional abundances of ancestral strains at each assembled *B. caccae* insertion site. Reads originating from strains without a given IS instance (ancestral strains) are recognized by having gapped alignments across the assembled insertion sequence. Ancestral strain fraction is expressed as the number of observed gapped alignments over the median sequence coverage within the neighboring 10kb of sequence. Also noted are adjacent genes mentioned in the main text that are downstream of the putative outward-facing promoter carried on the insertion sequence.

Supplementary Table 2 (separately attached)

Assembly statistics, completeness metrics, tRNA and rRNA loci counts, total annotated genes, and coverage depths for all annotated species draft genomes in short-read and read cloud libraries from each time point. Results are shown for the largest bin of each species.

Supplementary Table 3 (separately attached)

Assembly statistics, completeness metrics, tRNA and rRNA loci counts, total annotated genes, and coverage depths for bins created by merging all those annotated as *B. caccae* in short-read and read cloud libraries at each time point.

Supplementary Table 7 (separately attached)

Coding sequences with RNA sequencing read counts and fold-change between time points in the neighboring 10kb around the five IS614 integration loci in *B. caccae* estimated to have large-scale ancestral strain shifts. Gene annotations were obtained using Prokka. The target gene downstream of the putative promoter as well as the upstream gene are highlighted (green: downstream, red: upstream).

Supplementary Table 8 (separately attached)

Total reads, sequencing coverage, assembled genome draft size and N50, and taxonomic annotation for all 53 isolates from stool samples of time points A, C, and D.

Supplementary Results

IS614 adjacent to *per1*

The rise in expression of *per1* coincided with a one-week course of cefepime followed immediately by a two week course of meropenem between timepoints B and C. *Per1* continues to exhibit high expression in time point D, 19 days after withdrawal of meropenem, despite the absence of any further beta-lactam antibiotic administration. Our drafts located an IS adjacent to this gene oriented correctly for IS-mediated transcription to occur. While estimating relative abundance of this insertion, we were unable to detect reads from the ancestral strain, and determined this insertion to be fixed within the population over the course of treatment. This particular instance of *per1* and neighboring sequence was only found using our read cloud approach and was not present in any of the six available reference isolate *B. caccae* genomes or in any of the short-read assembled *B. caccae* drafts.

Gram-positive enrichment in DNA extractions with enzymatic lysis

The short-read libraries and previous read cloud libraries, which were prepared with the now discontinued 10X Genomics Gemcode platform, all utilized DNA extracted with mechanical lysis. These libraries displayed concordant species-level community composition across all samples (Supplementary Figure 1). Our primary read cloud libraries prepared with the current 10X Genomics Chromium platform, were prepared from DNA extracted with enzymatic lysis and show a greater representation of gram positive bacteria. This suggests that the differing extraction protocols used for the primary read cloud and short-read libraries, not the library preparation method, are the main source of discrepancies in community composition.