Phase-variable capsular polysaccharides and lipoproteins modify bacteriophagesusceptibility in Bacteroides thetaiotaomicron

Nathan T. Porter1,4, Andrew J. Hryckowian2,4✉, Bryan D. Merrill2, Jaime J. Fuentes1, Jackson O. Gardner2, Robert W. P. Glowacki1, Shaleni Singh1, Ryan D. Crawford3, Evan S. Snitkin1, Justin L. Sonnenburg2 and Eric C. Martens2,4✉

A variety of cell surface structures dictate interactions between bacteria and their environment, including their viruses (bacteriophages). Members of the human gut Bacteroidetes characteristically produce several phase-variable capsular polysaccharides (CPSs), but their contributions to bacteriophage interactions are unknown. To begin to understand how CPSs have an impact on Bacteroides–phage interactions, we isolated 71 Bacteroides thetaiotaomicron-infecting bacteriophages from two locations in the United States. Using B. thetaiotaomicron strains that express defined subsets of CPSs, we show that CPSs dictate host tropism for these phages and that expression of non-permissive CPS variants is selected under phage predation, enabling survival. In the absence of CPSs, B. thetaiotaomicron escapes bacteriophage predation by altering expression of eight distinct phase-variable lipoproteins. When constitutively expressed, one of these lipoproteins promotes resistance to multiple bacteriophages. Our results reveal important roles for Bacteroides CPSs and other cell surface structures that allow these bacteria to persist under bacteriophage predation, and hold important implications for using bacteriophages therapeutically to target gut symbionts.

The human gut microbiome is dominated by a diverse population of bacteria, with hundreds of different species typically coexisting within an individual1,2. Frequent diet changes, host immune responses and bacteriophage infections are among the many intermittent perturbations to the gut ecosystem. Despite these perturbations, an individual’s microbiome generally remains stable over long time periods3, suggesting that bacteria have evolved strategies to persist despite frequent disturbances. One mechanism that may promote ecosystem resilience is the ability of some bacteria to produce multiple capsular polysaccharides (CPSs), cell surface components that have been diversified in the genomes of gut-dwelling Bacteroidetes and other phyla4,5. Previous work showed that CPSs from Bacteroidetes and other phyla play roles in evading or modulating host immunity6–10, but the diversity of CPS synthesis loci in gut bacteria suggests that they could fill other roles5,6,11,12.

The phylum Bacteroidetes—within which members of the genus Bacteroides are typically the most abundant Gram-negative gut symbionts in industrialized human populations5,13—provides excellent models to study persistence and competition mechanisms, including CPSs. The type strains of the well-studied species Bacteroides thetaiotaomicron and Bacteroides fragilis each encode eight different CPSs14,15 and there is broad genetic diversity of cps loci among different strains within these species5. In Bacteroides spp., CPS structures appear to surround the entire cell16,17 and the biosynthetic loci that encode these surface coatings are often under the control of phase-variable promoters18,19. Together with other regulators, phase-variable CPS expression generates phenotypic heterogeneity within an otherwise isogenic population that may facilitate survival during ecological disturbances6,15,16,20.

Bacterial viruses or bacteriophages (phages), similar to the bacteria on which they prey, vary greatly across individual gut microbiomes and are responsive to host dietary changes and disease states21–23. Compared with bacteria, far less is understood about the phages of the gut microbiome, especially the mechanisms governing phage–bacteria interactions. In the present study, we tested the hypothesis that CPSs mediate Bacteroides spp.–phage interactions. Our results support this hypothesis, but also reveal that B. thetaiotaomicron possesses additional phase-variable, phage-evasion strategies in addition to CPSs. Our results provide a glimpse into the intricacy of bacteria–phage interactions in the human gut, and provide a foundation for future work to leverage these interactions to manipulate the gut microbiome.

Results

Bacteriophages infect B. thetaiotaomicron in a CPS-dependent fashion. The genomes of human gut Bacteroidetes frequently encode multiple CPSs5 (see Extended Data Fig. 1). To test the hypothesis that Bacteroides CPSs mediate interactions with phages, we isolated 71 phages that infect B. thetaiotaomicron VPI-5482 from two locations in the United States (Ann Arbor, MI and San Jose, CA). Phages were isolated using the wild-type strain that encodes eight genetically distinct CPSs and other phyla play roles in evading or modulating host immunity6–10, but the diversity of CPS synthesis loci in gut bacteria suggests that they could fill other roles5,6,11,12.

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To determine whether infection by these phages was impacted by CPSs, we tested each phage against each of the 10 host strains in a plaque assay to measure host range. Hierarchical clustering of the infection profiles revealed a cladogram with three main branches that each encompasses phages from both collection sites (Fig. 1 and see also Extended Data Fig. 3). Phages in branches 1 and 2 generally infect the acapsular strain and are blocked by some, but not all, CPSs. ARB154 exclusively infected cps8, an uncommon CPS among B. thetaiotaomicron strains that appears to be contained in a mobile element. Phages in branch 3 generally infect wild-type, cps1, cps2 and cps3 well, and this branch contained most of a subset of phages that fail to infect the acapsular strain, suggesting that they require CPSs for infection.

**Specific CPS elimination alters bacterial phage susceptibility.** For the phages described that robustly infect the acapsular strain, resistance in only a subset of a single cps strain indicates that capsule-independent receptor(s) mediate(s) infection and only a subset of ‘non-permissive’ CPSs block it. For phages that inefficiently infect the acapsular strain, one or more CPSs may serve as a direct phage receptor. To further define these roles, we investigated a subset of six phages (ARB72, ARB78, ARB82, ARB101, ARB105 and ARB25; marked in blue text in Fig. 1). These phages infect wild-type B. thetaiotaomicron and five of them infect the acapsular strain poorly or not at all. We first tested the hypothesis that some CPSs are required for infection by deleting only the subsets of CPSs encoding permissive capsules based on our previous experiments. For ARB72, simultaneous elimination of the two most permissive capsules, CPS1 and CPS3, from wild-type B. thetaiotaomicron reduced infection below the limit of detection (Fig. 2a). Likewise, elimination of the most permissive CPS for ARB78, ARB82, ARB101 and ARB105 significantly reduced infection by these phages, in some cases only after multiple CPS were eliminated. In some cases, this occurred even when other permissive CPSs were still present (Fig. 2b–c).

For ARB25, which promiscuously infects B. thetaiotaomicron CPS variants (Fig. 1), some single and compounded cps deletions reduced infection rates (Fig. 2f). Although individual deletions of four permissive CPSs (CPS1, -6, -7, -8) led to partially reduced infection, so did single eliminations of either of two CPSs that were initially determined to be non-permissive (CPS3 and CPS4). Moreover, combinatorial deletion of non-permissive CPS4 and permissive CPS1 completely eliminated detectable infection, suggesting more complicated regulatory interactions, which are known to occur with Bacteroides CPSs. It is interesting that strains lacking CPS4 or CPS1/CPS4 compensated by significantly increasing relative expression of the non-permissive cps2 locus, which could contribute to ARB25 resistance (Fig. 2g).

A strain expressing only two non-permissive CPSs (CPS2 and CPS3) could not be detectably infected by ARB25 (Fig. 2f, 2, 3 only). However, a strain expressing CPS2, -3 and -4 regained some susceptibility (Fig. 2f, 2, 3, 4 only), indicating that, when CPS4 is present, it is capable of mediating some infection by ARB25, which is different from the observation made in Fig. 1. In contrast to sole expression of CPS2 and CPS3, deletion of just the cps2 and cps3 loci led to dominant expression of cps1 and cps4, which increased infection efficiency and production of clearer plaques (Fig. 2f–h). ARB25 formed smaller and more turbid plaques on the Δcps4 strain, demonstrating that loss of CPS4 expression alone modifies ARB25 infection (Fig. 2h). Additional experiments with another B. thetaiotaomicron strain that encodes homologues of cps2, cps5 and cps6 support the elimination of these permissive capsules, reducing, in some cases, bacteria–phage interaction (see Extended Data Fig. 4). Moreover, the exogenous presence of a stoichiometric excess of purified, non-permissive CPS2 did not inhibit ARB25 infection of the acapsular strain, suggesting that CPS does not block infection in trans (see Extended Data Fig. 5).

Liquid cultures of each of the CPS-expressing strains inoculated with live or heat-killed ARB25 largely replicated our initial plaque assays (Fig. 3). Initial growth perturbations were characterized by extended lag or drop in density, but liquid cultures of ARB25-susceptible strains eventually either re-grew to high (wild-type, acapsular, cps1, cps5) or intermediate (cps4, cps7, cps8) densities, suggesting outgrowth of resistant bacteria that was not attributable to inactivity of the phage present (see Extended Data Fig. 5). In contrast to plate-based assays, liquid cultures of cps4 were sensitive to growth inhibition by ARB25, whereas cultures of cps6 were not. A similar correlation between plaque assays and liquid infections was observed with SJC01, a branch 2 phage with an infection profile similar to ARB25 (see Extended Data Fig. 5). Importantly, re-isolation of ARB25-free isolates and re-infection with the same phage resulted in most (69%) of the re-isolates exhibiting susceptibility, revealing that resistance is not predominantly caused by permanent mutations and is reversible (see Supplementary Table 2).

**Phage-resistant, wild-type B. thetaiotaomicron exhibits altered cps locus expression.** We hypothesized that wild-type cells that are pre-adapted by expressing non-permissive capsules would be positively selected in the presence of phage. We infected wild-type B. thetaiotaomicron with ARB25 and monitored bacterial growth. Cultures treated with a high multiplicity of infection (MOI ≈ 1) displayed similar growth kinetics as observed previously, with an apparently resistant population emerging after 3–4 h (Fig. 4a). Notably, cultures originating from different single colonies displayed variable growth kinetics in the presence of ARB25. Next, we measured whether infection with ARB25 resulted in altered CPS expression by the surviving bacteria. B. thetaiotaomicron exposed to heat-killed phage predominantly expressed CPS3 and CPS4. Treatment with live ARB25 resulted in a significant decrease of cps1 and cps4 expression and a significant increase in expression of the non-permissive cps3 locus (Fig. 4b, Dirichlet’s regression P < 0.01). Similar growth and expression phenotypes occurred in cultures treated with a low (~10−7) MOI (see Extended Data Fig. 6). Notably, the most resistant bacterial clones in both experiments (as evidenced by faster outgrowth post-infection; Fig. 4 and see also Extended Data Fig. 6) expressed lower levels of permissive cps1 and cps4 and higher levels of non-permissive cps3 in heat-killed phage treatment groups (see Extended Data Fig. 6). Therefore, pre-existing variation in CPS expression may contribute to the ability of some clones to mount resistance during phage challenge.

**Multiple phase-variable features equip B. thetaiotaomicron to survive phage predation.** The results described in the “Phage-resistant, wild-type B. thetaiotaomicron exhibits altered cps locus expression” section show that variations in CPSs can pre-adapt a subpopulation to survive phage challenge. However, B. thetaiotaomicron Δcps also grows after infection by ARB25 in liquid culture (Fig. 3) and most recovered Δcps clones regained susceptibility to ARB25 (see Supplementary Table 2), suggesting that reversible, CPS-independent resistance mechanisms exist. We therefore performed RNA-sequencing (RNA-seq) to measure differences in gene expression between ARB25 post-infected, wild-type B. thetaiotaomicron and acapsular B. thetaiotaomicron.

As expected, the transcriptomes of wild-type bacteria surviving ARB25 infection (n = 3) were largely characterized by alterations in CPS expression (Fig. 5a and see also Supplementary Table 3). Of 83 genes with significant expression changes of threefold or more, 63 belonged to 4 cps loci, with cps1/cps4 decreased and cps2/cps3 increased. Two additional gene clusters encoding different, outer-membrane ‘Sus-like systems,’ which are responsible for import and degradation of carbohydrates and other nutrients, were also decreased after infection. Notably, these loci encode TonB-dependent transporters (similar to Escherichia coli TonA,
the first described phage receptor\textsuperscript{[9]}, suggesting that the proteins encoded by these genes might be part of the receptor for ARB25.

In acapsular \textit{B. thetaiotaomicron}, 118 genes showed significant expression changes after ARB25 challenge and most (85\%) were upregulated (Fig. 5b and see also Supplementary Table 4). One of the two Sus-like systems (\textit{BT2170-73}) that was decreased in ARB25-exposed wild-type was similarly decreased in acapsular \textit{B. thetaiotaomicron}. Among the most highly upregulated genes
after infection (28 genes with ≥10-fold increase and an adjusted $P \leq 0.01$), 6 genes in the well-characterized, starch-utilization system (Sus)$^{37}$ were increased, suggesting that surviving bacteria consume glycogen released from lysed siblings.

An additional 17 upregulated genes belong to 8 loci that encode predicted, outer-membrane S-layer lipoproteins and OmpA β-barrel proteins. One of these (BT1927) was previously found to be phase variable and to increase $B.\ thetaiotaomicron$ resistance.
to complement-mediated killing when locked in the ‘on’ state. The remaining S-layer clusters share both syntenic organization and weak homology to BT1927-25. The promoter regions of all 8 loci are also flanked by a pair of imperfect 17-nucleotide palindromic repeats (Fig. 5c). Three of these repeats are identical to those that mediate recombination of the BT1927 promoter and the remaining four vary only by a trinucleotide in the middle of each imperfect palindrome (Fig. 5c). Finally, amplicon sequencing of the promoter regions, using directionally oriented primers, confirmed recombination in five of the seven loci, whereas two did not generate PCR products (see Extended Data Fig. 7).

Among the genes that were differentially regulated in ARB25-infected acapsular B. thetaiotaomicron, we identified a recombinational shufflon similar to systems described in B. fragilis. Two genes in this shufflon encode TonB-dependent transporters (BT1040, BT1046) with truncated 5’-ends relative to BT1042 (Fig. 5d,e, and see also Extended Data Fig. 8). We detected, using PCR and amplicon sequencing, the presence of all five predicted alternative recombination states (Fig. 5d,e, and see also Extended Data Fig. 8) and mutation of the associated tyrosine recombinase (BT1041) locked the corresponding mutant into the native genomic architecture. It is of interest that deletion of BT1033-52 led to variable plaquing efficiency by ARB25, compared with the acapsular parent strain (see Extended Data Fig. 8), suggesting that loss of these genes may exert indirect global effects that mediate phage susceptibility while revealing that it is not the only receptor.

We gained additional insights into the complexity of the transcriptional response of B. thetaiotaomicron to phage infection by performing RNA-seq on B. thetaiotaomicron cps1 challenged with ARB25. Interestingly, the cps1 strain that is forced to express a permissive capsule can also survive ARB25 infection (Fig. 3) and mainly does so by increased expression of the S-layer proteins identified (see Extended Data Fig. 9 and Supplementary Table 5). This suggests that, at least for CPS1, co-expression of capsule and S-layer proteins is not mutually exclusive.

We also performed RNA-seq on B. thetaiotaomicron wild-type and acapsular strains challenged with phage SJC01. Wild-type bacteria infected with SJC01 exhibited similar alterations in CPS expression to those seen with ARB25 (see Extended Data Fig. 9 and Supplementary Table 6). In wild-type bacteria infected with SJC01, 61 of 67 differentially expressed genes belonged to CPS1 and CPS4 (both downregulated) or CPS3 (upregulated), which is consistent with CPS3 being non-permissive for SJC01. As observed with ARB25, nutrient-utilizing, Sus-like systems were downregulated in SJC01-infected cells, including previously described systems for ribose and fungal cell wall α-mannan use. In SJC01-infected acapsular cultures, expression of four of the eight S-layer proteins

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**Fig. 3 | Effects of ARB25 phage infection on growth of bacteria expressing different CPSs.** Ten strains—the wild-type (WT), the acapsular strain (Δcps) or the eight single cps-expressing strains—were infected with either live or heat-killed ARB25. Growth was monitored via OD600 on an automated plate-reading instrument as described in Methods, and individual growth curves for live and heat-killed phage exposure are shown separately. Growth curves represent the mean of three biological replicates performed with similar results on separate days. Results from a minimum of three 200-μl technical replicate cultures were averaged to generate each biological replicate.

**Fig. 4 | ARB25 infection of wild-type B. thetaiotaomicron causes altered cps gene expression.** Wild-type B. thetaiotaomicron was infected with live or heat-killed ARB25 at an MOI of 1. a, Growth was monitored by measuring OD600 every 15-30 min and individual growth curves for live and heat-killed phage exposure are shown separately (n = 3). The experiment was conducted three times as shown, plus a separate high MOI, with similar results. b, The cps gene transcript analysis was carried out by qPCR. The end of the growth curve in a represents the point at which cultures were harvested for qPCR analysis (that is, the first observed time point where culture surpassed an OD600 of 0.6). Significant changes in cps1, cps3 and cps4 expression were observed between groups treated with live or heat-killed ARB25 (P < 0.01 determined by the z-test for each change, using Dirichlet’s regression; bars represent the mean and error bars the s.e.m.; n = 3). Please see the Source data for all actual P values. Individual replicates for high and low MOI experiments are displayed in Extended Data Fig. 6.
was prominent, with the BT1927–25 locus being the most highly expressed. In addition, two genes (BT4014–13) encoding predicted phase-variable restriction endonucleases were upregulated between 6- and 16-fold (see Extended Data Fig. 9 and Supplementary Table 7).

**S-layer expression promotes resistance to multiple phages and is prominent in vivo.** The gene encoding the canonical outer membrane S-layer protein (BT1927), and its downstream genes were among the most highly activated in acapsular *B. thetaiotaomicron* after ARB25 or SJC01 challenge (Fig. 5b and see also Extended Data Fig. 9). We therefore focused on the effects of these proteins on phage infection. We mutated the recombination site upstream of the phase-variable promoter as described previously to create S-layer ‘off’ and S-layer ‘on’ variants of acapsular *B. thetaiotaomicron*. Growth of acapsular S-layer off cells was more effectively inhibited by ARB25 relative to acapsular S-layer on cells (Fig. 6a). The strength of this effect was altered by the age of the colonies used for subsequent liquid culture experiments to test phage infectivity (see Extended Data Fig. 10), suggesting hysteretic effects on the expression or function of this S-layer. In addition to ARB25, constitutive expression of the BT1927 S-layer promotes resistance to three additional phage isolates (Fig. 6b–d). In combination with previous findings that BT1927 promotes complement resistance, these observations suggest that BT1927 and perhaps the seven other *B. thetaiotaomicron* S-layers (Fig. 5c) promote resistance to a variety of disturbances.

Based on our results, phase variants in CPSs, S-layers, nutrient receptors and restriction endonucleases are all selected for during phage predation. These mechanisms may help to explain previous observations that *Bacteroides* spp. can coexist with phages in vitro. We hypothesized that, if the phase-variable systems that promote resistance also spontaneously revert some cells to a susceptible state, the population could generate enough susceptible bacteria to maintain a phage population. To test this in an in vivo model, we colonized germ-free Swiss Webster mice separately with either wild-type or acapsular *B. thetaiotaomicron* for 7 d, then introduced ARB25 by oral gavage. Both bacterial populations reached high colonization levels, but were not noticeably perturbed on addition of ARB25 and, for 72 d, bacteria and phage coexisted at high levels (Fig. 6e).

As the Δcps strain cannot evade ARB25 through alterations in CPS expression, we hypothesized that it would accrue mutations that promote full resistance after several weeks of constant ARB25 pressure. However, after isolating phage-free *B. thetaiotaomicron* from the intestinal contents of mice, we noted that all the Δcps clones regained susceptibility to ARB25 whereas 5 of 13 wild-type isolates remained resistant (see Methods and Supplementary Table 8). These results suggest that longer-term (perhaps permanent) resistance can occur after prolonged exposure to ARB25 in vivo, but this requires the presence of CPSs.

Last, we performed RNA-seq on bacteria recovered from the caecal contents of mice after 72 d of in vivo co-culture with ARB25. Compared with the corresponding in vitro transcriptomes, many additional genes were induced in vivo, as expected based on previous studies of *B. thetaiotaomicron* metabolic adaptation to the gut. Surprisingly, wild-type *B. thetaiotaomicron* that coexisted with ARB25 for 72 d in vivo exhibited lower expression of non-permissive CPS (on average fivefold lower than uninfected wild-type *B. thetaiotaomicron* grown in vitro). Although the expression of non-permissive CPS2 was increased, so was the expression of several permissive CPSs (CPS5, CPS6, CPS7 and CPS8; see Extended Data Fig. 9 and Supplementary Table 9), which may have been influenced by the in vivo environment that was previously shown to select for expression of CPS4, CPS5 and CPS6 (ref. ). Wild-type *B. thetaiotaomicron* also increased expression of six of the S-layer loci, while repressing one.

As expected, acapsular *B. thetaiotaomicron* altered expression of some of its S-layers after 72 d of ARB25 exposure in vivo (see Extended Data Fig. 9 and Supplementary Table 10). Notably, one of the S-layers (BT1826) showed dominant expression (2,738-fold increased expression), suggesting that BT1826 confers optimal resistance to ARB25 in this strain background and in vivo growth condition. Surprisingly, acapsular *B. thetaiotaomicron* displayed high expression of another set of three genes (BT0292 to BT0294; increased 79- to 156-fold) and one of these genes, BT0294, encodes a predicted lipoprotein. Adjacent to this locus is a predicted recombinase and we identified a near-consensus promoter, in the ‘off’ orientation upstream of BT0292 and flanked by 18-bp repeats. We demonstrated via PCR/sequencing that this promoter is capable of undergoing phase variation (see Extended Data Fig. 9), bringing the total number of *B. thetaiotaomicron* phase-variable loci that show selection in response to phages to 19. Finally, the phase-variable restriction endonuclease system identified in vitro in SJC01-infected cells was also upregulated in wild-type and acapsular *B. thetaiotaomicron* after prolonged coexistence with ARB25 in vivo (see Extended Data Fig. 9).
of their dynamics during health and disease. However, metagenomics-based approaches do not generate information on the definitive hosts or the mechanisms of individual bacteria–phage interactions, limiting dissection of their ecological roles in the gut. By isolating phages for a host of interest, experiments can be done to complement metagenomics-based studies, and synergistically build a foundation for understanding phage–bacteria interactions in the gut and other complex ecosystems.

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**Figure a**

Wild-type *B. thetaiotaomicron*

**Figure b**

Acapsular *B. thetaiotaomicron*

**Figure c**

| Genes                | Arrangement (number in gene is ARB25/HK fold-change) | Recombination sequence |
|----------------------|------------------------------------------------------|------------------------|
| BT1032–53 locus      | BT1032–53 locus variant 1:                          | 5'-gttact tag gtaac     |
| BT1042–45 locus      | BT1046–50 locus variant 2:                          | 5'-gttact tag gtaac     |
| BT1042–45 locus      | BT1046–50 locus variant 3:                          | 5'-gttact tag gtaac     |

**Figure d**

*BT1032–S3 locus published genome architecture:*

*BT1032–S3 locus variant 1:*

*BT1032–S3 locus variant 2:*

*BT1032–S3 locus variant 3:*

**Figure e**

MW (bp) 4 5 6 7 8

Wild type

MW (bp) 1 2 3

Wild type

WBT1041
serve as obligate receptors\(^1\) or increase the affinity of a phage for the bacterial cell surface. This latter type of adherence to CPSs might increase the likelihood of phage–receptor contact, similar to previous observations of phages in host mucus\(^39\).

Using ARB25 and SJC01 as representatives from our larger collection, we demonstrate that infection with these phages does not fully eradicate their target \textit{B. thetaiotaomicron} populations in vitro and in vivo. Phage–bacteria coexistence was previously observed in other \textit{Bacteroides}–phage systems\(^35\). Given the prevalence and diversity of CPSs in gut-resident Bacteroidetes (see Extended Data Fig. 1), our results suggest that CPSs influence phage–host coexistence across this prominent phylum. Analysis of transiently phage-resistant subpopulations of the acapsular and \textit{cps}I strains that emerge after ARB25 or SJC01 infection revealed additional phase-variable surface proteins, at least one of which (\textit{BT1927-26}) confers increased resistance to phage infection when constitutively expressed (see Fig. 6a–d and Extended Data Fig. 10). Previously, it was shown that 1 in 1,000 \textit{B. thetaiotaomicron} cells in a phage-free environment expresses \textit{BT1927} (ref. \(^43\)). In contrast to non-permissive CPSs, which can comprise up to 40\% of the expressed CPSs (for example, CPS3 in Fig. 4b), spontaneous \textit{BT1927-ON} cells may emerge only after longer periods of phage exposure, such as those we modelled in vivo (see Extended Data Fig. 9). Taken together, our data on CPSs, surface proteins and restriction enzymes, as they relate to phage infection in \textit{B. thetaiotaomicron}, collectively reveal that there are at least 19 independent loci that equip \textit{B. thetaiotaomicron} to survive phage predation.

The present study points to the existence of complex relationships between bacteria and phages in the gut microbiome. Considering that these interactions probably differ by bacterial species/strain and evolve differently within individuals or regionally distinct global populations, the landscape becomes even more complex. Given the diverse adaptive and counteradaptive strategies present in \textit{B. thetaiotaomicron} and its relatives, our findings hold important implications for using phages to therapeutically alter the composition or function of the gut microbiota. Although a cocktail of multiple phages could theoretically be harnessed to elicit more robust alteration of target populations within a microbiome, the complexity of host tropisms and bacterial countermeasures that exist for \textit{B. thetaiotaomicron} imply that a deliberate selection of complementary phages would be needed. Given these considerations, our findings contribute an important early step towards building a deep functional understanding of the bacterium–virus interactions that occur in the human gut microbiome.

### Methods

**Bacterial strains and culture conditions.** The bacterial strains used in the present study are listed in Supplementary Table 1. Frozen stocks of these strains were maintained in 25\% glycerol at \(-80^\circ\)C and were routinely cultured in an anaerobic chamber or in anaerobic jars (using GasPak EZ anaerobe container system sachets w/indicator, BD) at 37°C in \textit{Bacteroides} phage recovery medium (BPRM), as described previously\(^{43}\): per 1 l broth, 10 g meat peptone, 10 g casein peptone, 2 g yeast extract, 5 g NaCl, 0.5 g l-cysteine monohydrate, 1.8 g glucose and 0.12 g MgSO\(_4\) heptahydrate were added; after autoclaving and cooling to approximately 55°C, 10 ml of 0.22-\(\mu\)-filtered haemin solution (0.1% w/v in 0.22% NaOH), 1 ml of 0.22-\(\mu\)-filtered, 0.05 g ml\(^{-1}\) CaCl\(_2\) solution and 25 ml of 0.22-\(\mu\)-filtered, 1 M Na\(_2\)CO\(_3\) solution were added. For BPRM agar plates, 15 g\(\ell\) of agar was added before autoclaving, and haemin and Na\(_2\)CO\(_3\) were added before pouring the plates. For BPRM top agar used in soft agar overlays, 3.5 g\(\ell\) of agar was added before autoclaving. Haemin, CaCl\(_2\), and Na\(_2\)CO\(_3\) were added to the top agar immediately before conducting the experiments. Bacterial strains were routinely recovered from the freezer stocks directly on to agar plates of brain–heart infusion (BHI) supplemented with 10% horse blood (Quad Five) (BHI-blood agar; or for the SJC strains used in Fig. 1, on BPRM agar), grown anaerobically for up to 3 d, and a single colony was picked for each bacterial strain, inoculated into 5 ml BPRM and incubated anaerobically for up to 3 d, and a single colony was picked for each bacterial strain, inoculated into 5 ml BPRM and grown anaerobically overnight to provide the starting culture for experiments (note that, for the \textit{BT1927} S-layer protein experiment shown in Fig. 6, 3 d of growth on BPRM medium was determined to promote the greatest phage resistance).

For the experiment described in Fig. 2g, liquid cultures of \textit{B. thetaiotaomicron} were grown in BPRM using the pyrogalol method\(^44\). Briefly, a sterile cotton
Ball was burned and then pushed midway into the tube, after which 200 µl of saturated NaHCO₃ and 200 µl of a 35% pyrogallol solution were added to the cotton ball. A rubber stopper was used to seal the tubes and the tubes were incubated at 37 °C.

**Bacteriophage isolation from primary wastewater effluent.** The bacteriophages described in the present study were isolated from primary wastewater effluent from two locations, at the Ann Arbor, Michigan Wastewater Treatment Plant and the San Jose-Santa Clara Regional Wastewater Treatment Facility. After collection, the primary effluent was centrifuged at 5,500 g for 10 min at room temperature to remove any remaining solids. The supernatant was then sequentially filtered through 0.45-µm and 0.22-µm polyvinylidene fluoride (PVDF) filters to yield “processed primary effluent.” Initial screening for plaques was done using a soft agar overlay method in which 10 ml of primary effluent was directly added to overnight culture before plating (2) enrichment, in which 10 ml processed primary effluent was mixed with 10 ml 2× BPRM and 3 ml exponential phase *B. thetaiotaomicron* culture, and grown overnight; the culture was centrifuged at 5,500 g for 10 min and filtered through a 0.22-µm PVDF filter, and a second enrichment, in which serial dilutions were concentrated up to 500-fold via 30- or 100-kDa PVDF or polyethersulfone size exclusion columns. Up to 1 ml processed primary effluent, enrichment or concentrated processed primary effluent was added to the culture before adding BPRM top agar. To promote a diverse collection of phages, no more than five plaques from the same plate were plated purified, and a diversity of plaque morphologies was selected as applicable. When using individual enrichment cultures, only a single plaque was purified. Single isolated plaques were picked into 100 µl phage buffer (prepared as an autoclaved solution of 5 ml of 1 M Tris, pH 7.5, 5 ml of 1 M MgSO₄, 2 g NaCl in 500 ml with double-distilled H₂O). Phages were successfully plaque purified using one of two methods: (1) a standard full-plate method, in which the diluted phage samples were combined with *B. thetaiotaomicron* overnight culture and top agar, and plated via soft agar overlay, or (2) a higher-throughput, 96-well plate-based method, in which serial dilutions were plated in 96-well plates and 1 µl of each dilution was spotted on to a solidified top agar overlay. This procedure was repeated at least three times to purify each phage.

High-titre phage stocks were generated by flooding a soft agar overlay plate that yielded a ‘lacy’ pattern of bacterial growth (near confluent lysis). After overnight incubation of each plate, 5 ml of sterile phage buffer was added to the plate to resuspend the phage. After at least 2 h of incubation at room temperature, the lysate was spun at 5,500 g for 10 min to clear debris and then filter sterilized through a 0.22-µm PVDF filter.

**Phylogenetic analysis of human gut Bacteroidales and enumeration of cps biosynthetic gene clusters.** Phylogenetic analysis was performed by creating a core gene alignment using a customized, publicly available software package, cognac, which is written in C++ with C* integration (ref. 46). Briefly, GenBank files for the 53 isolates were parsed to extract the amino acid sequences and orthologous genes were identified with cd-hit v4.7 requiring at least 70% amino acid identity and ensuring that genes were of similar length (ref. 46). The cd-hit output was parsed and core genes were identified as those present in a single copy in all genomes. Amino acid sequences were concatenated and aligned with MAFFT v.7.310 (refs. 47, 48). The concatenated gene alignment was then used as the input for FastTree v.2.1.10 to generate an approximate maximum likelihood phylogeny (ref. 49). The tree created from the core genome alignment was then midpoint rooted and visualized using phytools v.0.6-99, ape v.5.3 and R packages, respectively (ref. 50, 51). The tree created from the core genome alignment was then midpoint rooted and visualized using phytools v.0.6-99, ape v.5.3 and R packages, respectively (ref. 50, 51).

**Quantitative host range assays.** To accommodate the large number of phages in our collection, we employed a spot titre assay for semiquantitative comparisons of infectivity on each bacterial strain. High-titre phage stocks were prepared on their ‘preferred’ host strain, which was the strain yielding the highest titre of phages in a pre-screen of phage–host range (see Fig. 1 and Supplementary Table 1). Lysates were then diluted to approximately 10^9 plaque-forming units (p.f.u.) ml⁻¹, added to the wells of a 96-well plate and further diluted to 10^6, 10^5 and 10^4 p.f.u. ml⁻¹ using a multichannel pipettor. Of each of these dilutions, 1 μl was plated on to solidified top agar overlays containing the single bacterial strains indicated in each figure. After the spots dried, plates were incubated anaerobically for 15–24 h before counting plaques. Phage titres were normalized to the preferred host strain. The heatmaps and dendrogram were generated using the heatmap function in the stats package of R v.3.4.0, which employs unsupervised hierarchical clustering (complete link) and諄ical similarity between bacterial phages. Each row of the dendrogram at the left of Fig. 1 indicating degree of similarity between infection profiles.

**Images of phage plaques.** To document the morphologies of phages formed by the purified phages, two sets of plaque pictures were captured: the first set was taken with a Color QCount Model 530 (Advanced Instruments) with a 0.01-s exposure. Images were cropped to 7.5 mm × 2.5 mm, but were otherwise unaltered. The second set of images was taken on a ChemiDoc Touch instrument (Bio-Rad) with a 0.5-s exposure. Images were cropped to 7.5 mm × 2.5 mm and unaturally high background pixels were then removed using ImageJ. Plates were visualized using phytools v.0.6.99, ape v.5.3 and R packages, respectively (ref. 50, 51). Each image in Extended Data Fig. 2 was taken from a ChemiDoc Touch instrument (Bio-Rad).

**Incubation of ARB25 phage with extracted CPS.** Approximately 50–100 p.f.u. of ARB25 in 50 µl phage buffer was mixed with an equal volume of H₂O or capsule (100 µg). Phages extracted by the method described above were then added at a concentration of 10^6 p.f.u. ml⁻¹ to 1 µl samples incubated at 37 °C for 30 min. Samples were then plated on to the acapsular strain, and plaques were counted after 15–24 h of anaerobic incubation at 37 °C. Counts from two replicates on the same day were averaged, and the experiment was performed three times. Although the size of individual CPS2 polymers is unknown, an estimate of 1,000 heptose sugars per molecule (180,000 Da) would be 9 × 10^10 CPS glycans at 1 mg ml⁻¹. If the CPSs were only 10% pure, incubation with 10^9 ARB25 p.f.u. ml⁻¹ was estimated to provide at least 10^-10 fold more CPS glycans than plaque-forming units.

**Bacterial growth curves with phages.** For growth curve experiments, three or four individual colonies of each indicated strain were picked from agar plates and grown overnight in BPRM. Then, for experiments in Figs. 3 and 6, and Extended Data Figs. 5 and 10 each clone was diluted 1:100 in fresh BPRM and 100 µl was added to a microtiter plate. Then 10 µl of approximately 5 × 10^6 p.f.u. ml⁻¹ live or heat-killed phage was added to each well, the plates were covered with an optically clear, gas-permeable membrane (Diversified Biotech), and the optical density at 600 nm (OD₆₀₀) values were measured using an automated plate-reading device (BioTek Instruments). Phages were heat killed by heating to 95 °C for 30 min, and heat-killed phage had no detectable p.f.u. ml⁻¹ with a limit of detection of 100 p.f.u. ml⁻¹ (ref. 52). Data Fig. 5, wild-type *B. thetaiotaomicron* was infected with live or heat-killed ARB25, and bacterial growth was monitored via the OD₆₀₀ on an automated plate reader for 12 h. At 0, 6, 0.2, 8.36 and 11.7 h post-inoculation, replicate cultures were vortexed in 1.5 (v:v) chloroform, centrifuged at 5,500 and 4 °C for 10 min, and the aqueous phase was titrated on the acapsular strain. No phages were detected in heat-killed controls.

**Generation of phage-free bacterial isolates and determination of their phage susceptibility.** To isolate phage-free bacterial clones from ARB25-infected cultures (see Supplementary Tables 2 and 8), each culture was plated on a BHI-blood agar plate using the single colony-streaking method. Of each of these dilutions, 1 µl of each culture was spun at 5,500 g for 10 min, and the aqueous phase was titrated on the acapsular strain. No phages were detected in heat-killed controls.

To determine whether cultures still contained viable phage, 50 µl of each culture was vortexed with 20 µl chloroform, centrifuged at 5,500 g for 10 min. Of the lysate 10 µl was spotted on BPRM top agar containing naive acapsular bacteria, and incubated anaerobically overnight at 37 °C. Loss of detectable phage in the twice-passaged clones was confirmed for most of the clones (79 of 89, 89%) by the absence of plaques on the naive acapsular strain. To determine whether the resulting phage-free isolates were resistant to ARB25 infection, each culture was re-isolated on a separate BHI-blood agar plate. One colony was picked from each of these secondary plates and was inoculated into 150 µl BPRM broth and incubated anaerobically at 37 °C for 2 d. Only one of the clones (a cps4 isolate) failed to grow in liquid medium. To determine whether cultures still contained viable phage, 50 µl of each culture was vortexed with 20 µl chloroform, centrifuged at 5,500 g for 10 min. Of the lysate 10 µl was spotted on BPRM top agar containing naive acapsular bacteria, and incubated anaerobically overnight at 37 °C. Loss of detectable phage in the twice-passaged clones was confirmed for most of the clones (79 of 89, 89%) by the absence of plaques on the naive acapsular strain.
Measurement of cps gene expression. For Figs. 2g and 4b, and Extended Data Fig. 6, overnight cultures were diluted into fresh BPRM to an OD_{600} of 0.01. For Fig. 4b, 200 µl of approximately 2 × 10^9 p.f.u. ml^-1 of live or heat-killed phage was added to 5 ml of the diluted cultures. For Extended Data Fig. 6, 260 µl of approximately 2 × 10^9 p.f.u. ml^-1 of live or heat-killed phage was added to 5 ml of the diluted cultures. Bacterial growth was monitored by measuring the OD_{600} every 15–30 min using a GENESYS 20 spectrophotometer (Thermo Fisher Scientific). Cultures were briefly mixed by hand before each measurement. For determination of relative cps gene expression, cultures were grown to an OD_{600} of 0.6–0.8, and total RNA was isolated using the RNeasy Mini Kit (Qiagen). RNA-stabilized cell pellets were stored at −80 °C. Total RNA was isolated using the RNeasy Mini Kit (Qiagen) and then treated with the TURBO DNA-free Kit (Ambion), followed by an additional isolation using the RNeasy Mini Kit. Complementary DNA was synthesized using SuperScript III Reverse Transcriptase (Invitrogen) according to the manufacturer’s instructions using random oligonucleotide primers (Invitrogen). Quantitative PCR (qPCR) analyses for cps locus expression were performed on a Mastercycler ep realplex instrument (Eppendorf). Expression of each of the eight cps synthesis loci was quantified using primers to a single gene in each locus (primers are listed in Supplementary Table 12) and normalized to a standard curve of DNA from wild-type B. thetaiotaomicron. The primers used were selected to target a gene specific to each cps locus and were previously validated against the other strains that lack the target cps locus for specificity. Relative abundance of each cps-specific transcript was then calculated for each locus. A customized SYBR-based master mix was used. Primer extensions were performed with Taq (New England Biolabs), and contained 2.5 mM MgSO_4, 0.125 mM dNTPs, 0.25μM each primer, 0.1 μl of a 100× stock of SYBR Green I (Lanza) and 500 U Hot Start Taq DNA Polymerase (New England Biolabs). Then 10 ng cDNA was used for each sample, and samples were run in duplicate. A touchdown protocol with the following cycling conditions was used for all assays: 95 °C for 3 min, followed by 40 cycles of 3 s at 95 °C, 20 s of annealing at a variable temperature and 20 s at 68 °C. The annealing temperature for the first cycle was 58 °C, then dropped 1 °C each cycle for the subsequent five cycles. The annealing temperature for the last 34 cycles was 52 °C. These cycling conditions were followed by a melting curve analysis to determine amplicon purity.

Transcriptomic analysis of B. thetaiotaomicron after phage infection. Whole-genome transcriptional profiling of wild-type and acapsular B. thetaiotaomicron infected with live or heat-killed ARB25 or SJC01, or from in vivo samples, was conducted using total bacterial RNA that was extracted as described in “Measurement of cps gene expression” (Qiagen RNAEasy Turbo DNA-free Kit) and then treated with Ribo-Zero rRNA Removal Kit (Illumina) and concentrated using RNA Clean and Concentrator-5 Kit (Zymo Research Corp.). Sequencing libraries were prepared using TruSeq barcoding adaptors (Illumina), and 24 samples were multiplexed and sequenced with 50-bp single-end reads in one lane of an Illumina HiSeq instrument at the University of Michigan Sequencing Core. Demultiplexed samples were analysed using SeqMan NGene and Arraystar software (DNASTAR) using EdgeR normalization and >98% sequence identity for read mapping. Changes in gene expression in response to live ARB25 infection were determined by comparison to the heat-killed retained strain: retention of triple or more expression changes up or down and EdgeR adjusted p value <0.01. RNA-Seq data have been deposited in the public repository at the National Institutes of Health (NIH) Gene Expression Omnibus (GEO) database as accession no. GSE147071.

PCR and sequencing of phase variable B. thetaiotaomicron chromosomal loci. We found that each of the eight chromosomal loci shown in Fig. 5c had almost identical 301-bp promoter sequences, including both of the imperfect palindromes that we predicted to mediate recombination and the intervening sequence at each locus. Although the eight S-layer genes and seven of the eight upstream regions encoding putative tyrosine recombinases (all but the BT1927 region) shared significant nucleotide identity and gene orientation, we were able to design primers that annealed to the regions upstream and downstream to the promoter variable, and used these to generate an amplicon for each locus that spanned the predicted recombination sites. After gel extracting a PCR product of the expected size for each locus, which should contain promoter orientations in both the on and off orientations, we performed a second PCR using a universal primer that lies within the 301-bp sequence of each phase-variable promoter, and extended to unique primers that anneal within each S-layer protein-encoding gene. Bands of the expected size were excised from agarose gels, purified and sequenced using the primer that anneals within each S-layer-encoding gene to determine whether the predicted recombined on promoter orientation is detected. (Note that the assembled B. thetaiotaomicron genome architecture places all of these promoters in the proposed off orientation. We were able to detect six out of eight of these loci in the on orientation in ARB25-treated cells by this method; see Extended Data Fig. 7.) Similar approaches were used to determine the reorientation of DNA fragments in the B. thetaiotaomicron PUL shuffled on Fig. 5d, and the restriction enzyme and additional lipoprotein system shown in Extended Data Fig. 9. For shuffled gene orientation, we used PCR primer amplicons positioned according to the schematic in Fig. 5d, followed by sequencing with the primer on the downstream end of each amplicon, according to its position relative to the shuffled promoter sequence. For a list of primers used, see Supplementary Table 12.

Construction of acapsular B. thetaiotaomicron S-layer on and off mutants. Acapsular B. thetaiotaomicron S-layer on and off mutants (Δcps BT1927-ON and Δcps BT1927-OFF, respectively) were created using the Δtdk allelic exchange method. To generate homologous regions for allelic exchange, use primers to an OD_{600} of 0.6–0.8. Two 100× solutions were used to amplify the BT1927-ON and BT1927-OFF promoters from the previously constructed BT1927-ON and BT1927-OFF strains via colony PCR using Q5 High Fidelity DNA polymerase (New England Biolabs). Candidate Δcps BT1927-ON and Δcps BT1927-OFF mutants were screened and confirmed by PCR using the primer pair BT1927_Diagnostic_R and BT1927_Diagnostic_F, and confirmed by Sanger sequencing according to these diagnostic primers. All plasmids and primers are listed in Supplementary Tables 11 and 12, respectively.

Construction of B. thetaiotaomicron mutants lacking one or more cps loci. All publicly available bacterial genomes in the National Center for Biotechnology Information (NCBI) GenBank were queried via MultiGeneBLAST to identify fully sequenced bacteria with B. thetaiotaomicron VPI-5482-like cps loci. B. thetaiotaomicron 7330 was identified as having syntonic copies of VPI-5482-like cps2, cps5 and cps6 loci with corresponding genes in the same order and ≥98% amino acid identity. Mutants of strain 7330 lacking one or more cps loci were constructed for the homologous recombination using the tdk allelic exchange method. The B. thetaiotaomicron 7330 tdk strain was generated by ultraviolet mutagenesis through exposure of a liquid culture of 7330 to 320 nm ultraviolet light from a VWR-20E transilluminator (VWR) for 60 s, and plating on to BH-blood agar supplemented with 200 μg ml^-1 of 5-fluoro-2’-deoxyuridine. All plasmids and primers used to construct these strains are listed in Supplementary Table 11 and 12, respectively.

Germ-free mouse experiments. All experiments were approved by the University of Michigan Institutional Animal Care and Use Committee and animals were monitored by a veterinarian. A group of six 6- to 8-week-old, germ-free, Swiss Webster mice were randomly assigned to two different groups containing two females and one male each, then gavaged with either wild-type or acapsular B. thetaiotaomicron for 7 d of monoclonalization. After 7 d, mice were gavaged with 1 mM sodium bicarbonate followed immediately by 2 × 10^9 p.f.u. of ARB25 as previously described. Faeces were monitored for both colony-forming units and plaque-forming units every 7 d by plating faecal homogenates in SM buffer or faecal homogeneate supernatant, and serial dilutions in SM buffer on BPRM top agar plates. Sample size was selected based on using the fewest animals required to observe significant effects on colonization and gene expression in previous, similar experiments. The experimenters were not blinded to the different treatment (B. thetaiotaomicron strain) that each group was receiving.

Data representation and statistical analysis. The heatmaps for Fig. 1 and Extended Data Fig. 3 and the dendrogram for Fig. 1 were generated using the heatmap function in the stats package of R v.3.4.0 which employs unsupervised hierarchical clustering (complete linkage method) to group similar phage infection. Other graphs were created in Prism software (GraphPad Software). Statistical significance in the present study is denoted as follows, unless otherwise indicated: *P < 0.05; **P < 0.01; ***P < 0.001; ****P < 0.0001. All precise P values are either provided on each figure or in the Source data. Statistical analyses other than Dirichlet's regression were performed in Graphpad Prism. Dirichle's regression was performed in R using the package DirichletReg v.0.6-3, employing the alternative parameterization used previously. Briefly, the parameters in this distribution are the proportions of relative cps gene expression and the total cps expression, with cps7 expression used as a reference because we previously determined this cps to be poorly activated and not subject to phase-variable expression. The variable of interest used in Fig. 4b is phage viability (live versus heat-killed phage). Precision was allowed to vary by group given that this model was superior to a model with constant precision, as determined by a likelihood ratio test at a significance level P < 0.05. P values for each condition were determined using the z-test.

Reporting summary. Further information on research design is available in the Nature Research Reporting Summary linked to this article.

Data availability. Source data for all the experiments, along with corresponding statistical test values, where appropriate, are provided. RNA-seq data for whole-genome transcriptional profiling is deposited in NCBI Gene Expression Omnibus database as accession no. GSE147071.

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Author contributions

N.T.P. and A.J.H. performed most of the experiments, including initial phage isolation, host range measurements, construction of additional mutants and subsequent testing. B.D.M. and J.O.G. assisted A.J.H. with the experiments listed. J.F.E. and S.S. performed...
and analysed RNA-seq experiments, except those shown in Fig. 5. N.T.P. and E.C.M. performed these experiments for Fig. 5. R.W.P.G. and A.J.H. constructed additional capsule mutants for ED4. N.T.P., A.J.H., J.J.F. and E.C.M. designed the experiments, and analysed and interpreted most of the data. R.D.C. and E.S.S. performed whole-genome phylogenetic analysis. J.J.F. conducted the corresponding cps locus search. J.L.S. and E.C.M provided tools and reagents. N.T.P., A.J.H., J.J.F., S.S. and E.C.M. prepared the display items and compiled the Source data. N.T.P., A.J.H., J.J.F. and E.C.M. wrote the paper. All authors edited and approved the manuscript before submission.

Competing interests
The authors declare no competing interests.

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Correspondence and requests for materials should be addressed to A.J.H. or E.C.M.
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Extended Data Fig. 1 | See next page for caption.
Extended Data Fig. 1 | Diversification and structure of \textit{cps} gene clusters in human gut Bacteroidetes. Diversification and structure of \textit{cps} gene clusters in human gut Bacteroidetes. \textbf{a}, The genomes of 53 different human gut Bacteroidetes (predominantly named type strains) were searched for gene clusters that contain two or more different protein families indicative of \textit{cps} loci (see Methods). The number of \textit{cps} loci detected in each genome is shown in the context of phylogenetic tree derived from the core genome of the 53 species used for this analysis; species for which \textit{cps} loci were not detected using our search criteria are marked with a red “X”. Due to gaps in several genomes, which often occur at \textit{cps} loci, the numbers shown are likely to be an underestimate. \textbf{b}, Schematics of the 8 annotated \textit{cps} loci in \textit{B. thetaiotaomicron} VPI-5482, which are singly present in the \textit{cps1-cps8} strains used in this study, or completely eliminated in the acapsular strain. Genes are color coded according to the key at the bottom and additional Pfam family designations are provided under most genes. The four main protein families used for informatics analysis are marked with asterisks and highlighted in bold in the key.
Extended Data Fig. 2 | Representative pictures of phage plaques for all phages in this study: (a) phages from Ann Arbor (ARB); (b) phages from San Jose (SJC). Representative pictures of phage plaques for all phages in this study: (a) phages from Ann Arbor (ARB); (b) phages from San Jose (SJC). The top row of images for each phage are unaltered; background and saturated pixels were removed from images in the bottom row to facilitate viewing of the plaques. Experiment was performed only once for photographing plaques, no major variations were observed in additional experiments with the same phage on the same host strain. Scale bar = 2 mm.
Extended Data Fig. 3 | Replication of a subset of host range assays of B. thetaiotaomicron-targeting phages on strains expressing different CPS types. Replication of a subset of host range assays of B. thetaiotaomicron-targeting phages on strains expressing different CPS types. Ten bacteriophages isolated and purified on the wild-type, acapsular, or the 8 single CPS-expressing strains were re-tested in a spot titer assay to determine phage host range. 10-fold serial dilutions of each phage ranging from approximately $10^6$ to $10^3$ plaque-forming units (PFU) / ml were spotted onto top agar plates containing the 10 bacterial strains. Plates were then grown overnight, and phage titers were calculated. Titers are normalized to the titer on the preferred host strain for each replicate. Each row in the heatmap corresponds to a replicate for an individual phage, whereas each column corresponds to one of the 10 host strains. One to three replicates of the assay were conducted for each phage by the two lead authors (AJH and NTP). Assays were carried out at the same time, and each author used the same set of cultures and phage stocks. For comparison, individual replicates from Fig. 1 are included (marked with *). Experiment was conducted once during a visit of AJH to the University of Michigan research site to compare reproducibility between experimenters with the replication described above.
Extended Data Fig. 4 | Effects of eliminating permissive CPS from another *B. thetaiotaomicron* strain. Effects of eliminating permissive CPS from another *B. thetaiotaomicron* strain. a, We identified *B. thetaiotaomicron* 7330\(^{10}\) as the only sequenced and genetically tractable strain that contains VPI-5482-like cps loci (*cps2*, *cps5*, and *cps6*). Gene colors illustrate syntenic genes with >98% amino acid identity but do not indicate function. Please see Extended Data Fig 1 for functional annotation of *B. thetaiotaomicron* VPI-5482 cps loci. We also observed that the Branch 2 phage SJC01 did not yield productive infection in *B. thetaiotaomicron* 7330, but could partially clear lawns of *B. thetaiotaomicron* 7330 at high titers. This ability to clear lawns is a previously described phenomenon known as “lysis from without\(^{57}\).” b, Deletion of permissive capsules (*cps2*, *cps5*, and *cps6*) either alone or in combination affects VPI-5482 infection by SJC01 (n = 7 biological replicates per strain; bars represent the geometric mean). c, While SJC01 plaques on WT *B. thetaiotaomicron* VPI-5482, it does not form plaques on wild-type *B. thetaiotaomicron* 7330. However, SJC01 does exhibit a “lysis from without” clearing phenotype at high densities of phage (top two spots, made with 1 microliter of 1e8 and 1e7 PFU per mL, according to titers observed on wild-type VPI-5482). Experiment was independently performed 7 times with VPI-5482 and 8 times with 7330 with similar results. d, *B. thetaiotaomicron* 7330 strains lacking *cps5* (with the exception of 7330 Δ*cps5 Δcps6*) show the lysis from without phenotype less frequently than strains that have intact *cps5*. Experiments were performed with the following number of replicates per strain with similar results: wild-type 7330 (n = 8), Δ2 (n = 8), Δ5 (n = 8), Δ6 (n = 8), Δ2,5 (n = 17), Δ2,6 (n = 5), Δ5,6 (n = 3), Δ2,5,6 (n = 19). For panel b, significant differences in phage titers on each mutant strain were compared to wild type via two-tailed Mann-Whitney test with actual P values shown. For panel c, scale bars = 0.5 cm.

56. Wu, M. et al. Genetic determinants of in vivo fitness and diet responsiveness in multiple human gut *Bacteroides*. *Science* **350**, aac5992 (2015).
57. Abedon, S. T. Lysis from without. *Bacteriophage* **1**, 46–49 (2011).
Extended Data Fig. 5 | See next page for caption.
Extended Data Fig. 5 | Free CPS does not inhibit ARB25 infection when provided in trans and effect of CPS to phage infection on bacterial growth. Free CPS does not inhibit ARB25 infection when provided in trans and effect of CPS to phage infection on bacterial growth. 

**a.** ARB25 was incubated with purified CPS1 or CPS2 (1 mg/ml, an estimated 10^3 molar excess of CPS molecules to phage, see Methods) before plating on the acapsular strain, and plaques were counted after overnight incubation. Titers are normalized to mock (H2O) treatment. No significant differences in titers were found compared to mock treatment, as determined by Welch’s t test, 2-tailed (n = 3 biological replicates, bars represent mean ± SEM). 

**b.** Post ARB25-infected, surviving cultures still contain infectious phages. Wild-type *B. thetaiotaomicron* was infected with live or heat-killed ARB25, and bacterial growth was monitored via optical density at 600 nm (OD600). At 0, 6.02, 8.36, and 11.7 h post inoculation, replicate cultures were removed and phage levels were titered (n = 3 and individual replicate curves are shown). No phages were detected in heat-killed controls. Note that the PFU/mL do not increase substantially after the initial “burst” corresponding to decreased bacterial culture density prior to re-growth. 

**c.** Ten strains: the wild-type (WT), the acapsular strain (Δcps), or the eight single CPS-expressing strains were infected with either live or heat-killed SJC01. 

**d.** 20 different colonies of cps4 or cps5 strains were infected with ARB25. Growth was monitored via optical density at 600 nm (OD600) on an automated plate reading instrument as described in Methods and individual growth curves for live and heat-killed phage exposure are shown separately.
Extended Data Fig. 6 | Infection of wild-type *B. thetaiotaomicron* at a low multiplicity of infection and subsequent effects on cps gene expression.

Infection of wild-type *B. thetaiotaomicron* at a low multiplicity of infection and subsequent effects on cps gene expression. **a.** The wild-type (WT) strain was infected at a low multiplicity of infection (MOI = 1 × 10⁻⁴) of live or heat-killed ARB25, and bacterial growth was monitored via OD₆₀₀ (n = 3 biological replicates and separate curves are shown). **b.** RNA was harvested from cultures after reaching an OD₆₀₀ of 0.6-0.7, cDNA was generated, and relative expression of the 8 *cps* loci was determined by qPCR (histogram bars are mean ± SEM of 3 biological replicates). Individual replicates of high MOI (c) and low MOI (d). In the high MOI experiment, replicate 2 showed higher starting expression of the non-permissive CPS3 compared to others. In the low MOI experiment, replicate 3 showed higher starting expression of the non-permissive CPS3. In both experiments, post phage-exposed replicates displayed nearly identical CPS expression profiles characterized by high expression of CPS3. The experiments in a-c were repeated one time with three parallel biological replicates started from single *B. thetaiotaomicron* wild-type colonies picked from the same plate.
Extended Data Fig. 7 | See next page for caption.
Extended Data Fig. 7 | Determination of phase-variable promoter switching for six loci encoding putative S-layer proteins. The hypothesis that the promoters associated with seven additional *B. thetaiotaomicron* S-layer like lipoproteins are phase variable was validated using a PCR amplicon sequencing strategy. Because of high nucleotide identity in both the regions flanking the 7 additional loci, a nested PCR approach was required to specifically amplify and sequence each site. In the first step, a primer lying in each S-layer gene (Supplementary Table 5 “S-layer gene” primers) was oriented towards the promoter and used in a PCR extension to a primer in the upstream recombinase gene (Supplementary Table 5 “recombinase gene 3” primer). The products of this PCR were purified without gel extraction and used in a second reaction with a nested primer that lies internal to the previous recombinase gene primer (Supplementary Table 5 “recombinase 2” primer). The expected PCR products from this reaction, which are ~1kb and span promoter sequences in both the ON and OFF orientations, were excised and used for an orientation-specific PCR using the original S-layer gene primer for each site and a universal primer (green schematic) that was designed for each promoter and is oriented to extend upstream of the S-layer gene (e.g., OFF orientation). Resulting products from this third reaction, which should correspond to the ON orientation if a promoter inversion has occurred in some cells, were obtained for 5/7 of the additional identified loci and the BT1927 S-layer locus as a control. In all cases in which an amplicon and sequence were obtained, the expected recombination occurred between the inverted repeat site proximal to the S-layer gene start (new DNA junction), which would orient the promoter to enable expression of the downstream S-layer gene. The sequences shown are the consensus between forward and reverse reads for each amplicon. The putative core promoter -7 sequence is shown in bold/red text, the coding region of each S-layer gene is shown in bold/blue text and the S-layer gene proximal recombination site is noted and highlighted in bold/gold text. Note that the 5′-end of the sequenced amplicon was not resolved for the BT2486 locus.
Extended Data Fig. 8 | See next page for caption.
Extended Data Fig. 8 | Recombination between the genes BT1040, BT1042, and BT1046 and effect of BT1033-52 locus. Recombination between the genes BT1040, BT1042, and BT1046 and effect of BT1033-52 locus. 

a, Pfam domain schematics of the amino acid sequences of these three genes highlighting that BT1040 and BT1046, as originally assembled in the *B. thetaiotaomicron* genome sequence, lack additional N-terminal sequences that are present on BT1042.

b, Sequencing of the 8 PCR amplicons schematized in Fig. 5d. Amplicons 1, 5 and 8 represent the original genome architecture, while the others represent inferred recombination events that are validated here by sequencing. The 5′ and 3′ ends of the BT1042, BT1040 and BT1046 genes are color-coded to assist in following their connectivity changes after recombination. A series of single-nucleotide polymorphisms (SNPs) present in BT1042, downstream of the proposed recombination site, are highlighted in yellow. The transfer of these SNPs to a fragment containing the 5′ end of BT1040 (Amplicon 4) was used to narrow the recombination region to the 7 nucleotide sequence highlighted in red. Additional SNPs that are specific to the regions upstream of this recombination site are shown in white text for each sequence. Susceptibility of acapsular *B. thetaiotaomicron* to ARB25 without the BT1022-52 locus is not affected.

c, Ten-fold serial dilutions of ARB25 were spotted onto lawns of *B. thetaiotaomicron Δcps* (n = 5) and *B. thetaiotaomicron Δcps ΔBT1033-52* (n = 5). Each of the 5 biological replicates contained 3 technical replicates from independent clones and all 15 replicates are shown individually. Plaques were determined by normalizing plaque counts on *B. thetaiotaomicron Δcps ΔBT1033-52* relative to plaque counts on *B. thetaiotaomicron Δcps* for each replicate. Statistical significance was determined using the 2-tailed Mann-Whitney test and bars represent the geometric mean.
Extended Data Fig. 9 | See next page for caption.
Extended Data Fig. 9 | Whole genome transcriptional analyses of several additional B. thetaiotaomicron strain and phage combinations. Whole genome transcriptional analyses of several additional B. thetaiotaomicron strain and phage combinations. 

**a.** Infection of the cps1 strain with ARB25, revealing a post-infection response that is largely characterized by increased expression of S-layer/OmpA proteins. 

**b.** Infection of wild-type B. thetaiotaomicron with SJC01, revealing that, as with ARB25/wild-type, the bacteria survive phage infection by mostly altering CPS expression. Expression of the non-permissive CPS3 is prominently increased. 

**c.** Infection of acapsular B. thetaiotaomicron with SJC01, revealing that in the absence of CPS survival is mostly promoted by increased S-layer/OmpA expression and expression of a phase-variable restriction enzyme system. Transcript abundance values in panels a-c were compared between live and HK treatments to generate fold change (x axis), which is plotted against the adjusted P value (edgeR) for each gene that was generated using an exact test adapted for overdispersed data (n = 3 biological replicates for each panel, performed with similar results). Please see Supplementary Tables 5-7 for actual adjusted P values for panels (a-c). 

**d.** Gene schematic of the phase-variable restriction enzyme system (top) and a lipoprotein contain locus (bottom) that is different from the 8 S-layer loci also revealed in this study. The inverted repeat sequence that was determined to mediate recombination in each locus is shown. 

**e.** PCR analysis of the restriction enzyme system and additional lipoprotein promoter orientations with primers designed to detect phase variation from off to on states. Amplicons were sequenced to confirm the re-orientation to the on orientation (not shown). Experiment was performed once. 

**f.** Global transcriptional responses of wild-type B. thetaiotaomicron in the ceca of mice after 72 d of co-existence with ARB25. Note that shifts in CPS expression are mostly characterized by increases in permissive CPS, which may be dictated by growth in vivo selecting for these capsules or against the non-permissive CPS3. Correspondingly, wild-type shows increased expression of some but not all S-layer/OmpA systems and the phase-variable restriction enzyme. 

**g.** Global transcriptional responses of acapsular B. thetaiotaomicron in the ceca of mice after 72 d of co-existence with ARB25. In the absence of CPS, surviving bacteria show increased expression of only a subset of the identified S-layer/OmpA proteins, with BT1826 expressed most dominantly, along with the BT0291-94 locus and expression of the restriction enzyme system. Experiments in panels f-g are the results of 3 separate biological replicates, statistical tests are identical to those described in panels (a-c). The dashed lines in panels a,b,c,f,g represent the adjusted P value cutoff (≥0.01) that was used to establish significance, which was generated using an exact test adapted for overdispersed data.
Extended Data Fig. 10 | ARB25 (a) or SJC01(b) infection of the acapsular BT1927 locked on and off strains after 1, 2 or 3 days of growth on BPRM. ARB25 (a) or SJC01(b) infection of the acapsular BT1927 locked on and off strains after 1, 2 or 3 days of growth on BPRM (n = 6 biological replicates per treatment, performed with similar results). Three separate colonies were picked each day as a biological replicate, grown overnight and used to setup infection cultures that were monitored for 24 h in an automated plate reader. Colonies picked after only 1 day show the least resistance to either phage when BT1927 is locked on. After 2 days, resistance is increased and this continues to increase after 3 days, becoming almost complete (compared to HK controls for ARB25). Growth curves represent the mean of 6 biological replicates ± standard error.
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- For null hypothesis testing, the test statistic (e.g. F, t, r) with confidence intervals, effect sizes, degrees of freedom and P value noted Give P values as exact values whenever suitable.
- For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
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- Estimates of effect sizes (e.g. Cohen's d, Pearson's r), indicating how they were calculated

Our web collection on statistics for biologists contains articles on many of the points above.

Software and code

Policy information about availability of computer code

Data collection No software was used for data collection

Data analysis R version 3.4.0; Package "DirichletReg" version 0.6-3 (for R); Lasergene SeqMan NGen and Arraystar 16 (DNAStar, Madison, WI)

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All RNA-seq data have been deposited in the publicly available NIH Gene Expression Omnibus (GEO) database on project number GSE147071.

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Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

| Sample size | No power calculations were made prior to experiments to arrive at sample size for assays, including in vivo experiments. Sample sizes for bacteria-phage infections studies were selected based on preliminary experiments in which significant variations between infection of different hosts (e.g., data in Fig. 1) were observed, including variation between experimenters at different institutions. For phage infection experiments, this was generally n=6 or greater. Mouse numbers for in vivo experiments were selected based on similar gnotobiotic colonization experiments in which significant changes in biological responses were observed. |
| Data exclusions | No data points were excluded |
| Replication | Key experimental assays (bacteria-phage infection interactions) were replicated between two independent research labs at Stanford University and the University of Michigan, including an in-person meeting at the UM to test methods side-by-side and resolve discrepancies. All attempts to replicate experiments were successful and this is stated as appropriate in corresponding figure legends along with a statement of replicate numbers and observation of similar results. |
| Randomization | For studies in Fig. 6, germfree mice were randomly assigned to the two treatment groups with the restriction that each group contain both male and female mice. |
| Blinding | No investigators were blinded to experimental data |

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| Materials & experimental systems | Methods |
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Animals and other organisms

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| Laboratory animals | Male and female 6-8 week old germfree Swiss Webster mice were utilized. This is stated in the corresponding section of Methods. |
| Wild animals | NA |
| Field-collected samples | NA |
| Ethics oversight | All animal experiments were approved by the University of Michigan Institutional Animal Care and Use Committee, which is approved by the Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC) and animals were monitored by a veterinarian. The authorizing entities are acknowledged in corresponding Methods. |

Note that full information on the approval of the study protocol must also be provided in the manuscript.