The Spatial Heterogeneity of the Gut Limits Predation and Fosters Coexistence of Bacteria and Bacteriophages

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Title

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Summary

The ecological dynamics underlying the coexistence between antagonistic populations of bacteria and their viruses, bacteriophages (phages) in the mammalian gut microbiota remain poorly understood. We challenged a murine synthetic bacterial community with phages to study the factors allowing phages-bacteria coexistence. Coexistence was not dependent on the development of phage-resistant clones, nor on the ability of phages to extend their host range. Instead, our data suggest that phage-inaccessible sites in the mucosa serve as a spatial refuge for bacteria. From there, bacteria disseminate in the gut lumen where they are predated by luminal phages fostering the presence of intestinal phage populations. The heterogeneous biogeography of microbes contributes to the long-term coexistence of phages with phage-susceptible bacteria. This observation could explain the persistence of intestinal phages in humans as well as the low efficiency of oral phage therapy against enteric pathogens in animal models and clinical trials.

Introduction

The mammalian gut is a highly complex and structured organ lined by a variety of eukaryotic cells types that serve the establishment of a mutualistic relationship between the host and different enteric microbes, including viruses. Bacteriophages (phages) are the most abundant viruses residing in the gut, but their precise role in shaping the microbiome remains unclear (Manrique et al., 2017). Changes in the viral and bacterial communities of the gut are increasingly reported to be associated with pathological conditions in humans, including diabetes, inflammatory bowel diseases and colorectal cancer (Hannigan et al., 2018b; Manrique et al., 2017; Zhao et al., 2017). Although fluctuations in the viral communities have been reported in a recent longitudinal study in humans, a large proportion of individual-specific viral contigs remain detectable over time (months to years), suggesting that individuals possess their own viral fingerprint (Manrique et al., 2016; Shkoporov et al., 2019). However, it remains understudied how phages and their corresponding bacterial targets persist together in the gut.

Using phage-bacterial model systems, dynamics of the coexistence of predators and preys have been the subject of theoretical and experimental studies, mostly performed *in vitro* and *in silico* (Betts et al., 2014; Brockhurst et al., 2006; Hannigan et al., 2018a; Lenski and Levin, 1985; Weitz et al., 2013). In the mammalian gut, the interaction of phages and bacteria has been explored in mice and pigs (Galtier et al., 2016; Looft et al., 2014; Maura et al., 2012a; Maura et al., 2012b; Reyes et al., 2013; Weiss et al., 2009; Yen et al., 2017), while human data are derived from metagenomics studies (Manrique et al., 2017; Shkoporov et al., 2019) and few clinical trials of phage therapy (Sarker and Brussow, 2016).

Studies in mice have shown that virulent phages have a limited effect on the targeted bacterial populations within the gut (Bhandare et al., 2019; Galtier et al., 2017; Maura et al., 2012a; Weiss et al., 2009). Nevertheless, both phage and bacterial populations could persist in the gut of animals for several weeks (Maura and Debarbieux, 2012; Maura et al., 2012b). Likewise, a large randomized phage therapy trial targeting *Escherichia coli* diarrhoea in Bangladeshi children showed no evidence for *in vivo* amplification of oral phages in the gut despite their persistence (Sarker et al., 2016). A similar situation was described in the human gut where the crAssphage coexists with its highly abundant Bacteriodetes bacterial host (Guerin et al., 2018; Shkoporov et al., 2018; Yutin et al., 2018). Three recent studies showed that administration of virulent phages can also strongly impact the intestinal colonization of their targeted bacteria and nevertheless, still support long-term coexistence (Duan et al., 2019a; Gogokhia et al., 2019; Hsu et al., 2019). The experimental settings

of these studies could account for differences in the amplitude of the impact, notably because of the use of axenic mice, characterized by an immature immune system and thus unstable colonization of bacteria of human origin. Moreover, the limited number of phages and bacteria couples that have been studied in the gut environment does not allow yet drawing conclusions on the type and prominence of factors that influence phage-bacterium interactions.

Since phages are proposed as a treatment for the major public health threat of antibiotic-resistant bacterial infections (Roach and Debarbieux, 2017), as well as means to precisely engineer the intestinal microbiota, further insight into the variety of phage-bacterium interactions and their coexistence in the mammalian gut are deeply needed (Brussow, 2017). Several factors were shown or proposed to be involved in this coexistence, such as (i) arms race dynamics with resistance development to phage infection and viral counter-resistance, (ii) the inherent or evolved ability of the phage to infect multiple hosts, and (iii) the distribution of these two antagonistic populations into distinct anatomical structures (Brockhurst et al., 2006; Doron et al., 2018; Galtier et al., 2017; Heilmann et al., 2012; Hilborn, 1975; Labrie et al., 2010). In this report, we investigated the contribution of these factors using the synthetic Oligo-Mouse-Microbiota comprising 12 distinct strains (OMM^{12}) (Brugiroux et al., 2016).

This animal model allows following defined pairs of phages and bacteria in the gut without the need of antibiotic treatments as a confounding factor commonly used to study enteric pathogens in murine models (Croswell et al., 2009). In addition, it provides more realistic conditions than mono-colonized mice that lack aspects of competitive and synergistic interspecies interactions of microbes (Weiss et al., 2009). We established stable colonization of two *E. coli* strains in gnotobiotic OMM^{12} mice and studied the population dynamics of these strains in the presence of virulent phages. We found that phages were less abundant in the mucosal part of the gut compared to *E. coli* levels. Our data are in agreement with the ecological theory of source-sink dynamics (Holt, 1985) providing an explanation for the lack for phage-resistant mutant selection and the limited efficacy of virulent phages in reducing intestinal bacterial loads.

Results

E. coli commensal strain Mt1B1 colonizes the gut of OMM^{12} mice

Mice harbouring the OMM^{12} consortium (*Acutalibacter muris, Akkermansia muciniphila, Bacteroides caecimuris, Bifidobacterium longum subsp. animalis, Blautia coccoides, Clostridium clostridioforme, Clostridium innocuum, Enterococcus faecalis, Flavonifractor plautii, Lactobacillus reuteri, Muribaculum intestinale, Turicimonas muris*) were exposed to the murine *E. coli* commensal strain Mt1B1 to test its capacity to establish in this synthetic community. Mice became colonized with this strain within two to three days. The fecal levels of strain Mt1B1 remained stable over a period of two weeks (Fig. 1A). Mice did not exhibit signs of discomfort or change in feces consistency. Twelve days after inoculation of strain Mt1B1, intestinal sections (ileum and colon) were examined and the location of strain Mt1B1 was determined by fluorescence *in situ* hybridization (Fig. 1B and Fig. S1). Strain Mt1B1 was found in all sections of the gut, including the ileum, consistent with the location from which it was isolated (mucosa from the ileum of conventional laboratory mice) (Garzetti et al., 2018; Lagkouvardos et al., 2016).

Selected virulent phages replicate only on strain Mt1B1 in the gut environment of OMM^{12} mice

We isolated several phages infecting strain Mt1B1 from the environment and selected three (Mt1B1_P3, Mt1B1_P10, Mt1B1_P17) with different characteristics (host range, adsorption and lysis kinetics, genomic content) (Table 1, Fig. S2 and Tables S1, S2, S3). Both, Podoviridae P3 and P10 are close to *E. coli* phages K1F and K1E, respectively, which are capsule-specific phages. Myoviridae P17 is closely related to *E. coli* phages phAPEC8 and ESCO13. Like ESCO13 and by contrast to phAPEC8, Mt1B1_P17 does not carry an endo-N-acetylneuraminidase gene involved in the degradation of the K1 capsule and therefore is most likely not capsule-specific (Trotereau et al., 2017). In liquid broth phages P3 and P10 displayed similar infection kinetics patterns on strain Mt1B1, with rapid lysis followed by a moderate bacterial regrowth at 1.5 hours. In contrast, phage P17 halted the growth of strain Mt1B1 for several hours and slow bacterial regrowth resumed only after more than 10 hours (Fig. 2A). When used in combination, these three phages caused rapid lysis of strain Mt1B1 followed by very slow regrowth (Fig. 2A). Then, we assessed the capacity of each phage to replicate in gut sections collected from Mt1B1-colonized OMM^{12} mice, in an *ex-vivo* assay previously used to reveal the activities of phages along different gut sections (Galtier et al., 2017; Maura et al., 2012a). We collected samples of the ileum and colon from Mt1B1-colonized OMM^{12} mice. We then separated out the mucosal and luminal parts of the ileum and colonic

tissues. We compared the replication of the three phages in these samples *ex vivo* with their replication on Mt1B1 planktonic cultures, at both exponential and stationary growth phases. All three phages displayed similar patterns with efficient replication in all tested gut sections and in exponential growth phase liquid culture, while no amplification was observed in cells at stationary phase (Fig. 2B). As strain Mt1B1 did not multiply in the stationary growth phase (grey bars in Fig. 2B), we concluded that Mt1B1 cell growth is necessary for amplification of these three phages.

Next, we evaluated the transit time of Mt1B1 phages at 6, 24, 48 and 72 hr in axenic mice that received a single oral dose ($6x10^7$ pfu) of a mixture of the three phages (equal amounts of each phage). The fecal level of Mt1B1 phages was below the threshold at 6hr, maximum at 24 hr and then decreased at 48 hr and finally reached the limit of detection at 72 hr (Fig. 3A). In gut sections, we found that the level of phages at 6 hr reached 10^6, 10^4 and 10^3 PFU/g in the luminal part of the ileum, the mucosal part of the ileum and the luminal part of the colon, respectively, while it remained undetected in the mucosal part of the colon. At 24 hr the level of phages in the ileum dropped while it increased in the colon and at 48 hr it was barely detectable in the ileum while only present in the luminal part of the colon. At 72 hr the level of phages was below the threshold of detection in all gut sections tested (Fig 3B). Then, we performed a similar experiment in OMM^{12} mice, not inoculated with strain Mt1B1, by taking samples at 24 and 48 hr post-gavage (same dose as above). Phage levels in feces were slightly lower than those observed in axenic mice. We could detect phages only in the luminal part of the colon at both 24 and 48 hr post-gavage as anticipated from experiments with axenic mice (Fig 3C). These findings indicate that none of the Mt1B1 phages amplified *in vivo* on any of the 12 strains ruling out a possible off-target amplification. These data are in agreement with previous results on phage safety from human volunteers and from conventional mice not colonized with the targeted bacteria (Bruttin and Brussow, 2005; Maura et al., 2012b; Weiss et al., 2009).

In vivo infection of Mt1B1-colonized OMM^{12} mice by a phage cocktail

We next investigated how Mt1B1-colonized OMM^{12} mice responded to an identical single oral dose of the three phages ($6x10^7$ pfu). The fecal levels of phages and bacteria were monitored at short (4 and 6 hours post oral dose) and long (during two weeks) periods of time (Fig. S3A). At all time points but one (day18) following phage administration, levels of Mt1B1 in the phage group were lower than in the control group, despite remaining within one-log of variation and not reaching significance. Within the two weeks of observation phage levels fluctuated within 2 logs (Fig. S3B).

Nevertheless, phage:bacteria ratios remained stable (less than 1 log variation) over time (Fig. S3C). These data show that a single dose of these three phages is sufficient to initiate their long-term replication in Mt1B1-colonized OMM^{12} mice indicating that this animal model is suitable to study the coexistence of phages and bacteria in the gut.

Afterwards, we asked whether repeating the phages administration during three consecutive days could disturb this coexistence between phages and strain Mt1B1. Such a setting mimics a phage therapy treatment targeting bacterial pathogens residing in the human gut (Corbellino et al., 2019). In two independent experiments, we observed a small but significant decrease in the fecal levels of strain Mt1B1 when comparing phage-treated and control groups (d15, p=0.0001; d16, p=0.002; d17, p=0.023) (Fig. 4A and Table S4). Despite this significant impact, phage levels and phage:bacteria ratios remained stable (within 1-log) (Fig. 4B, 4C) and were comparable to data obtained with a single dose of phages showing that the three consecutive phage administrations did not destabilize phage bacteria coexistence (Fig S3).

Phage replication does not trigger shifts in the OMM^{12} bacterial community

We next assessed the stability of the gut microbiota of both phage treated (n=6) and non-treated (n=5) mice groups by two methods, 16S rRNA specific qPCR (Brugiroux et al., 2016) and 16S amplicon sequencing. This quantification was performed at day0 (before colonization with strain Mt1B1), day14 (before the gavage with the three phages) and day17 (one day after the third phages gavage) and both methods showed similar results (Fig. S4). Changes in the bacterial community (qPCR data) were analysed by performing a between-class principal component analysis (PCA), taking into account days, cages and phage inoculation. Results identified daily fluctuations as the main source of the observed variations regardless of the presence or absence of phages (Fig. 4D, Table S5). This finding is also consistent with the passive transit of these phages in OMM^{12} devoid of *E. coli* strain Mt1B1 (Fig. 3C). Therefore, the replication of phages observed in Mt1B1-colonized OMM^{12} mice result exclusively from their capacity to infect strain Mt1B1 in the gut environment, confirming the lack of a possible off-target amplification to support phage coexistence.

Maintenance of bacterial populations is not caused by the emergence of phage-resistant clones

Next, we tested whether the emergence of phage-resistant clones could explain how bacteria can coexist with phages. A total of 280 isolated fecal clones of strain Mt1B1 from 13 mice exposed to

198 the three phages cocktail (from both short and long-term colonization) were susceptible to each of
199 the three phages. We also tested for the emergence of phage-resistant clones from several gut
200 sections (luminal and mucosal parts from ileum and colon). Again, all of these clones (n=800) were
201 susceptible to each of the three phages. Thus, phage resistance development does not explain the
202 maintenance of susceptible bacterial populations in presence of phages.
203
204 **Heterogeneous spatial localization of bacteria in the gut can explain phage-bacteria coexistence**
205 In the absence of evidence for off-target phage amplification on heterologous bacteria and a lack of
206 support for the emergence of phage-resistant bacteria, we wondered whether the spatial
207 heterogeneity of the mammalian gut might create niches where bacteria remain protected against
208 phage predation (Heilmann et al., 2012). We tested this hypothesis by examining luminal and
209 mucosal parts from ileum and colon sections from Mt1B1-colonized OMM^{12} mice exposed or not to
210 phages. Compared to Mt1B1 titers in control mice, phage cocktail application was associated with
211 small but significant Mt1B1 titer decreases in both, the luminal and mucosal parts of the ileum and
212 the mucosal part of the colon (Fig. 4E and Table S4). This indicates that these regions harbour
213 Mt1B1 cells susceptible to phage infection. We observed an average of 2-log and 1-log lower phage
214 titers in the mucosal compared to the luminal parts of the ileum and colon, respectively (Fig. 4F).
215 Consequently, in mucosal parts we found a parallel reduction of the density of both phages and
216 bacteria. However, phage:bacteria ratios revealed a significant ($p=0.006$) lower relative abundance
217 of phages in the mucosal part of the ileum suggesting that at this location some Mt1B1 bacteria
218 might not be exposed to phages (Fig 4G and Table S4). We concluded that a heterogeneous spatial
219 localization of strain Mt1B1 in the ileum reduces accessibility to phages by providing phage-free
220 refuges.
221
222 **Coexistence of a single phage (CLB_P2) with its targeted bacterium (*E. coli* strain 55989) is also**
223 **explained by spatial refuges**
224 To challenge and strengthen the causal role of spatial localization of bacteria in their coexistence
225 with virulent phages, we investigated the behaviour of the Myoviridae phage CLB_P2 infecting the
226 pathobiont enteroaggegative *E. coli* strain 55989. This phage was previously shown to efficiently
227 infect strain 55989 in gut sections and to coexist with it over 3 weeks (Maura and Debarbieux,
228 2012; Maura et al., 2012b). First, we showed that strain 55989 colonizes the gut of OMM^{12} mice
229 (Fig. S5A) as previously reported in conventional mice (Maura et al., 2012b), and did likewise not

result in any sign of intestinal disease. Second, when 55989-colonized OMM^{12} mice received a single oral dose of phage CLB_P2 (1x10^{8} pfu) we observed that fecal levels of strain 55989 in presence or absence of phage CLB_P2 were similar around 10^{8} cfu/g and the phage titers remained stable around 10^{7} pfu/g (Fig 5A, 5B), showing that this model reproduces conditions for stable phage:bacteria ratios (Fig 5C). No significant difference in the fecal levels of strain 55989 was observed on individual days between phage CLB_P2-treated and control groups (Table S6). However, the ANOVA comparison between these two groups, when including the three days, reached significance (p=0.0268) (Table S7). Third, we examined the luminal and mucosal parts of both ileum and colon from 55989-colonized OMM^{12} mice three days after the administration of the single dose of phage CLB_P2. In all sections, but the mucosal part of the colon, the level of strain 55989 was significantly lower in the phage CLB_P2 group than in the control group (Fig. 5D and Table S6). Phage CLB_P2 levels were similar in all sections except in the mucosal part of the ileum in which it was about 2-log lower (Fig. 5E). Phage CLB_P2:strain 55959 ratios in gut sections indicate a significant difference between luminal and mucosal parts of the ileum, while no such difference was observed in the colon (Fig 5F and Table S6). Fourth, we tested 165 and 822 colonies isolated from feces and gut sections, respectively, for their susceptibility to phage CLB_P2 and found that they were all susceptible, showing that development of phage resistance to a single phage could not explain the maintenance of susceptible bacterial populations in presence of this phage (Fig. S5B). In addition, we determined the frequency of phage-resistant clones in fecal pellets from 55989-colonized OMM12 mice that received either phage CLB_P2 or PBS and found that they were at the same range for both groups and equivalent to the frequency obtained with a fresh culture of strain 55989 (Table S7).

To support further our conclusions, we quantified the abundance of strain 55989 in unwashed gut sections using immunohistochemistry (Fig. 6A and Fig. S6). We found no difference in the abundance of strain 55989 in the mucosal parts of both ileal and colonic sections from phage CLB_P2-treated mice compared to controls, which is in agreement with the presence of bacterial refuges on the mucosal parts of the gut (Fig. 6B and Table S8). In the luminal parts of both ileum and colonic sections the abundance of strain 55989 (quantified by fluorescence intensity) was not different between the two conditions, while a significant difference was reached when comparing CFU data (Fig. 5D). This underlines the difficulty to compare indirect (microscopy) with direct (plating) quantifications. To conclude, the lower abundance of phages in the mucosal parts of the ileum provides opportunities for bacteria to reside in phage-free niches by escaping to phage

predation. Taken together, data from two independent models (Mt1B1- and 55989-colonized OMM^{12} mice) using different phages show that the biogeography of bacteria participates to the coexistence of phages and phage-susceptible bacteria in the gut.

Discussion

The intestinal microbiota of mammals is relatively stable over time in healthy subjects while it contains large antagonistic populations of bacteria and phages. The mechanisms that underlie this apparently peaceful coexistence are unknown. Several hypotheses can be drawn from years of *in vitro* studies of isolated systems (one phage/one bacterium) or more recently from environmental studies (Buckling and Rainey, 2002; Fortuna et al., 2019; Horne, 1970; Laanto et al., 2017; Meyer et al., 2016). However, none of them has yet been tested in a relevant intestinal environment. Using a gnotobiotic mouse model, two *E. coli* strains and four different virulent phages, we showed that the emergence of phage-resistant bacteria and the possibility to infect off-target bacteria can be excluded as major factors supporting coexistence. Instead, we found that the spatial distribution of phages and bacteria along the radial axis of the gut fits the classical ecological theory of source-sink dynamics (Holt, 1985). Our data suggest that refuges for bacteria in the mucosal layer serve as a source, with the lumen acting as a sink in which the phages can infect their target. As phages do not exert a major direct selective pressure on the source, the bacteria reaching the gut lumen remain susceptible enabling the phages to maintain their density in the lumen over time (Fig 6C). This different luminal/mucosal distribution of phages was significant in ileal but not in colonic sections. This might not be the case in the human colon that displays organisational differences compared to mice colon, the latter being essentially filled with structured fecal pellets in which phage-bacteria interactions are likely to be less intense (Nguyen et al., 2015).

In phage bacteria interaction studies, the prey-predator dynamics have been classically studied through the prism of phage-resistant bacteria and counter resistant phages. Recently, an *in vitro* study has proposed the model named “leaky resistance” to explain the maintenance of virulent phages by the high rate of transitions between phage-resistant and phage-susceptible bacteria within the populations (Chaudhry et al., 2018; Silveira and Rohwer, 2016). However, we failed to isolate phage-resistant bacteria within the entire gut during this work performed with two couples of phages and bacteria. This suggests that phage-resistant bacteria may, *in vivo*, either carry a high cost or have a low selective value in both single and repeated phage application (Gomez and

Buckling, 2011; Koskella et al., 2012). Coupled with the concept of costly phage resistance mutations, our results are in agreement with observations from *in silico* individual based stochastic spatial models, which showed that structured environments can create spatial refuges that lead to coexistence between bacteria and phages without the emergence of resistant clones (Heilmann et al., 2012; Sousa and Rocha, 2019).

The lower abundance of phages in the mucosal parts could be triggered by the reduced bacterial density compared to luminal parts, according to a density-dependent phenomenon that was previously observed in several *in vitro* structured environments (Eriksen et al., 2018). This density-dependent phenomenon was linked to the "proliferation threshold" that proposes that a minimum number of bacteria is needed for the phage to initiate infection and amplify (Payne et al., 2000; Wiggins and Alexander, 1985). Note that the source-sink and the "proliferation threshold" hypotheses are not mutually exclusive to support the presence of bacterial refuges driving the long-term coexistence of phages and susceptible bacteria. We propose that the gut displays a mucosal-luminal gradient of phage density exposing bacterial populations to different phage concentrations (Fig. 6C). Such a gradient can explain the persistence for months and years of phage populations in human fecal samples without major variations of the microbiota composition.

Nevertheless, the heterogeneous spatial distribution of bacteria in the gut does not exclude other factors or processes that could also participate to the long-term coexistence of phage with susceptible bacteria. Some of these processes were not directly tackled in this study like the influence of abiotic factors (Lourenco et al., 2018; Scanlan et al., 2019). In particular, phenotypic resistance that is described as the ability of bacteria to oscillate between phage-susceptible and phage-resistant cells could be involved in some of our observations. This phenotypic resistance is led by environmental or cellular stochasticity that modulate the expression of genes, for example those encoding for phage receptors (Bull et al., 2014; Chapman-McQuiston and Wu, 2008). Phage diffusion may be limited by mucins and other glycoproteins, lipids and DNA molecules (Johansson et al., 2011; Qi et al., 2017). Immunoglobulin motifs in structural phage proteins were also shown to favour phage binding to mucus (Barr et al., 2013). However, only ORF118 of phage Mt1B1_P17 possesses such a motif (identified by a specific *in silico* search) homologous to the bacterial Ig-like domain (Big_2) (bit score=29.5, individual E-value=5.1×10^{-9} and conditional E-value=2.8×10^{-11}) (Fraser et al., 2006). The absence of such motifs in three out of the four phages and in particular in

CLB_P2 is consistent with our *in vivo* observation that these phages are less abundant in the mucosal parts of gut sections.

Our data show that the gnotobiotic OMM^{12} model is particularly well suited to study the mechanisms involved in phage bacteria dynamics in the gut. OMM^{12} community establishes a long-term stable composition over several mouse generations and can be used as platform to flexibly incorporate additional bacterial strains (Brugiroux et al., 2016; Herp et al., 2019; Studer et al., 2016). Interestingly, Hsu and colleagues recently reported the use of similar synthetic microbiota models to study phage bacteria interactions with two major differences. First, axenic mice were inoculated with bacterial strains during two weeks before the inoculation of phages, compared to stably colonized OMM^{12} mice. Second, the 10 bacterial strains chosen are from human origin, instead of mouse origin for OMM^{12}, and may rapidly undergo genetic adaptation to the mouse environment (Barroso-Batista et al., 2014; Lourenco et al., 2016). Despite these differences, both mouse models confirm that the inoculation of virulent phages in the gut generally leads to the coexistence of phages and bacteria. Hsu et al. found that *Enterococcus faecalis* phage resistant mutants increased over time and that the microbial community structure was affected. Here, we could not detect any of these two events. In addition, they found that some phages have strong impact on abundance of cognate host bacteria, while such impact was not previously observed in several mouse models (Maura et al., 2012a; Weiss et al., 2009). This suggests that different phage-bacteria couples may exhibit distinct eco-evolutionary dynamics, increasing the complexity of studying these interactions in natural environments (Shkoporov and Hill, 2019). A recent example of such complexity was reported when studying the *in vivo* prophage induction of *Roseburia intestinalis*, a dominant bacterium of the human gut, which leads to the coexistence of phages and bacteria with the selection of CRISPR-mediated bacterial resistance along with hypervirulent phage variants (Cornuault et al., 2019).

Deciphering the dynamics of phage bacteria interactions in the gut is required to develop efficient phage-guided therapeutic strategies and ultimately obtain firm clinical evidences that are still lacking for intestinal infections (Brussow, 2017; Duan et al., 2019b; Sarker et al., 2016). A recent case report showed that the *in vitro* isolation of a single virulent phage, used to target a multidrug resistant strain of *Klebsiella pneumoniae*, led to the eradication of this pathogen from the patient's

gut (Corbellino et al., 2019). While lacking mechanistic insights, this work confirms the medical potential of phages to selectively target bacteria residing in the gut.

Author contribution

Conceptualization, L.D., L.D.S. and M.L.; Methodology, B.S., L.D., L.D.S., M.B. and M.L.; Investigation, C.E., L.C., L.D.S., M.L., Q.L.B. and T.P.; Formal Analysis, L.C., M.L. and P.C.; Writing – Original Draft, L.D., L.D.S. and M.L.; Writing – Review & Editing; B.S., L.C., L.D., L.D.S., and M.L.; Funding Acquisition B.S., L.D. and M.B. Resources; B.S., M.B., L.D. and P.C.; Supervision, L.D. and L.D.S.

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Declaration of Interests

The authors declare no competing interests.

Main figures titles and legends

Figure 1. The *E. coli* strain Mt1B1 stably colonizes the gut of the OMM^{12} mice.

A. Fecal levels of the *E. coli* strain Mt1B1 at the indicated time points for each OMM^{12} mouse (n=11) receiving a single dose of 10^8 cfu by oral gavage at day 0. Red dots, individual values; horizontal bar, median; box, 25th-75th quantiles, vertical bars, min/max values (within 1.5 x interquartile interval). **B.** Localization by FISH of the strain Mt1B1 in the ileal section of Mt1B1-colonized OMM^{12} mice. Intestinal cells (nuclei) were stained with DAPI (purple), and Mt1B1 (red+green=yellow) and Eubacteria (red) were stained with specific FISH probes. A representative image from a group of four mice is presented. Scale bar, 50µm. See also Figure S1.

Figure 2. Mt1B1 phages P3, P10 and P17 infect strain Mt1B1 both *in vitro* and *ex-vivo*.

A. Growth curves for strain Mt1B1 (n=3 for each condition) measured via OD_{600nm} reading in liquid broth in the absence (grey) or presence of phage P3 (orange), P10 (blue) or P17 (green), or of a cocktail of these three phages (purple; equal proportions of each) added at t=0 at MOI of 1 x 10^{-2}. The inset shows an enlargement for early time points. (error bars represent standard error of the mean, SEM). **B.** Amplification over 5h (n=3 biological replicates) of individual phages (P3, orange; P10, blue; P17, green; each at an MOI of 1 x 10^{-2}) and Mt1B1 cells (grey) in indicated homogenized gut sections (lu., luminal; mu, mucosal) from Mt1B1-colonized OMM^{12} mice (*ex vivo*) and flasks with Mt1B1 cells in exponential (exp.) (OD_{600nm}=0.5) or stationary (stat.; 24hr) growth phases (*in vitro*). N-fold multiplication relative to the initial number of phages added at t=0, which was approximately 100 fold lower than the amount of Mt1B1 cells, is shown. The n-fold multiplication of strain Mt1B1 was calculated by plating samples before and after the 5hr incubation (means with standard deviation).

Figure 3. Passive phage transit in axenic and OMM^{12} mice.

Axenic (n=8) and OMM^{12} (n=4) mice received a single dose of the three Mt1B1 phages ($3x10^7$ pfu; phages mixed in equal proportions). Phage titers were determined in fecal pellets and intestinal sections at indicated time points. **A.** Fecal phage titers from axenic mice. **B.** Phage titers in organs from axenic mice. **C.** Phage titers from OMM^{12} mice feces and organs. Dots represent individual data and horizontal bars represent the average.

Figure 4. Virulent Mt1B1 phages do not affect the microbiota composition of the OMM^{12} mice and display an heterogeneous repartition in gut sections supporting their coexistence with strain Mt1B1.

A. Mt1B1-colonized OMM^{12} mice (n=25) received at day14, 15 and 16 PBS (red, n=11) or the three phages P3, P10 and P17 together (blue, n=14; 6x10^{7} pfu per dose made of the same amount of each phage) by oral gavage. Levels of *E. coli* strain Mt1B1 in the feces. **B.** Phage titers from the fecal samples reported in panel A. **C.** Phage:bacteria ratios for fecal samples collected on days15, 16 and 17. Dots, individual values; horizontal bar, median; box, 25th-75th quantiles, vertical bars, min/max values (within 1.5 x interquartile interval). **D.** Between-group PCA (BCA, axes 1 and 2) for the 16S rRNA qPCR data for mice receiving PBS (n=5, filled circle) or the phage cocktail (n=6, filled triangle) by oral gavage at the indicated time points (see the colors indicated) for 10 bacteria from the fecal microbiota of OMM^{12} mice (strains YL2 and KB18 were not detected). On day 17 mice were sacrificed and gut section analyzed. **E.** Titers of strain Mt1B1 in indicated gut sections (lu., luminal; mu., mucosal). **F.** Titers of Mt1B1 phages in the samples reported in panel E. **G.** Phage:bacteria ratios for the indicated gut sections in panels A and B. Dots, individual values; horizontal bar, median; box, 25th-75th quantiles, vertical bars, min/max values (within 1.5 x interquartile interval). See also Table S4 and S5.

Figure 5. Spatial heterogeneity of the *E. coli* strain 55989 in the gut sections participates to the coexistence with virulent phage CLB_P2.

A. 55989-colonized OMM^{12} mice (n=28) were colonized during 7 days before receiving a single administration of PBS (red, n=12) or phage CLB_P2 (blue, n=16; 1x10^{8} pfu) by oral gavage. **A.** Fecal titers of strain 55989. **B.** Fecal titers of phage CLB_P2. **C.** Phage:bacteria ratios for fecal samples collected on days 8, 9 and 10. On day 10 mice were sacrificed and gut section analyzed. **D.** Titers of strain 55989 in indicated gut sections. **E.** Titers of phage CLB_P2 in the samples reported in panel D. **F.** Phage:bacteria ratios for the indicated gut sections in panels D and E. Dots, individual values; horizontal bar, median; box, 25th-75th quantiles, vertical bars, min/max values (within 1.5 x interquartile interval). See also Table S6.

Figure 6. Source-sink dynamics support the coexistence of virulent phages and bacteria in the gut. **A.** Representative photograph (scale bar, 200µm) of immunofluorescence staining of strain 55989 (red) and mucus (green) in unwashed ileum section with nuclei of epithelial cells stained with DAPI (blue). **B.** Quantification of the red fluorescence per pixel in mucosal and luminal parts of ileal and colonic sections from 55989-colonized OMM^{12} mice at day 10 (n=4 to 5). **C.** Schematic representation of the source-sink dynamics between virulent phage and bacteria populations in the gut. Bacteria located close to the mucosal layer (in yellow) form refuges that phages cannot reached. Bacteria located in the intestinal lumen are killed (dotted lines) by phages that can persist through the gut. Bacteria and phage populations coexist without the strong selection of phage-resistant bacteria.

Main tables and legends

Table 1. Main characteristics of Mt1B1 phages P3, P10 and P17

STAR Methods

RESOURCE AVAILABILITY

Lead contact

Further information and requests for resources and reagents should be directed to and will be fulfilled by the Lead Contact, Laurent Debarbieux (laurent.debarbieux@pasteur.fr).

Materials Availability

This study did not generate new unique reagents

Data and Code Availability

Accession numbers for phage genomes are: MT496969 for Mt1B1_P3, MT496971 for Mt1B1_P10 and MT496970 for for Mt1B1_P17.

EXPERIMENTAL MODEL AND SUBJECT DETAILS

Microorganisms

Bacterial strains including Mt1B1 (DSM-28618) and 55989 are listed in Table S1. Bacteriophage CLB_P2 isolation and characterization is described on Maura *et al*. (Maura et al., 2012b).

Bacteriophage Mt1B1_P3, P10 and P17 were isolated and purified from sewage water, with an enrichment technique (explained in method details below) using E. coli strain Mt1B1. All phages used were amplified in exponential growing cultures of the respective host strain for approximately 4 hours. Cell lysate supernatants containing amplified phages were after 0.22 µm filter sterilized and stored at 4°C.

Strains were routinely cultured in lysogeny broth (LB), or on LB agar or Drigalski agar (Bio-Rad, Hercules, CA) plates, at 37°C.

Animals and ethics

C57Bl/6J mice (seven to nine-week-old) OMM^{12} were bred at Institut Pasteur (Paris, France). A total of 72 OMM^{12} and 8 C57Bl/6J axenic healthy mice were used. For all the experiments littermates of the same sex were randomly assigned to experimental groups, being used both female and male mice. Food and drinking water were provided ad libitum. All mice were housed in isocages and in an animal facility in accordance with Institut Pasteur guidelines and European recommendations. All animal experiments were approved by the committee on animal experimentation of the Institut Pasteur and by the French Ministry of Research.

METHOD DETAILS

Phage isolation

First, sewage water from four locations was filtered at 0.45µm and mixed with an equal volume of 2X Luria- Bertani (LB) medium. Second, these four mixtures were inoculated with a fresh growing culture of Mt1B1 OD_{600nm} of 0.4 at 600nm, final dilution 1/200) and incubated on a shaker at 37°C overnight. The next day chloroform (1/10 vol./vol.) was added to the flasks and incubated at room temperature for one hour. Following centrifugation at 8000g for 10min 1 mL of the supernatant was mixed with 1/10 vol./vol. of chloroform and centrifuged at 8000g for 5min. A 100-fold dilution in TN buffer (10mM Tris HCl pH7.5, 100mM NaCl) of the aqueous phase was prepared. 10µL of the undiluted and diluted solutions were spread with an inoculation loop on the top of two separate LB agar plates and allowed to dry for 30min under a safety cabinet. Subsequently, 1mL of an exponentially growing culture of Mt1B1 was applied to fully cover each plate; the excess of liquid culture was removed and plates were incubated at 37°C overnight. The next day, individual plaques were picked and resuspended in tubes containing 200µl TN buffer. 1/10 vol/vol. chloroform was mixed and tubes were centrifuged at 8000g for 5min. These steps of plaque purification were performed three times. Finally, 10µL of the last resuspended plaque were added to 1mL of a liquid culture of Mt1B1 (OD_{600nm} of 0.4 at 600nm) and incubated at 37°C in a shaker for 5hr. 1/10 vol./vol. of chloroform was mixed and after centrifugation at 8000g for 5min this stock was stored at 4°C and served as starting solution for large scale lysates (Henry et al., 2013).

Host range tests

Host range tests were performed as follows: 3µL of PBS-diluted phage solutions (0.2µm filtered sterilized crude lysates adjusted to 10^7 pfu/mL) were deposited side by side on the lawn of each tested bacterium on agar LB plates. Plates were incubated at 37°C overnight. Phages were grouped according to their host range and three representative phages (Mt1B1_P3, Mt1B1_P10 and Mt1B1_P17) of the main groups were chosen (Table S1).

Adsorption assays and phage growth

Three independent adsorption assays were performed for each phage according to the protocol previously described (Chevallereau et al., 2016). Data were fitted using an exponential function and adsorption times were defined as the time required to reach a threshold of 10% of non-adsorbed

phage particles. To record phage growth and bacteria lysis, an overnight culture of strain Mt1B1 was diluted in LB broth and grown to an OD_{600nm} of 0.2 from which 150µL were distributed into each of the wells of a 96-well plate (Microtest 96 plates, Falcon). 10 µL of sterile phage lysates diluted in PBS to obtain a multiplicity of infection (MOI) of 1×10^{-2} in each well. Plates were incubated in a microplate reader at 37°C, with a shaking step of 30sec before the automatic recording of OD_{600nm} every 15min over 20hr (Glomax MultiDetection System, Promega, USA).

Phage genomes sequencing and analysis

Sterile phage lysates were treated by DNase (120 U) and RNase (240 µg/mL) and incubated for 30 min at 37°C before adding EDTA (20 mM). Lysates were treated with proteinase K (100 µg/mL) and SDS (0.5%) and incubated at 55°C for 30min. DNA was extracted by a phenol-chloroform protocol modified from Pickard (Pickard, 2009). Sequencing was performed using Illumina technology (Illumina Inc., San Diego, CA) MiSeq Nano with paired-end reads of 250bp. Quality of reads was visualised by FastQC v0.10.1 Brabraham Bioinformatics (http://www.bioinformatics.babraham.ac.uk/projects/fastqc/). Assembly was performed using a workflow implemented in Galaxy-Institut Pasteur using clc_assembler v4.4.2 and clc_mapper v4.4.2 (CLC Bio, Qiagen). Phage termini were determined by PhageTerm (Garneau et al., 2017) and annotations were performed by the RAST v2.0 server (Aziz et al., 2008). According to the nomenclature proposed for naming viruses (Adriaenssens and Brister, 2017), the full names for phages P3, P10 and P17 are vB_EcoP_Mt1B1_P3, vB_EcoP_Mt1B1_P10 and vB_EcoM_Mt1B1_P17, respectively. The Accession numbers for the phage genomes are pending.

Search for Ig-like domains on phage genomes

Protein sequences of the four phages were scanned for homologs on Pfam database using the HMMER website (Potter et al., 2018). The results were compared to a comprehensive HMM (Hidden Markov Models) database of Ig-like domains found on Pfam that was kindly provided by Dr. Sean Benler.

***Ex vivo* phage replication assay**

Oligo-MM^{12} mice received 200µL of strain Mt1B1 (10^7 CFU prepared from an overnight culture in LB at 37°C) in sterile sucrose sodium bicarbonate solution (20% sucrose and 2.6% sodium bicarbonate, pH 8) by oral gavage and three days after were sacrificed to collect and weight intestinal sections

(ileum and colon). PBS was added to each sample (1.75mL) before homogenization (Oligo-Macs, Milteny Biotech). A volume of 150µL of each homogenized gut sample was distributed in the wells of a 96-well plate and 10µL of each individual phage were added to reach an MOI of 1 x 10^{-2}, and the plate was incubated at 37°C. A fraction of the homogenized samples was also serially diluted in PBS and plated on Drigalski medium to count Mt1B1 colonies at t=0. Following five hours of incubation, samples were serially diluted in PBS and plated on Drigalski medium as well as on LB agar plates overlaid with strain Mt1B1. Both set of plates were incubated at 37°C overnight. The same procedure was followed for *in vitro* growth assays with bacteria taken during exponential (OD_{600nm} 0.5) or stationary (24hr) growth phase at 37°C with shaking.

Passive phages transit in axenic and OMM^{12} mice

Axenic (n=8 divided in 4 cages) or OMM^{12} (n=4 divided in 2 cages) mice were orally gavaged once by 200µL of a cocktail of Mt1B1 phages (1x10^7 pfu of each of the three phages) diluted in PBS. Fecal pellets were collected before phage gavage and at 6, 24, 48 and 72hr post-gavage for axenic mice and at 24 and 48hr post-gavage for OMM^{12} mice. Pellets were transferred in pre-weighted, sterile, 2mL tubes, weighted and resuspended in 1mL of PBS. Serial dilutions in PBS were performed and plated onto LB plates overlaid with strain Mt1B1 to assess phage titers. Two mice were sacrificed on each time point to collect intestinal sections, which were homogenized in PBS using gentleMACS^{TM} OCtoDissociator (Miltenyi Biotec) and then plated on LB plates overlaid with strain Mt1B1. The luminal part corresponds to the gut content that was recovered by squeezing the intestinal tube with the back of a scalpel, which was subsequently homogenized in 1mL of PBS. The mucosal part corresponds to the remaining tissues (empty intestinal tube) that were washed in 10mL of PBS before being transferred into a new tube with 1.75mL of fresh PBS and then homogenized.

Murine model of *E. coli* colonization

The long-term coexistence experiment with strain Mt1B1 included 7 mice (5 that received phages and 2 that did not) and lasted 23 days. At day0 mice feces were collected prior to Mt1B1 administration by oral gavage (200 µL of bacteria resuspended in sodium bicarbonate buffer (see above). Fecal pellets were transferred in pre-weighted, sterile, 2mL tubes, weighted and resuspended in 1mL of PBS. Serial dilutions in PBS were performed and plated onto Drigalski plates. The three phages (2x10^7 pfu of each phage in 200µL of PBS) were administered as a mixture once

by oral gavage at day9. The level of phages was assessed from serial dilutions in PBS spotted on LB plates overlaid with strain Mt1B1.

Two shorter independent experiments with repeated phage administration were performed with 11 (6 with phages and 5 without divided in 4 cages) and 14 (8 with phages and 6 without divided in 4 cages) Mt1B1-colonized mice respectively. At day0 mice feces were collected prior to Mt1B1 administration (as described above) by oral gavage. Fecal pellets were prepared as above. The three phages ($2x10^7$ pfu of each phage in 200µL of PBS) were administered altogether once by oral gavage at day14, 15 and 16. The level of phages was assessed as above. Each mouse was sacrificed at day17 to collect feces and intestinal sections, the latest being homogenized in PBS using gentleMACS^{TM} OCtoDissociator (Miltenyi Biotec) and all samples were plated on both Drigalski plates and LB plates overlaid with strain Mt1B1. Luminal and mucosal part of gut sections were recovered as described above.

For experiments with *E. coli* strain 55989 fecal samples were collected on days0, 1, 3, 7, 8, 9 and 10. On day0, after collecting fecal samples, oral gavage of *E. coli* strain 55989 was performed as described above for strain Mt1B1. At day7, after collecting fecal sample, 200µL of either phage CLB_P2 ($2x10^8$ pfu/mL) (16 mice divided in 6 cages) or PBS (12 mice divided in 5 cages) were administered to the mice by oral gavage (two independent experiments were performed). At day10, feces were collected before sacrifice and dissection of the mice and assessment of the levels of bacteria and phages as above.

Identification of resistant clones

For the experiments with Mt1B1-colonized OMM^{12} mice and the phage cocktail, 20 clones from each gut section from mice (n=8) exposed to the cocktail during three days and sacrificed at day17, as well as from fecal samples from mice (n=5) exposed to a single dose of the cocktail at days10, 16 and 24, were streaked vertically in LB agar plates and subsequently each of the three phages was horizontally streaked across. Plates were incubated at 37°C and phenotype was checked after 5h and overnight.

For the experiments using 55989-colonized OMM^{12} mice and phage CLB_P2, 75 to 94 isolated colonies per sample (gut sections and feces from n=8 mice sacrificed at day10) were randomly chosen by a robot (Qpix 420; Molecular Devices, Sunnyvale, USA) and cultured overnight in 150µL of LB in 96 well microplates. The next day, 10µL were added to a new plate filled with 140µL of LB and incubated at 37°C for 2hr. Then, 8µL of each clone were spotted on two separated LB agar

plates and let dry for 20 minutes. Then, on one plate spots of 4µL of phage CLB_P2 (400 pfu) were spotted on top of each bacterial spot. On the second plate phage spots were 100 fold more concentrated (40,000 pfu). Susceptible clones are defined by the clearance of the bacterial spot at one or both phage concentrations (often full clearance was observed with 40,000 pfu and partial clearance with 400 pfu), while clones not affected by the phage at any concentration are defined as resistant. The frequency of phage-resistant clones in fecal samples from 55989-colonized OMM^{12} mice was assessed by plating 2.25x10^4 to 3.42x10^5 55989 cells on Drigalski plates inundated with 3x10^7 pfu of phage CLB_P2. The number of phage-resistant clones was recorded after overnight incubation at 37°C and compared to plates inundated with the same amount of phage CLB_P2 and on which 3.83x10^6 cells from an exponential growing culture of strain 55989 was spread.

Histological analysis by FISH and immunochemistry

Fluorescence in situ hybridization was performed as previously described on intestinal samples from *E. coli* Mt1B1 colonized OMM^{12} mice bred at the LMU Munich where the fecal level of strain Mt1B1 reached 10^9 CFU/g (Brugiroux et al., 2016). Ileal and colonic tissues were fixed in 4% paraformaldehyde (4°C overnight), washed in 20% sucrose (4°C overnight), embedded in O.C.T (Sakura), and flash frozen in liquid nitrogen. FISH was performed on 7µm sections, using double 3´and 5´-labelled 16S rRNA targeted probes specific for Enterobacteriaceae (Ent186-2xCy3 (CCC CCW CTT TGG TCT TGC)) and Eubacteria (1:1 mix of Eub338I-2xCy5 (GCT GCC TCC CGT AGG AGT) and Eub338III-2xCy5 (GCT GCC ACC CGT AGG TGT)). 1µg/mL–1 4',6-diamidino-2-phenylindole (Roth) was used for DNA staining. Images were recorded with a Leica TCS SP5 confocal microscope (Leica, Wetzlar).

For immunofluorescence staining unflushed ileal and colonic tissues from *E. coli* 55989-colonized OMM^{12} mice bred at Institut Pasteur were fixed in Carnoy (ethanol/chloroform/acetic acid 60:30:10) for mucus preservation, dehydrated, and embedded in paraffin according to the standard protocol. All the stainings were done on dewaxed 8µm sections. Antigen retrieval was performed with Retrievagen A (BD BIOSCIENCES) during 10min at 97°C, followed by 20min at room temperature. After blocking with Protein Block Serum Free (DAKO) during 15min at room temperature, immunofluorescence staining was performed using mouse monoclonal antibody anti Muc2-A488, 1/100 (Santa Cruz Biotechnology) and rabbit anti *E. coli* O104, 1/50 (SSI diagnostica) during 4hr at room temperature, then overnight at 4°C. After washings in PBS, the following fluorescence-labeled secondary antibodies were used: anti-mouse-A488, 1/200 (Life Technologies)

and anti-rabbit-A555, 1/200 (Life Technologies) during 4.30hr at room temperature, and nuclei stained with DAPI. Slides were examined under an Olympus IX81 microscope equipped with a charge-coupled device (CCD) camera, and red fluorescence (*E. coli* O104) per pixel was quantified with ImageJ software. An example of the different regions delimited for the quantifications is shown in Fig. S6.

OMM^{12} community composition

From homogenized fecal samples, 500µL were centrifuged at 8.000g for 10min and the supernatant was removed. Pellets were diluted in 500µL of lysis buffer (500 mM NaCl, 50 mM Tris-HCl, pH 8.0, 50 mM EDTA, 4% sodium dodecyl sulfate (SDS) and incubated for 15min at 50°C (Yu and Morrison, 2004). Then, 100µL of lysozyme (25mg/ml) was added and samples were incubated at 37°C for 2 hr. DNA extraction was performed using the Maxwell Cell tissue kit (Promega) and samples were frozen at -20°C until use. The primers, probes and qPCR protocol were used in conformity with previously described methods (Brugiroux et al., 2016) with the exception of the SsoAdvancedTM Universal Probes Supermix (BioRad). The qPCR reactions were performed in duplicate and in two independent runs using MasterCycler realplex4 from Eppendorf. Amplicon libraries targeting the V3-V4 16S region were amplified by PCR (25 cycles) using Illumina primers (forward primer: 5'-TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGCCTACGGGNGGCWGCAG-3', reverse primer: 5'-GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGACTACHVGGGTATCTAATCC-3') and then sequenced (Illumina MiSeq 2x300 bp). Sequences and statistical analyses were performed with SHAMAN (shaman.c3bi.pasteur.fr) (Quereda et al., 2016).

QUANTIFICATION AND STATISTICAL ANALYSIS

For the growth curves for strain Mt1B1 (n=3 for each condition) error bars represent standard error of the mean (SEM). The n-fold multiplication of strain Mt1B1 on the *ex vivo* experiment, represented on figure 2 was calculated by plating samples before and after the 5hr incubation and the error bars represent means with standard deviation.

Statistical analysis on the number of bacteria and phages generated by the animal experiments were carried out using the lme4, lmerTest and car packages of R (Bates et al., 2015; Fox and Weisberg, 2019; Kuznetsova et al., 2017). Both cfu and pfu were log10-transformed prior to analysis. In each experiment, two groups of mice were considered, a group exposed to phages and

an unexposed control group. The impact of phages could be assessed based on the abundance of phages (log-pfu). Given the non-linearity of responses, the day at which a measure was performed was considered as a categorical variable. Linear mixed-models were used to account for random experimental effects (i.e., individuals, experiments and cage effects).

Overall effects were assessed with Analysis of Variance (ANOVA) and post-hoc Tukey's comparisons and were performed using the lsmeans R package (Lenth, 2016). $p < 0.05$ was considered statistically significant.

16S-quantification data were analysed using multivariate analysis after standard normalization. A principal component analysis (PCA) was performed with the R package ade4 on the matrix of ΔCt values of 10 bacterial strains (strains YL2 and KB18 were not detected) (Dray and Dufour, 2007). In addition, a between-group PCA was done in order to assess experimental effects, based on 12 groups of observations: 3 days (0, 14, and 17) and 4 cages (2 exposed, 2 unexposed).

Supplemental Table

Table S3, related to Table 1. Genome annotations of Mt1B1 phages P3, P10 and P17.

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