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1 **The gut environment regulates bacterial gene expression which modulates susceptibility to**
2 **bacteriophage infection**

3

4 **Authors**

5 Marta Lourenço^{1,2#}, Lorenzo Chaffringeon^{1,3,4}, Quentin Lamy-Besnier¹, Marie Titécat^{1,5}, Thierry
6 Pédrón¹, Odile Sismeiro⁶, Rachel Legendre^{6,7}, Hugo Varet^{6,7}, Jean-Yves Coppée⁶, Marion Bérard⁸, Luisa
7 De Sordi^{1,3,4} and Laurent Debarbieux^{1*}

8 **Affiliations**

9 1 Institut Pasteur, Université de Paris, CNRS UMR6047, Bacteriophage Bacterium Host, Paris F-75015
10 France

11 2 Sorbonne Université, Collège Doctoral, F-75005 Paris, France

12 3 Sorbonne Université, INSERM, Centre de Recherche St Antoine, UMRS_938, Paris, France

13 4 Paris Center for Microbiome Medicine (PaCeMM) FHU, AP-HP, Paris, Ile-de-France, France

14 5 Université de Lille, INSERM, CHU Lille, U1286-INFINITE-Institute for Translational Research in
15 Inflammation, F-59000 Lille, France

16 6 Transcriptome and EpiGenome Platform, Biomics, Center for Technological Resources and Research
17 (C2RT), Institut Pasteur, Université de Paris, Paris F-75015 France

18 7 Bioinformatics and Biostatistics Hub, Department of Computational Biology, Institut Pasteur,
19 Université de Paris, Paris F-75015 France

20 8 Institut Pasteur, Université de Paris, DT, Animalerie Centrale, Centre de Gnotobiologie, 75724 Paris,
21 France

22 # present address: Department of Global Health, Institut Pasteur, Université de Paris, Paris F-75015
23 France

24

25 *** Lead author and correspondence**

26 laurent.debarbieux@pasteur.fr

27 **Summary**

28 Abundance and diversity of bacteria and their viral predators, bacteriophages (phages), in the
29 digestive tract are associated with human health. Particularly intriguing is the long-term coexistence
30 of these two antagonistic populations. We performed genome-wide RNA sequencing on a human
31 enteroaggregative *Escherichia coli* isolate, to identify genes differentially expressed between *in vitro*
32 conditions and in murine intestines. We experimentally demonstrated that four of these
33 differentially expressed genes modified the interactions between *E. coli* and three virulent phages by
34 either increasing or decreasing its susceptibility/resistance pattern and also by interfering with
35 biofilm formation. Therefore, the regulation of bacterial genes expression during the colonization of
36 the digestive tract influences the coexistence of phages and bacteria, highlighting the intricacy of
37 tripartite relationships between phages, bacteria and the animal host in intestinal homeostasis.

38

39 **Introduction**

40 The microbiota of the mammalian gut includes bacteria and their viral predators, bacteriophages
41 (phages). In healthy individuals, these two antagonistic populations generally remain stable over
42 time, but variations in their density and diversity have been associated with several human diseases
43 or disorders (Gregory et al., 2020; Manrique et al., 2017; Mirzaei and Maurice, 2017; Shkoporov et
44 al., 2019; Zuo et al., 2020). The long-term coexistence of populations of phages and bacteria *in vivo*
45 probably results from a combination of mechanisms deployed by each of the three partners: phages,
46 bacteria and the host (Kirsch et al., 2021; Mirzaei and Maurice, 2017).

47

48 Multiple molecular mechanisms regulate the dynamics of phage-bacterium interactions. These
49 include mutations of genes encoding phage receptors to prevent attachment, restriction-
50 modification enzymes and CRISPR systems, to degrade phage genomes, mutations of genes encoding
51 proteins essential for phage infection and replication, abortive infection and superinfection exclusion
52 systems (Labrie et al., 2010). Genome data mining has recently identified a growing number of

53 bacterial genes involved in phage defence systems experimentally tested only *in vitro* (Bernheim and
54 Sorek, 2020; Millman et al., 2020; Rousset et al., 2021). In addition, other mechanisms, such as
55 phenotypic resistance, a reversible state providing bacteria with temporary resistance to phages (Bull
56 et al., 2014; Chapman-McQuiston and Wu, 2008; Levin et al., 2013), and leaky resistance (Chaudhry
57 et al., 2018), have been proposed as means of prolonging the coexistence of phages and bacteria in
58 microcosms *in vitro*. Moreover, abiotic (temperature, oxygen, nutrients) and biotic (microbial and
59 mammalian cells) factors have been shown to influence phage-bacterium interactions in both *in vitro*
60 and *in vivo* environments (Alseth et al., 2019; Golec et al., 2014; Hadas et al., 1997; Labedan, 1984;
61 Lourenço et al., 2020; Scanlan et al., 2019; Sillankorva et al., 2004). However, the relevance and
62 preponderance of these mechanisms in the mammalian gut remain poorly explored (Cornuault et al.,
63 2020; De Sordi et al., 2019).

64

65 Bacteria modulate gene expression during their establishment in the digestive tract of mammals.
66 Whether and how this could affect their susceptibility to phages remains unknown. However, there
67 have been several observations indicating that phages with a similar efficacy *in vitro* behave
68 differently in the gastrointestinal tract of mice (Maura and Debarbieux, 2012; Weiss et al., 2009).
69 Likewise, we showed that phage replication in homogenized intestinal sections from colonized mice
70 (*ex vivo*) is different not only from that *in vitro* but also between different gut sections (Galtier et al.,
71 2017; Lourenço *et al.*, 2020; Maura et al., 2012a). These findings suggest that the gut environment
72 modulates phage-bacterium interactions.

73

74 Here, we aimed to identify bacterial genes for which regulation within the digestive tract modifies
75 bacterial susceptibility to phages. We performed a genome-wide transcriptomic analysis comparing
76 *in vitro* and *in vivo* conditions for the *Escherichia coli* strain 55989. This strain is an enteroaggregative
77 O104:H4 clinical isolate (EAEC pathotype frequently associated with traveller's diarrhoea) (Lääveri et
78 al., 2020) that is known to form biofilms and that carries a pAA plasmid encoding virulence

79 determinants (Touchon et al., 2009). We previously isolated and characterized three virulent phages
80 (*Podoviridae* CLB_P1, *Myoviridae* CLB_P2 and *Siphoviridae* CLB_P3) infecting strain 55989 (Maura et
81 al., 2012b). Associated in a cocktail, these three phages rapidly decreased the intestinal load of strain
82 55989 colonizing the gut of conventional mice (Maura *et al.*, 2012a), before to promote a long-term
83 coexistence with this strain (Maura *et al.*, 2012b).

84

85 We identified chromosome- and plasmid-encoded genes differentially expressed by the *E. coli* strain
86 55989 during gut colonisation, encoding proteins with functions relating to iron acquisition, aerobic
87 and anaerobic respiration, sugar metabolism, motility, adhesion, aggregation, biofilm regulation and
88 LPS biosynthesis. We studied four of these genes (*bssR*, *fliA*, *lscC* and *rfaL*), encoding proteins with
89 functions expected to affect phage-bacterium interactions. Using a combination of phenotypic assays
90 we experimentally confirmed that each of these four genes modulated phage-bacterium interactions
91 for at least one of the phages. This study reveals a molecular mechanism, ie the modification of
92 bacterial gene expression by the gut environment, which influences the susceptibility of bacteria to
93 phages, thereby contributing to the coexistence of these two antagonistic populations.

94

95 **Results**

96 **The efficacy of phage replication on *E. coli* cells grown *in vitro* differs from homogenized *E. coli*-** 97 **colonized gut samples**

98 We previously tested individually the ability of phages CLB_P1, CLB_P2 and CLB_P3 to establish a
99 long-term coexistence with the strain 55989 colonizing conventional mice. Each of these phages
100 behave differently. Phage CLB_P2 lasted over four weeks, while CLB_P3 and CLB_P1 fell below the
101 threshold of detection after eleven and four days, respectively (Maura and Debarbieux, 2012). These
102 data together with *in vitro* and *ex vivo* phage replication assays performed on 55989 cells (Maura *et*
103 *al.*, 2012a) supported the hypothesis that the regulation of bacterial genes may influence phage-
104 bacteria interactions. To test this hypothesis, we aimed to perform *in vivo* transcriptomics on

105 samples from monocolonized mice (axenic mice colonized only with strain 55989). First, we assessed
106 the individual phage replication on 55989 cells recovered from homogenized gut sections (ileum and
107 colon) of monocolonized mice, as well as on 55989 cells collected from liquid medium during the
108 exponential and stationary growth phases (Fig. 1). As a control, we tested the stability of the three
109 phages in absence of strain 55989 and found that during the assay (5 h) the number of phages
110 decreased by about 1-log in LB and gut samples from axenic mice (Fig. 1, left). We also evaluated the
111 behaviour of exponential and stationary grown 55989 cells when added to gut homogenates from
112 axenic mice during 5 h. We found that exponentially grown cells were capable to grow by about 2-log
113 in both ileum and colon sections, while stationary grown cells could not (Fig 1, right).

114 The three phages displayed an amplification of three to four orders of magnitude relative to the
115 initial phage dose on exponentially growing 55989 cells (Fig. 1, central). In sharp contrast, the level of
116 phages CLB_P1 and CLB_P3 recovered after incubation with cells collected during the stationary
117 growth phase was several orders of magnitude lower than the initial dose, showing that these
118 phages did not amplify, whereas phage CLB_P2 displayed a moderately impaired amplification (two
119 orders of magnitude lower compared to the exponential growth condition) (Fig. 1 central part). The
120 strong reduction of the amount of recovered phage CLB_P1 upon incubation with stationary cells
121 suggests that it binds to cells but is unable to infect them. A similar situation was previously reported
122 for phage T7 exposed to *E. coli* cells lacking *flhDC* and overexpressing curli in biofilms (Vidakovic et
123 al., 2018). Perhaps, the inability of phage CLB_P1 to persist in the murine gut could be linked to its
124 inability to infect 55989 cells colonizing the gut of mice.

125 On homogenized ileal samples from monocolonized mice, the three phages amplified to a similar
126 degree to cells collected during exponential growth. On colon samples from monocolonized mice,
127 CLB_P2 and CLB_P3 amplification were similar to that on ileal samples, but the amplification of
128 CLB_P1 was strongly reduced by 4-log (Fig. 1 central part). These data are consistent with a previous
129 assessment of the amplification of these phages in intestinal sections collected from conventional
130 mice colonized with strain 55989, demonstrating that the presence of other microbes was not

131 involved in the differences observed between *in vitro* and *ex vivo* conditions (Maura *et al.*, 2012a).
132 Therefore, the replication of each of the three phages was differentially affected by the growth of
133 strain 55989 in different environments. Since the regulation of gene expression is a hallmark of the
134 adaptation of bacteria to changing environments, we next looked for relationships between bacterial
135 gene expression and phage-bacterium interactions.

136

137 ***E. coli* strain 55989 experiences metabolic shifts during gut colonisation**

138 For identification of the set of genes from strain 55989 specifically expressed in the gut, we
139 compared the genome-wide pool of mRNAs extracted in three sets of growth conditions: i) *in vitro*
140 exponential growth (OD₆₀₀=0.5; “exponential” samples *n*=4); ii) *in vitro* stationary growth (equivalent
141 OD₆₀₀=5; “stationary” samples *n*=4); iii) *in vivo* growth in the colon of monocolonized mice (“colon”
142 samples *n*=4; see methods). A principal component analysis (PCA) showed that the three conditions
143 were very different and, within each condition, the replicates were very similar resulting in a
144 remarkable differentiation and supporting further analysis (Fig. 2A). Interestingly, the global gene
145 expression pattern of colon samples was more similar to exponential samples than to stationary
146 samples (Fig. 2B). We then compared the transcriptomes of the colon with exponential and
147 stationary samples separately, to identify the set of genes differentially expressed between these
148 sets of conditions (Fig. 2C). Next, we cross-checked genes from these comparisons with the
149 differential expressed genes between exponential and stationary samples to remove those in
150 common and identify genes differentially expressed during the growth of strain 55989 in the colon.
151 We obtained a list of 156 over and 53 underexpressed genes, the functions of which were
152 determined using a Gene Ontology analysis (<http://geneontology.org/>) and the EcoCyc database
153 (Keseler *et al.*, 2011) (Tables S1 and S2).

154

155 A large proportion of the functions of genes overexpressed in the colon concerned sugar metabolism
156 and transport (Fig. 3A and Table S1A). The most significantly overexpressed gene, *ompG*, encodes a

157 specific porin involved in carbohydrate transport (Fajardo et al., 1998). Several carbohydrate
158 pathways (sucrose, gluconate, glucurate, galactarate and galactonate) were overexpressed,
159 consistent with previous reports of *E. coli* adaptation to the gut environment (Conway and Cohen,
160 2015; Fabich et al., 2008; Lourenço et al., 2016). We also found that genes involved in carnitine
161 metabolism (*fixA*, *fixB*, *fixC* and *caiT*), were overexpressed. Furthermore, genes involved in the
162 utilization of ethanolamine (*eutN*, *eutG*, *eutJ* and *eutP*) were overexpressed.

163 As expected, we observed differential expression for genes involved in the transition from aerobic to
164 anaerobic environments. Together with the genes involved in carnitine metabolism, the *frdBCD*
165 genes encoding the fumarate reductase, and *fumB*, encoding a fumarase were all found to be
166 overexpressed, as previously reported (Condon and Weiner, 1988; Meadows and Wargo, 2015;
167 Woods and Guest, 1987). Conversely, the full operon *cyoABCDE*, encoding the subunits of the
168 cytochrome bo terminal oxidase and haem O synthase, was strongly underexpressed, a hallmark of
169 decreased oxygen availability (Fig. 3B and Table S1B) (Cotter et al., 1990). The second most
170 significantly underexpressed set of genes was related to iron acquisition and included enterobactin
171 biosynthesis and export genes (*entC*, *entS*, *fepD*, *fhuD*), together with *fecI*, *yncD* and *yncE* genes, all of
172 which encode proteins with functions relating to iron transport. These observations validated our
173 approach to identify bacterial genes whose expression could affect phage-bacteria interactions in the
174 gut, but also highlighted a number of specific features putatively involved in the intestinal
175 colonization process of this enteroaggregative *E. coli* strain and that require further investigations.

176

177 **Plasmid-encoded virulence determinants are upregulated during *E. coli* growth in the gut**

178 The EAEC strain 55989 carries a plasmid (p55989) from the pAA family (Croxen and Finlay, 2010) that
179 harbours several genes encoding factors involved in adhesion to epithelial cells (Boll et al., 2017;
180 Weintraub, 2007). In our analysis of *in vivo* differentially expressed plasmid-encoded genes, we
181 identified seven overexpressed genes, four of them with functions relating to adhesion (*agg3B*,
182 *agg3C*, *agg3D* and *p55989_0069*) (Fig. S1; Table S1C), consistent with the phenotype of EAEC strains,

183 which form aggregates at the surface of intestinal cells. We also found two plasmid genes encoding
184 putative transposases to be overexpressed, consistent with the mechanisms involved in the genetic
185 adaptation of *E. coli* cells to the gut environment (Barroso-Batista et al., 2014; Lourenço *et al.*, 2016).
186 Regarding other mobile genetic elements, the *in silico* analysis of the genome of strain 55989
187 revealed that it contains seven intact prophage regions, one incomplete and one questionable (see
188 methods). None of these regions were differentially expressed in our RNA-Seq data.

189

190 **Comparative transcriptomic analysis highlights bacterial genes related to phage infection**

191 Since phage replication is altered in both stationary phase and colon compared to exponential phase
192 samples (Fig. 1), we analysed the above transcriptomics dataset by performing pairwise comparisons
193 between exponential phase with either colon or stationary phase samples in order to identify genes
194 potentially linked to these changes in phage replication. Then, we removed genes differentially
195 expressed between colon and stationary phase samples (see methods and Fig. 2D). In total, 238
196 genes (73 overexpressed and 143 underexpressed) were identified, about 50% of which have no
197 known function (Table S1D and S1E, respectively). The underexpressed genes (Table S1E) with known
198 functions included genes involved in the biosynthetic pathway for flagella (including *fliA*) and LPS
199 (including *rfaL*), both of which act as cell wall receptors for phages (Choi et al., 2013; Feugeas et al.,
200 2016; Shin et al., 2012). All genes encoding proteins involved in the flagellum apparatus were indeed
201 underexpressed, suggesting that phages relying on this structure for entry would less efficiently
202 infect strain 55989 in both stationary phase and colonic samples. None of the overexpressed genes
203 with known functions were previously associated with phage infection (Table S1D). However, the
204 most significantly overexpressed gene, *bssR*, has been implicated in biofilm formation, and another
205 two genes (*lsrC* and *ego*) are involved in the import of autoinducer-2, a quorum sensing molecule.
206 Interestingly, the function of these genes is related to bacterial community lifestyle and could
207 possibly affect phage-bacterium interactions as previously shown for *Vibrio cholera* (Hoque et al.,

208 2016; Pires et al., 2021). Therefore, we experimentally tested the contribution of these four gene
209 (*bssR*, *fliA*, *lsrC* and *rfaL*) to the infectivity of each of the three phages.

210 The impact on phage-bacterium interactions of the two underexpressed genes, *fliA*, the master
211 regulator of flagellum assembly (Helmann and Chamberlin, 1987) and *rfaL* (also known as *waal*),
212 encoding the O-antigen ligase (Klena et al., 1992), was assessed using 55989 strains in which either
213 gene was deleted (55989 Δ *fliA* and 55989 Δ *rfaL*). To investigate the role of the two overexpressed
214 genes, *bssR*, which encodes a global transcriptional regulator involved in biofilm formation (Domka et
215 al., 2006), and *lsrC*, which encodes a subunit of the autoinducer-2 membrane transporter (Xavier and
216 Bassler, 2005), we introduced a plasmid carrying either of these genes, under the control of an IPTG-
217 inducible promoter, into strain 55989.

218

219 **The lack of *rfaL* decreases the susceptibility of strain 55989 to phage CLB_P1**

220 We first assessed the efficiency of plating (EOP) of phage CLB_P1 and found that it dropped by 3-log
221 (2.10×10^{-3} +/- 3.11×10^{-4}) on the 55989 Δ *rfaL* strain, but remained unchanged in the 55989 Δ *fliA*
222 strain (1.19 +/- 0.28) as well as in the strain 55989 overexpressing either *bssR* (1.08 +/- 0.33) or *lsrC*
223 (1.18 +/- 0.45) genes. The growth and lysis of cells over time in liquid medium following exposure to
224 phage CLB_P1 confirmed these results with notably the lack of lysis of the 55989 Δ *rfaL* strain (Fig.
225 4A,B). As *rfaL* encodes the O-antigen ligase we hypothesized that the O-antigen could act as a
226 receptor for this phage. In fact, phage CLB_P1 could not bind to the 55989 Δ *rfaL* strain compared to
227 either the WT or the 55989 Δ *fliA* strain (Fig 4C,D,E). Moreover, cells aggregation by a O104 serotype
228 specific antibody was prevented when 55989 cells were incubated with phage CLB_P1 but not with
229 another unrelated phage, showing that phage CLB_P1 recognizes the O-antigen part of the LPS as a
230 receptor (Fig 4F). We then evaluated the impact of phage CLB_P1 when added to 24 h-old biofilms
231 formed by either 55989 Δ *fliA*, 55989 Δ *rfaL*, 55989-*pbssR* or 55989-*plsrC* strains (Fig. S2). The
232 overexpression of either *bssR* or *lsrC* genes had no significant effect on the amount of biofilm
233 produced compared to the strain 55989 carrying the empty plasmid between 24 and 48 h in absence

234 or presence of phage CLB_P1 as assessed by crystal violet quantification (Fig. S2A and Table S2A and
235 S2B). Likewise, the amount of CFUs recovered at 48 h from the corresponding experiments did not
236 revealed any significant differences (Fig. S2B and Table S2A and S2B). We obtained similar results
237 with 55989 Δ *fliA* and 55989 Δ *rfaL* strains (Fig. S2C,D and Table S2A and S2B). In conclusion, these data
238 show that the downregulation of *rfaL* when 55989 cells inhabit the colon of mice likely impairs their
239 infection by phage CLB_P1, which by itself does not affect biofilm formation.

240

241 **Phage CLB_P2 infection is insensitive to the lack of *rfaL* or *fliA* genes as well as the overexpression**
242 **of *bssR* or *IsrC* genes**

243 Neither the EOP of CLB_P2 (Table S2C) nor the lysis of 55989 Δ *fliA* or 55989 Δ *rfaL* strains, or the lysis
244 of 55989 cells carrying the plasmids *pbssR* or *pIsrC*, was affected in comparison to their
245 corresponding control strains (WT and *empty*), showing that none of these genes interfere with
246 CLB_P2 infection in these growth conditions (Fig. 5A,B). Unexpectedly, the presence of CLB_P2
247 significantly increased the amount of biofilm when either *bssR* ($p=0.0029$) or *IsrC* ($p=0.0007$) were
248 IPTG-induced, in comparison with the control strain (Fig 5C and Table S2A). This was not correlated
249 to changes of CFUs in the overexpressing strains compared to the control (Fig 5D and Table S2B). We
250 even noticed that the presence of CLB_P2 led to a significant reduction of the level of CFUs of all
251 strains compared to its absence ($p=0.005$ and below) (Fig. 5D and Table S2B). This moderate drop of
252 1 to 2-logs of CFUs indicates that some phage CLB_P2 infected cells embedded in the biofilm matrix.
253 We hypothesize that this phage displays some affinity to the biofilm matrix, which leads to carry over
254 some particles during the washing steps before cells are resuspended and mixed with this residual
255 phage population. This was further supported by the data obtained from biofilm assays performed
256 with 55989 Δ *fliA* or 55989 Δ *rfaL* strains and their plasmid-complemented counterparts. For all of
257 these strains phage CLB_P2 did not change the amount of biofilms produced but its presence led
258 again to a significant reduction of the level of CFUs recovered (Fig. S3AB and Table S2A and S2B).
259 Therefore, none of the four candidate genes were affecting the capacity of phage CLB_P2 to infect

260 55989 cells, but we uncovered that the presence of CLB_P2 increases biofilm formation by cells
261 overexpressing genes related to community lifestyle, perhaps as a defence response against this
262 phage.

263

264 **Phage CLB_P3 strongly promotes biofilm formation of 55989 Δ *fliA* and 55989 Δ *rfaL* strains**

265 The EOP of phage CLB_P3 (Table S2C) on strain 55989 Δ *rfaL* was slightly higher compared to the
266 control, while it remains unchanged for the strains 55989 Δ *fliA*, 55989*pbssR* and 55989*p/srC*. The lysis
267 kinetics of strain 55989 Δ *rfaL* by CLB_P3 was not affected during early time points in contrast to late
268 time points, compared to all the other strains tested that were not different from their respective
269 controls (WT and *empty*) (Fig 6A,B). We then observed that the presence of CLB_P3 significantly
270 increased the amount of biofilm formed by both the 55989 Δ *fliA* ($p=0.0040$) and 55989 Δ *rfaL*
271 ($p<0.0001$) strains (Fig 6C and Table S2B). Particularly surprising was the amount of biofilm formed by
272 the 55989 Δ *rfaL* strain that increased by a factor of 5 on the OD scale compared to the highest values
273 observed in all the other conditions tested (0.1 vs 0.02). When *trans*-complemented with a plasmid
274 expressing either *fliA* or *rfaL*, the corresponding defective strains formed as much biofilm as the wild
275 type strain (Fig. 6C). This large increase in biofilms was not correlated to an increase in CFUs (Fig 6D
276 and Table S2C). Moreover, adsorption assays of phage CLB_P3 revealed that its affinity to each of the
277 55989 Δ *fliA* and the 55989 Δ *rfaL* strains was nearly 1-log lower compared to wild type strain,
278 excluding the hypothesis that a stronger binding of this phage elicits an elevated biofilm formation
279 (Fig. S4). In contrast, no significant impact of phage CLB_P3 was observed on the amount of biofilms
280 formed by strains overexpressing either *bssR* or *IsrC* (Fig. S5A and Table S2A). Nevertheless, the
281 amount of CFUs recovered from 55989 *p/srC* biofilms exposed to CLB_P3 was significantly higher
282 compared to the corresponding control cells ($p=0.0016$), a result that is the opposite of those
283 obtained with phage CLB_P2 (Fig. S5B and Table S2B). Here we found that cells that are lacking either
284 *fliA* or *rfaL* genes strongly increase biofilm formation only in presence of phage CLB_P3, perhaps
285 eliciting a phage defence system.

286

287 **The replication of phage CLB_P1 is abolished in the ileum of 55989 Δ *rfaL*-colonized mice.**

288 The two strongest phenotypes associated to the four gene candidates were observed with phage
289 CLB_P1 that is severely impaired in infecting 55989 Δ *rfaL* cells, and phage CLB_P3 that is strongly
290 promoting biofilm formation of both 55989 Δ *fliA* and 55989 Δ *rfaL* cells. We then asked to which
291 extent these phenotypes would translate into modifications of phage-bacteria interactions in the
292 mouse gut. For this we tested the *ex vivo* replication of the three phages (CLB_P2 serving as a control
293 phage) in intestinal organs from 55989 Δ *fliA*- and 55989 Δ *rfaL*-colonized mice. As expected, we found
294 that the amplification of phage CLB_P1 is strongly affected on ileal and colonic homogenates from
295 55989 Δ *rfaL*-colonized mice, compared to phages CLB_P2 and CLB_P3 (Fig. 7). These data
296 demonstrate that a 3-log reduction of the EOP observed *in vitro* translates into a weak replication in
297 organ homogenates, preventing the further *in vivo* evaluation of phage CLB_P1. Results obtained
298 with homogenates from 55989 Δ *fliA*-colonized mice were not dissimilar to those obtained from wild-
299 type-colonized mice for the three phages (Fig. 1) showing that this gene has no impact on phage
300 infection in organs (Fig. 7). Therefore, the increased biofilm formation promoted by the presence of
301 CLB_P3 during the *in vitro* assays does not translate into an impaired phage infection in organs.
302 Nevertheless, during this assay we observed that the number of CFUs of the 55989 Δ *fliA* strain did not
303 increase in the colon compared to the ileal samples. This suggests that some genes under *fliA*
304 regulation may be required for optimal growth in this gut section. This could also explain the trend of
305 a lower amplification in colon compared to ileal sections observed with the three phages.

306

307 **Discussion**

308 Bacteria and virulent phages coexist over time in the digestive tract of mammals, raising questions
309 about the predator-prey dynamics of these two antagonistic populations (Lourenço *et al.*, 2020;
310 Mirzaei and Maurice, 2017). Here, we investigated whether the regulation of bacterial gene
311 expression occurring during their colonisation of the gut environment affects their susceptibility to

312 phages (Lourenço et al., 2018). We performed a genome-wide transcriptomic analysis of *E. coli* strain
313 55989, an enteroaggregative pathogenic strain for which we had already characterised three virulent
314 phages, CLB_P1, CLB_P2 and CLB_P3.

315 Comparisons of the transcriptomics data from colon samples with exponentially and stationary
316 grown cells revealed the differential expression of several genes. Besides expected genes related to
317 anaerobic growth as well as sugar metabolism and transport in the gut, we identified some genes
318 related to host adaptation. For instance, the overexpression of *ompG* implies the activation of the
319 transcriptional regulator *ycjW* (Luhachack et al., 2019), which in turns also regulates the production
320 of H₂S, which has been shown to protect pathogens against the host immune response (Toliver-
321 Kinsky et al., 2019). We also report the overexpression of genes involved in the metabolism of
322 carnitine and ethanolamine, which are compounds derived from the host cells membrane. Both are
323 known to play roles in the gut microbiota with carnitine being used for osmoprotection or as a source
324 of nutrients (Meadows and Wargo, 2015), while ethanolamine has been previously shown to be used
325 by enteroaggregative *E. coli* for improving its ability to outcompete commensal *E. coli* (Bertin et al.,
326 2011). Amongst underexpressed genes we found several iron acquisition systems, including
327 enterobactin. This counterintuitive observation, since it is well established that iron acquisition
328 systems are required for gut colonisation (Deriu et al., 2013), must be counterbalanced by the
329 diversity of multiple iron acquisition systems identified in *E. coli* genomes, some of which, like Feo,
330 are expressed in anaerobic conditions (Lau et al., 2016). Moreover, *fumB*, the expression of which
331 decreases in conditions of iron limitation, was found to be overexpressed in the colon, suggesting
332 that iron supply was not limiting in our experimental conditions (Fig. 3A and Table S1A). In addition,
333 the analysis of the 55989 plasmid-encoded genes identified four overexpressed genes with functions
334 related to adhesion. Together with the concomitant overexpression of *uspF* (Table S1A) and
335 underexpression of *ompX* (Table S1B), both located on the chromosome, these observations support
336 that bacterial motility is reduced in the colon (Nachin et al., 2005; Otto et al., 2001). Beyond the
337 specificities linked to this particular enteropathogenic strain of *E. coli*, which would deserve further

338 investigations, our data are congruent with similar studies using comparative transcriptomics
339 between *in vitro* and *in vivo* conditions for intestinal bacteria (Denou et al., 2007).

340 The untargeted approach (whole intestinal sections) used for this work has some limitations despite
341 its success for identifying genes involved in phage susceptibility. For instance, the transcriptomic
342 profiles were treated as belonging to a single homogeneous bacterial population, whereas bacteria
343 within an intestinal section actually face different physiological conditions (Li et al., 2015). Moreover,
344 about 50% of the candidate genes identified had no predicted functions. Some may then encode
345 proteins involved in phage-bacterium interactions, warranting additional studies to determine their
346 mode of action.

347 We showed that four of the genes identified from transcriptomic comparisons alter the interactions
348 of strain 55989 with at least one of the three phages, CLB_P1, CLB_P2 or CLB_P3. When
349 overexpressed or deleted, these genes led to phenotypes that could be related to a global response
350 protecting cells against phages. However, the elicited response was specific to each of the three
351 phages (only 55989 Δ *rfaL* cells for CLB_P1, both 55989*pbssR* and 55989*plsrC* cells for CLB_P2 and
352 both 55989 Δ *fliA* and 55989 Δ *rfaL* cells for CLB_P3), highlighting the complexity of predicting the *in*
353 *vivo* efficacy of phages from *in vitro* experiments.

354 In *E. coli*, LPS is one of the receptors most frequently used by phages (Hantke, 2020). However, as
355 K12 strains (the most frequently used in laboratories) do not synthesise O-antigen (Liu and Reeves,
356 1994), *rfaL* (the gene encoding the O-antigen ligase) has not previously been identified as a gene
357 involved in phage infection including a recent systematic study (Maffei et al., 2021). Here we
358 demonstrated that phage CLB_P1 recognizes the O104 antigen as a receptor, consistent with its host
359 range towards the ECOR collection (Maura *et al.*, 2012b). Interestingly, the lack of *rfaL* does not
360 completely abolish the capacity of phage CLB_P1 to form plaques on the 55989 Δ *rfaL* lawns,
361 suggesting that CLB_P1 recognizes a secondary receptor. However, neither in liquid broth with
362 agitation nor in gut homogenates this secondary receptor was sufficient to allow phage CLB_P1
363 infection. Therefore, the low rates of replication of phage CLB_P1 on cells in the stationary phase or

364 in colon samples (Fig. 1) can be attributed to the underexpression of *rfaL* identified during the
365 comparative analysis of transcriptomics data. More broadly, when considering application in human,
366 our data suggest that a phage that displays a 3-log reduction of the EOP on a clinical isolate would
367 likely be inefficient *in vivo*.

368 The strong stimulation of biofilm formation by the CLB_P3 phage in the 55989 Δ *fliA* and 55989 Δ *rfaL*
369 strains was both striking and puzzling, as the functions of these genes are unrelated to each other.
370 Furthermore, these two strains remained as susceptible to CLB_P3 as the WT on both solid and liquid
371 media. It is therefore tempting to speculate that the presence of CLB_P3 led to an increase in the
372 production of an extracellular polysaccharide matrix in these two mutant strains but not in the wild-
373 type, perhaps as a defence against phage CLB_P3. Since *fliA* is a sigma factor, we hypothesize that its
374 absence unlocks a genetic regulation that is otherwise inactive, only in the presence of phage CLB_P3
375 but not of CLB_P1 or CLB_P2. As the lack of *rfaL* affects the structure of the LPS, we hypothesize that
376 the presence of CLB_P3, but not of CLB_P1 or CLB_P2, uncovers a bacterial receptor not involved in
377 phage infection but instead in a signalling process promoting matrix production, perhaps as a phage
378 defence system. Interestingly, these two hypotheses are not exclusive.

379 The presence of phage CLB_P2 increased the formation of biofilms by strains overexpressing either
380 *bssR* or *lscC* with a similar amplitude. Again, it is tempting to suggest that the increase in biofilm
381 formation is linked to the induction of a phage defence mechanism, as exopolysaccharide production
382 has been shown to protect cells from phage infection in many bacterial species (Darch et al., 2017; de
383 Sousa et al., 2020; Radke and Siegel, 1971). It is worth noting that the presence of CLB_P2, compared
384 to its absence, resulted in the recovery of significantly fewer cells from biofilm for all strains tested.
385 This observation suggests that CLB_P2 may be the most potent of the three phages at killing cells
386 embedded in biofilms. Alternatively, this phage may have a loose affinity for the biofilm matrix (Barr
387 et al., 2013) and decrease the number of CFUs at the plating step instead of within the biofilm.

388 The phenotypes observed with the four genes converged to lower bacterial susceptibility to phages,
389 in other words increasing phage resistance, in both the presence (*in vitro*) and absence (*in vivo*) of

390 phages. This suggests that bacteria become less susceptible to phages when they colonize the gut.
391 Here, the formation of biofilms at the surface of epithelial cells could be invoked as a phage-defence
392 response that could have a negative impact on health when such biofilms are linked to virulence as
393 for the enteroaggregative strain 55989. Moreover, gene regulation entails a lesser fitness cost than
394 loss-of-function due to genetic mutations, which may be crucial to ensure persistence within a
395 fluctuating environment including the competition between bacteria.

396 Experiments in animals with a cocktail of these three phages, or other phage cocktails, have shown
397 that despite initially reduction of bacterial levels in the gut, coexistence of phages with their hosts is
398 rapidly established (Hsu et al., 2019; Maura *et al.*, 2012b). This coexistence is supported by multiple
399 overlapping mechanisms, including the regulation of bacterial gene expression, as shown here
400 (Javaudin et al., 2021; Lourenço *et al.*, 2018). Aligning the composition of phage cocktails to the
401 environmental conditions in which they are to be used, may be the best option for optimizing phage
402 applications. In addition, repeated phage applications, prescribed like a standard anti-microbial drug,
403 might result in more effective treatments by preventing the establishment of coexistence.

404 In conclusion, tripartite interactions occur between phages, bacteria and the host, all of which
405 influence each other. The mammalian gut environment and its response to bacterial colonisation
406 affects bacterial gene expression, which in turns influences phage infection. Conversely, phage
407 variants may be selected to improve efficacy against established bacterial clones by adaptation (De
408 Sordi et al., 2017; Mathieu et al., 2020), potentially inducing a response in the bacteria (De Sordi *et*
409 *al.*, 2019) affecting their relationship with the host (intestinal epithelial and immune cells) (Diard et
410 al., 2017; Sausset et al., 2020). Globally, the balance between these equilibria governs gut
411 homeostasis and health.

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414 between lists of genes from the different samples and for critically reading the manuscript. We thank
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427

428 **Author contributions**

429 Conceptualization and Methodology, L.D., L.D.S. and M.L. Investigations, L.C., M.L., M.T., O.S., Q.L.B.
430 and T.P. Formal analysis, J.Y.C., R.L., M.L. and H.V. Resources, MB. Supervision and Funding
431 Acquisition L.D. Writing - Original Draft, M.L. and L.D. Writing – Review and Editing, L.C., L.D., L.D.S.,
432 Q.L.B., M.L. and T.P.

433

434 **Declaration of interest**

435 None to declare

436

437 **Main figure titles and legends**

438 **Figure 1. The replication of phages CLB_P1, CLB_P2 and CLB_P3 on strain 55989 is affected by** 439 **growth conditions *in vitro* and *ex vivo***

440 Phages CLB_P1, CLB_P2 or CLB_P3 were added to LB (green) or to homogenized gut sections (ile,
441 ileum, dark blue; col, colon, light blue) from axenic mice and incubated during 5 h before
442 quantification (left part). The amplification of phages (CLB_P1, CLB_P2, CLB_P3, each added at an
443 MOI of approximately 0.01) is reported after 5 h of incubation with strain 55989 (central part) in the
444 indicated conditions: exponentially growing cells (exp, dark green; $OD_{600}=0.5$); cells in stationary
445 phase (sta, light green; equivalent $OD_{600}=5$); homogenized gut sections from monoclonized mice
446 (ile, ileum, dark blue; col, colon, light blue). 55989 cells collected from exponential or stationary
447 growth conditions were incubated with homogenized gut sections from axenic mice during 5 h and
448 their density was reported (right part). Median n-fold multiplication is shown relative to the initial
449 number of plaque-forming units (PFUs) or colony-forming units (CFUs). n=2 to 5 biological replicates
450 with 1 to 3 technical replicates.

451

452 **Figure 2. The mRNA content of *E. coli* cells growing in the gut is different from that of cells grown *in*** 453 ***vitro***

454 **A.** Cells of *E. coli* strain 55989 were collected from *in vitro* cultures (LB medium, 37°C with shaking) at
455 an OD_{600nm} of 0.5 (exponential, $n=4$) and an equivalent OD_{600nm} of 5 (stationary, $n=4$) or from *in vivo*
456 tissues (colon of monoclonized mice, $n=4$) and their mRNA was extracted, sequenced and subjected
457 to principal component analysis (biological variability was the main source of variance). **B.** Heatmap
458 and dendrogram obtained for VST-transformed data for sequenced mRNAs from the samples
459 analysed in A (see methods). **C.** Numbers of overexpressed (O/E) and underexpressed (U/E) genes
460 from pairwise comparisons between colon and either exponential or stationary phase samples. **D.**
461 Numbers of overexpressed (O/E) and underexpressed (U/E) genes from pairwise comparisons
462 between exponential and either stationary or colon samples.

463

464 **Figure 3. The growth of *E. coli* strain 55989 in the colon involves the activation of carbohydrate**
465 **metabolism and a decrease in aerobic respiration**

466 A network analysis was performed, based on translated protein–protein interactions, with the
467 STRING webserver (string-db.org) using strain MG1655 as template, on genes overexpressed (**A**) and
468 underexpressed (**B**) in the colon of monocolonized mice relative to cells in the stationary and
469 exponential growth phases. The full list of genes analysed is available in Tables S1 and S2. The large
470 closed circles (orange for overexpressed and blue for underexpressed genes) correspond to pathways
471 indicated in bold and discussed in the text.

472

473 **Figure 4. Phage CLB_P1 does not infect 55989 Δ *rfaL* cells and recognizes the O104-antigen moiety of**
474 **the LPS.**

475 **A.** Growth curves in LB of the *E. coli* strains 55989 (WT), 55989 Δ *rfaL* and 55989 Δ *fliA* (supplemented
476 with kanamycin for Δ *rfaL* and Δ *fliA* strains), in the presence and absence of phage CLB_P1, added at
477 $t=0$, at an MOI of 0.01 ($n=2$ to 3 for each set of conditions). Error = standard error of the mean.

478 **B.** Growth curves in LB of *E. coli* strain 55989 (WT), WT+*pbssR* (supplemented with 0.05 mM IPTG)
479 and WT+*plsC* (supplemented with 0.01 mM IPTG) in presence or absence of phage CLB_P1 added at
480 $t=0$ and a MOI of 0.01 ($n=2$ to 3 for each condition). Errors = standard error of the mean.

481 **C, D, E.** The adsorption of phage CLB_P1 on strains 55989 Δ *rfaL* (C), 55989 (D) and 55989 Δ *fliA* (E) was
482 evaluated according to standard procedures (see methods). Adsorption constants and adsorption
483 kinetics (90% of phages bound) were calculated by applying an exponential decay function to the
484 corresponding data.

485 **F.** Microscopic observations of 55989 cells (1×10^7 CFU) mixed with either PBS or phage CLB_P1
486 (2.5×10^7 PFU) or a *Pseudomonas aeruginosa* phage (2.5×10^7 PFU) during 10 min, followed by the
487 addition of either O104 anti-serum or PBS. Scale bar, 20 μ m

488

489 **Figure 5. Phage CLB_P2 increases the formation of biofilms in 55989 cells overexpressing either**
490 ***bssR* or *lsrC* genes.**

491 **A.** Growth curves in LB of *E. coli* strain 55989 (WT), WT+*pbssR* (supplemented with 0.05 mM IPTG)
492 and WT+*pIsrC* (supplemented with 0.01 mM IPTG) in presence or absence of phage CLB_P2 added at
493 $t=0$ and a MOI of 0.01 ($n=2$ to 3 for each condition). Errors = standard error of the mean.

494 **B.** Growth curves in LB of the *E. coli* strains 55989 (WT), 55989 Δ *rfaL* and 55989 Δ *fliA* (supplemented
495 with kanamycin for Δ *rfaL* and Δ *fliA* strains), in the presence and absence of phage CLB_P2, added at
496 $t=0$, at an MOI of 0.01 ($n=2$ to 3 for each set of conditions). Error = standard error of the mean.

497 **C.** Biofilm formation, reported as variations of OD_{570nm} recorded at 48 h relative to 24 h for the
498 indicated *E. coli* strains in the presence or absence of phage CLB_P2 added at 24 h (1×10^7 PFU/mL)
499 and IPTG at 0.05 mM for empty and *bssR* plasmids or 0.01 mM for the *lsrC* plasmid, as well as
500 kanamycin; $n=3$ to 4 independent experiments. The boxes indicate the interquartile range; the
501 horizontal bars correspond to the median and the vertical bars indicated the minimum and maximum
502 values (within 1.5 interquartile intervals), $p=p$ -value of the Tukey post-hoc tests (Table S2A).

503 **D.** Number of CFU resuspended from biofilms recovered from microplates set up in parallel to those
504 used for biofilm quantification shown in panel C (see methods).

505

506 **Figure 6. Phage CLB_P3 increases biofilms formation in 55989 cells deleted for either *fliA* or *rfaL***
507 **genes.**

508 **A.** Growth curves in LB of *E. coli* strain 55989 (WT), WT+*pbssR* (supplemented with 0.05 mM IPTG)
509 and WT+*pIsrC* (supplemented with 0.01 mM IPTG) in presence or absence of phage CLB_P3 added at
510 $t=0$ and a MOI of 0.01 ($n=2$ to 3 for each condition). Errors = standard error of the mean.

511 **B.** Growth curves in LB of the *E. coli* strains 55989 (WT), 55989 Δ *rfaL* and 55989 Δ *fliA* (supplemented
512 with kanamycin for Δ *rfaL* and Δ *fliA* strains), in the presence and absence of phage CLB_P3, added at
513 $t=0$, at an MOI of 0.01 ($n=2$ to 3 for each set of conditions). Errors = standard error of the mean.

514 **C.** Biofilm formation, reported as change in OD_{570nm} at 48 h relative to 24 h for the indicated *E. coli*
515 strains in the presence or absence of phage CLB_P3 added at 24 h (1x10⁷ PFU/mL); *n*=3 to 4
516 independent experiments. When necessary IPTG was added at 0.05 mM. The boxes represent the
517 interquartile range; the horizontal bars represent the median and the vertical bars represent the
518 minimum and maximum values (within 1.5 interquartile intervals), *p*=*p*-value for post hoc Tukey tests
519 (Table S2A).

520 **D.** Number of CFU resuspended from biofilms recovered from microplates set up in parallel with
521 those used to quantify biofilms shown in panel C (see methods).

522

523 **Figure 7. Phage CLB_P1 cannot replicate on intestinal homogenates of 55989Δ*rfaL*-colonized mice.**

524 Amplification (*n*=2 biological replicates) of phages (CLB_P1, CLB_P2, CLB_P3, each added at an MOI
525 of approximately 0.01) after 5 hours of incubation with either strain 55989Δ*rfaL* or 55989Δ*fliA* from
526 homogenized gut sections from either 55989Δ*rfaL*- or 55989Δ*fliA*-monocolonized mice (ile, ileum,
527 dark blue; col, colon, light blue). The median *n*-fold multiplication is shown relative to the initial
528 number of PFUs or CFUs.

529

530

531 **STAR Methods**

532 **Resource Availability**

533 **Lead Contact**

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

536

537 **Materials Availability**

538 The bacterial strains 55989 Δ *rfaL* and 55989 Δ *fliA* as well as phages are available from the lead
539 contact upon completion of a Materials Transfer Agreement (MTA).

540

541 **Data and Code Availability.**

542 RNA-Seq data have been deposited at GEO and are publicly available as of the date of publication.

543 Accession numbers are listed in the key resources table.

544 This paper does not report original code.

545 Any additional information required to reanalyze the data reported in this paper is available from the

546 lead contact upon request.

547

548 **Experimental models and subject details**

549 **Ethics statement**

550 C3H axenic mice (seven to nine weeks old) reared at Institut Pasteur (Paris, France) were housed in
551 an animal facility in accordance with Institut Pasteur guidelines and European recommendations.

552 Food and drinking water were provided *ad libitum*. Protocols were approved by the veterinary staff
553 of the Institut Pasteur animal facility (Ref.#18.271) and the National Ethics Committee
554 (APAFIS#26874-2020081309052574 v1). We used 17 mice (15 males and 2 female) for this study.

555

556 **Phages and bacterial strains**

557 The *Escherichia coli* strain 55989 was previously described (Mossoro et al., 2002) and other strains
558 are listed in the key resources table.

559 Phages CLB_P1 (KC109329.1), CLB_P2 (OL770107) and CLB_P3 (OL770108) have been described
560 elsewhere (Maura *et al.*, 2012b). The 55989 mutants were obtained by a three-step PCR in which
561 each gene (*rfaL* and *fliA*) was disrupted by the insertion of a kanamycin resistance marker gene by
562 lambda Red-mediated homologous recombination (Chaveroche et al., 2000). Primers used for this
563 study are listed in key resources table. ASKA plasmids (chloramphenicol resistant) were used for the
564 overexpression of *bssR* and *IsrC* and the complementation of 55989 Δ *rfaL* and 55989 Δ *fliA* strains
565 (Kitagawa et al., 2005).

566 Strains were routinely cultured in lysogeny broth (LB Lennox - BD), or on LB Lennox agar (BD) or
567 Drigalski agar (lactose agar with bromothymol blue and crystal violet- CONDA) plates, at 37°C. When
568 required for selection, streptomycin (100 μ g/mL) or kanamycin (100 μ g/mL) or chloramphenicol (30
569 μ g/ml) (Sigma) was added.

570

571 **Methods details**

572 ***Ex vivo* assay**

573 C3H axenic mice received 200 μ L of PBS or strains 55989 or 55989 Δ *fliA* or 55989 Δ *rfaL* (10^7 CFU
574 prepared from an overnight culture in LB at 37°C) in sterile sucrose sodium bicarbonate solution
575 (20% sucrose and 2.6% sodium bicarbonate, pH 8) by oral gavage. Three days later, they were killed,
576 and intestinal sections (ileum and colon) were collected and weighed. PBS was added to each sample
577 (1.75 mL for ileum and colon) before homogenisation (Oligo-Macs, Miltenyi Biotec). We dispensed
578 140 μ L of each homogenized sample into the wells of a 96-well plate and 10 μ L of each individual
579 phage was added, to reach an MOI of 1×10^{-2} , and the plate was incubated at 37°C. A fraction of the
580 homogenized samples was also serially diluted in PBS and plated on Drigalski medium for the
581 counting of *E. coli* colonies at $t=0$. Following five hours of incubation, samples were serially diluted in

582 PBS and plated on Drigalski medium and on LB agar plates overlaid with strain 55989. Both sets of
583 plates were incubated at 37°C overnight. The same procedure was followed for *in vitro* assays with
584 bacteria collected during the exponential (OD₆₀₀=0.5) or stationary (24 h; equivalent OD₆₀₀=5) growth
585 phases, at 37°C, with shaking. These cells were also incubated during 5 h with homogenized gut
586 samples from axenic mice as a control of experiments performed with samples from monocolonized
587 mice.

588

589 **Transcriptomics**

590 Strain 55989 was grown overnight at 37°C with shaking (n=4 replicates). Cultures were diluted in
591 fresh medium and incubated until they reached an OD_{600nm} of about 0.5, at which point, half the
592 volume was collected for RNA extraction, the other half being maintained at 37°C, with shaking, up
593 to 24 h at which time point they reached an equivalent OD_{600nm} of 5, corresponding to stationary
594 phase. RNA was extracted by centrifuging the cells and incubating them with TRIzol (Sigma T-9424)
595 for lysis.

596 For intestinal samples, axenic mice received 200 µL of strain 55989 (10⁷ CFU prepared from an
597 overnight culture in LB at 37°C) in sterile sucrose sodium bicarbonate solution (20% sucrose and 2.6%
598 sodium bicarbonate, pH 8) by oral gavage. Three days later, the mice were killed, and intestinal
599 sections were collected and immediately frozen in liquid nitrogen (ileum and colon). TRIzol was then
600 added to the frozen samples, which were homogenized (Oligo-Macs, Miltenyi Biotec). Total RNA
601 from both *in vitro* and *in vivo* samples was purified by standard organic extraction
602 (phenol/chloroform) followed by ethanol precipitation. It was then treated with the RNeasy mini kit
603 (Qiagen) for final purification, and the remaining genomic DNA was removed with an on-column
604 RNase-free DNase set protocol (Qiagen). RNA integrity was assessed with the Bioanalyser system
605 (Agilent) and RNA integrity number (RIN) and ribosomal ratio (23S/16S) were determined. We
606 obtained rRNA-depleted RNA with the Ribo-Zero rRNA depletion kit for eukaryotic and prokaryotic
607 RNA (Illumina). Libraries were prepared with the TruSeq Stranded RNA LT prep kit (Illumina), with

608 final validation on the Bioanalyser system. Final DNA quantification was performed with sensitive
609 fluorescence-based quantification assays ("Quant-It" assay kit and a QuBit fluorometer, Invitrogen).
610 Libraries were then normalised to a concentration of 2 nM and multiplexed. The samples were then
611 denatured at a concentration of 1 nM with 0.1 N NaOH at room temperature, and were finally
612 diluted to 9.5 pM for loading onto the sequencing flowcell. Sequencing was performed on an Illumina
613 HiSeq 2500 machine, producing 65 bp single-reads. A mean of 266 M and 2 M of reads was obtained
614 for colonic and in vitro samples, respectively.

615

616 **Data analysis**

617 After sequencing, we performed a first quality check with fastQC
618 (<https://www.bioinformatics.babraham.ac.uk/projects/fastqc/>), and then cleaned up the reads with
619 cutadapt (Martin, 2011). Bowtie was used for read alignment and files were transformed into BAM
620 and SAM formats with samtools software (Li et al., 2009). Finally, reads were counted with
621 featureCounts and the statistical analysis was performed with R software ([https://www.r-](https://www.r-project.org/)
622 [project.org/](https://www.r-project.org/))(Gentleman et al., 2004) packages including DESeq2 (Anders and Huber, 2010; Love et
623 al., 2014) and the SARTools package (Varet et al., 2016). Normalisation and differential analysis were
624 performed with the DESeq2 model and package. This report comes with additional tab-delimited text
625 files containing lists of differentially expressed features. A gene ontology analysis was performed on
626 the lists of under- and overexpressed genes (<http://geneontology.org/>). Gene functions were verified
627 with the EcoCyc database (Keseler *et al.*, 2011). A comparative analysis was performed with a
628 customised R script performing all possible pairwise comparisons between the gene lists for the
629 different samples. A variance stabilizing transformation (VST) representation of the data was
630 obtained with the DESeq2 package (Love *et al.*, 2014). Protein-protein association networks were
631 generated with the STRING webserver (<https://string-db.org/network/>) using the MG1655 strain as a
632 template (Szklarczyk et al., 2019). Putative prophages in the *E. coli* 55989 genome were identified
633 using PHASTER (PHAge Search Tool - Enhanced Release) (Arndt et al., 2016) and are listed below.

prediction	start	end	size (kb)
questionable	821138	866847	45.7
intact	1093207	1140290	45.0
incomplete	1408037	1417268	9.2
intact	1420060	1456075	36.0
intact	1758317	1808332	50.0
intact	2141235	2180631	39.3
intact	2664274	2710113	45.8
intact	3339788	3384869	45.0
intact	4784434	4810665	26.2

634

635

636 **Phage efficiency of plating (EOP) test**

637 The efficiency of plating (EOP) was calculated (ratio of the number of plaques formed by the phage
638 on each strain tested to the number of plaques formed on the host strain 55989) for each phage.

639 Three independent replicates were performed using bacterial cultures grown to an OD of
640 approximately 0.2 and spread on LB plates onto which phage dilutions were spotted. Plates were
641 incubated at 37°C overnight.

642

643 **Adsorption assays and phage growth**

644 Three independent adsorption assays were performed for each phage, in accordance with a
645 previously described protocol (Chevallereau et al., 2016). Data were approximated with an
646 exponential decay function and adsorption times were defined as the time required to reach a
647 threshold of 10% non-adsorbed phage particles. Phage growth and bacterial lysis were assessed by
648 diluting an overnight culture of each strain in LB broth and culturing the cells to an OD_{600nm} of 0.2. We
649 then dispensed 140 µL of this culture into each of the wells of a 96-well plate (Microtest 96 plates,

650 Falcon). We added 10 μL of sterile phage lysate diluted in PBS to obtain a multiplicity of infection
651 (MOI) of 1×10^{-2} to each well. Plates were incubated in a microplate reader at 37°C , with shaking 30 s
652 before the automatic recording of $\text{OD}_{600\text{nm}}$ at 15-minute intervals over a period of 20 hours
653 (GloMax[®]-Multi Detection System, Promega, USA).

654

655 **Biofilm formation and quantification**

656 Overnight bacterial cultures were diluted 1:100 in LB medium with, when required, chloramphenicol
657 or kanamycin, and dispensed into UV-sterilised 96-well PVC microplates. The wells located at the
658 edge of the microplates were filled with 200 μL water to prevent evaporation during incubation in a
659 static chamber, at 37°C , for 24 h and 48 h. Two microplates were used after 24 h, for biofilm staining
660 and CFU counts. Biofilm staining was performed by adding 125 μL of 1% crystal violet (V5265; Sigma-
661 Aldrich) to wells previously washed twice with water. After 15 minutes of staining, the crystal violet
662 solution was removed by flicking, and biofilms were washed three times with water. For CFU counts,
663 aggregates were homogenized within the wells by repeated pipetting and serial dilutions were plated
664 on LB agar plates. For the other eight microplates, the LB medium was removed at 24 h by pipetting.
665 Two microplates were filled with LB and incubated for a further 24 h and the treated as described
666 above for the 24 h time point. A similar pair of microplates was filled with one of the three phages
667 (2×10^6 PFU in 200 μL in LB) and incubated for a further 24 h. These microplates were treated as
668 described above for the 24 h time point. Stained microplates were left to dry overnight under a hood
669 and the crystal violet was then resuspended in a 1:4 acetone:ethanol mixture, for the reading of
670 absorbance at 570 nm (Tecan Infinite M200 PRO). For each microplate, we measured the OD for five
671 technical replicates (five wells), and three to five independent experiments were performed for each
672 strain.

673

674 **Agglutination assay**

675 The O104 immun-serum (Monospecific O Rabbit antiserum, réf #45840, lot O104L11H11, SSI
676 Diagnostica) was used as recommended by the provider. Briefly, a volume of 700 μL of a stationary
677 phase liquid culture of strain 55989 grown in LB at 37°C under agitation was centrifuged 10 min at
678 5.000g and after discarding the supernatant, the pellet was resuspended in 700 μL of PBS. This
679 sample was heated during 80 minutes at 99°C and then cooled to room temperature as
680 recommended. Then 5 μL (1×10^7 CFU) of this sample was introduced in different wells of a 96-well
681 plates (Microtest 96 plates, Falcon). Then either 5 μL of PBS, or phage CLB_P1 ($2.5 \cdot 10^7$ PFU) or phage
682 PAK_P3 ($2.5 \cdot 10^7$ PFU). After 10 minutes at room temperature either 10 μL of PBS or O104 immun-
683 serum was added and the plate was incubated 1 h at 37°C. Next, a drop (5 μL) of each condition was
684 put on a glass slide and covered with a cover slip for direct examination under a phase contrast
685 microscope (Olympus IX81). Pictures were taken at X20 magnification.

686

687 **Quantification and statistical analysis**

688 Quantification and statistical analysis of the transcriptomics data are reported in the corresponding
689 Methods section and in the Table S1.

690 For the growth curves of bacterial strains ($n=2$ to 3 for each condition) error bars represent standard
691 error of the mean (SEM) as indicated in the legends of Figures 4, 5 and 6. Statistical analysis on the
692 number of bacteria and the OD_{570nm} generated by the biofilm experiments were carried out using
693 the lme4, lmerTest and car packages of R (Bates et al., 2015; Fox and Weisberg, 2018; Kuznetsova et
694 al., 2017). CFU were log₁₀-transformed prior to analysis. Linear mixed-models were used to account
695 for random experimental effects (i.e. the effect of the plate and experiments). Prior analysis,
696 normality was assessed with a QQ-plot. Overall effects were assessed with Analysis of Variance
697 (ANOVA) and post-hoc Tukey's comparisons and were performed using the lsmeans R package
698 (Lenth, 2016). $p < 0.05$ was considered statistically significant. The results of the comparisons are
699 recapitulated in Table S2A and S2B.

700

701 **Declaration of Interests**

702 The authors declare no competing interests.

703

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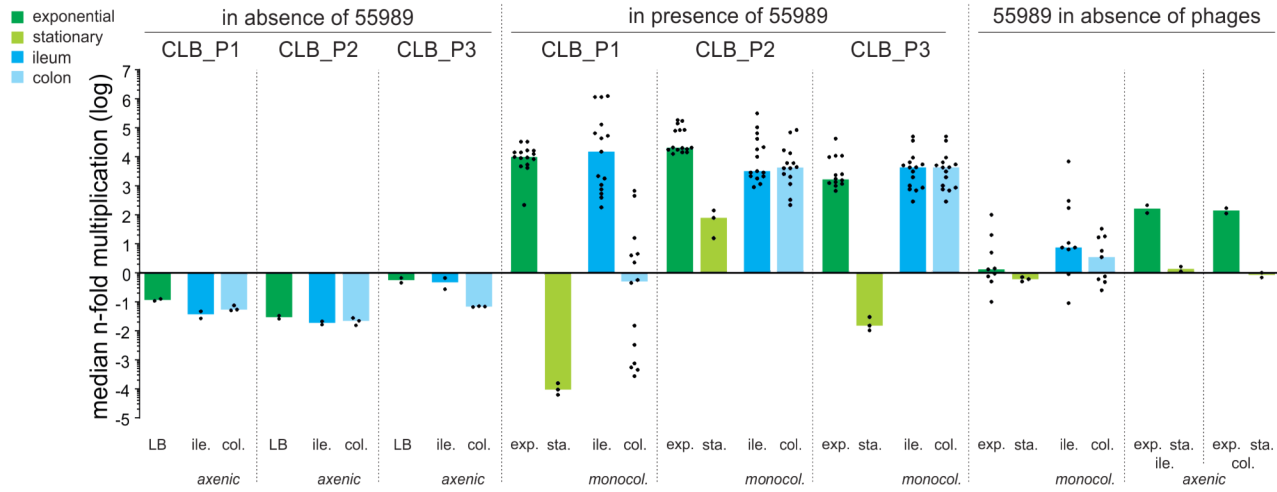
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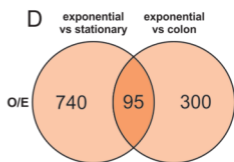
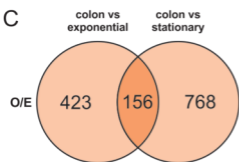
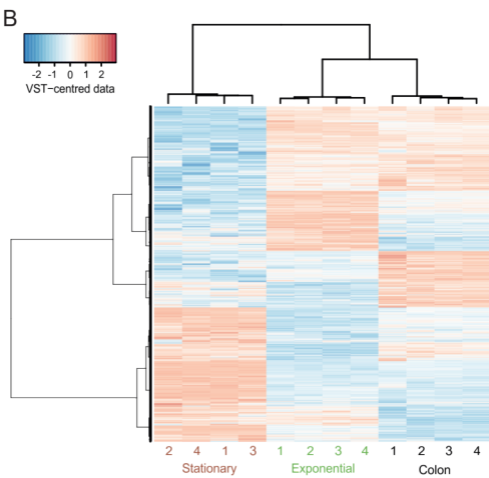
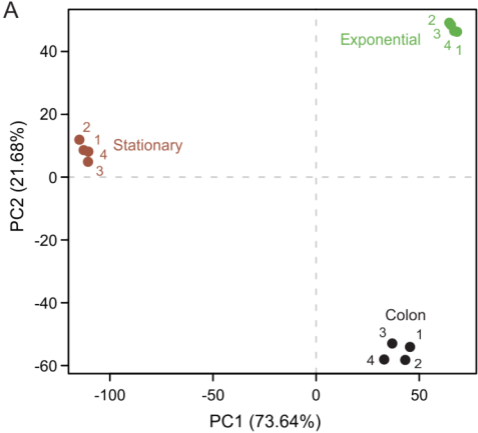
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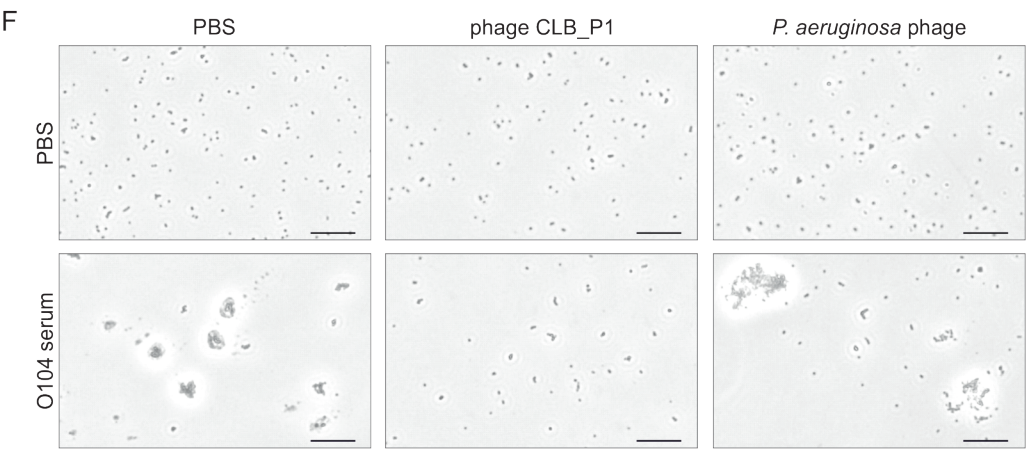
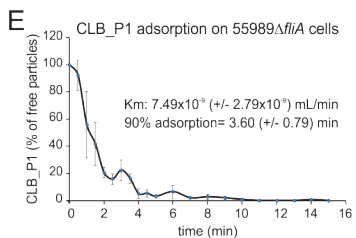
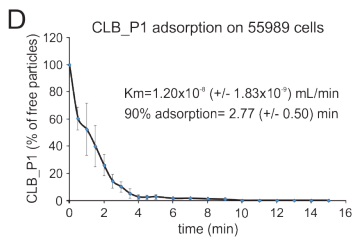
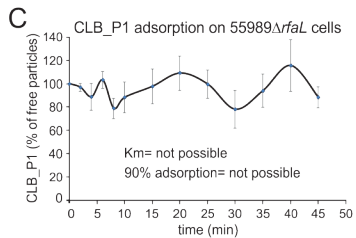
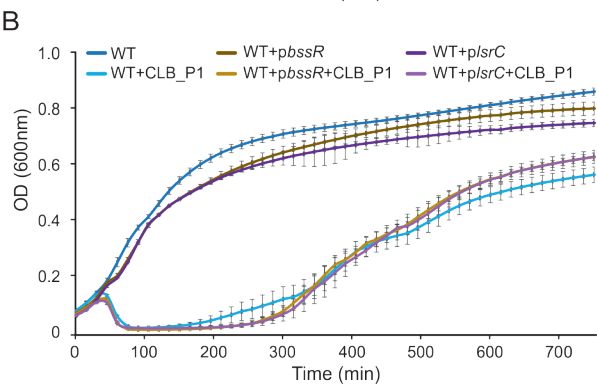
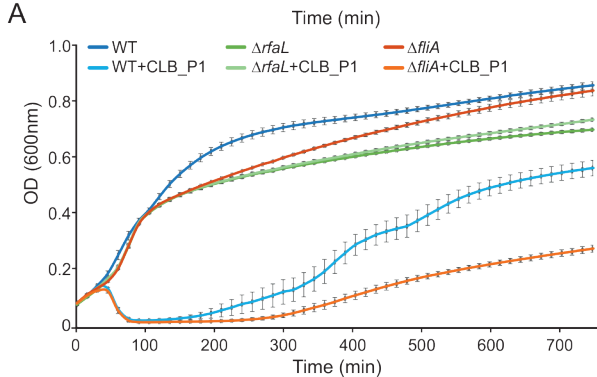
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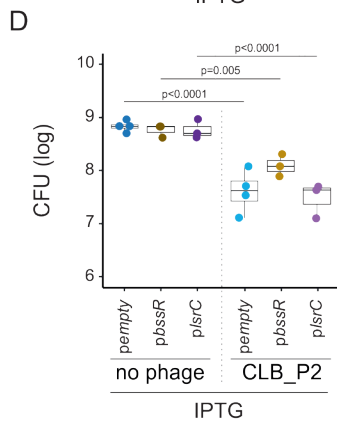
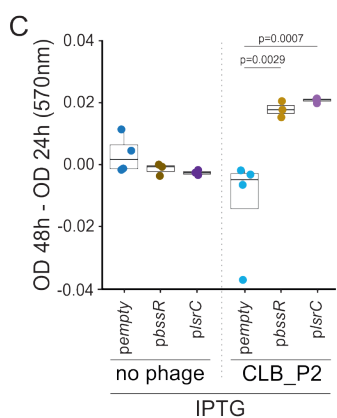
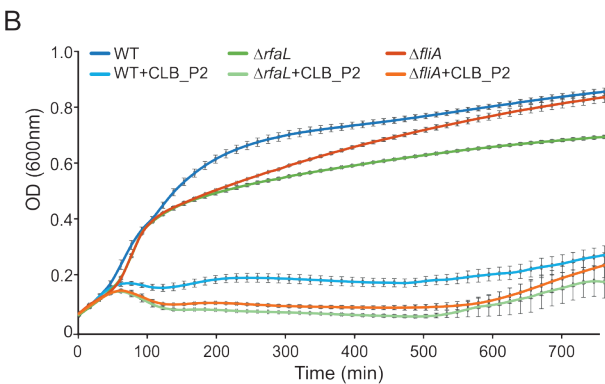
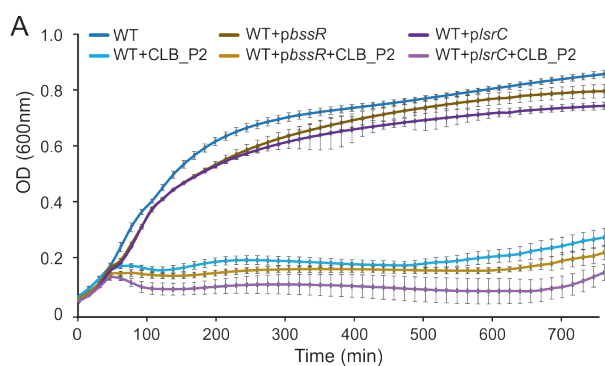
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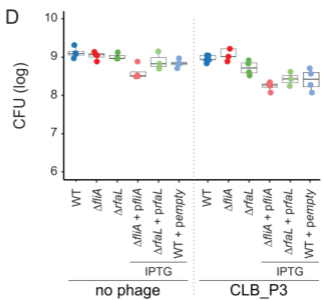
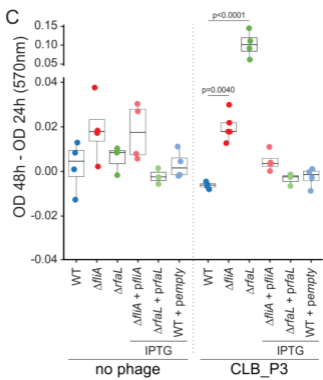
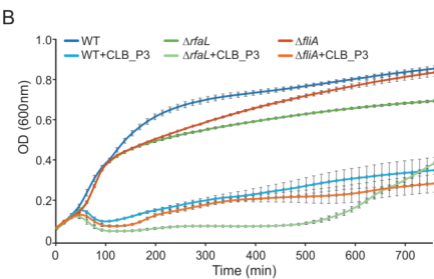
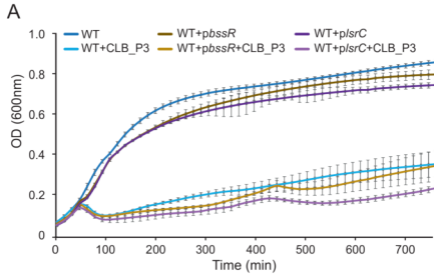
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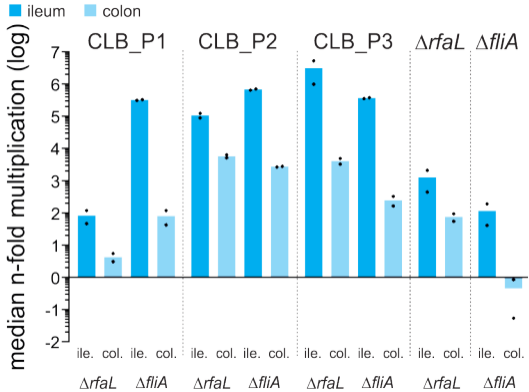












in vitro

in vivo



phage replication efficacy

exponential

stationary

ileum

colon

comparative transcriptomics

+++

receptor

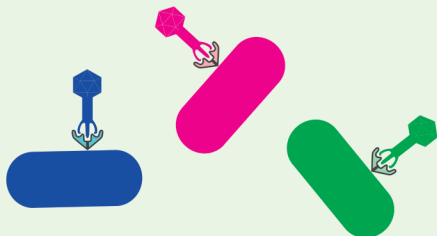
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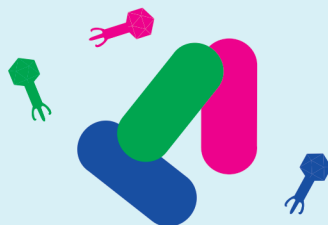
biofilm

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phage bacteria interactions



infection



no infection