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# The gut environment regulates bacterial gene expression which modulates susceptibility to bacteriophage infection

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

3

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## 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 (OD<sub>600</sub>=0.5; “exponential” samples *n*=4); ii) *in vitro* stationary growth (equivalent  
141 OD<sub>600</sub>=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 *fecl*, *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 $\Delta$ *fliA* and 55989 $\Delta$ *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 ( $2.10 \times 10^{-3}$  +/-  $3.11 \times 10^{-4}$ ) on the 55989 $\Delta$ *rfaL* strain, but remained unchanged in the 55989 $\Delta$ *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 $\Delta$ *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 $\Delta$ *rfaL* strain compared to  
227 either the WT or the 55989 $\Delta$ *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 $\Delta$ *fliA*, 55989 $\Delta$ *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 $\Delta$ *fliA* and 55989 $\Delta$ *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 $\Delta$ *fliA* or 55989 $\Delta$ *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 $\Delta$ *fliA* or 55989 $\Delta$ *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 $\Delta$ *fliA* and 55989 $\Delta$ *rfaL* strains**

265 The EOP of phage CLB\_P3 (Table S2C) on strain 55989 $\Delta$ *rfaL* was slightly higher compared to the  
266 control, while it remains unchanged for the strains 55989 $\Delta$ *fliA*, 55989*pbssR* and 55989*p/srC*. The lysis  
267 kinetics of strain 55989 $\Delta$ *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 $\Delta$ *fliA* ( $p=0.0040$ ) and 55989 $\Delta$ *rfaL*  
271 ( $p<0.0001$ ) strains (Fig 6C and Table S2B). Particularly surprising was the amount of biofilm formed by  
272 the 55989 $\Delta$ *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 $\Delta$ *fliA* and the 55989 $\Delta$ *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 $\Delta$ *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 $\Delta$ *rfaL* cells, and phage CLB\_P3 that is strongly  
290 promoting biofilm formation of both 55989 $\Delta$ *fliA* and 55989 $\Delta$ *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 $\Delta$ *fliA*- and 55989 $\Delta$ *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 $\Delta$ *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 $\Delta$ *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 $\Delta$ *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 H<sub>2</sub>S, 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 $\Delta$ *rfaL* cells for CLB\_P1, both 55989*pbssR* and 55989*plsrC* cells for CLB\_P2 and  
352 both 55989 $\Delta$ *fliA* and 55989 $\Delta$ *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 $\Delta$ *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 $\Delta$ *fliA* and 55989 $\Delta$ *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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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<sub>600</sub>=0.5); cells in stationary  
445 phase (sta, light green; equivalent OD<sub>600</sub>=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<sub>600nm</sub> of 0.5 (exponential, n=4) and an equivalent OD<sub>600nm</sub> 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 $\Delta$ *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 $\Delta$ *rfaL* and 55989 $\Delta$ *fliA* (supplemented  
476 with kanamycin for  $\Delta$ *rfaL* and  $\Delta$ *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 $\Delta$ *rfaL* (C), 55989 (D) and 55989 $\Delta$ *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 ( $1 \times 10^7$  CFU) mixed with either PBS or phage CLB\_P1  
486 ( $2.5 \times 10^7$  PFU) or a *Pseudomonas aeruginosa* phage ( $2.5 \times 10^7$  PFU) during 10 min, followed by the  
487 addition of either O104 anti-serum or PBS. Scale bar, 20  $\mu$ 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 $\Delta$ *rfaL* and 55989 $\Delta$ *fliA* (supplemented  
495 with kanamycin for  $\Delta$ *rfaL* and  $\Delta$ *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<sub>570nm</sub> 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 ( $1 \times 10^7$  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 $\Delta$ *rfaL* and 55989 $\Delta$ *fliA* (supplemented  
512 with kanamycin for  $\Delta$ *rfaL* and  $\Delta$ *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<sub>570nm</sub> 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 (1x10<sup>7</sup> 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 $\Delta$ *rfaL* and 55989 $\Delta$ *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 $\Delta$ *rfaL* and 55989 $\Delta$ *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  $\mu$ g/mL) or kanamycin (100  $\mu$ g/mL) or chloramphenicol (30  
569  $\mu$ g/ml) (Sigma) was added.

570

## 571 **Methods details**

### 572 ***Ex vivo* assay**

573 C3H axenic mice received 200  $\mu$ L of PBS or strains 55989 or 55989 $\Delta$ *fliA* or 55989 $\Delta$ *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  $\mu$ L of each homogenized sample into the wells of a 96-well plate and 10  $\mu$ L of each individual  
579 phage was added, to reach an MOI of  $1 \times 10^{-2}$ , 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 ( $OD_{600}=0.5$ ) or stationary (24 h; equivalent  $OD_{600}=5$ ) 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  $\mu$ L of strain 55989 ( $10^7$  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.

<b>prediction</b>	<b>start</b>	<b>end</b>	<b>size (kb)</b>
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<sub>600nm</sub> 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  $\mu\text{L}$  of sterile phage lysate diluted in PBS to obtain a multiplicity of infection  
651 (MOI) of  $1 \times 10^{-2}$  to each well. Plates were incubated in a microplate reader at  $37^\circ\text{C}$ , 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<sup>®</sup>-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  $\mu\text{L}$  water to prevent evaporation during incubation in a  
659 static chamber, at  $37^\circ\text{C}$ , 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  $\mu\text{L}$  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 ( $2 \times 10^6$  PFU in 200  $\mu\text{L}$  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  $\mu\text{L}$  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  $\mu\text{L}$  of PBS. This  
679 sample was heated during 80 minutes at 99°C and then cooled to room temperature as  
680 recommended. Then 5  $\mu\text{L}$  ( $1 \times 10^7$  CFU) of this sample was introduced in different wells of a 96-well  
681 plates (Microtest 96 plates, Falcon). Then either 5  $\mu\text{L}$  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  $\mu\text{L}$  of PBS or O104 immun-  
683 serum was added and the plate was incubated 1 h at 37°C. Next, a drop (5  $\mu\text{L}$ ) 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<sub>570nm</sub> 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 log<sub>10</sub>-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

704 **References**

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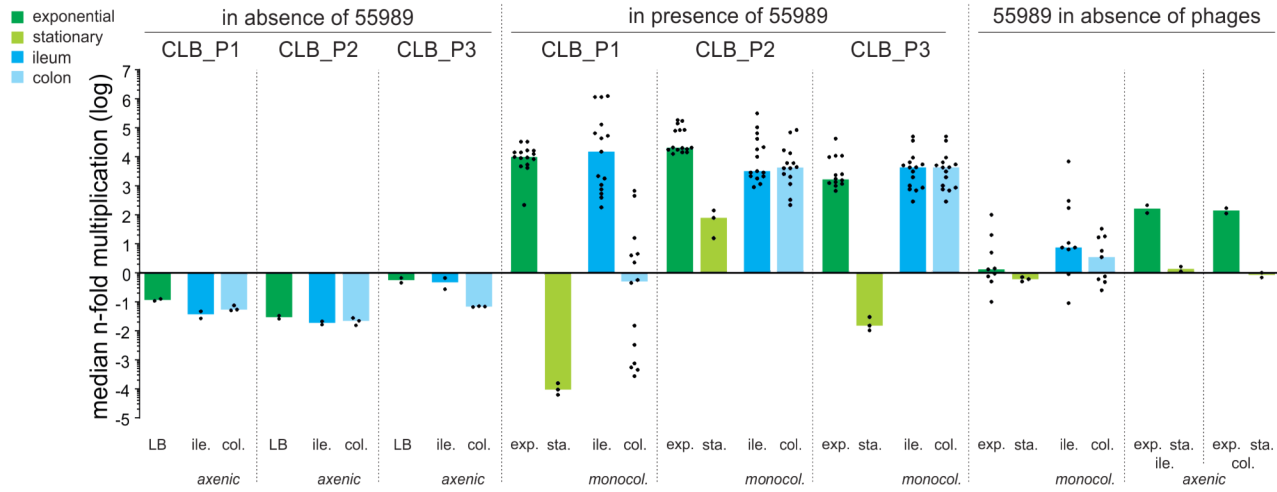
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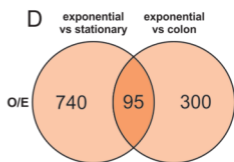
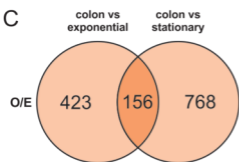
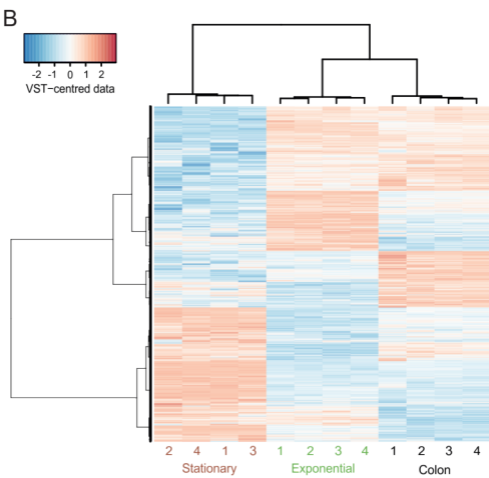
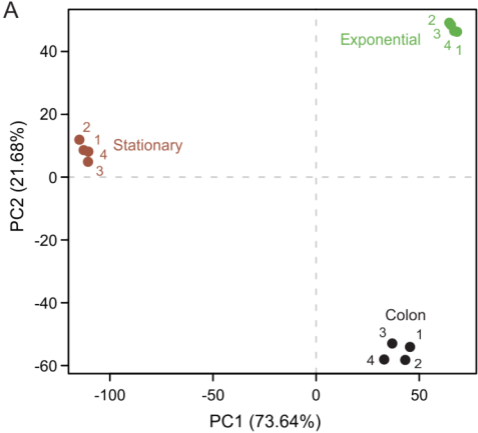
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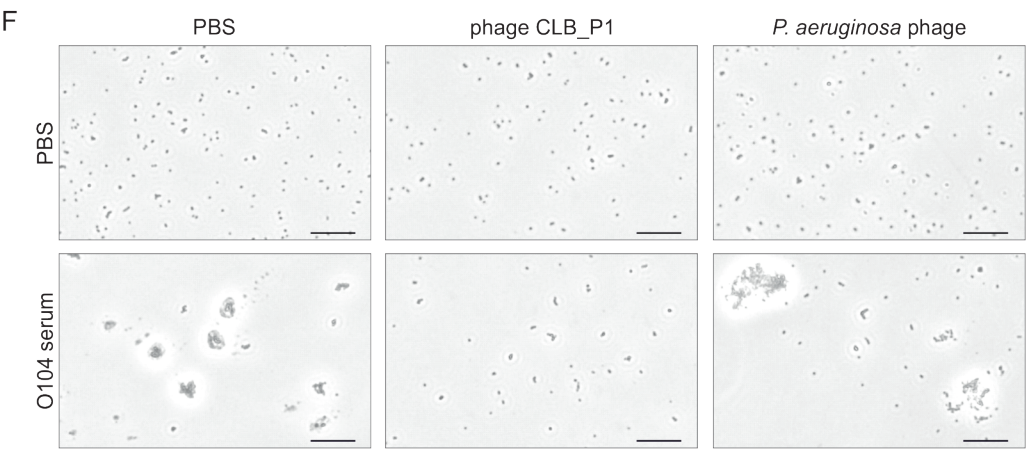
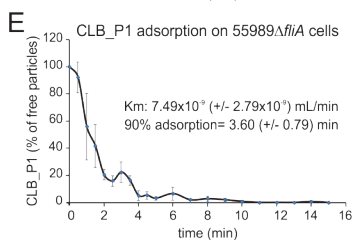
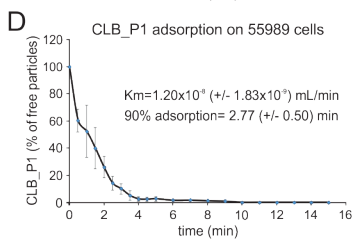
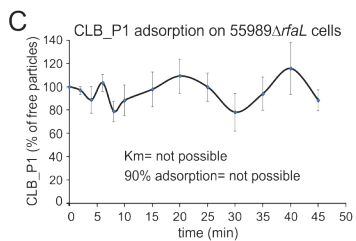
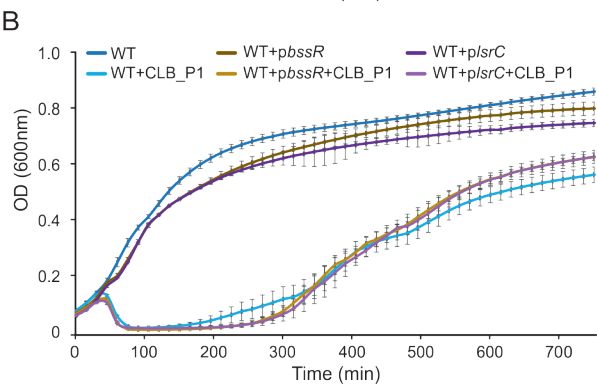
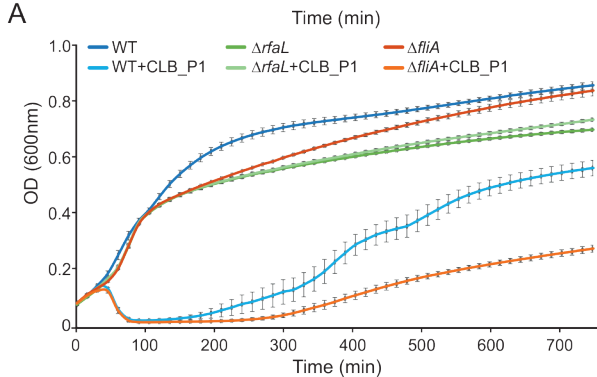
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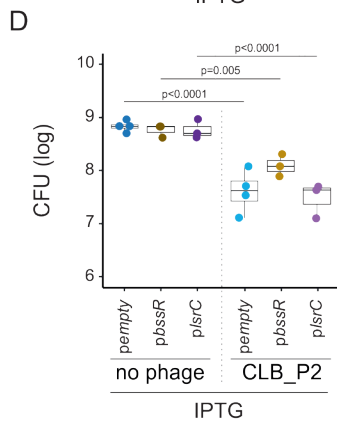
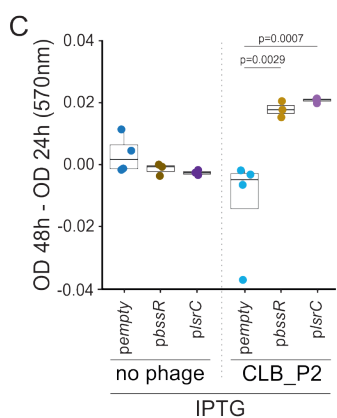
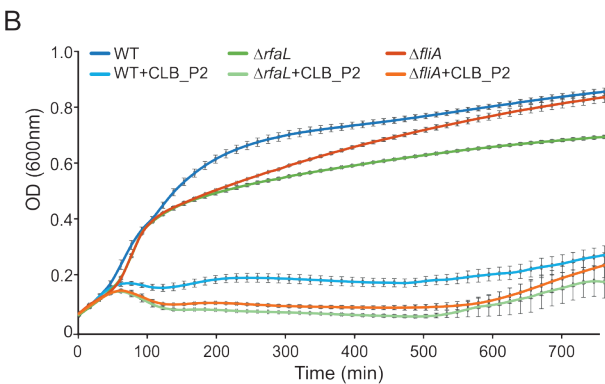
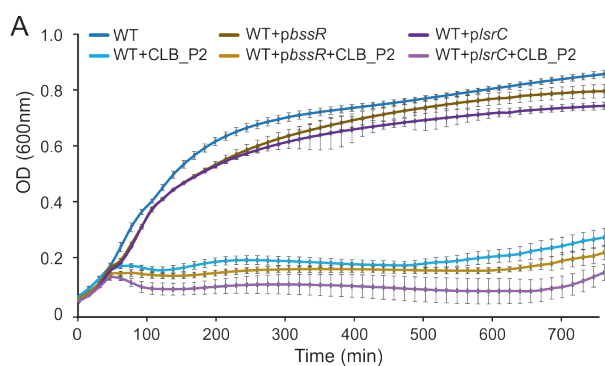
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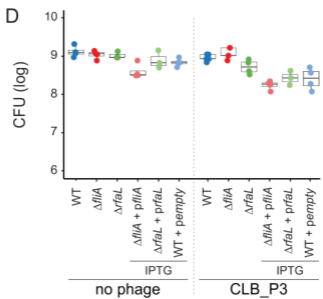
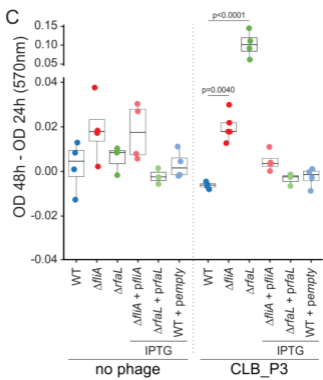
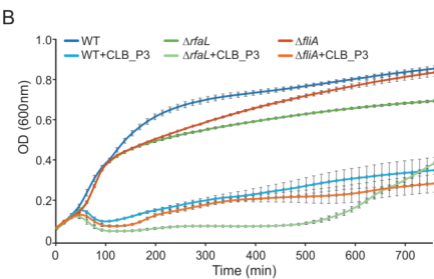
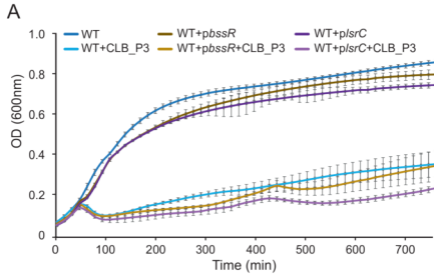


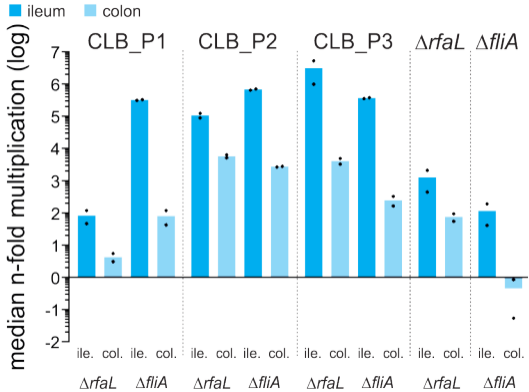










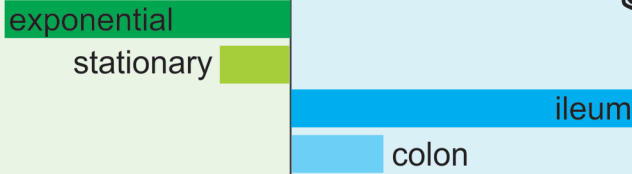


*in vitro*

*in vivo*



phage replication efficacy



comparative transcriptomics

+++

receptor

+

-

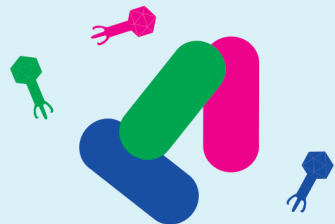
biofilm

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phage bacteria interactions



infection



no infection