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The Gut Microbiota Facilitates Drifts in the Genetic Diversity and Infectivity of Bacterial Viruses

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1 **Title:**

2 **The gut microbiota facilitates drifts in the genetic diversity and infectivity of bacterial viruses**

3

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6

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14

15 **Summary**

16 The intestinal microbiota and human health are intimately linked but interactions between
17 bacteria and bacteriophages in the context of the mammalian intestine remain largely
18 unexplored. We used comparative population genomics to study a tripartite network consisting
19 of a virulent bacteriophage, its bacterial host, and a phage-insensitive bacterial strain both *in*
20 *vitro* and within the murine gut. The bacteriophage adapted to infect the insensitive strain
21 when the three partners co-existed in the gut of conventional mice, but not dioxenic mice or in
22 planktonic cultures. The molecular changes associated with modifications in the bacteriophage
23 host spectrum included single amino-acid substitutions and an unusual homologous intra
24 genomic recombination event within the genome of the bacteriophage. An intermediate
25 bacterial host isolated from the murine microbiota mediated bacteriophage adaptation. Our
26 data indicate that by offering access to new hosts, the microbiota shifts the genetic diversity of
27 bacteriophages thereby promoting long-term persistence of bacteriophage populations.

28

29 **Introduction**

30 Viruses are the most abundant biological entities in the intestinal microbiome, with a marked
31 predominance of bacteriophages (Minot *et al.*, 2013; Norman *et al.*, 2014; Virgin, 2014).

32 Culture-independent metagenomic approaches have been successful in characterising viral
33 communities, but these methods are largely uninformative about their relationships with the
34 matching bacterial hosts in the gut (Breitbart *et al.*, 2003; Minot *et al.*, 2011; Mirzaei and
35 Maurice, 2017). This is particularly true for virulent bacteriophages: their sequences, unlike
36 those of temperate ones, cannot be deduced from bacterial genomes and identifying their
37 vehicles of replication in the intestinal microbiome is often unachievable. For instance, the
38 CrAssphage is the most widespread bacteriophage in the human population, but its host is still
39 not known (Dutilh *et al.*, 2014).

40 All humans carry a unique set of intestinal bacteriophages (Faith *et al.*, 2013). However, healthy
41 individuals share a pool of conserved bacteriophages, which are different in type, number and
42 abundance, from those found in patients suffering from Inflammatory Bowel Disease (IBD)
43 (Manrique *et al.*, 2016; Norman *et al.*, 2015). More, significant bacteriophage changes in
44 relative abundance, richness and diversity were recently reported to be associated with the
45 development of human autoimmune disease (Zhao *et al.*, 2017). Indeed, there is increasing
46 evidence that bacteriophages make a substantial contribution to the eubiosis of the human
47 microbiome, although the mechanisms are yet to be defined (De Paepe *et al.*, 2014; Manrique
48 *et al.*, 2017). In that perspective, it is no wonder that viral communities homogenise between
49 donors and patients undergoing successful faecal microbiota transplant (FMT) (Chehoud *et al.*,
50 2016; Zuo *et al.*, 2017). However, it remains remarkable that filtered (bacteria-free) FMT

51 provides curative results comparable to those of traditional FMT in resolving gut infections by
52 *Clostridium difficile* (Ott *et al.*, 2016). This implies that the viral community can re-establish
53 itself by replicating on the new hosts available and thereby contribute to reshaping the
54 bacterial population towards a healthy condition.

55 Bacteriophage survival depends on the ability to perpetuate the antagonistic coevolution with
56 bacterial hosts. This requires maximising the viral infection capacity towards each new
57 population encountered (Paterson *et al.*, 2010). When the ecological conditions are perturbed,
58 this relationship can be destabilised with consequent increased extinction risk for the
59 bacteriophage (Wright *et al.*, 2016). To limit this risk, viral genome plasticity generates genomic
60 diversity to face the spatial and temporal variations typical of antagonistic interactions
61 (Buckling and Rainey, 2002; Vos *et al.*, 2009).

62 We hypothesised that the bacterial heterogeneity in the intestinal tract, would expand the
63 genomic diversification of the coevolving viral population, promoting bacteriophage adaptation
64 to different hosts. Using three experimental conditions (*in vitro* and in dioxenic and conventional
65 mice) we identified molecular events allowing one virulent bacteriophage to adapt to
66 previously insensitive host bacteria, a phenomenon observed exclusively in the intestine.

67 We propose for bacteriophages to persist within this highly competitive environment they
68 exploit the microbiota as a base to jump between different bacterial hosts.

69

70

71 Results

72 The intestinal environment promotes bacteriophage host-jump

73 Our experimental approach involved a tripartite network consisting of: (i) a novel virulent
74 bacteriophage, P10, related to the myovirus Felix O1; (ii) its host, the pathogenic *Escherichia*
75 *coli* strain LF82 and (iii) the commensal *E. coli* strain MG1655, to which P10 cannot bind, and is
76 therefore non-infective (up to 10^{10} pfu were tested) (Figure S1). The behaviour of these three
77 partners was monitored for 24 days in parallel in three conditions: *in vitro* (planktonic cultures),
78 in the gut of mice only colonised with strains LF82 and MG1655 (dixenic) and in the gut of
79 conventional mice (Figure 1A and see Figure S2 for bacteriophage population densities). We
80 screened for “host-jumps” or, in other words, a gain of function by P10; the function being the
81 ability to infect the previously insensitive host, strain MG1655. No endogenous bacteriophage
82 infecting either of the strains was isolated from mouse intestine from the control group.
83 Samples were analysed by plaque assay and sequencing of total bacteriophage populations (see
84 below). Interestingly, bacteriophage P10 variants adapted to strain MG1655 (hereafter named
85 ad_P10) and still infecting strain LF82 were isolated, but only from the gut of conventional mice
86 (20% rate). No P10 variants able to jump hosts were obtained from dixenic mice or *in vitro*
87 (Figure 1A and S2).

88

89 Adapted bacteriophages have extensively diversified host range

90 We investigated whether the ability of ad_P10 in infecting a previously insensitive host was
91 associated with a more generalised effect on the bacteriophage host range. We isolated 16
92 ad_P10 bacteriophages and tested them on a collection of 105 Enterobacteriaceae strains

93 (Figure 2 and Table S1). Almost half of the ad_P10 isolates had a reduced host range than the
94 wild type strain (up to 50% reduction), and most others showed marginal difference; one
95 exceptional ad_P10 variant had expanded its host range to 11 more strains than the wild-type
96 P10 (Figure 2).

97

98 **An unusual genome recombination event associated with host jump in virulent** 99 **bacteriophages**

100 To gain insight into the host jump mechanism and its variable effect on the host range of
101 adapted bacteriophages, the genomes of two ad_P10 isolates were sequenced. In both
102 genomes, a stretch of 216 or 280 nucleotides of the hypervariable region of the tail fiber gene
103 *gp91* underwent duplication, resulting in two interspersed repeats. The segment recombined
104 within the hypervariable domain of the adjacent long tail fiber gene *gp90*, causing the deletion
105 of either 237 or 273 nucleotides. This recombination was probably promoted by the presence
106 of homologous flanking regions of 17 to 22 base pairs (Figure 3A). We named this event a
107 “homologous intra genomic recombination” (hIGR) and investigated its frequency by
108 sequencing the corresponding region in 20 randomly chosen ad_P10 isolates: it was present in
109 10 of the 20 isolates tested. Recombination is a major factor in genomic evolution, so we tested
110 whether similar events had taken place in other bacteriophage genomes. A search in the
111 database of all annotated bacteriophage tail fibers revealed that three other bacteriophages of
112 the FelixO1-like family displayed the same tail fiber protein organisation as ad_P10: 60- to 70-
113 amino acids in the C-terminal hypervariable region were duplicated in the adjacent tail fiber-

114 encoding gene sequence (Figure 3B). Similar hIGR events were also found, but at different
115 positions, in the tail fiber genes of other bacteriophages (Figure 3B and Table S2).

116

117 **Population genomic analyses indicates that the patterns of mutation differ between *in vitro***
118 **and *in vivo* populations**

119 We used population genomics analyses to compare P10 populations evolving *in vitro*, in dixeric
120 mice and in conventional mice. The number of single-nucleotide polymorphisms (SNPs)
121 accumulated over 24 days (17 to 28 SNPs, at frequency higher than 1%; Table S3) did not differ
122 significantly ($p=0.67$) and did not depend on the presence of strain MG1655 in the system
123 ($p=0.1$) or on the host-jump event ($p=0.93$) (Mann-Whitney test). This suggests that the
124 adaptation was not a consequence of a higher mutation rate in the intestinal environment than
125 in laboratory cultures.

126 We found that gene *gp37*, which is homologous to *rIIA* (one of the *r* genes for lysis inhibition in
127 T4 (Paddison *et al.*, 1998)) was a hotspot for mutations in all populations, implicating this gene
128 in general viral fitness that was not investigated further (Figure 1B-C). Some genomic regions
129 were differentially mutated *in vitro* versus *in vivo*. In liquid cultures, P10 persistently infected
130 only one host, strain LF82, and none of the SNPs in these populations were highly frequent (<
131 20%), suggesting that they had a limited role in viral fitness. The genes most frequently affected
132 in this condition included those encoding enzymes involved in nucleic acid metabolism, possibly
133 fine-tuning the infection mechanism (Figure 1B-C and Table S3).

134 By contrast, numerous mutations were only found in bacteriophages replicating in the

135 intestinal tract. Many were present at high frequency and were located in the genomic region

136 predicted to encode for structural proteins. Most of the high-frequency SNPs, like the HIGR
137 described above, were in the two tail fiber genes (*gp90* and *gp91*).

138

139 **A single point mutation in a tail fiber gene is sufficient for a host jump**

140 Tail fiber gene *gp91* was only modified in ad_P10 populations (those that showed a host-jump)
141 with a particular SNP (A→G at position 55079) at a frequency of 100% (confirmed by PCR
142 sequencing of 20 individual ad_P10 bacteriophages; Figure 1B-C and Table S3). It was absent
143 from non-adapted populations from all three conditions tested. This variant results in a single-
144 amino acid change at position 284 (Y284H). We tested its involvement in host specificity and
145 constructed a recombinant P10 bacteriophage (rP10) carrying this mutation (Y284H): this single
146 SNP was sufficient to confer the ability to infect strain MG1655 (Figure S3). However, this
147 happened at the expense of bacteriophage replication in its original host, strain LF82 (decrease
148 of nearly 100-fold) without conferring highly efficient replication in strain MG1655 (Figure S3).
149 However, consecutive replication in this new host rapidly led to adaptation such that rP10
150 replication in strain MG1655 was ten times higher than P10 in strain LF82 (Figure S3). As few as
151 four replication passages *in vitro* were sufficient for this massive gain of infectivity on the new
152 host. Genomic sequencing showed that this improvement was accompanied by additional
153 mutations in the genome of rP10 that likely contributed to increasing phage replication
154 efficiency on the new host. We counted 7 additional SNPs, of which 4 were non-synonymous
155 and one deletion (1bp) (Table S4). Two of the non-synonymous mutations were located within
156 each of the tail fiber genes, one in the DNA polymerase gene and the others in genes of
157 unknown function (Table S4).

158

159

160 **Bacterial diversity within the microbiota favours viral host-jump**

161 We investigated why host-jumps were only observed for bacteriophages replicating in the gut
162 of conventional mice. We hypothesised that this event is mediated by the infection of one or
163 more intermediate bacterial hosts within the intestinal microbiota leading to the observed
164 modifications of the bacteriophage genetic diversity. We isolated a gut bacterial strain that was
165 infected by ad_P10 at high efficacy of plating (EOP) of 1 from faeces of conventional mice not
166 exposed to any bacterial inoculum (see methods). By contrast, wild-type P10 was only poorly
167 able to form plaques (EOP=10⁻²) on this strain. This supports the idea that the P10 host-jump is
168 concomitant with an increased lytic efficacy against an alternative host in the same
169 environment. Sequence analysis identified this bacterium as a murine *E. coli* strain that we
170 named Mouse *E. coli* (MEc) 1. We studied the role of this putative intermediate host in the
171 host-jump of P10 resulting in ad_P10 by using co-cultures *in vitro*: we mixed P10 with strains
172 MEc1 or MG1655, with or without the presence of strain LF82, or with a mix of the three *E. coli*
173 strains. After seven days of incubation, the efficacy with which the wild-type P10 population
174 infected MEc1 increased 10-fold whenever this strain was present (Figure 4A). If strain MG1655
175 was also present, the host jump was observed, although it was delayed to day 24 when the
176 preferential host of P10, LF82, was in the system (Figure 4A-B). By contrast, no host-jump was
177 observed when P10 was mixed *in vitro* in the presence of LF82 and MEc1, alone or in
178 combination, nor, as expected, in the presence of MG1655 alone (Figure 4A-B). This indicates
179 that host-jumps become prevalent when there is a strong pressure conferring advantage on the

180 new infecting population, such as in conditions where the density of the preferential hosts is
181 low or null.

182 **Discussion**

183 To persist in the environment, obligate parasites, such as viruses, rely on the availability of
184 suitable hosts. Genetic mutations during viral replication can generate viral variants that can
185 acquire the ability to infect new hosts (Poirier and Vignuzzi, 2017). This is illustrated by several
186 human pandemics caused by viruses originally incapable of infecting human cells (for example
187 HIV, flu virus H1N1, and Ebola virus). Our work demonstrates that the abundance of potential
188 hosts favours shifts in viral infectivity: this suggests that the diversity of intestinal microbiota
189 enables the persistence of bacteriophages by promoting diversification due to the availability of
190 divergent infection paths.

191 Numerous studies have described the behaviour of bacteria and bacteriophages *in silico* and *in*
192 *vitro*, defining population dynamics, emergence of bacterial resistance and bacteriophage
193 adaptation (Betts *et al.*, 2014; Kerr *et al.*, 2006; Morgan *et al.*, 2005; Weitz *et al.*, 2013;
194 Wichman *et al.*, 2005). However, how these phenomena apply to the homeostasis of human
195 viral communities remains largely unknown (Scanlan, 2017).

196 The host jump that we observed in the intestinal tract had a large effect on the host spectrum
197 of the P10 population. In the murine intestinal tract, this resulted in a host expansion from one
198 to two or three (possibly more) bacterial strains coevolving with P10. However, the infectivity
199 towards a set of strains that has not coevolved with the bacteriophage, was very variable and
200 often restricted, compared to the wild-type P10. There are at least two possible explanations
201 for these observations: either the host-jump emerged several times in different bacteriophage
202 variants, or a single event was followed by additional mutations affecting their host range
203 phenotype.

204 Environmental studies have shown that the structure of bacteriophage-host infection networks
205 are typically nested, providing a stable community at the site of sampling (Beckett and
206 Williams, 2013; Flores *et al.*, 2011; Koskella and Brockhurst, 2014). Our results confirm that this
207 pattern is not limited to bacterial hosts isolated from the same environment; it also applies to
208 the ability to infect other potential host strains, reflecting a more generalised, space- and time-
209 independent pattern of behaviour.

210 Host-jumps were concomitant with the accumulation of mutations in the P10 genome. These
211 mutations largely differed from those found *in vitro* and were mostly in the tail fiber genes.
212 Amongst those, we report a previously undescribed hIGR and provide evidence for similar
213 hIGRs in other bacteriophages, with implications for viral fitness and possibly adaptation to
214 different hosts. Modular exchange between different bacteriophages is known to drive genomic
215 evolution by homologous, illegitimate or site-specific recombination (Brussow *et al.*, 2004;
216 Ignacio-Espinoza and Sullivan, 2012; Yap and Kreuzer, 1991). Here we provide a demonstration
217 of intra-genomic recombination in bacteriophages not harbouring genes homologous to known
218 recombinases, such as all FelixO1-like bacteriophages. Possibly, these bacteriophages exploit
219 the host recombinase machinery, for example that associated with integrated prophages (LF82
220 harbours four of them (Miquel *et al.*, 2010)), to mediate the evolution of their own genomes
221 which contain “ready-to-use” recombination sites. Such recombination would be a novelty in
222 virulent bacteriophages and further investigation is required to determine whether it can be
223 found elsewhere than in tail fiber genes.

224 In all adapted bacteriophages, host-jump was associated to accumulation of point mutations.
225 Although many localized in the tail fiber genes, one particular SNP was sufficient in itself to

226 drive host-jump. However, it did not confer a selective infectious advantage over the wild-type
227 bacteriophage population which continues to replicate on the available original host (optimal
228 fitness). This provides an explanation of why it was not selected and amplified in planktonic
229 cultures or in dixeric mice in the presence of only two bacterial strains. This is consistent with
230 the results of previous experimental and theoretical coevolutionary studies (Ashby *et al.*, 2014).
231 In antagonistic coevolution, the arms-race dynamics between viruses and hosts lead to
232 increased viral infectivity via a constant supply of genomic mutations. However this comes at a
233 cost in terms of fitness, preventing widely infective variants from fixing (Buckling and Rainey,
234 2002; Gandon *et al.*, 2008; Poullain *et al.*, 2008) and favouring fluctuating selection on
235 alternative host genotypes (Gomez and Buckling, 2011; Hall *et al.*, 2011). Our study widens this
236 concept to infection of different host strains (host-jump) generating viral diversity directly
237 proportional to host diversity.

238 The kinetics of events leading to host jump is not known. The single SNP we report might have
239 appeared first, followed by other adaptive mutations, as we observed with the specific
240 recombinant variant we generated, or it could have been the other way around. These events
241 are likely to be affected by the uneven spatial distribution of bacteriophage hosts in the
242 intestine. Indeed, their accessibility differs between sites and their density and diversity can
243 vary providing different opportunities for bacteriophage spatial adaptation. Here, the available
244 hosts can drive fluctuating selection of differentially infectious bacteriophage variants resulting
245 from the accumulation of genomic mutations. A schematic representation of fitness landscape
246 showing viral subpopulations and the available hosts is presented in Figure 4C. The extreme
247 endpoints are the extinction of the least fit and least competitive variants, and the expansion,

248 and probably improved persistence, of those acquiring the ability to infect previously
249 insensitive hosts. Although we did not find significant differences in the accumulation of SNPs
250 when comparing our three experimental conditions, our results should not suggest that the
251 surrounding environment has no impact on genomic evolution. Accumulation of deleterious
252 mutation leads to inevitable extinction, but this happens at a more rapid pace *in vivo* (in the
253 gut), being this an “open” system where the least fit can be eliminated, compared to *in vitro*
254 closed conditions (in flasks). Therefore, mutations found in populations isolated from the
255 intestinal tract are more likely to only reflect advantageous genomic variants.

256 We propose that these interactions play key roles in maintaining bacteriophage and microbial
257 diversities in the intestinal tract. In this context, disruption of such bacteriophage-bacteria
258 interactions may play crucial role in causing dysbiosis associated to antibiotic treatments or
259 inflammatory disease. Our work shows that the microbiota is a neglected source of
260 evolutionary pressure: it promotes drifts in viral genomic diversity and host ranges by
261 facilitating host jumping and providing an opportunity to persist in this ecological niche.

262

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264 Investigation, L.D.S.; Formal Analysis, L.D.S. and V.K.; Writing, L.D.S., V.K. and L.D.; Funding
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266

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279

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417

418

419 **FIGURE LEGENDS**

420 **Figure 1. Bacteriophage host-jump only occur in the gut of conventional mice.**

421 (A) Schematic representation of three experimental conditions, where bacteriophage P10 was
422 mixed with two *E. coli* strains (MG1655 and LF82), *in vitro* (IV), *in vivo* in dioxenic mice (DM) or
423 conventional mice (CM) for 24 days. Inoculation with bacteria and P10 is shown as black
424 arrows. Grey arrows indicate control (Ct) sampling prior to inoculation, followed by sampling of
425 bacteria and P10. The orange box shows recovery of adapted (ad_) bacteriophage only in
426 conventional mice. The relative bacteriophage densities are shown in Figure S2.

427 (B) Comparative population genomics showing diversity of bacteriophage mutations. Symbols
428 show a map of SNPs present at a frequency higher than 5% in P10 populations after 24 days *in*
429 *vitro* (IV), in conventional mice (CM) and in dioxenic mice (DM), in the presence of one strain (1S),
430 LF82, or two strains (2S), LF82 and MG1655. Two adapted (ad_) populations were sequenced
431 separately, only one is shown here for clarity. These showed the highest frequency of
432 mutations, mainly gathered in the tail fiber genes *gp90* and *gp91*. For a full list of SNPs and
433 positions in all populations refer to table S3. Relevant genes names are indicated along with
434 functional clusters (NA, nucleic acids) whose positions have been deduced from genes
435 annotation.

436 (C) Mutation Y284H in gene *gp91* (position 55079; highlighted in orange) is the only genomic
437 modification common to all ad_P10 clones. The figure shows a genomic map of bacteriophage
438 P10 with enlargement of the terminal region of the Gp91 protein. The alignment of sequences
439 from P10 and 20 isolated ad_P10 (numbered 1 to 20) is deduced by PCR amplification of the
440 genomic region encompassing all amino-acid substitutions (highlighted in pink and yellow)
441 found within this protein.

442 **Figure 2. Broad host range variations are associated to bacteriophage adaptation.**

443 Ad_P10 (n=16) were tested for infectivity (light blue) versus 105 Enterobacteriaceae (see Table
444 S1) and compared to the ancestral P10 bacteriophage (purple). The shared acquired infectivity
445 towards strain MG1655 has a very variable effect on the global host range. Only positive results
446 are shown for clarity.

447

448 **Figure 3. Homologous intra genomic recombination (hIGR) events are widespread in**
449 **bacteriophage tail fibers.**

450 (A) Schematic representation of the interspersed duplication (red arrow) of the hypervariable
451 region of *gp91* (red box) in *gp90* coupled by the deletion of the corresponding region in *gp90*
452 (orange box) in ad_P10 compared to the wild type P10. Sequences of recombination sites (rs) of
453 *gp90* (black) and *gp91* (green) are indicated as well as their location (black arrows). (B)
454 Examples of hIGR from annotated tail fibers of other bacteriophages with duplicated regions
455 shown in yellow (for a full list of local alignments see Table S2).

456

457 **Figure 4 Intermediate hosts from the microbiota alter bacteriophage infectivity.**

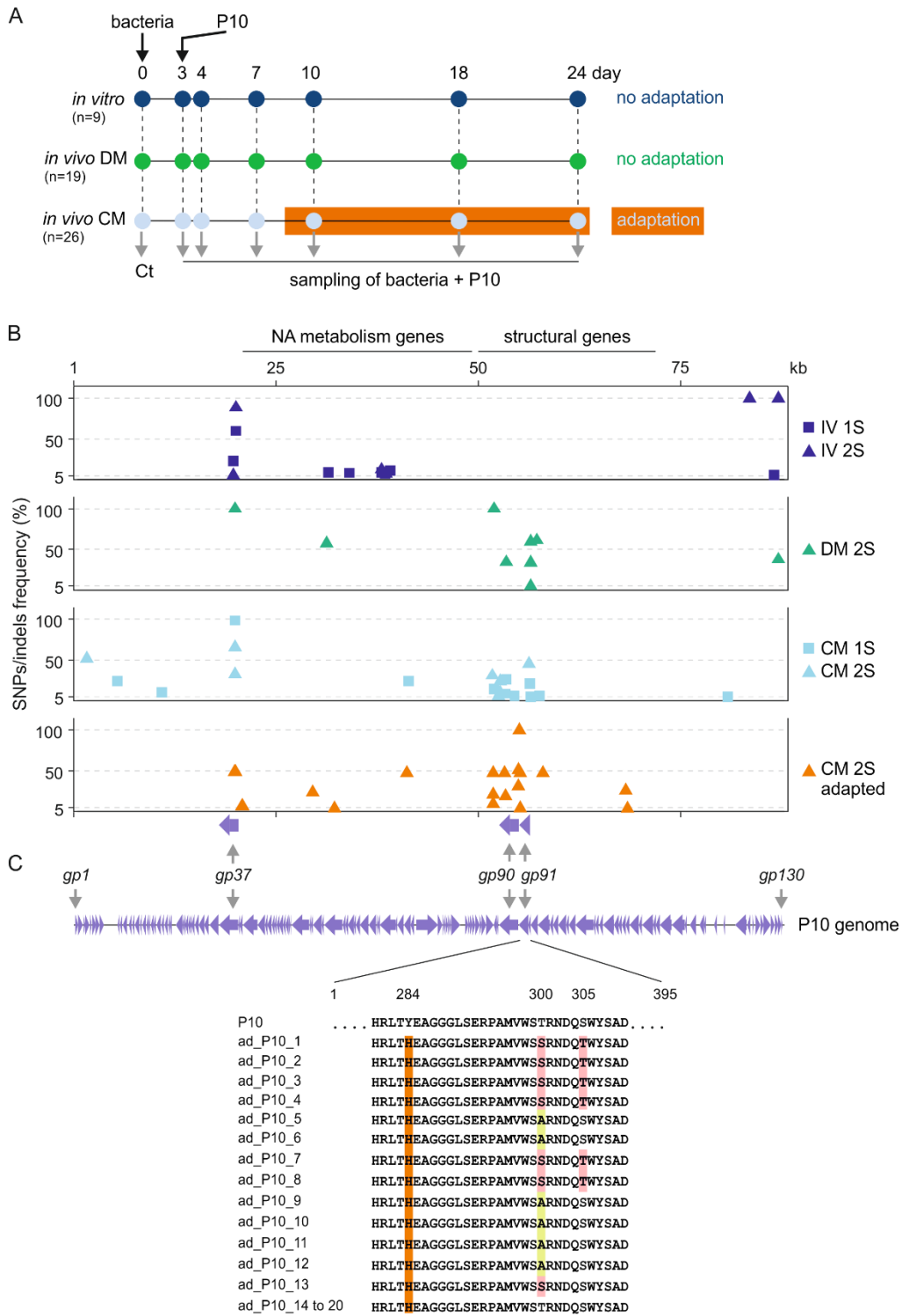
458 (A-B) The intermediate host MEc1 is sufficient to promote P10 host-jump *in vitro*. Data are
459 shown as titers of P10 bacteriophages recovered at day 7 (A) or day 24 (B) following their
460 incubation in flasks in presence of two strains LF82 + MG1655 (black), or LF82 + MEc1 (blue), or
461 MEc1 + MG1655 (green) or the three of them (red) (see methods). Samples were enumerated
462 on three bacterial hosts, LF82 (circles), MEc1 (square) and MG1655 (triangle). Adaptation of
463 P10 to infect MG1655 only happened when MEc1 is in the mix. The initial dose of

464 bacteriophage was 2×10^6 pfu added to a three-days-old bacterial culture (see methods). Scales
465 are logarithmic on the base 10. Bars show the mean of three independent experiments \pm s.e.m.
466 (C) Schematic representation of a theoretical fitness landscape showing the dynamic
467 fluctuations in bacteriophage infectious populations relative to host abundance. Peaks
468 represent bacteriophage sub-populations with fitness shown as a coloured gradient reflecting
469 the viral infectivity and consecutive abundance. Increased bacterial diversity impacts on viral
470 population sizes and diversity, favouring host jumps (black arrow).

471

472

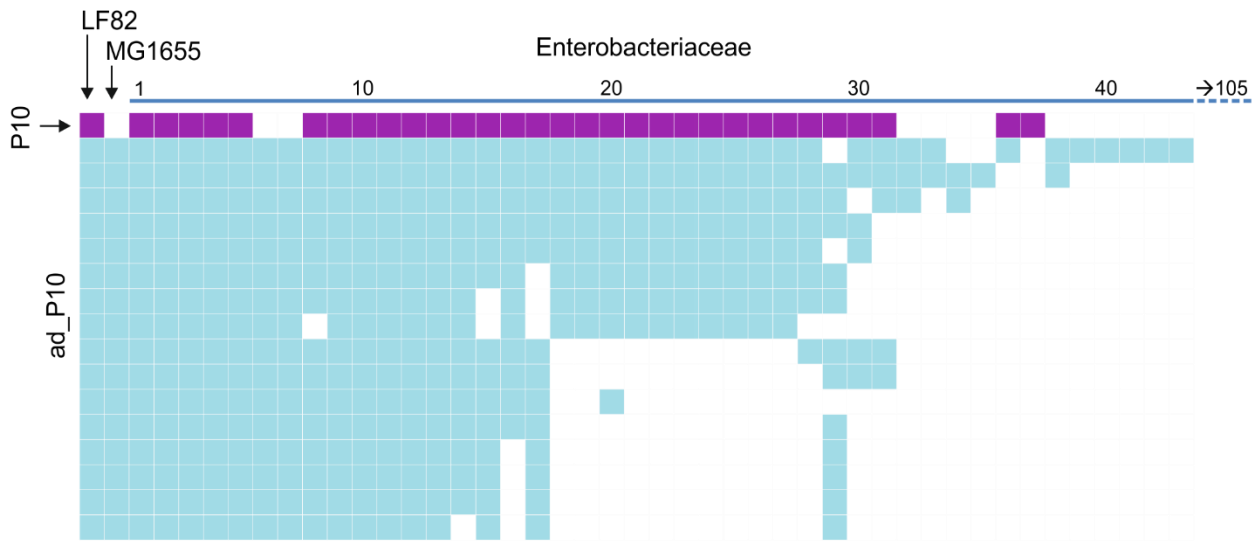
473 Figure 1



474

475

476 Figure 2



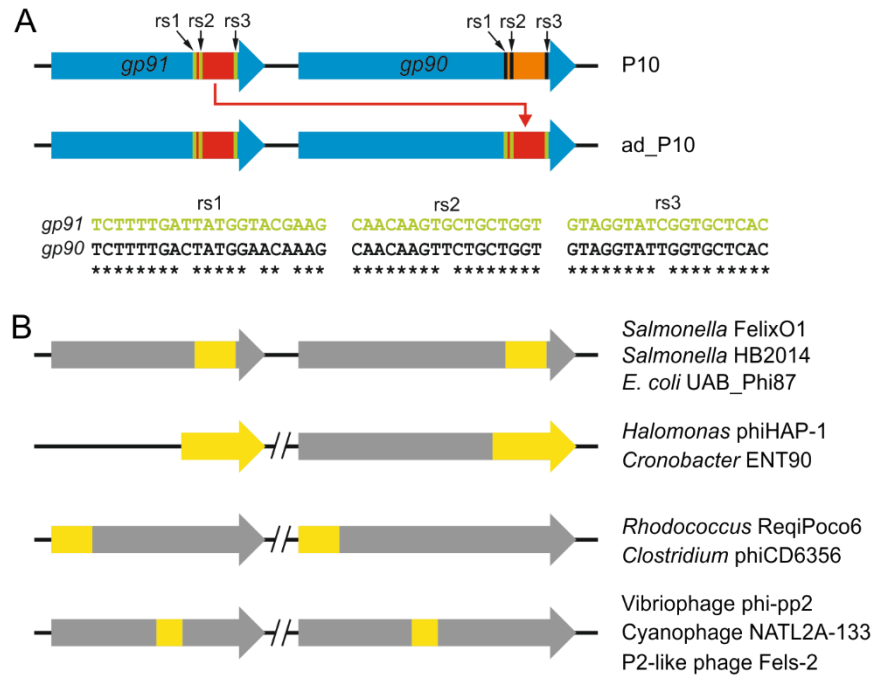
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481 Figure 3

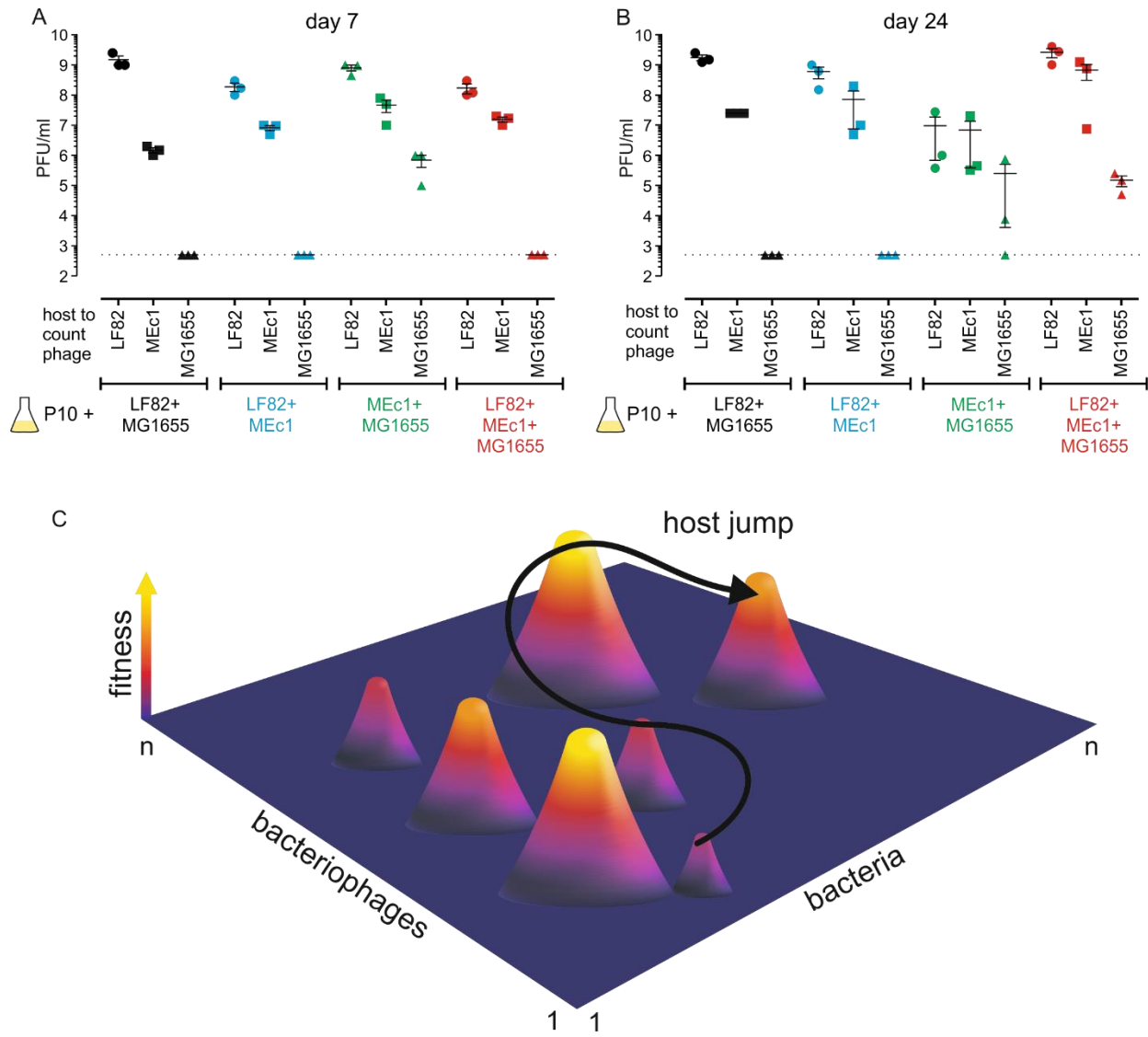


482

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484

485 Figure 4



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487

488

489 **STAR METHODS**

490 **CONTACT FOR REAGENT AND RESOURCE SHARING**

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

493

494 **EXPERIMENTAL MODEL AND SUBJECT DETAILS**

495 Microorganisms

496 Bacteriophage P10 (its full name is LF82_P10 – study accession number PRJEB18073) was
497 isolated and purified from wastewater, with an enrichment technique (Debarbieux *et al.*, 2010)
498 using *E. coli* strain LF82. Adsorption of P10 to strains LF82 and MG1655 was measured by
499 adding 2×10^5 pfu to exponentially growing cultures ($OD_{600}=0.3$) and redrawing aliquots every
500 60 seconds during 10 minutes to titer the unabsorbed bacteriophage (Hyman and Abedon,
501 2009). To distinguish both *E. coli* strains we introduced two different antibiotic and fluorescent
502 markers. Strain LF82S (Galtier *et al.*, 2016) was disrupted at the *ampC* locus by insertion of a
503 DsRed fluorescence marker gene along with a kanamycin cassette by lambda Red mediated
504 homologous recombination to produce strain LF82 DsRed, named LF82 in this manuscript.
505 Ampicillin resistance and GFP marker genes were P1 transduced from *E. coli* MG1655 $\lambda att-$
506 *ampgfp* (Da Re *et al.*, 2007) into strain *E. coli* MG1655 resistant to streptomycin (Da Re *et al.*,
507 2013) producing strain MG1655 GFP (named MG1655 in this manuscript). The techniques used
508 have been described in detail elsewhere (Datsenko and Wanner, 2000; Thomason *et al.*, 2007).
509 Strains were routinely cultured in lysogeny broth (LB), or on LB agar or Drigalski agar (Bio-Rad,

510 Hercules, CA) plates, at 37°C. When required, streptomycin (100 µg/mL), kanamycin (50 µg/mL)
511 or ampicillin (50 µg/mL) (Sigma, St. Louis, MO) was added.

512

513 Animals and Ethics

514 BALB/cYJ mice (seven-week-old females) supplied by Charles River Laboratories and CHO axenic
515 mice bred at the Institut Pasteur (Paris, France) were housed in an animal facility in accordance
516 with Institut Pasteur guidelines and European recommendations. Groups of co-housed mice are
517 indicated in the method details section. Food and drinking water were provided ad libitum.
518 Protocols were approved by the veterinary staff of the Institut Pasteur animal facility (Ref.
519 #10.565) and the National Ethics Committee (Ref. #2015-0040).

520

521 **METHOD DETAILS**

522 Experimental conditions of coevolution

523 Bacteriophage P10, strains LF82 and MG1655 were mixed in planktonic cultures (*in vitro*
524 setting) or introduced in mice (*in vivo* settings) as indicated in Figure 1A and as detailed below.

525 *In vitro*

526 Approximately 10⁸ cfu of each bacterial strains grown to stationary phase were inoculated both
527 individually and combined, in 1ml of LB supplemented with streptomycin (50 µg/µL) incubated
528 statically at 37°C. Three days later 2 x 10⁶ pfu of P10 were added to the mix. Every seven days
529 bacterial and bacteriophage titers and infectivity towards both initial bacterial strains were
530 obtained from 100 µL of the sample and cultures were refreshed starting from a 10-fold

531 dilution in fresh medium. Three technical replicates of three independent experiments were
532 performed.

533 *In vivo*

534 Ten groups of 1 to 5 mice for each *in vivo* model (dixenic and conventional mice) were force-fed
535 strains LF82 and MG1655 (approximately 10^8 cfu of each strain per mouse, grown to stationary
536 phase), followed, three days later, by a single bacteriophage administration (2×10^6 pfu per
537 mouse). In conventional mice, streptomycin (5 g/L) was added to drinking water from three
538 days before bacterial administration when it was lowered to 0.5 g/L and renewed weekly.
539 Absence of detectable endogenous *E. coli* and bacteriophages infecting LF82 or MG1655 was
540 confirmed prior to bacteriophage administration by plaque assay. A control group consisted in 5
541 mice caged together and receiving only streptomycin. Whenever a mouse displayed an
542 adaptation event, those sharing the same cage also carried such bacteriophage variants. Due to
543 likeliness of coprophagy in these animals, each cage was considered to have hosted a single
544 adaptation event.

545 Bacteriophage P10 was also independently mixed with strain LF82 or MG1655 *in vitro* and in
546 conventional mice during 24 days and no plaques were ever recovered on lawns of MG1655.

547

548 Bacteria and bacteriophage quantification

549 Bacterial and bacteriophage samples from liquid cultures or freshly collected fecal samples
550 were weighed and homogenized in PBS, serially diluted in PBS and 4 μ L of each dilution were
551 spotted onto LB or Drigalski agar supplemented with streptomycin and kanamycin or ampicillin
552 for the quantification of strains LF82 or MG1655, respectively. Bacteriophage titers were

553 determined by spotting the same dilutions onto LB agar plates covered with a bacterial lawn of
554 the host strain and supplemented with the appropriate antibiotics. The plates were incubated
555 for 18 hrs at 37°C. The detection limit was 5×10^2 cfu or pfu per millilitre of culture or gram of
556 faeces.

557

558 Host range of adapted bacteriophages

559 Ad_P10 (n=16) were tested for infectivity, as described above, against different *E. coli* strains
560 (n=105) listed in Table S1 and compared to the profile of wild type P10.

561

562 Analysis of genomic sequences

563 Sequencing of bacteriophages and bacteriophage populations (study accession number
564 PRJEB18073) was performed using Illumina sequencing technology (Illumina Inc., San Diego,
565 CA). Bacteriophage DNA was extracted from a sterile bacteriophage solution, using DNase and
566 RNase pretreatments followed by a phenol-chloroform extraction, modified from Pickard
567 (Pickard, 2009). For bacteriophage sequence analysis, the quality of Illumina reads was
568 visualised by FastQC v0.10.1 Brabraham Bioinformatics
569 (<http://www.bioinformatics.babraham.ac.uk/projects/fastqc/>). Bacteriophage assembly was
570 performed using a workflow implemented in Galaxy-Institut Pasteur using *clc_assembler* v4.4.2
571 and *clc_mapper* v4.4.2 (CLC Bio, Qiagen). P10 annotation was performed by the RAST v2.0
572 server (Aziz *et al.*, 2008) followed by manual curation. For synteny analysis, we aligned all the
573 contigs of phage genomes using *blastn* v2.2.18 (Altschul *et al.*, 1990) (with option `-F F`) against
574 the sequence of P10. Artemis Comparison Tool (Carver *et al.*, 2005) was used to visualize the

575 synteny and for detection of recombination. No other bacteriophage than P10 was ever
576 purified and sequenced from amplification on strains LF82 or MG1655.
577 For researching hIGR events in other bacteriophage genomes, we downloaded the annotation
578 of all bacteriophages available on PhAnToMe (<http://www.phantome.org/>) (September 2016)
579 and looked for homologous regions in all annotated tail fiber sequences within each
580 bacteriophage genome using megablast blastn v2.2.18 (with option -F F). Genome mutations
581 were identified using the BRESEQ Variant Report - v0.26 pipeline (Barrick *et al.*, 2009) and the
582 annotation of identified variants was processed using SnpEff v4.1 (Cingolani *et al.*, 2012).

583

584 Bacteriophage recombination

585 An adenine to guanidine substitution at nucleotide position 55079 of P10 genome was
586 introduced in the *gp91* gene by site-directed mutagenesis using Polymerase Chain Reaction
587 (PCR). The mutated gene was cloned as an *EcoRI-XbaI* fragment in plasmid pUC18 (Norlander *et*
588 *al.*, 1983) and transformed into LF82. P10 was added to a culture of LF82, carrying such plasmid
589 (or an empty plasmid as control), in early logarithmic growth phase, at an M.O.I. of 0.1, co-
590 growing with strain MG1655 in equal proportions to enrich the recombinant population. A
591 lysate recovered after 4 hrs incubation at 37°C was plated on a lawn of either strain LF82 or
592 MG1655 to recover ad_P10 plaques. Presence of the mutation was confirmed by PCR in 20
593 plaques isolated on MG1655 and following three rounds of purification. No plaques on MG1655
594 were observed for the control group.

595

596 Bacteriophage fitness test

597 The infection efficiency of the recombinant P10 bacteriophage (rP10) carrying the Y284H
598 mutation in the Gp91 protein compared to the wild type P10 bacteriophage was measured as
599 follows. First, rP10 was replicated on the MG1655 strain during four cycles of four hours at
600 multiplicity of infection (M.O.I.) = 0.001. The resulting bacteriophage lysate was named rP10
601 [MG]. Then, P10, rP10 and rP10 [MG] were added to exponentially growing cultures at
602 $OD_{600}=0.2$ of either strains LF82 + MG1655, LF82 + MEc1 or MEc1 + MG1655 mixed in equal
603 proportions and at M.O.I. = 0.01. A negative control included the same amount of
604 bacteriophages mixed to growing medium only. Co-cultures were incubated at 37°C statically
605 during 4 hrs and bacteriophages were titered on each of the bacterial strains. Data were
606 normalized as coefficient of multiplication compared to the initial bacteriophage inoculum.
607 Experiments were independently replicated three times and means were compared by
608 unpaired, one-tailed, t-test.

609

610 MEc1 isolation and sequencing

611 Frozen fecal samples from streptomycin-treated control mice (not exposed to P10 or LF82 or
612 MG1655) were serially diluted and plated aerobically onto Drigalski plates to select for possible
613 Enterobacteriaceae. Isolated positive colonies were replicated in liquid cultures to approximate
614 $OD_{600}=0.2$, and 6 μ L of culture were spotted onto agar plates and exposed to 1×10^5 pfu of P10
615 in a 4 μ L double spot assay (Saussereau *et al.*, 2014). MEc1 was chosen as representative of 50
616 bacteriophage-sensitive colonies out of 60. Bacteriophage-bacteria co-culture was performed
617 as detailed above in the experimental conditions of coevolution. Genomic sequencing (study

618 accession number PRJEB21810) was performed using Illumina technology (Illumina Inc., San
619 Diego, CA) and analyzed as above.

620

621 **QUANTIFICATION AND STATISTICAL ANALYSIS**

622 Statistical analysis

623 Statistical analyses (Mann Whitney test and Student t-test) were conducted with Prism 5
624 software (GraphPad). $p < 0.05$ was considered statistically significant.

625 Sample size was determined by the “resource equation method” (Mead, 1988).

626

627 **DATA AND SOFTWARE AVAILABILITY**

628 The genomic sequences of bacteriophage P10, ad_P10 and rP10 as well as bacteriophage
629 populations have been deposited in the ENA under study accession number PRJEB18073.

630 The genomic sequence of strain MEc1 has been deposited in the ENA under study accession
631 number PRJEB21810.

632