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1 **Molecular approaches to uncover phage-lactic acid bacteria**
2 **interactions in a model community simulating fermented**
3 **beverages.**

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23 **Abstract**

24 Food microbial diversity and fluxes during the fermentation processes are well studied
25 whereas phages-bacteria interactions are still poorly described in the literature. This is
26 especially true in fermented beverages, and especially in cider, which is an alcoholic
27 fermented apple beverage. The transcriptomic and proteomic responses of the lactic acid
28 bacterium (LAB) *Liquorilactobacillus mali* UCMA 16447 to a lytic infection by phage
29 UCMA 21115, both isolated from cider, were investigated, in order to get a better
30 understanding of phages-bacteria interactions in such fermented beverage. During phage
31 infection, 122 and 215 genes were differentially expressed in *L. mali* UCMA 16447 strain at
32 T₁₅ and T₆₀ respectively, when compared to the uninfected condition. The same trends were
33 confirmed by the proteomic study, with a total of 28 differentially expressed proteins found at
34 T₆₀. Overall, genes encoding cellular functions, such as carbohydrate metabolism, translation,
35 and signal transduction, were downregulated, while genes involved in nucleotide metabolism
36 and in the control of DNA integrity were upregulated in response to phage infection. This
37 work also highlighted that phage infection repressed many genes involved in bacterial cell
38 motility, and affected glycolysis.

39 **Keywords:** Phage, fermented food, fermented beverage, microbial interactions, cider

40

41 **1. Introduction**

42 The diversity of bacteriophage (phage) communities, known as phageomes and to a larger
43 extent as viromes, in fermented foods and beverages is poorly described, as reviewed by
44 Ledormand et al., 2020. If the occurrence of phages in such environments has been
45 demonstrated decades ago, the data which illustrate their impact on mixed communities and
46 their ecological roles are scarce when compared to other microbial ecosystems (Paillet and
47 Dugat-Bony, 2021). Phages are often studied for their negative impact on bacteria with
48 technological interests like starter cultures, while they also play a major role as drivers of
49 microbial communities (Mills et al., 2013). The behaviour of their bacterial hosts during
50 phage infection has been reported in a few studies. This is especially true for a few potentially
51 pathogenic bacteria such as *Pseudomonas aeruginosa* (Chevallereau et al., 2016; Lavigne et
52 al., 2013; Zhao et al., 2016), *Yersinia enterocolitica* (Leskinen et al., 2016), and *Enterococcus*
53 *faecalis* (Chatterjee et al., 2019), whose the transcriptomic response during phage infection
54 was evaluated.

55 Lactic acid bacteria (LAB) are of great importance in the making and preservation of
56 fermented foods and beverages. Knowledge about phages-LAB interactions remains to be
57 developed. Some studies focusing on the response of the well-known dairy starter species
58 *Lactococcus lactis* during phage infection have been carried out at the transcriptomic and the
59 proteomic levels (Fallico et al., 2011; Ainsworth et al., 2013; Lemay et al., 2019, 2020). Apart
60 from these data on *Lc. lactis*, little is known about the mechanisms of phages-LAB
61 interactions in fermented foods and beverages. Cider, which is an alcoholic fermented apple
62 beverage, is a typical example of a ‘black box’ ecosystem when it comes to phages-LAB
63 interactions within the microbial communities throughout the fermentation process. Cider
64 microbiota has the particularity to be less diverse than in other fermented foods, due to harsh
65 physico-chemical conditions related in part to the low pH and the presence of ethanol (Misery

66 et al., 2021). Yeasts and LAB are the two types of microorganisms that contribute to the
67 fermentation process of cider. Yeasts include *Saccharomyces* (*S. cerevisiae*, *S. uvarum*) and
68 non-*Saccharomyces* (*Hanseniaspora*, *Candida*) genera, and are responsible for the alcoholic
69 fermentation (Misery et al., 2021). The malolactic fermentation (MLF) is performed by LAB
70 from *Oenococcus* sp., *Leuconostoc* sp., *Pediococcus* sp. and other *Lactobacillaceae* genera
71 (members of the former *Lactobacillus* genus) (Cousin et al., 2017). Among these LAB,
72 *Liquorilactobacillus mali* is a species which is commonly isolated from apple juice and cider.
73 *L. mali* cells are Gram positive rods (0.6 μm \times 1.8-4.2 μm), catalase positive,
74 homofermentative, potential biogenic amine productive and motile (Carr and Davies, 1970;
75 Coton et al., 2010; Cousin et al., 2015; Neville et al., 2012). To date no phage targeting *L.*
76 *mali* is known.

77 More generally, the diversity of phages in cider has not been described yet, and much less
78 how phage predation could influence cider fermentation (Ledormand et al. 2020).
79 Deciphering the role of phages in cider microbial communities would contribute to get a
80 better understanding of the microbial dynamics involved in fermentation processes. The aim
81 of the present study was to assess the interactions between phage UCMA 21115 and its LAB
82 host strain, *L. mali* UCMA 16447, both originating from cider, by means of transcriptomic
83 and proteomic methods.

84 **2. Materials & Methods**

85 ***2.1 Bacterial strains and growth conditions***

86 Five strains, *Liquorilactobacillus mali* UCMA 16447 strain, *L. mali* UCMA 19420 strain,
87 *Secundilactobacillus collinoides* UCMA 16566 strain, *S. collinoides* UCMA 20009 strain, and
88 *Lactobacillaceae sp. nov.* UCMA 15818 strain (unpublished data), were used in this study.
89 They were from the UCMA collection (Université de Caen Microbiologie Alimentaire, Caen,
90 France) and all originated from cider. Strains were routinely aerobically grown in de Man
91 Rogosa & Sharpe (MRS; Difco) broth adjusted to pH 5.5, supplemented with 5 g/L of
92 fructose and 0.5 g/L of cysteine, and incubated at 30°C for 24 hours.

93 ***2.2 Phage isolation, propagation and titration***

94 Phage UCMA 21115 was isolated from a traditional French cider sample collected in the
95 Normandy region (France). Its isolation and propagation were performed against the *L. mali*
96 UCMA 16447 strain. Phage isolation was performed with an enrichment step where 1 mL of
97 cider was added to a 10 mL-culture of *L. mali* UCMA 16447 at the exponential growth phase,
98 and incubated overnight at 30°C. The phage enrichment in MRS medium was centrifuged at
99 $4,700 \times g$ for 20 min and filtered sterile using 0.45 μm filters, and stored at 4°C. Phage
100 isolation and propagation were carried out using the classical double layer plate technique
101 using MRS agar 0.5% (w/v) supplemented with 10 mM CaCl_2 and 10 mM MgSO_4 , and
102 incubated at 30°C for 24 h to allow the formation of plaques. Phage titration was expressed as
103 plaque-forming units per mL (PFU/mL).

104 ***2.3 Electron microscopic analysis***

105 Phage lysates (10^9 PFU/mL) were purified on a caesium chloride gradient by
106 ultracentrifugation (Dalmaso et al., 2016). The phage phase was then collected and dialyzed

107 in phage buffer (100 mM Tris-HCl pH 7.5; 100 mM NaCl, 10 mM MgCl₂) overnight at 4°C.
108 The phages were then observed with the transmission electron microscope JEOL 1011
109 (accelerating voltage of 80 kV), after a negative staining with uranyl acetate (1.5%).

110 **2.4 Adsorption rate of phage UCMA 21115**

111 Phage UCMA 21115 was added to *L. mali* UCMA 16447 at a multiplicity of infection (MOI)
112 of 0.1 and incubated at 30°C for 30 min. Every 5 min, 2 mL were collected and filtered
113 through a 0.45 µm filter in order to assess the phage titer in the supernatant by counting the
114 plaques using the double layer agar method. The adsorption rate was calculated with the
115 formula $(N_0 - N_t / N_0) * 100$ where N_0 represents the phage titer used before the co-incubation,
116 and N_t the phage titer at time t in the supernatant after filtration (Feng et al., 2020). The
117 amount rate of non-adsorbed phages compared to the amount of phages for the infection is
118 represented with the standard deviations of three independent experiments.

119 **2.5 One-step-growth experiments (OSGC)**

120 The OSGC was performed as described by Jaomanjaka et al., 2016, in triplicate. Briefly, the
121 *L. mali* UCMA 16447 strain was cultured until an OD_{600nm} value of 0.2, and 1 mL was
122 centrifuged at 10,000 × g for 2 min. The pellet was re-suspended in 1 mL of phage UCMA
123 21115 lysate in order to yield a MOI of 0.1, and was then incubated for 30 min at 30°C to
124 allow phage adsorption. The sample was centrifuged at 10,000 × g for 2 min to eliminate
125 unabsorbed phages. The pellet was then diluted (10^{-3}) in MRS broth and incubated at 30°C.
126 Some samples were collected every 30 min, filtered sterile with 0.45 µm filters, and
127 enumerated using the double layer plate count agar method. The burst size, the latency and
128 rise phases of phage UCMA 21115 were estimated. The OSGC is presented with the standard
129 deviations of three independent experiments.

130 **2.6 DNA extraction, genome sequencing and genome analysis**

131 Phage DNA was extracted as described by Dalmasso et al., (2012) after phage fraction
132 recovering issued from an ultracentrifugation at $35,000 \times g$ for 3 h on a caesium chloride
133 gradient. Whole phage genome sequencing was performed on an Illumina sequencer
134 producing paired-end reads 2×150 bases in length. Reads were assembled into a contig using
135 Unicycler (Wick et al., 2017). An *in silico* genome analysis was performed using RAST
136 (Rapid Annotation using Subsystem Technology) for ORFs and putative proteins prediction
137 (Aziz et al., 2008). The genome of phage UCMA 21115 is available under the accession
138 number: ON117153.

139 ***2.7 Constitution of a LAB model community for studying phage-bacteria interactions***

140 The five LAB strains were grown separately overnight in MRS broth at 30°C as precultures.
141 One litre of fresh MRS broth was inoculated with 10 mL of each of the four strains making up
142 the model community (i.e. UCMA 19420, UCMA 16566, UCMA 20009, and UCMA 15818
143 strains), and incubated at 30°C until an $\text{OD}_{600\text{nm}}$ value of 0.2. In the same way, 500 ml of
144 MRS broth were inoculated with 5 mL of *L. mali* UCMA 16447 strain as preculture. Once the
145 *L. mali* UCMA 16447 culture had reached an $\text{OD}_{600\text{ nm}}$ value of 0.2, it was divided into two
146 fractions: one fraction was infected by phage UCMA 21115 at a MOI of 0.1, and the other
147 was not infected, and used as a growth control. The two fractions were immediately placed
148 into cellulose dialysis tubes (MWCO 12000 – 14000, ROTH) (Saraoui et al., 2013), and
149 immersed into the growth medium inoculated with the synthetic bacterial community.
150 Dialysis tubing was made to contain *L. mali* UCMA 16447 strain but allows the exchange of
151 solutes with the synthetic bacterial community. Incubation was carried out at 30°C . The
152 samples from the dialysis tubes were taken at 0, 15 and 60 min after phage infection
153 corresponding to the early infection phase (10 and 30 mL for RNA and protein extractions,
154 respectively). The cells were harvested by centrifugation at $4,700 \times g$ for 10 min and cell
155 pellets were stored at -80°C prior to RNA and protein extraction. Three independent

156 biological replicates per growth condition were performed, which represented a total of 15
157 samples (3 replicates at T0, 3 replicates at T15 with and without phages, and 3 replicates at
158 T60 with and without phages). At T0 and T60, 30 mL of the model community were also
159 sampled for protein extraction.

160 ***2.8 RNA extraction***

161 The pellets were re-suspended in 500 μ L of TE buffer (0.01M Tris pH 7.5; 0.01M EDTA) and
162 the cells were lysed at 6,000 \times g at 4°C with a Precellys® (Bertin Technologie, France) using
163 a mix of glass beads (1.5 mm, Dutsher, France) and zirconium beads (0.5 and 0.1 mm,
164 Dutsher, France). Total RNA extraction was performed with TRIzol Reagent (Fisher
165 Scientific, Illkirch, France) and chloroform/isoamyl alcohol (24:1) (Sigma Aldrich, USA)
166 separation. RNA was then purified using Direct-Zol RNA Miniprep (ZymoResearch, Irvine,
167 CA, USA) according to the manufacturer's instructions, and RNA integrity was checked on a
168 denaturing agarose gel as described by Aranda et al., 2012. RNA quantification and
169 qualification was performed using a Nano TECAN spectrophotometer (Life Science,
170 Switzerland).

171 ***2.9 cDNA synthesis, RNA-Seq, reads mapping and data analyses***

172 rRNA depletion (Fastselect kit, Qiagen), cDNA library construction and RNA-Seq
173 sequencing were carried out at Genewiz, Inc. facilities (South Plainfield, NJ, USA). RNA
174 sequencing was performed on an Illumina sequencer, producing paired-end reads of 2x150
175 bases in length. In order to eliminate Illumina adaptors from sequencing, FastP processor
176 (Chen et al., 2018) was used before checking the quality of sequencing data with FastQC
177 v.0.11.8 (de Sena Brandine and Smith, 2019). The reads were mapped against the genome of
178 *L. mali* UCMA 16447 (Bioproject PRJNA727965) strain using Bowtie 2 (Langmead and
179 Salzberg, 2012) with a minimal fragment size of 50 pb and counted using HTSeq v.0.9.1

180 processor (Anders et al., 2015). Reads matching with coding DNA sequences (CDS) were
181 retrieved from the raw data set for subsequent analyses. The data were filtered to eliminate the
182 genes displaying an average of less than 10 reads per sample for all data (15 samples). The
183 statistical analysis of RNA-Seq data was performed using SARTools DESeq2 tool v.1.7.3
184 (Varet et al., 2016) with default settings. Gene transcripts with an adjusted p-value ($p < 0.05$)
185 and a fold change of ≥ 2 and ≤ -2 (in absolute values) were considered to be differentially
186 expressed between different conditions. The functional annotation of the transcripts was done
187 using the Kyoto Encyclopedia of Gene and Genomes (KEGG).

188 The RNA-Seq data used in this study are available in NCBI SRA repository, under the
189 reference number PRJNA804221.

190 ***2.10 Protein extraction***

191 The cell pellets were re-suspended in 2 mL of wash buffer (50 mM TrisHCl pH 8.0, 1 mM
192 PMSF), lysed at 6,000 x g with a Precellys® (Bertin Technologie, France) using a mix of
193 glass beads (1.5 mm) and zirconium beads (0.5 and 0.1 mm), and centrifuged at 10,000 x g
194 for 10 min at 4°C. The supernatant was recovered and the proteins were subsequently
195 precipitated with four volumes of precipitation buffer (80% of acetone, 20% of 50 mM
196 TrisHCl pH 8.0), and incubated for 1h at -80°C, then overnight at -20°C. The tubes were
197 centrifuged at 13,000 x g for 10 min at 4°C. The supernatant was removed, and the pellets
198 were dried for 30 min under a chemical hood. Finally, the proteins were re-suspended in a
199 protein buffer (50 mM TrisHCl pH 8.0, 5% glycerol, 1 mM EDTA, 1 mM PMSF, 100 mM
200 NaCl), and quantified using the Bradford method (Bradford, 1976). The proteins were
201 prepared from three independent biological replicates.

202 ***2.11 Mass-spectrometry analysis***

203 Protein preparation and nLCMS analysis were performed as previously described (Aubourg et
204 al., 2020). Before post-process, the samples were analysed using the Preview software
205 (ProteinMetrics) in order to estimate the quality of the tryptic digestion and predict any post-
206 translational modifications. The result presented below, is used for the “bank research /
207 identification” part. The fragmentation pattern was used to determine the sequence of the
208 peptide. Database searching was performed using the Peaks X+ software. A homemade
209 database composed of *L. mali* UCMA 16447, *L. mali* UCMA 19420, *S. collinoides* UCMA
210 16566, *S. collinoides* UCMA 20009 and strain UCMA 15818, was used. The variable
211 modifications allowed were as follows: Nterm-acetylation, methionine oxidation,
212 Deamidation (NQ). In addition, C-Propionamide was set as fix modification. “Trypsin” was
213 selected as Specific. Mass accuracy was set to 30 ppm and 0.05 Da for MS and MS/MS mode,
214 respectively. The data were filtered according to a FDR of 1%.

215 ***2.13 Identification of differentially expressed proteins***

216 To quantify the relative levels of protein abundance between the different groups, the samples
217 were analysed using the label-free quantification feature of the PEAKS X+ software. The
218 features of the same peptides from all replicates of each sample were aligned through the
219 retention time alignment algorithms. The mass error tolerance was set at 30 ppm, Ion Mobility
220 Tolerance (1/k0) at 0.05 and the retention time tolerance at 7 min. The normalization factors
221 of the samples were obtained by the total ion current (TIC) of each sample. The quantification
222 of the protein abundance level was calculated using the sum area of the top three unique
223 peptides. A 1.5-fold increase in relative abundance and a Peak significance ≥ 15 using
224 ANOVA as significance method were used to determine the enriched proteins.

225 3. Results

226 3.1 Characterization of phage UCMA 21115

227 Phage UCMA 21115 was isolated from a French cider sample, and infected *L. mali* UCMA
228 16447 strain. This phage has a small-isometric head and a long non-contractile tail, which
229 indicates that it belongs to the *Siphoviridae* family of the order Caudovirales (**Fig. 1**). The
230 head diameter was $60.18 \text{ nm} \pm 3 \text{ nm}$ ($n = 3$) and the tail length was $223.68 \text{ nm} \pm 5 \text{ nm}$ ($n = 3$).
231 The lytic activity of phage UCMA 21115 was assessed in MRS broth pH 5.5 at different MOI
232 values (1, 0.1 and 0.01) by performing $\text{OD}_{600\text{nm}}$ measurements (**Fig. S1**). Phage UCMA 21115
233 lysed its hosts in 4 h at a MOI of 1. A lytic activity was observed up to a MOI of 0.01 (**Fig.**
234 **S1**). As it was isolated from cider, which is an acidic fermented beverage, phage UCMA
235 21115 was also checked for its ability to resist to acidic pH values ranging from pH 3.0 to pH
236 5.5 (**Fig. S2**). Phage UCMA 21115 showed a strong resistance at pH values above 4.0 (**Fig.**
237 **S2**). Phage UCMA 21115 targeted only *L. mali* UCMA 16447 strain among the 120 tested
238 LAB strains (including strains of *Leuconostoc* sp., *Oenococcus* sp., *Pediococcus* sp. and
239 *Lactobacillaceae* members of the former *Lactobacillus* genus) (data not shown).

240 3.2 Kinetics profile of phage UCMA 21115

241 To establish the binding affinity of phage UCMA 21115 to its host strain *L. mali* UCMA
242 16447, the phage adsorption to the bacterial cells was estimated during an incubation period
243 of 30 min. After 5 min, nearly 80% of the phage particles adsorbed to the bacterial cells (**Fig.**
244 **2A**). After 20 min, more than 95% of the phage particles were attached to the host cells.

245 To better understand the population dynamics of phage UCMA 21115, a one-step growth
246 curve (OSGC) experiment was performed in the presence of *L. mali* UCMA 16447 strain at a
247 MOI of 0.1. The latent period lasted approximately 150 min, after 30 min of adsorption (T_0
248 is after 30 min of adsorption), before reaching the plateau phase after 240 min (**Fig. 2B**). The

249 burst size, which is the number of particles being released per cell, was of about 22 ± 2
250 phages produced per host cell.

251 ***3.3 Genome analysis of phage UCMA 21115***

252 Phage UCMA 21115 has a genome of 27,915 bp with a G+C content of 36.3%. A total of 42
253 open reading frames (ORFs) were predicted from the genome with no tRNA found (**Table**
254 **S1**). Among the 42 ORFs, 21 were predicted on the forward strand, 21 on the reverse
255 orientation, and 7 out of the 42 ORFs were assigned to a predicted function by RAST. The
256 strictly lytic activity was confirmed since no module associated with lysogeny was found
257 inside the genome. The predicted functions found inside the genome were in relation to
258 packaging (terminase subunit), replication (DNA polymerase subunit, DNA primase),
259 structure protein (tail protein, phage protein) and lysis (hydrolase, lysin) (**Table S1**). The
260 genome was also BLAST against the NCBI virus database to identify the most related phages.
261 Phage UCMA 21115 was close to *Leuconostoc* phages, particularly phage P974 (accession
262 number MN552147.1, identity = 99.65 %, coverage = 99%) and phage phiLNTR3 (accession
263 number KC013029.1, identity = 99.45 %, coverage = 99%).

264 ***3.4 Global overview of the transcriptional response of L. mali UCMA 16447 when infected*** 265 ***by phage UCMA 21115***

266 In order to study the impact of the lytic phage UCMA 21115 on *L. mali* UCMA 16447 strain,
267 a transcriptomic approach based on RNA-Seq was followed. As the first minutes after a stress
268 are crucial for bacterial survival, three time points were chosen after phage infection: 0, 15
269 and 60 min (the adsorption time was not taken into account here). The gene levels of
270 expression in cells subjected to phage infection were compared to uninfected cells.

271 About 115 millions of sequencing reads were generated for all conditions, and the number of
272 reads per sample varied from 4.4×10^6 to 1×10^7 (**Fig. S3**).

273 Read counts were established for each CDS available on the reference genome. The threshold
274 for a comparative classification of gene expression was set to 2-fold with $p < 0.05$ being
275 considered as a significant differentially expressed gene. This fold-change corresponds to a
276 change that is higher than 2 or lower than 0.5 in absolute values. **Table 1** presents the number
277 of up or down regulated genes for each comparison. When comparing the infected conditions
278 (P) to the control experiment, a total of 122 genes were differentially expressed (DE) after 15
279 min (T15 vs T15P). This number almost doubled after 60 min, with 215 DE genes (T60 vs
280 T60P). It appeared that translation, cell motility, signal transduction and carbohydrates
281 metabolism were the most affected KEGG functional categories of genes that were repressed
282 (**Fig. 3**). After 15 min of phage infection, 10, 8 and 7 genes involved in translation, cell
283 motility and signal transduction, respectively, were repressed (**Fig. 3A**). After 60 min of
284 infection, the same categories were affected, with an increase in the number of DE genes, and
285 especially in repressed genes. For example, 22 genes involved in cell motility, 22 involved in
286 translation, 15 involved in signal transduction and 15 involved in carbohydrates metabolism
287 were repressed (**Fig. 3B**). The number of induced genes after 60 min of infection increased in
288 the same functional categories than after 15 min, with 10 and 5 genes induced in the
289 nucleotide metabolism and in the carbohydrates metabolism, respectively (**Fig. 3B**). Three
290 genes involved in amino acid metabolism, replication and repair or folding, and sorting and
291 degradation were also induced (**Fig. 3B**).

292 *3.5 Identification of the induced and repressed genes of L. mali UCMA 16447 during phage* 293 *infection*

294 All the functions impacted by phage infection are depicted on **Fig. 4**.

295 *3.5.1 Drastic modifications of cell motility and chemotaxis during phage infection*

296 Cellular motility was one of the main repressed function during phage infection. After 60 min,
297 26 genes were repressed encoding proteins for flagellar assembly such as basal body
298 components (*fliE*; fold change of 0.46; *fliR*; fold change of 0.41), hook (*flgB*; fold change of
299 0.29; *flgD*; fold change of 0.31), and filament (*fliS*; fold change of 0.13) (**Table S2**). In
300 addition to cell motility, 11 genes involved in chemotaxis belonging to the signal transduction
301 category were repressed. Among these genes involved in chemotaxis for the regulation of the
302 flagellar system, a methyl-accepting chemotaxis protein (MCP) (fold change of 0.15) and
303 genes encoding for two-component systems like *cheA*, *cheB* and *cheW* (fold change of 0.27;
304 0.17 and 0.18, respectively) were repressed (**Table S2**). Genes encoding in the stator of the
305 system (*MotA*; fold change of 0.13, and *MotB*; fold change of 0.18) were also repressed
306 (**Table S2**).

307 *3.5.2 Modification of the expression of translation-coding genes during phage infection*

308 The cellular translation was also repressed as evidenced by the downregulation of many of the
309 genes coding for ribosomal subunits. More than 20 of these genes were repressed after 60 min
310 of infection (fold change between 0.23 and 0.49) (**Table S2**). On the opposite, the genes
311 involved in the synthesis of amino acids like glycine, serine and threonine (homoserine
312 deshydrogenase; fold change of 2.11) (**Table S2**) were overexpressed during the infection.

313 *3.5.3 Large induction of functions for DNA replication during infection*

314 The machinery necessary for the replication and the synthesis of the phage genome was
315 induced. Genes encoding the biosynthesis of purines and pyrimidines were more expressed in
316 the infected condition than in the uninfected condition. For example, the ribonucleoside-
317 diphosphate reductase (*rNDP*; fold change of 3.82) and the orotidine-5'-phosphate
318 decarboxylase (*pyrF*; fold change of 2.07) genes were induced (**Table S2**). Genes coding for
319 enzymes essential to DNA replication, like DNA polymerase (*DNApol* subunit gamma; fold

320 change of 5.86) and DNA gyrase subunit A (*gyrA*; fold change 2.18) (**Table S2**), were
321 overexpressed. Another gene coding for an enzyme which is probably involved in the
322 recombination of DNA was also overexpressed (*RecR*; fold change of 3.12) (**Table S2**).

323 *3.5.4 Inhibition of the glycolysis pathway during phage infection*

324 Another gene functional class affected by phage infection was the metabolism of
325 carbohydrates. The phosphotransferase system (PTS) involved in the mannose uptake was
326 repressed during the infection (fold change of 0.40) (**Fig. 5 & Table S2**). Several genes
327 coding for enzymes involved in the Embden-Meyerhof pathway (glycolysis) were repressed
328 (**Table S2**). It was the case for the phosphofructokinase gene (*pfk*; fold change of 0.42), the
329 glyceraldehyde 3-phosphate dehydrogenase gene (*gpdh*; fold change of 0.43), the
330 phosphoglycerate kinase gene (*pgk*; fold change of 0.38), the phosphoglycerate mutase gene
331 (*pgm*; fold change of 0.49) and the triose phosphate isomerase gene (*tpi*; fold change of 0.42)
332 (**Fig. 5**). Conversely, the D-lactate dehydrogenase coding gene (*ldh*; fold change of 2.52) was
333 highly induced during phage infection (**Fig. 5**), suggesting an impact on the lactate
334 metabolism. The overexpression of the fructose 1,6 biphosphatase coding gene (*fbp*; fold
335 change of 3.99) (**Fig. 5**), which has an antagonistic activity to PFK, comes to reinforce the
336 idea of an inhibition of the glycolysis pathway. Other genes encoding enzymes involved in
337 carbohydrate metabolism were differentially expressed like the malate dehydrogenase gene
338 (*mdh*; 2.80 fold) and the acetate kinase gene (*ack*; fold change of 0.47).

339 *3.5.5 Other functions affected by phage infection*

340 The genes involved in lipid metabolism, and more specifically in fatty acid biosynthesis, like
341 the acetyl-CoA biotin carboxylase gene (*accC*; fold change of 0.32), the oxoacyl reductase
342 gene (*fabG*; fold change of 0.35) and the hydroxyacyl dehydratase gene (*hcd*; fold change of
343 0.40) (**Table S2**) were repressed during phage infection.

344 Genes coding for membrane transporters like the ATP-binding cassette (ABC), the phosphate
345 transport system ATP-binding protein (fold change of 0.46) and the energy transport system
346 ATP-binding protein (fold change of 0.46) were downregulated, hence demonstrating a
347 decline in nutrient uptakes from the cell environment (**Table S2**).

348 Genes encoding stress response proteins like chaperones GroES, GroEL (*groES*; *groEL*; fold
349 change of 2.34 and 2.69, respectively) and DnaJ (*dnaJ*; fold change of 2.10) were induced
350 during phage infection (**Table S2**). The largest group of induced genes encoded hypothetical
351 proteins and proteins of unknown function.

352 As *L. mali* UCMA 16447 strain was part of a four-strain model community closely related at
353 the genus level, the existence of possible signal exchanges during phage infection were also
354 sought at the transcriptomic level in *L. mali* UCMA 16447 strain. No evidence of this
355 phenomenon were found.

356 ***3.6 Changes in the proteome of L. mali UCMA 16447 strain during phage infection***

357 In addition to the RNA-Seq analysis, a proteomic approach to identify the major proteins
358 impacted by phage infection was carried out. Comparisons were made for *L. mali* UCMA
359 16447 strain between T0 and after 60 min with phage (T60P) and without phage (T60), and
360 between T60 and T60P. It appeared that 229 proteins were differentially expressed between
361 T0 and T60P (**Fig. S4**), and 227 of them were overexpressed at T60P. When comparing the
362 proteomes at T60 and T60P (**Fig. 6**), 28 proteins were differentially expressed, and only one
363 was under-expressed. The energy production and conversion category counted 5 DE proteins,
364 followed by carbohydrate transport and metabolism, nucleotide transport and metabolism, and
365 replication, recombination and repair, and translation (3 DE proteins for each category).

366 ***3.6.1 Confirmation of motility repression at the proteome level***

367 The only protein that was less abundant during phage infection was the flagellin protein (fold
368 change of -1.17) (**Table S3**). This observation followed the same pattern as RNA-Seq data
369 analysis, indicating the repression of cell motility during phage infection.

370 *3.6.2 Overproduction of DNA replication associated proteins during phage infection*

371 An increase in purine biosynthesis-related proteins during phage infection was observed. For
372 example, the hypoxanthine-guanine phosphoribosyltransferase (fold change of 1.48), an
373 enzyme involved in this function was more present in the infected condition (**Table S3**).
374 Proteins involved in nucleotide metabolism (D-amino acyl-tRNA deacylase, cytidylate kinase,
375 and deoxyguanosine kinase; fold change of 1.69, 1.68 and 1.87, respectively) (**Table S3**) and
376 in the replication and repair of DNA (DNA polymerase III, Sak2, and ribonucleoside
377 diphosphate reductase; fold change of 1.65, 5.96 and 3.20, respectively) (**Table S3**) were
378 more abundant in the infected condition than without phages, thus confirming the
379 transcriptomic results.

380 *3.6.3 Other proteins*

381 Other DE proteins, especially the proteins involved in amino-acid transport and metabolism,
382 were also identified. The tRNA adenine-methyltransferase, involved in amino acid
383 biosynthesis, and a proline iminopeptidase, involved in the release of amino acids (fold
384 change of 2.53 and 1.34, respectively) (**Table S3**), were more abundant in the phage infection
385 condition than in the control condition. A universal stress protein not clearly identified was
386 more abundant during phage infection than in the control condition (fold change of 1.77)
387 (**Table S3**).

388 Overall, all these proteomic results followed similar patterns as the results obtained with the
389 transcriptomic approach, with the exception of the L-2-hydroxyisocaproate dehydrogenase,
390 that has a catalytic activity close to the phosphofructokinase and the triose phosphate

391 isomerase (fold change of 1.71 and 1.18, respectively) (**Table S3**). Indeed, these proteins
392 were more abundant with the proteomic approach whereas their encoding genes were
393 repressed with the transcriptomic method.

394 In parallel with these comparisons, a proteomic study was performed on the proteome of the
395 synthetic bacterial community, *i.e.* on *L. mali* UCMA 19420, *S. collinoides* UCMA 16566, *S.*
396 *collinoides* UCMA 20009 and *L. sp. nov* UCMA 15818. No differences were found between
397 the conditions with and without phages (data not shown).

398 4. Discussion

399 Exploring the response of a food bacterium to phage infection is essential to understand what
400 happens at the population level, and more generally at the community level during
401 fermentation processes. Phage-LAB interactions in fermented foods are poorly described
402 using omics methods compared to phage-pathogenic bacteria interactions. In this context,
403 phage UCMA 21115, the first lytic phage isolated from cider, was isolated and characterized.
404 It targeted *L. mali* UCMA 16447 strain, also originating from cider. Phage UCMA 21115
405 belongs to the *Siphoviridae* family like other phages isolated from fermented foods and
406 beverages. For example, phage Vinitor targeting *O. oeni* (Philippe et al., 2021), phage Φ 1-A4
407 targeting *Ln. mesenteroides* (Lu et al., 2010), phage TP901-1 targeting *Lactococcus lactis*
408 (Mahony et al., 2016) and phage ATCCB targeting *Levilactobacillus brevis* (Feyereisen et al.,
409 2019) all belong to the *Siphoviridae* family. Phage UCMA 21115 has a genome of 27,915 bp,
410 which is smaller than other *Lactobacillus Siphoviridae* like, for example, *L. plantarum* phage
411 ATCC8014 displaying a 38,002 bp genome (Briggiler Marcó et al., 2019) and *L. delbrueckii*
412 subsp. *bulgaricus* phage PMBT4 displaying a 31,399 bp genome (Sprotte et al., 2022). Phage
413 UCMA 21115 was closer to *Leuconostoc* phages like phiLNTR3 (99.45 % of identity)
414 isolated from dairy products, than to other *Lactobacillus* phages (Kot et al., 2014). The lytic
415 potential of phage UCMA 21115 was characterized. The replication rate of a phage is
416 proportional to the growth rate of the bacterial strain. Thus, cell lysis appears more or less
417 quickly depending on the bacterial species and strain, and depending on the phage and its
418 MOI. For example, it appeared after 24 hours for *O. oeni* IOEB S 277 strain during infection
419 by phage Φ OE33PA (Jaomanjaka et al., 2016), and after 120 minutes for *Salmonella*
420 *enterica* subsp. *enterica* serovar Enteritidis ATCC 13076 strain during infection by phage
421 BPSELC-1 (Li et al., 2020). In the current study, the complete lysis of *L. mali* UCMA 16447
422 strain by phage UCMA 21115 occurred after 240 minutes.

423 Phage UCMA 21115 was then used in a model of infection of *L. mali* UCMA 16447 strain in
424 order to comprehensively investigate the transcriptional and proteomic response of the host
425 strain to phage infection, when placed within a bacterial community. To date, the classical
426 approaches to investigate phage-host interactions, particularly using transcriptomic and
427 proteomic methods, are based on single phage-strain couple models, as it was the case, for
428 example, in the transcriptomic study of *Lc. lactis* UC509.9 strain and phages Tuc2009 and c2
429 (Ainsworth et al. 2013). In the current study, placing *L. mali* UCMA 16447 strain in dialysis
430 tubing (Saraoui et al. 2013) immersed into a model community of four strains belonging to
431 the *Lactobacillaceae* family was intended to create conditions that possibly favour
432 interactions between strains. In artificial co-cultures, many positive interactions may occur, as
433 reviewed by Canon et al., 2020. For example, co-cultivation promotes interactions like
434 commensalism, cooperation, mutualism or syntrophy to enhance bacterial fitness or substrate
435 utilization. In co-cultivation, a wide variety of molecules can be exchanged, like extracellular
436 enzyme sharing, cross-feeding or quorum sensing signalling molecules. No differentially
437 expressed proteins were found in the model community whether *L. mali* UCMA 16447 strain
438 was infected or not. This may be the result of proteins being the last level of cell expression.
439 Furthermore, the 60 minutes of the experiment may not be extended enough to observe
440 significant differences in protein expression at the community level, if any. Studying the
441 metatranscriptomic response of the community could be more informative in future works
442 (Monnet et al., 2016).

443 The transcriptomic and proteomic results of *L. mali* UCMA 16447 response to phage
444 infection tended to follow the same patterns with a few discrepancies that can be explained by
445 the time span for protein expression regulation being longer than for transcription regulation.
446 For example, more than 20 genes were downregulated in the cell motility functional category
447 with RNA-Seq study whereas only two proteins were retrieved and differentially expressed

448 for this functional category with the proteomic approach. Such differences between
449 transcriptomic and proteomic results are often observed (Dalmasso et al., 2012).

450 As in other phage-bacteria transcriptomic studies, the current work showed that the genes
451 involved in DNA replication, transcription, nucleotide metabolism and amino acid biogenesis
452 were overexpressed to allow phage replication and virion synthesis (Ainsworth et al., 2013;
453 Danis-Wlodarczyk et al., 2018; Lavysh et al., 2017; Leskinen et al., 2016; Zhao et al., 2016).

454 Translation was another repressed function during phage infection, suggesting that phage
455 infection led to a decline in the translation of bacterial proteins. On the opposite, genes and
456 proteins involved in the metabolism of amino acids like glycine, serine and threonine were
457 overexpressed during the infection, presumably useful for the assembly of phage elements or
458 as an additional energy source. All these main metabolic functions are usually affected by
459 stress conditions as reported in other works, and especially for the members of the former
460 *Lactobacillus* genus (actual members of the *Lactobacillaceae* family) (Angelis et al., 2016).

461 The major overexpressed proteins during a phage infection globally belonged to categories
462 associated to DNA replication, nucleotide metabolism and the production of energy as has
463 been shown for *Salmonella* (Weintraub et al., 2019). In the present work, other DE proteins
464 involved in signal transduction and cell envelope were also found differentially expressed in a
465 study of *Lc. lactis* (Lemay et al., 2019). Namely, the proteins involved in the multiplication of
466 phage particles were retrieved as the most abundant ones.

467 The expression level of the genes involved in the carbohydrate metabolism was also modified
468 during the infection. The *pfk*, *gpdh*, *pgk* and *pgm* genes were repressed during infection, thus
469 suggesting an inhibition of the Embden-Meyerhof pathway (glycolysis) while the *ldh* gene
470 was overexpressed. The overexpression of the *ldh* gene was also observed during the phage
471 infection of a *Lc. lactis* strain, and the authors speculated that the NAD⁺ formed during the
472 transformation of pyruvate to lactate served to the glycolysis pathway (Ainsworth et al.,

473 2013). Yet, in our study we showed that the glycolysis enzymes were repressed, and thus that
474 glycolysis is likely to be severely impacted, and most probably slowed down. A previous
475 work on a *Vibrio* phage showed that NAD⁺ was necessary to phage DNA replication and that
476 the phage had a pathway of NAD⁺ salvage (Lee et al., 2017a). In the current work, it might
477 put forward the hypothesis that a mechanism of inhibition of glycolysis combined with a high
478 activity of the lactate dehydrogenase and malate dehydrogenase (during the conversion of
479 oxaloacetate into malate) is triggered by phage UCMA 21115 in order to spare NAD⁺, as has
480 been described in another study conducted in *Vibrio* phages (Lee et al., 2017b).

481 Cell motility was another major function affected by phage infection in *L. mali* UCMA 16447
482 strain. Some genera and species belonging to the former *Lactobacillus* genus are motile, and
483 *L. mali* is one of them (Cousin et al., 2015; Neville et al., 2012). The flagellar system of *L.*
484 *mali* UCMA 16447 strain was repressed during the infection. Several assumptions can be
485 made to explain this phenomenon, but remain speculative at this stage of the study. Some
486 phage receptors are located on bacterial flagella, as has been shown in previous works on
487 *Campylobacter jejuni* (Baldvinsson et al., 2014), *Salmonella* (Baldvinsson et al., 2014; Choi
488 et al., 2013), and *Erwinia* phages (Evans et al., 2010). Some mechanisms may be triggered in
489 *L. mali* UCMA 16447 strain to reduce flagella production to thwart the infection, if phage
490 infection is related to the flagellum receptor in the current study. In the same way, it has been
491 shown for *Agrobacterium* sp. (Gonzalez et al., 2018) that phage transfer to a receptor on the
492 cell membrane requires motility. The repression of the motility system in *L. mali* UCMA
493 16447 strain could perhaps contribute to a defence-like mechanism to prevent or, at least, to
494 slow down the infection, and thus to resist to phage attack. If the mechanisms that led to the
495 down-regulation of the genes encoding motility are still unknown, it is also possible to assume
496 that this phage-driven phenomenon helps the phage in save energy for its replication within its
497 host. The flagellar system is energy-consuming and its down-regulation would consequently

498 be of benefit to the phage as suggested by a previous work where a similar regulation of the
499 flagellar system was observed in *Bacillus thuringiensis* during phage infection (Wu et al.,
500 2014).

501

502 **5. Conclusion**

503 In conclusion, this study made it possible to describe for the first time the transcriptomic and
504 proteomic response of a LAB isolated from a fermented beverage (cider) to phage infection
505 within a bacterial community model. This study has revealed a fairly large disturbance in the
506 cell metabolism at the transcriptomic and proteomic levels, with specific responses to phage
507 infection. Some impacts on the host response were observed in different functional categories
508 like in cell motility and in the carbohydrate, amino acid and nucleotide metabolisms. Gaining
509 knowledge of host response to phage infection is crucial to better understand and even control
510 microbial equilibria throughout fermentation processes. A better understanding of phage-
511 bacteria interactions in fermented foods is crucial in order to continue to providing consumers
512 with sustainable products.

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521 **7. Disclosure statement**

522

523 No potential conflict of interest was reported by the authors.

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726 **Tables:**

727 **Table 1: Number of down- or up-regulated genes for each comparison, fold change > 2 and <**
728 **0.5, p-value < 0.05.**

729

730	Comparison	Level	Number of genes
731	T0 vs T15	up	0
732		down	0
733	T0 vs T15P	up	45
734		down	67
735	T15 vs T15P	up	36
736		down	86
737	T0 vs T60	up	0
738		down	1
739	T0 vs T60P	up	103
740		down	147
741	T60 vs T60P	up	92
742		down	123
743	T15 vs T60	up	0
744		down	0
745	T15 vs T60P	up	95
		down	167
	T15P vs T60	up	43
		down	42

746 T0 correspond to samples taken just before the infection with phage UCMA 21115. T15 and
747 T60 refers to 15 and 60 minutes after T0, respectively. P means that it is the infected
748 condition.

749

Figures captions:

Figure 1: Transmission electron micrographs of phage UCMA 21115.

Figure 2: Adsorption and one step growth curve of phage UCMA 21115 with *L. mali* UCMA 16447 in MRS broth at 30°C.

A. Adsorption of phage UCMA 21115 on *L. mali* 16447 during 30 minutes. **B.** One step growth curve of phage UCMA 21115 with *L. mali* UCMA 16447 during 300 minutes, where T0 is after 30 minutes of adsorption. Three independent experiments were carried out for each experiment and error bars indicate standard deviation.

Figure 3: Overview of the major functions of *L. mali* UCMA 16447 impacted during infection by phage UCMA 21115 at two time points by RNA-Seq analysis.

Bar graph displaying differentially expressed genes of *L. mali* UCMA 16447 during the infection by the phage UCMA 21115 at 15 (**A**) and 60 (**B**) minutes, respectively. Black bars indicate genes with an induced expression and white bars genes with a repressed expression in comparison to uninfected condition at the same time.

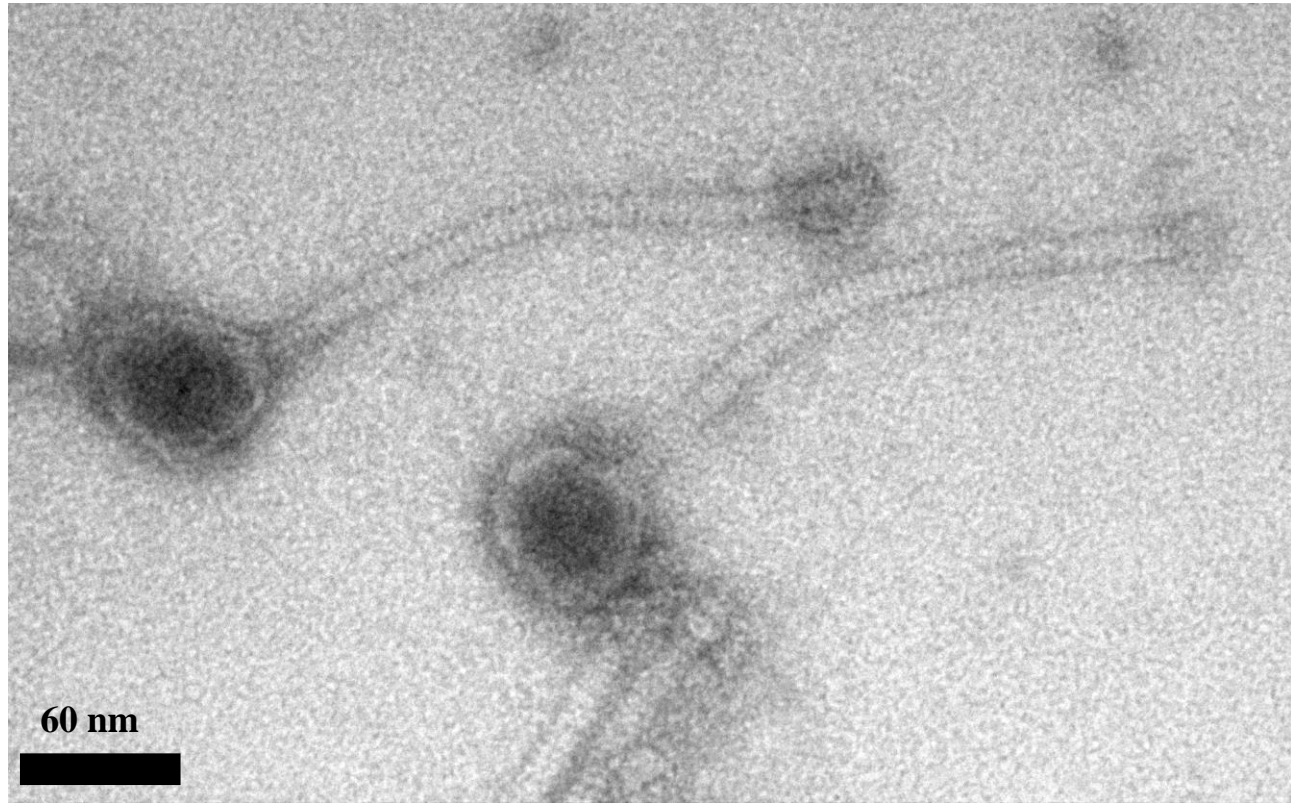
Figure 4: Simplified summary of principal functions with a level of expression induced (green) or repressed (red) in the transcriptomic study.

Figure 5: Embden-Meyerhof pathway of *L. mali* UCMA 16447 strain during infection by phage UCMA 21115, adapted from Muller *et al.*, 2015.

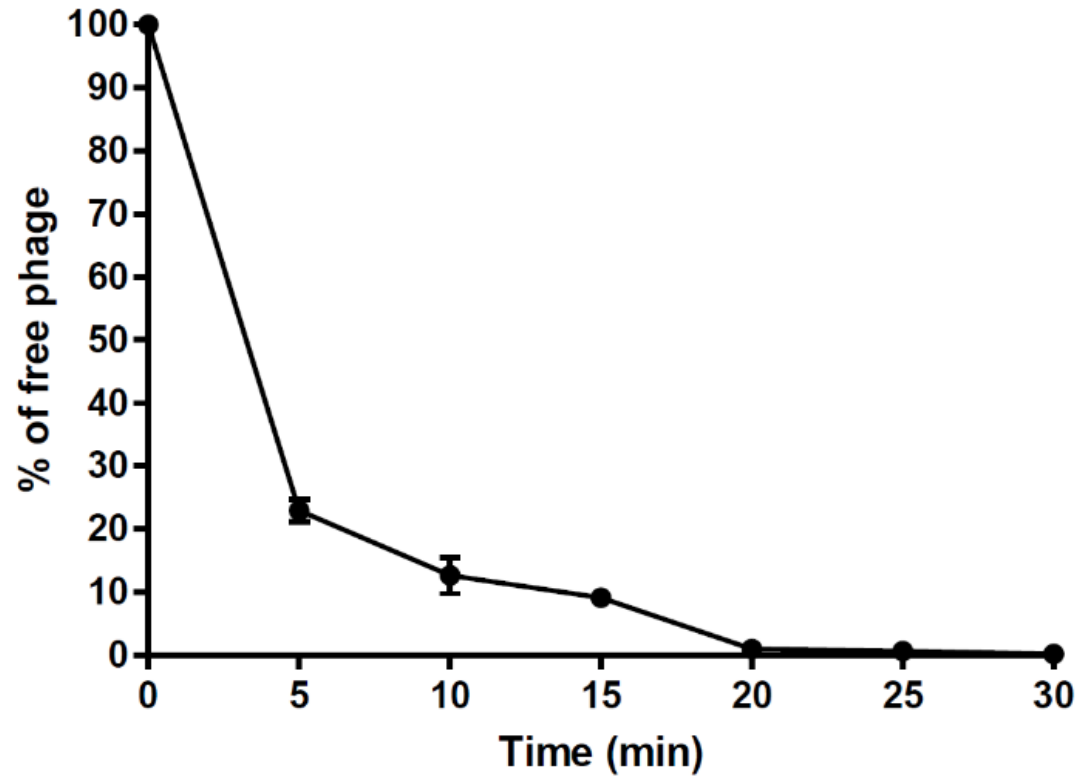
Genes impacted during the infection by phage UCMA 21115: repressed genes are mentioned in red and induced genes in green.

Figure 6: Number of differentially expressed proteins according to their functional classification during phage infection after 60 minutes of infection.

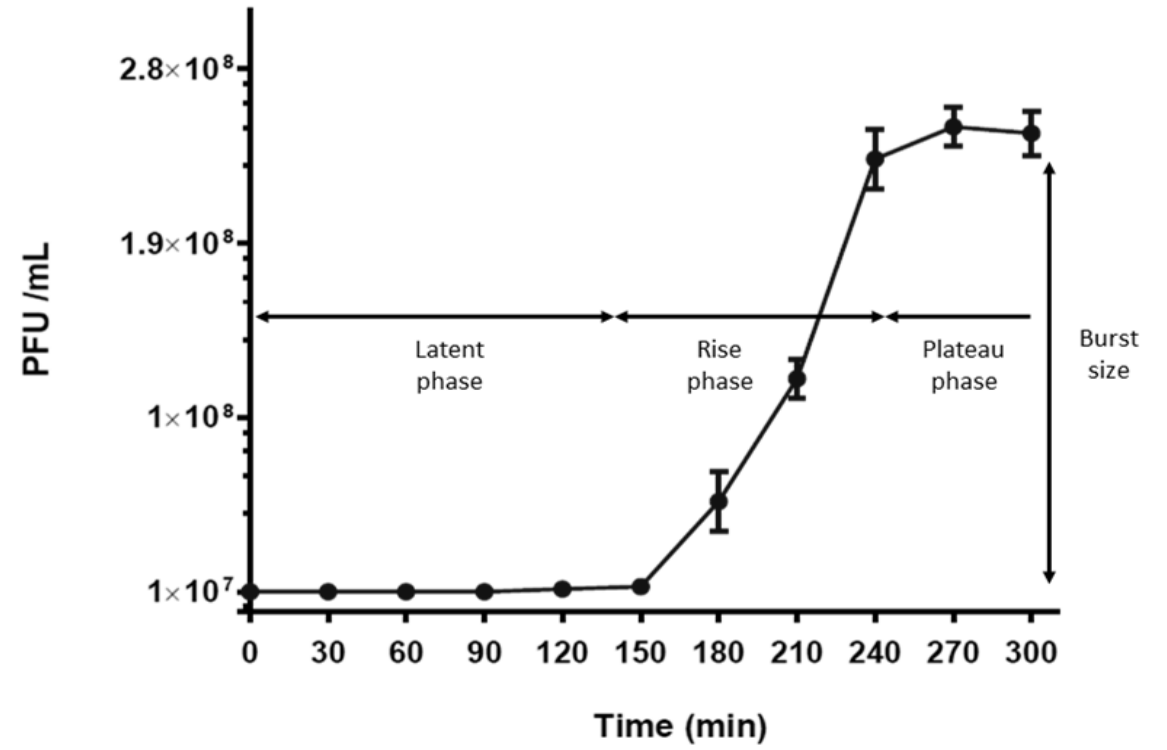
Comparison between T60 (60 min without phage) and T60P (60 min after phage infection). Black bars indicate proteins overexpressed and white bars proteins under expressed compared to uninfected condition.



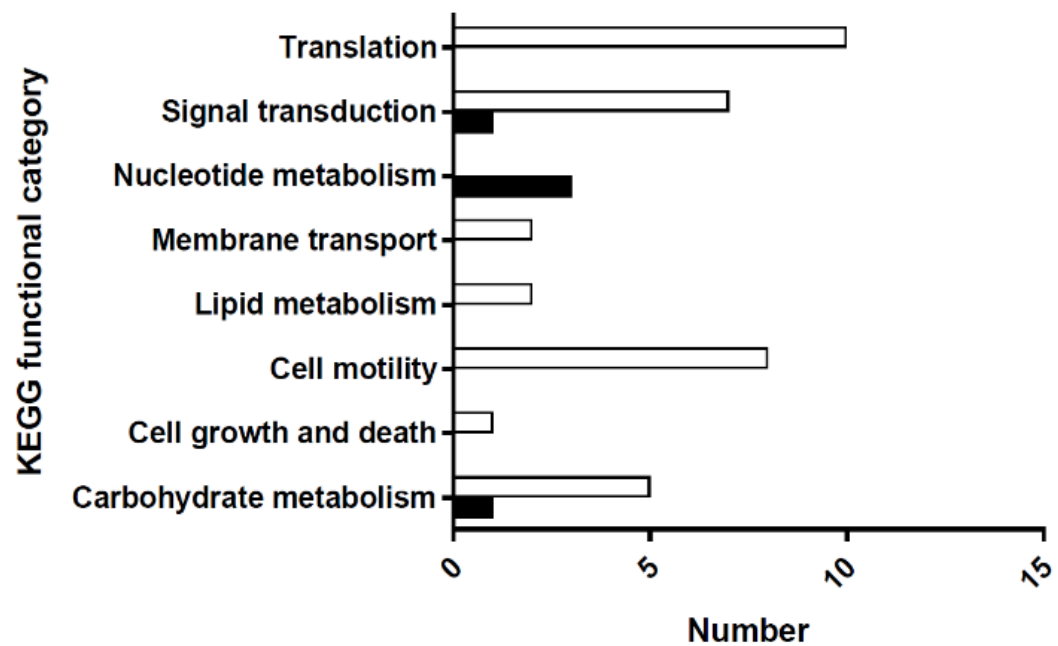
A.



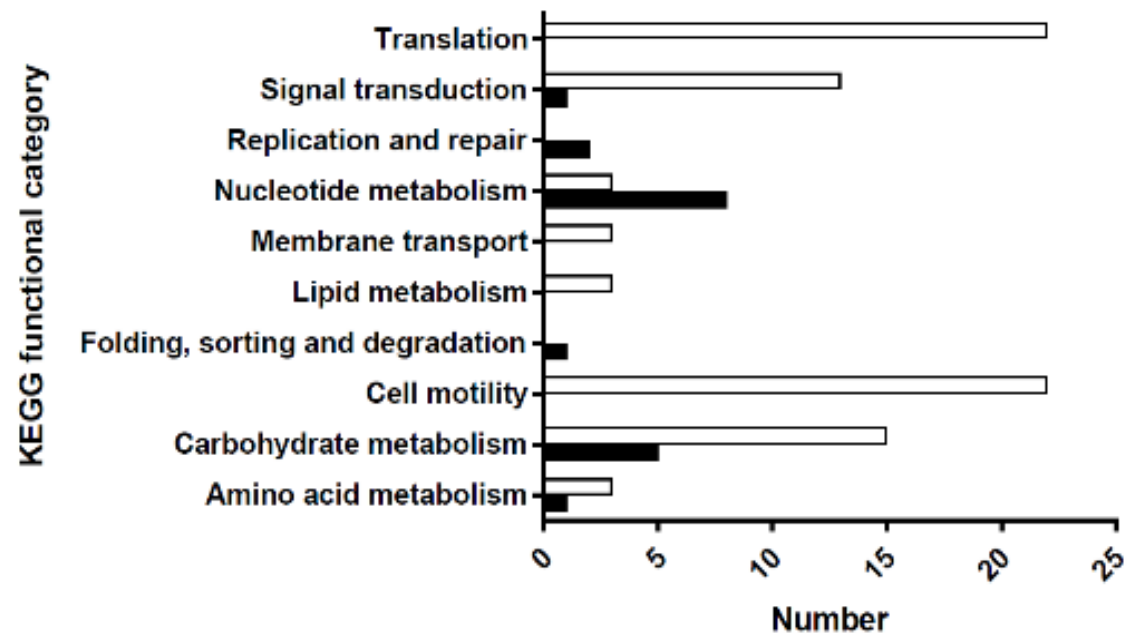
B.

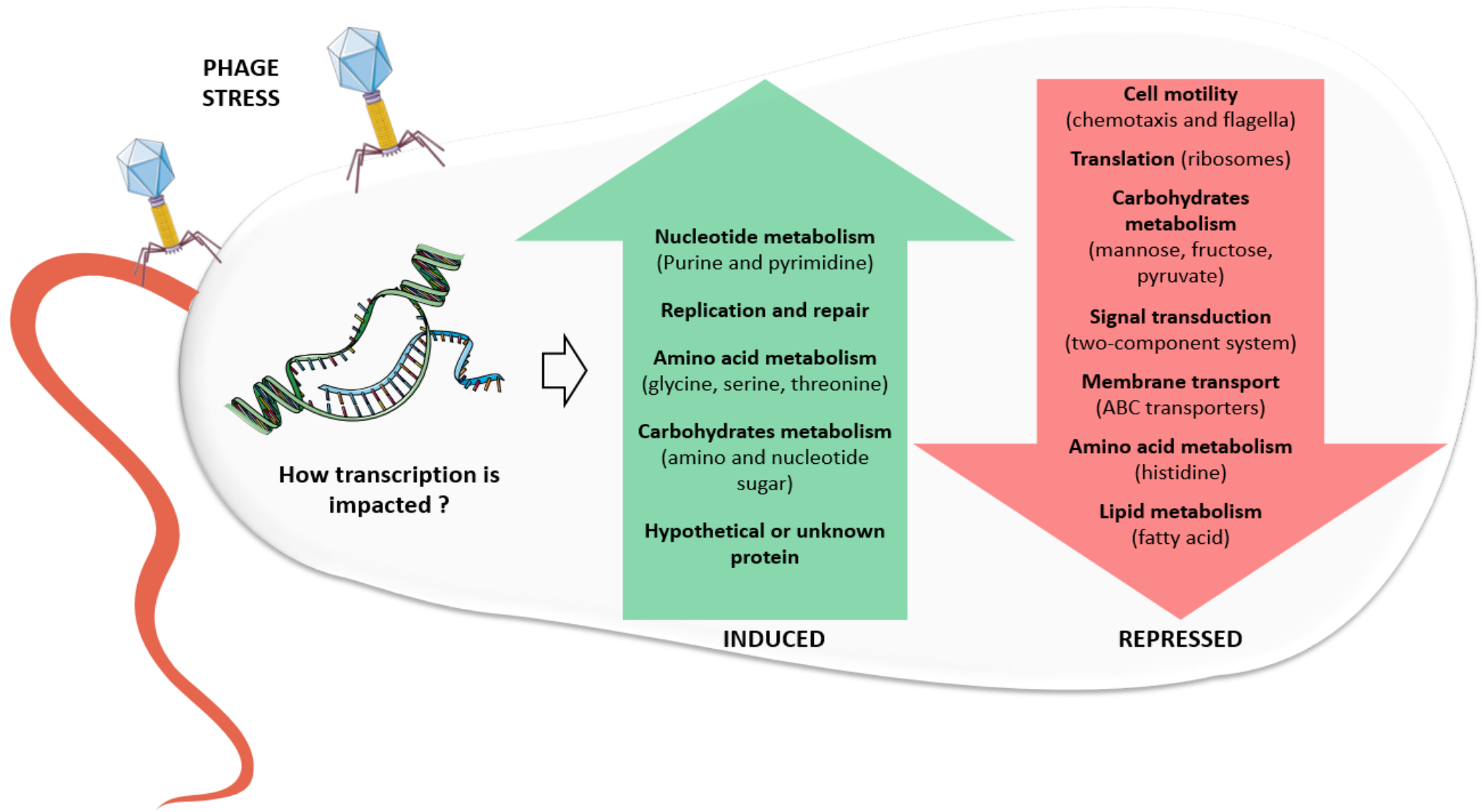


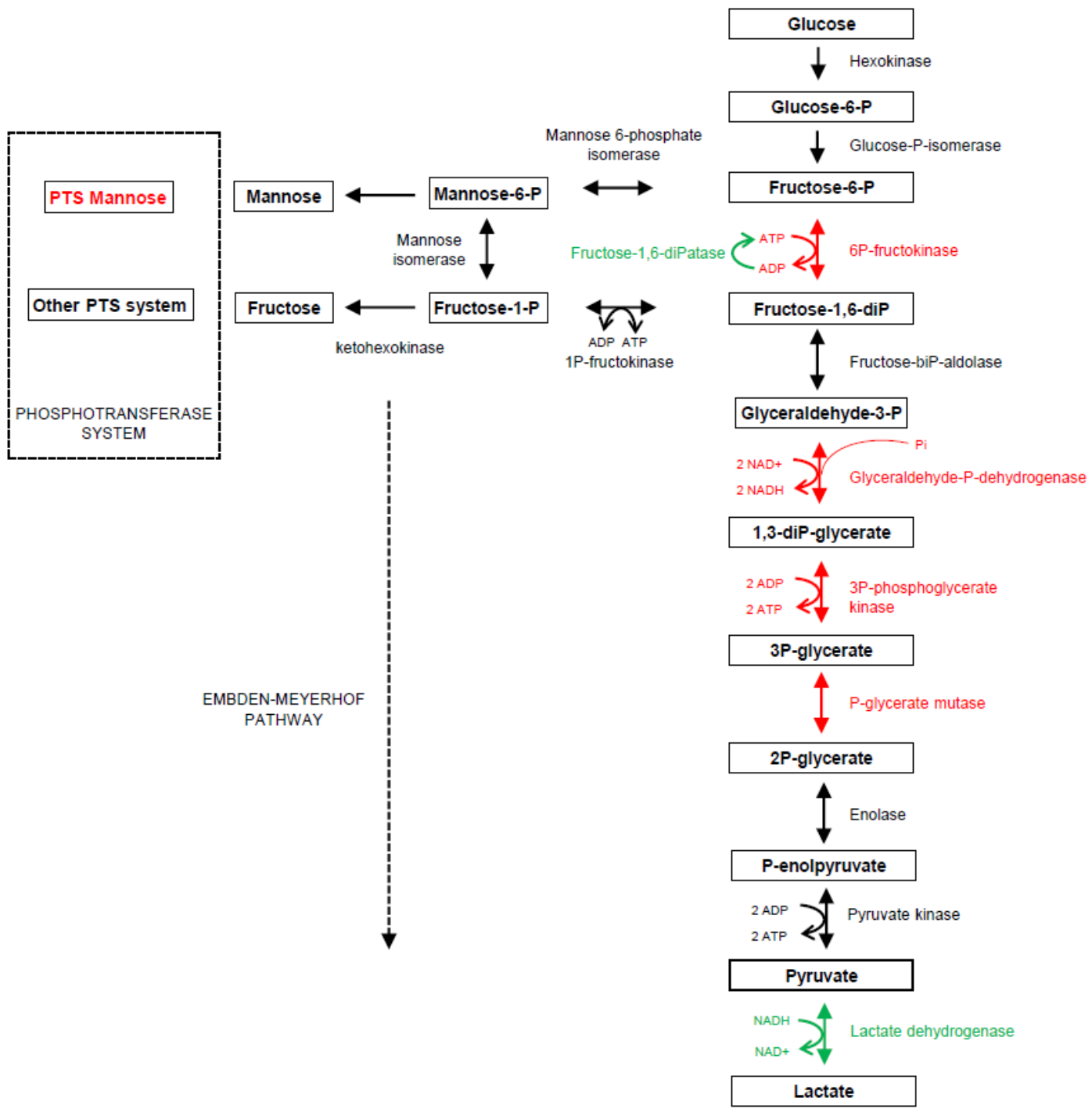
A.



B.







Functionnal classification

