AEM Accepted Manuscript Posted Online 29 September 2021
Appl Environ Microbiol doi:10.1128/AEM.01424-21
Copyright © 2021 American Society for Microbiology. All Rights Reserved.

Accepted Manuscript Posted Online

Applied and Environmental Microbiology

AEM

Applied and Environmental Microbiology

1 **The lytic siphophage vB_StyS-LmqsSP1 reduces *Salmonella* Typhimurium isolates on chicken**
2 **skin**

3 Golshan Shakeri[1], Jens A. Hammerl[2], Abdollah Jamshidi[1], Kiarash Ghazvini[3], Manfred Rohde[4], Istvan
4 Szabo[2], Corinna Kehrenberg[5], Madeleine Plötz[6], Sophie Kittler[6]*

5 [1]Department of Food Hygiene and Aquaculture, Faculty of Veterinary Medicine, Ferdowsi University
6 of Mashhad, Azadi Square, 9177948974 Mashhad, Iran

7 [2]German Federal Institute for Risk Assessment, Department of Biological Safety, Max-Dohrn Str. 8-
8 10, 10589 Berlin, Germany

9 [3]Department of Microbiology and Virology, Faculty of Medicine, Mashhad University of Medical
10 Sciences, Mashhad, Iran

11 [4]Central Facility for Microscopy, Helmholtz Centre for Infection Research GmbH, Inhoffenstraße 7,
12 38124 Braunschweig, Germany

13 [5]Institute for Veterinary Food Science, Justus-Liebig-University Giessen, Frankfurter Straße 92, 35392
14 Giessen, Germany

15 [6]University of Veterinary Medicine Hannover, Foundation, Institute for Food Quality and Food Safety,
16 Bischofsholer Damm 15, 30173 Hannover, Germany
17 *Corresponding author: Sophie Kittler

18 **Keywords:** *Salmonella* Typhimurium, phage biocontrol**,** phage therapy, foodborne zoonoses,
19 chicken

20

Downloaded from https://journals.asm.org/journal/aem on 02 October 2021 by 188.159.185.175.

Accepted Manuscript Posted Online

Applied and Environmental Microbiology

AEM

Applied and Environmental Microbiology

21 **Abstract**

22 Phage-based biocontrol of bacteria is considered as a natural approach to combat food-borne
23 pathogens. *Salmonella* spp. are notifiable and highly prevalent pathogens that cause
24 foodborne diseases globally. In this study, six bacteriophages were isolated and further
25 characterized that infect food-derived *Salmonella* isolates from different meat sources. The
26 siphovirus VB_StyS-LmqsSP1, which was isolated from a cow´s nasal swab, was further
27 subjected to *in-depth* characterization. Phage-host interaction investigations in liquid medium
28 showed that vB_StyS-LmqsSP1 can suppress the growth of *Salmonella* spp. isolates at 37 °C
29 for ten hours and reduce the bacterial titer at 4 °C significantly. A reduction of 1.4 to 3 log
30 units was observed in investigations with two food-derived *Salmonella* isolates and one
31 reference strain under cooling conditions using MOIs of 10^4 and 10^5. Phage application on
32 chicken skin resulted in a reduction of about 2 log units in the tested *Salmonella* isolates from
33 the first three hours throughout a one-week experiment at cooling temperature and an MOI of
34 10^5. The one-step growth curve analysis using vB_StyS-LmqsSP1 demonstrated a 60-min
35 latent period and a burst size of 50-61 PFU/infected cell for all tested hosts. Furthermore, the
36 genome of the phage was determined to be free from genes causing undesired effects. Based
37 on the phenotypic and genotypic properties, LmqsSP1 was assigned as a promising candidate
38 for biocontrol of *Salmonella* Typhimurium in food.

39 Importance: *Salmonella enterica* is one of the major global causes of foodborne enteritis in
40 humans. The use of chemical sanitizers for reducing bacterial pathogens in the food chain can
41 result in the spread of bacterial resistance. Targeted and clean label intervention strategies can
42 reduce *Salmonella* contamination in food. The significance of our research demonstrates the
43 suitability of a bacteriophage (vB_StyS-LmqsSP1) for biocontrol of *Salmonella enterica*
44 serovar Typhimurium on poultry due to its lytic efficacy under conditions prevailing in food
45 production environments.

Downloaded from https://journals.asm.org/journal/aem on 02 October 2021 by 188.159.185.175.

Accepted Manuscript Posted Online

Applied and Environmental Microbiology

AEM

Applied and Environmental Microbiology

46 **Introduction**

47 *Salmonella* (*S.*) *enterica* is one of the major global causes of foodborne enteritis in humans
48 (1). In 2018, it was the leading cause of notified foodborne outbreaks in the European Union
49 (EU), leading to more than 91,000 human cases of salmonellosis. Up to now, more than 2,500
50 serovars of *S. enterica* have been notified (1), but foodborne outbreaks in the United States
51 and Europe are mainly caused by *S.* Enteritidis and *S.* Typhimurium (2, 3). The serovar
52 Enteritidis is predominantly associated with chicken, whereas *S.* Typhimurium derives from a
53 wide variety of food-animal sources, among them poultry, pig and cattle (2). The highest
54 prevalence of *Salmonella*-positive single samples in the EU was reported for poultry meat,
55 minced meat and meat preparations (2).

56 The use of chemical sanitizers and preservatives for reducing pathogenic bacteria in the food
57 chain can result in the spread of bacterial resistance (4) and in the emergence of undesirable
58 allergenic effects, generation of byproducts and negative impact on the environment (5). Due
59 to these reasons, natural agents for biocontrol of pathogenic bacteria in foodstuff have gained
60 further attention in recent years (6).

61 Bacteriophage-based biocontrol is considered as an alternative and natural intervention
62 strategy for reducing bacterial contamination in food (7-9). While conventional strategies
63 combat both pathogens and the commensal microflora indiscriminately, the use of phages
64 allows a specific reduction in undesirable bacteria (9). Additionally, bacteriophages do not
65 affect organoleptic properties such as flavor, color and/or aroma of the treated food products
66 (9, 10).

67 Phage application in livestock before slaughter (pre-harvest) and in foods (post-harvest) is
68 permitted under FSIS Directive 7120.1 in the US (9, 11), and commercial phage preparations
69 have been approved as "Generally Recognized as Safe" (GRAS) for application in raw and
70 ready-to-eat meat and poultry products (9). In Europe, approval of phage products for food

Downloaded from https://journals.asm.org/journal/aem on 02 October 2021 by 188.159.185.175.

Accepted Manuscript Posted Online
Applied and Environmental Microbiology
AEM
Applied and Environmental Microbiology

71 applications is still in the preparatory stage due to open regulatory and scientific issues (9, 12,
72 13). Further phage preparations are needed for reducing foodborne zoonoses and especially
73 multidrug-resistant pathogens. Since they can be produced by relatively easy and economical
74 processes (14), phages are a very suitable measure for this purpose especially in developing
75 countries (15, 16).

76 Phage application was investigated for biocontrol of *S. enterica* in different food products as
77 reviewed by Moye et al. (9). Studies on bacteriophage application for control of *Salmonella* in
78 food products have shown promising results (6, 17-19). Some studies investigated the effect
79 of phages on chicken skin. However, only few studies determined the phage-based reduction
80 under practical conditions like temperatures of cooled storage and against *Salmonella* field
81 isolates from food samples (17, 20, 21). These factors can have a high impact on the reduction
82 of target bacteria in commercial food production settings. Testing phages only against
83 laboratory strains and under laboratory settings that do not resemble conditions during food
84 production might thus lead to biased results and the selection of unsuitable phages (22, 23).
85 For bacteriophage efficacy testing, phages should be tested under realistic conditions, using
86 strains most similar to those found on the targeted product (17, 21). Testing of phages in
87 commercial production plants is laborious, time-consuming and the results can sometimes
88 hardly be interpreted due to the complexity of influencing factors. Additionally, these factors
89 might lead to scientific results that are hard to reproduce, and thus, cannot be extrapolated to
90 other production plants or settings without further experimental analysis (24, 25). *In vitro*
91 models combining environmental factors of commercial food production settings with
92 controlled experimental conditions should be used to allow and extrapolation of the collected
93 data. Cooling conditions and food-derived *Salmonella* isolates on food matrices need to be
94 used for testing lytic phage efficacy of promising candidates *in vitro*. It has been reported that
95 clinically or environmentally isolated *Salmonella* can show insensitivity against phage

Downloaded from https://journals.asm.org/journal/aem on 02 October 2021 by 188.159.185.175.

Accepted Manuscript Posted Online
Applied and Environmental Microbiology
AEM
Applied and Environmental Microbiology

96 infection that suggest promising results on laboratory *Salmonella* and higher efficacy in
97 reduction experiments using laboratory strains compared to field isolates (17, 21). The former
98 study reported that meat-derived *Salmonella* isolates were less sensitive to phage infection
99 compared to laboratory strains (17). Killing of bacteria by phages can be achieved by different
100 mechanisms, e.g., passive inundation or a lytic infection cycle resulting in phage progenies.
101 The first infection step of phage binding to the bacterial cell surface is important for both
102 mechanisms, and requires attachment of phages to the bacterial cell by specific recognition
103 between the phage's receptor binding protein at the tip of the phage tail and a receptor located
104 on the bacterial cell surface. If a multitude of phages bind to the bacterial cell, destabilization
105 can lead to destruction without production of phage progenies (lysis from without), which is
106 often referred to as passive inundation (26). On the other hand, injection of the phage´s
107 nucleic acids followed by production of structural proteins and phage morphogenesis may
108 lead to lysis of the bacterial cell, releasing a variable number of progenies. For this complex
109 process, several metabolic prerequisites are necessary and internal resistance mechanisms of
110 the bacterial cell, destroying of the phage´s nucleic acids or interfering with phage replication,
111 need to be absent. These prerequisites and the general layout of phage defense mechanisms in
112 bacteria may vary between strains from different environments (27). Relatively few studies
113 have investigated the efficacy of *Salmonella* phages using food-derived strains on food
114 matrices under cooling conditions (17, 20, 28).

115 The present study aims to isolate bacteriophages suitable for reducing *Salmonella* on poultry
116 skin and to evaluate their efficiency as potential measures for biocontrol in commercial broiler
117 meat production systems. Six *Salmonella*-specific phages were investigated regarding their
118 host range on field isolates. Based on its broad host range, one of these phages, vB_StyS-
119 LmqsSP1 (LmqsSP1), was selected for the analysis of morphological and genomic properties
120 as well as burst size and latent period. For potential application purposes in the food chain, the

Downloaded from https://journals.asm.org/journal/aem on 02 October 2021 by 188.159.185.175.

Accepted Manuscript Posted Online
Applied and Environmental Microbiology
AEM
Applied and Environmental Microbiology

121 lytic efficacy against different *Salmonella* isolates was determined at 37 °C and 4 °C on
122 chicken skin and in culture media.

123 **Results**

124 **Six isolated phages form plaques on *Salmonella* isolates from meat products**

125 Overall, ten bacteriophages were isolated from 52 investigated samples (Table 1). Nine
126 bacteriophages were recovered from fecal samples of chicken flocks located in different parts
127 of northern Germany, one bacteriophage was isolated from a nasal swab of a cow housed at
128 the University of Veterinary Medicine Hannover, Germany, while no phages originated from
129 cecal content of various commercial poultry flocks when using the *Salmonella* host strain LT2
130 for phage isolation or alone. Based on the higher stability and reliable replication under
131 laboratory conditions, six bacteriophages were selected for further examination, while the
132 others were rejected due to their low concentration increase during propagation (< 10^6
133 PFU/mL after two propagations) and instability at 4 °C (Table 1).
134 The host range of the six phages was tested on 77 food-derived *Salmonella* isolates (Table 2)
135 representing 26 different serotypes derived from different food sources. Among these isolates,
136 60 were provided by the national reference laboratory on *Salmonella*, hosted at the German
137 Federal Institute for Risk Assessment (BfR, Berlin, Germany), 17 isolates originated from the
138 laboratory collection of the University of Veterinary Medicine Hannover, and two *E. coli*
139 O157:H7 strains were obtained from the German collection of microorganisms and cell
140 cultures (Leibnitz Intstitute DSMZ, Braunschweig, Germany). *Salmonella* isolates S1 and S2
141 were characterized as monophasic *S.* Typhimurium. S1 was further identified as the most
142 susceptible host, while S2 was very susceptible to four of the phages and exhibited a non-
143 wildtype phenotype against ampicillin (minimum inhibitory concentration (MIC) > 64 µg/ml),
144 gentamicin (MIC 16 µg/mL), sulfamethoxazole (MIC > 1024 µg/mL) and tetracycline (MIC >
145 64 µg/mL). The *Salmonella enterica* subsp. *enterica* rough variant S17, which was O-antigen-

Downloaded from https://journals.asm.org/journal/aem on 02 October 2021 by 188.159.185.175.

Accepted Manuscript Posted Online

Applied and Environmental Microbiology

AEM

Applied and Environmental Microbiology

146 deficient, showed susceptibility towards all examined phages and exhibited phenotypic
147 resistance against ampicillin, chloramphenicol, ciprofloxacin, trimethoprim,
148 sulfamethoxazole, and tetracycline. Efficient plaque production was shown on the *Salmonella*
149 spp. I rough variant S18 from poultry meat. Furthermore, the phage LmqsSP2 showed highly
150 efficient plaque formation on the *S*. Livingstone isolate S21, while LmqsSP1 only showed a
151 weak lytic effect on this isolate. High plaque formation by LmqsSP2 was also observed on the
152 *S*. Typhimurium isolate S3 and the *S*. Derby isolate S29. Both LmqsSP1 and LmqsSP2
153 showed efficient plaque production on the *Salmonella enterica* subsp. *enterica* rough variant
154 S19. The efficiency of plating was calculated in relation to the plating efficiency on their host
155 strain *S*. Typhimurium LT2 as shown in Figure 1. The six phages showed lytic ability on 30
156 (LmqsSP1), 13 (LmqsSP3), 10 (LmqsSP2), eight (LmqsSP4) and five (LmqsSP5 and
157 LmqsSP6) of the examined *Salmonella* isolates, covering 13 of 26 examined serotypes. The
158 phage LmqsSP1 expressed the broadest host range, covering 39 % of the investigated
159 *Salmonella* isolates and both tested *E. coli* strains. It showed a lytic effect on more than half
160 of the examined *S*. Typhimurium and *Salmonella enterica* subsp. *enterica* rough variants,
161 respectively. Furthermore, lysis by LmqsSP1 was observed on *Salmonell*a serogroup B and
162 the serotypes Schwarzengrund, Saintpaul, Paratyphi B, Newport, Livingstone, Indiana,
163 Enteritidis, Derby, and Bovismorbificans. No lytic ability was observed on 47 of the 77
164 examined isolates (Figure 1). Based on its inhibitory effect on the broadest spectrum of
165 *Salmonella* isolates, we selected the phage LmqsSP1 for further characterization and efficacy
166 testing at different temperatures and on food matrices.
167
168 **LmqsSP1 exhibited a morphology of the *Siphoviridae* family and forms plaques on lawns**
169 **of food derived *Salmonella* isolates**
170 The phage LmqsSP1 was isolated from the nasal swab of a cow and was classified as
171 Siphovirus, based on its morphology with an average head width of 76 nm (standard deviation

Downloaded from https://journals.asm.org/journal/aem on 02 October 2021 by 188.159.185.175.

Accepted Manuscript Posted Online

Applied and Environmental Microbiology

AEM

Applied and Environmental Microbiology

172 3), an average head length of 80 nm (standard deviation 4), and an average tail length of 191
173 nm (standard deviation of 3) (Figure 2B). LmqsSP1 produced clear plaques (Figure 2A) on *S.*
174 Typhimurium LT2 as well as on the three food-derived *S.* Typhimurium isolates S1, S2, and
175 S3, and low EOP with turbid plaques on the food-derived *Salmonella* isolates S4, S5, S6, and
176 S7. On *S.* Typhimurium S1 and S2, LmqsSP1 showed an EOP of ≥ 1, while on the isolate S3,
177 EOP was determined to be **<** 0.1 (Figure 1).

178 **One-step growth curves show low variance of LmqsSP1 propagation on different**
179 **susceptible *Salmonella* isolates**

180 Growth experiments were performed using the *S.* Typhimurium strain LT2 and the food-
181 derived isolates S1 and S2 as hosts (Figure 3). The latent period of LmqsSP1 was determined
182 to be ~60 min in all tested isolates. The burst sizes were determined to be 50 PFU/cell
183 (ranging between 42 and 56 in the three experiments) in *Salmonella* strain LT2, 55 PFU/cell
184 (between 55 and 58 in the three experiments) in the food-derived isolate S1 and 61 PFU/cell
185 (between 52 and 80 in the three experiments) in the food-derived isolate S2 (Figure 3).
186

187 **Dose-dependent inhibition of bacterial growth at 37 °C**

188 Results of tests on inhibiting growth of *Salmonella* populations at 37 °C demonstrated a
189 significant time- and dose-dependent effect of the phage (Figure 4). Even at an MOI of
190 0.0001, LmqsSP1 significantly inhibited bacterial growth compared to the control. Lower
191 optical density compared to the control was observed after 8.5 h in cultures with added phages
192 and *Salmonella* LT2 (P = 0.04), after 6.5 h in cultures with field isolate S1 (P = 0.03) and
193 after 4.5 h in cultures with field isolate S2 (P = 0.03) (Figure 4). Higher MOIs resulted in
194 faster and more efficient inhibition of bacterial growth compared to lower MOIs. When
195 LmqsSP1 was applied at an MOI of 10, growth of *Salmonella* strain LT2 (P = 0.001) and of
196 field isolates S1 (P = 0.05) and S2 (P = 0.009) was completely suppressed (Figure 4).
197 However, field isolate S2 showed regrowth after 7.5 h and reached an OD_{600} of 0.19 after 10 h

Downloaded from https://journals.asm.org/journal/aem on 02 October 2021 by 188.159.185.175.

Accepted Manuscript Posted Online

Applied and Environmental Microbiology

AEM

Applied and Environmental Microbiology

198 (Figure 4C). When LmqsSP1 was applied at MOIs equal to or greater than 0.001, growth
199 inhibition was more effective on the field isolates than on LT2. At MOIs of 0.01 after 10 h,
200 the optical density of S1 and S2 cultures was 0.2 and that of LT2 0.3 (Figure 4).

201 **LmqsSP1 reduced the *Salmonella* titer in liquid cultures at 4 °C**

202 When LmqsSP1 was inoculated into lysogeny broth (LB) containing approximately 10^3
203 CFU/mL of the respective *Salmonella* isolate or strain (MOI of 10^5), significant reductions
204 were observed after 24 h in all experiments. Compared to the control, a reduction of 3 log
205 units was observed in isolate S1, 2.6 log units in isolate S2, and 1.8 log units in *Salmonella*
206 LT2 (Figure 5, significance level P <0.0001 for all reductions). A higher bacterial density of
207 approximately 10^4 CFU/mL (MOI of 10^4) resulted in significant reductions of 3 log units in
208 isolate S2, 2.6 log units in isolate S1, and 1.4 log units in *Salmonella* LT2 (Figure 5,
209 significance level P <0.0001 for all reductions).

210 **Phage treatment significantly reduced the concentration of *Salmonella* isolates on**
211 **chicken skin at 4°C**

212 *Salmonella* contaminated chicken skin was treated with LmqsSP1 at an MOI of 10^5 to
213 examine the efficiency and stability of the phage on food samples at cooling temperature for
214 one week. Results of this experiment are shown in Figure 6. Concentrations of *Salmonella*
215 LT2 and the food-derived isolates S1 and S2 on treated chicken skin samples were reduced by
216 more than 1.8 log units compared to the control after 3 h. After 24 h, bacterial counts on
217 phage-treated samples were reduced by 2.2 log units when containing the food-derived
218 isolates S1 (P = 0.021) or S2 (P = 0.032) and by 1.9 log units on samples containing
219 *Salmonella* LT2 (P = 0.0007) (Figure 6). In subsequent experiments on phage susceptibility
220 of recovered *Salmonella* colonies from the tested skin pieces, no phage-resistant colonies
221 were observed. The numbers of plaques on the lawns from those colonies did closely
222 resemble those of the original strains or isolates (data not shown). A mean phage
223 concentration of $\log_{10}$ 9.6 ± 0.08 PFU/mL (mean±SD) was measured on chicken skin

Downloaded from https://journals.asm.org/journal/aem on 02 October 2021 by 188.159.185.175.

Accepted Manuscript Posted Online
Applied and Environmental Microbiology
AEM
Applied and Environmental Microbiology

224 contaminated with the food-derived *Salmonella* S1 and of $\log_{10}$ 9.5 ± 0.18 PFU/mL when
225 contaminated with S2 after 24 h. A mean concentration of $\log_{10}$ 9.3 ± 0.56 PFU/mL was
226 detected on the controls containing phages only. In the samples containing *Salmonella* LT2
227 and its control, mean phage concentrations of $\log_{10}$ 9.2 ± 0.11 PFU/mL and $\log_{10}$ 9.4 ± 0.07
228 PFU/mL were detected, respectively. The measured concentrations represented an increase in
229 phage concentration of 51 % and 44 % during the experiments with S1 and S2 and a decrease
230 of 51 % in the experiment with *Salmonella* LT2.

231 In an additional experiment, a lower MOI of 10 was used for treatment of contaminated skin
232 pieces (2.5 x 10^4 PFU/cm^2). No significant reduction in *Salmonella* counts was observed in
233 this experiment (data not shown).

234 **LmqsSP1 is a new member of the T5-like phages (*Tequintavirus* genus)**

235 Whole-genome sequencing and *de novo* assembly of the extracted DNA from purified virions
236 resulted in a single contig of ~110 kb with an average coverage of 200 per consensus base.
237 Manual curation of the assembled consensus sequence resulted in a determination of a final
238 genome size of 109,938 bp. The LmqsSP1 genome exhibited a GC content of 38.8 %, which
239 was significantly lower than the GC content of its host bacteria (~52 %). Further DNA-
240 alignments against available virus genomes of the GenBank database, conducted using
241 BLASTn of the National Center for Biotechnology Information (NCBI), showed that the
242 *Escherichia coli* (*E. coli*) phages slur09 (total score: 1.478e+05; query cover: 87 %; E-value:
243 0.0; identity: 95.34 %; accession no.: LN887948.1), vB_EcoS_EASG3 (total score:
244 1.631e+05; query cover: 89 %; E-value: 0.0; identity: 95.13 %; accession no.: MK373799.1)
245 and vB_EcoS_HASG4 (total score: 1.620e+05; query cover: 89 %; E-value: 0.0; identity:
246 95.30 %; accession no. MK373797.1), exhibited a close relationship to LmqsSP1. However,
247 the phylogenetic analysis of the phages showed that despite their close relationship, LmqsSP1
248 clustered independently from slur09 and the almost identical phages vB_EcoS_EASG3 and

Downloaded from https://journals.asm.org/journal/aem on 02 October 2021 by 188.159.185.175.

Accepted Manuscript Posted Online

Applied and Environmental Microbiology

AEM

Applied and Environmental Microbiology

249 vB_EcoS_HASG4 (Figure 7B and C). Nevertheless, due to the fact that all related phages
250 belong to the same taxonomical lineage (*Viruses; Caudovirales; Siphoviridae; Tequintavirus*
251 (synonym T5-like viruses)), we suggest allocating LmqsSP1 to the same viral genus.
252 Further bioinformatic analysis revealed that the LmqsSP1 genome exhibited 161 putative
253 open reading frames (ORFs), 15 transfer RNAs (tRNAs) and 64 rho-independent transcription
254 terminators (TTs) (Supplemental Material Dataset S1 and S2). Overall, many of the predicted
255 LmqsSP1 gene products showed a close relationship to gene products of phage T5, which
256 represents the prototype and the most prominent member of the Tequintaviruses, or other
257 members of T5-like phages (Supplemental Material Dataset S1). In general, LmqsSP1
258 possesses the typical components of T5-like phages. An overview of the functional prediction
259 of the phage-encoded gene products is provided in Figure 7A. Overall, the majority of the
260 gene products are organized in complex units (i.e., units for DNA metabolism and genome
261 replication lysis of the host cells and virion assembly), but some genes are localized far away
262 from their functional units. However, as this organization is in good agreement with other
263 members of T5-like phages (i.e., *E. coli* phages slur09 and EASG3, Figure 7C), the
264 delocalization of the genes might not affect their intended function or their concerted activity
265 (i.e. for the generation of virion particles). To assess the suitability of LmqsSP1 for biocontrol
266 or therapeutical applications, *in-depth* functional predictions of the individual gene products
267 were made. However, we found no gene product involved in a lysogenic behavior (i.e.,
268 prophage repressor) and/or chromosomal integration (i.e., phage integrase), supporting the
269 assumption that LmqsSP1 only performs a lytic lifestyle. Furthermore, there was no
270 indication that any LmqsSP1 gene product might be associated with virulence or resistance
271 development. Nevertheless, some gene products showed a structural and functional
272 relationship to homing endonucleases (Supplemental Material Table S1). As the genes of
273 these nucleases are described as being mobile, copies of them can be scattered on a phage
274 genome and/or within its bacterial host. Genome analysis of DNA from the phage LmqsSP1

Downloaded from https://journals.asm.org/journal/aem on 02 October 2021 by 188.159.185.175.

Accepted Manuscript Posted Online
Applied and Environmental Microbiology
AEM
Applied and Environmental Microbiology

275 after 90 consecutive inoculation cycles with *Salmonella* host strain LT2 revealed no changes
276 in its genomic traits and thus demonstrated distinct genome stability of the phage.
277

278 **Discussion**

279 According to the World Health Organization (29), *Salmonella* is one of four key global causes
280 of diarrheal diseases, and approximately one million human cases caused by *Salmonella* spp.
281 in the United States are food derived (30).

282 As shown in Figure 1, the tested bacteriophages LmqsSP1, SP2, SP3, SP4, SP5 and SP6 were
283 able to form plaques on four of 76 food-derived *Salmonella* isolates with similar or higher
284 efficacy compared to the laboratory host strain LT2. LmqsSP1 showed a 10 to 100-fold
285 reduced plaque formation on 11 *Salmonella* isolates (Figure 1). On the tested food-derived
286 isolates, the *Salmonella* phages in this study showed a moderate range of lysed host bacteria,
287 which is comparable to the host range reported for other phages of the *Siphoviridae* family
288 (31, 32). Highly efficient plaque formation on a broad range of bacterial hosts is known to be
289 an uncommon feature among most phages, especially when bacterial field isolates are tested
290 (33). Thus, phages with different host ranges can be applied simultaneously to increase the
291 range of affected bacterial strains. This application of phage cocktails by adding phages with
292 different host ranges and receptors is especially recommended in commercial settings when
293 the number and susceptibility of bacterial pathogens cannot be determined prior to treatment.
294 Synergistic effects of phage cocktails containing different phages compared to single phages
295 were reported in previous studies (18, 34-36). Phages showing a narrow host range might be
296 especially suitable for targeted reduction of previously identified bacterial strains (37).

297 Mean burst sizes of LmqsSP1 on the three tested *Salmonella* were measured to be 50 to 61
298 PFU/cell. This is in accordance with results from previous studies (20, 38-40), while the
299 measured latent periods of approximately 60 min were longer than previously reported for

Downloaded from https://journals.asm.org/journal/aem on 02 October 2021 by 188.159.185.175.

Accepted Manuscript Posted Online

Applied and Environmental Microbiology

AEM

Applied and Environmental Microbiology

300 *Salmonella*-specific phages, which showed latent periods of 15-25 min (20, 38-40). However,
301 results were consistent with those from studies on the *Salmonella*–specific siphoviruses
302 STm101, STm118 and PHB06 (19, 39). Comparable with LmqsSP1, STm118 showed a burst
303 size of 48 PFU/cell (39). A phage infecting *S*. Typhimurium that showed a latent period of 50
304 ± 5 min was reported by Carey-Smith et al. However, this phage failed to lyse its host strain at
305 5 °C even when the MOI exceeded 10^4 (31).

306 A short latent period and a large burst size were suggested as indicators for highly efficient
307 phages (22). Nonetheless, the results showed that LmqsSP1 reduces the *Salmonella*
308 concentration at 4 °C on chicken skin samples and in broth if a sufficient concentration of
309 phage was applied.

310 LmqsSP1 demonstrated a dose-dependent effect in broth, as shown in Figure 4. This is
311 consistent with the finding that bacterial reduction by LmqsSP1 on chicken skin was
312 dependent on its concentration. For a significant reduction of more than 1 log unit, other
313 authors suggested that a minimum MOI of 10^3or 10^4 is required (42, 43).

314 After 3 h at 4 °C, LmqsSP1 reduced food-derived salmonellae on chicken skin by 1.9 log
315 units. The reduction lasted for the entire storage time. To our knowledge, a limited number of
316 studies with conditions similar to our experiments have been published to date (40, 44-46). A
317 study using a siphovirus at a concentration of 10^7 PFU/mL at 4 °C on *S. enterica*
318 contaminated chicken skin found that it reduced *Salmonella* contamination by 2 log units
319 from day 1 to day 7 (44). However, another study using a myovirus at a concentration of 10^{10}
320 PFU/mL reduced *S*. Typhimurium contamination on chicken skin by only 1.2 log units from 6
321 h to 24 h after treatment (40). Other studies applying phages at cooling temperatures on
322 chicken skin detected reductions equal to or less than one log unit (45, 46). At a higher
323 temperature of 8 °C, that allows for the growth of *Salmonella*, application of a siphovirus at a
324 MOI of 10^4 on chicken skin resulted in a decrease in *Salmonella* by 3 log units after 24 h but

Downloaded from https://journals.asm.org/journal/aem on 02 October 2021 by 188.159.185.175.

Accepted Manuscript Posted Online

Applied and Environmental Microbiology

AEM

Applied and Environmental Microbiology

325 after seven days the reduction accounted for 2.43 log units only (20). Other studies applied
326 phages on chicken meat instead of skin to test their efficacy at retail level. Two phages from
327 the *Myoviridae* family individually and as a cocktail decreased *S*. Enteritidis levels on chicken
328 breast by 1.5 and 2.5 log units after 1 h (34). Another phage at an MOI of 10^4 caused a
329 reduction of about 1.7 log10 CFU/g in *S*. Typhimurium LT2 counts following 24 h of
330 treatment (28). A cocktail of a myovirus and a siphovirus eradicated *S*. Typhimurium and *S*.
331 Enteritidis within 15 min when chicken breast samples were wrapped in plastic coated with
332 ~10^{12} PFU/mL (10^8 PFU/cm^2), and this effectiveness remained stable for one week (47).
333 Complete elimination of *S*. Typhimurium and *S*. Enteritidis on chicken breast meat was also
334 observed at lower MOIs of 10^4 to 10^2 from 3 h to 16 h after phage treatment when phage
335 cocktails were used (48, 49). However, other studies reported that *S*. Typhimurium and *S*.
336 Enteritidis populations were reduced by 0.53 and 1.39 log units only, after using a phage
337 cocktail on chicken breast meat in other studies (50). These results show that the food matrix
338 and temperature at which phages are applied can have a major impact on the observed
339 reduction level.

340 Phage concentrations on chicken skin after treatment in our study indicated that phage
341 numbers increased by $\log_{10}$ 0.3 and 0.2 PFU/ml (51 % and 44 %), respectively during the
342 experiments with S1 and S2 and decreased by $\log_{10}$ 0.2 PFU/ml (minus 51 %) with
343 *Salmonella* strain LT2 compared to the controls. Considering the high MOI applied, this
344 might indicate that reduction of the strain LT2 may have occurred by inundation without
345 phage replication. In the experiments with field isolates S1 and S2, replication occurred. The
346 resulting increase in total phage numbers exceeded the expected increase after one replication
347 cycle in all available bacterial cells (10^4 CFU/chicken skin 10,000-fold, based on the
348 determined burst size of the phages). However, considering the high number of spatial
349 segments on the chicken skin and the likely non-uniform distribution of phages and bacteria
350 in these segments (41), killing by phage inundation without phage replication and killing by a

Downloaded from https://journals.asm.org/journal/aem on 02 October 2021 by 188.159.185.175.

Accepted Manuscript Posted Online

351 phage infection that resulted in final phage progeny release might have occurred
352 simultaneously in different segments of the matrix. The outcome of the experiments might
353 thus be rather a result of the predominating process than of killing by either inundation or
354 productive phage infection (25).

355 In poultry meat production, processes are tightly connected and time intervals for treatment of
356 meat by using phages are limited. However, some studies reported that reduction in
357 *Salmonella* concentrations was dependent on extended times of incubation (36, 54, 55). The
358 characteristics of chicken skin include feather follicles, high fat content and large folded
359 surfaces, providing protection for *Salmonella* from eradication by antimicrobial treatments
360 (41, 44, 51). The recovered colonies from treated skins were susceptible to LmqsSP1 and thus
361 full reduction by the phages would have been expected if no protection by the matrix
362 occurred. In addition, the reducing effect on *Salmonella* load on chicken skin was
363 significantly smaller than in broth medium at the same temperature, indicating that the
364 efficiency of phages was negatively affected on chicken skin. Short periods of phage activity
365 may limit the efficiency of bacterial reduction under commercial conditions when lytic phage
366 infection is required for reduction (17). This practical aspect suggests that using a high titer of
367 bacteriophage is crucial for achieving efficient results by passive inundation or a combination
368 of lytic infection and passive inundation under commercial conditions of poultry meat
369 production. It has also been emphasized that a homogeneous application of phages is
370 necessary for a good coverage of all host bacteria present (20, 46, 52). To achieve this, an
371 appropriate spray equipment (46) and high volumes of liquid (52) have been recommended.
372 However, it has been proposed that phage application in chilling water at chicken slaughtering
373 plants could be more efficient than spraying (20). Whilst extended use of current chemical
374 treatments such as chlorine can lead to a decline in the organoleptic quality of the meat (45),
375 application of phages does not affect the organoleptic properties of chicken carcasses (9). The

Downloaded from https://journals.asm.org/journal/aem on 02 October 2021 by 188.159.185.175.

Accepted Manuscript Posted Online
Applied and Environmental Microbiology
AEM
Applied and Environmental Microbiology

376 latter could, additionally increase the possibility of bacterial reduction through the mechanism
377 of passive inundation, particularly for low bacterial concentrations on chicken skin, and could
378 consequently restrict the emergence of BMIs throughout the process until use when followed
379 by refrigeration (53).

380 Results suggest that bacteriophage insensitive mutants did not occur after application of
381 LmqsSP1 under the applied conditions. However, while none of the investigated *Salmonella*
382 isolates recovered from phage-treated skin pieces after phage treatment showed reduced
383 susceptibility to LmqsSP1, all tested re-isolates from liquid culture were insensitive after an
384 extended one-week incubation of LmqsSP1 with *S*. Typhimurium LT2 at 37 °C. Bacterial
385 growth and long storage, optimal nutrient supply and growth temperatures do not reflect
386 conditions of practical application settings. These conditions might enhance the spread of
387 phage-resistant isolates due to an increased number of replication cycles compared to
388 practical settings or a short incubation period (27, 38). However, further research on
389 conditions favoring the occurrence of reduced phage-susceptibility and influencing the fitness
390 of resistant isolates is urgently needed (27). After application on chicken skin, few sensitive
391 bacteria remaining after treatment with phages might result from the lacking adsorption of
392 phages to bacteria hidden in holes and pores in the chicken skin. This is in accordance with
393 other studies (9, 44, 52). In agreement with these results, growth of the tested *Salmonella* in
394 liquid medium at 37 °C was inhibited for a minimum 7.5 h without regrowth as shown in
395 Figure 4. No isolates were recovered for susceptibility testing after this experiment, but results
396 indicate that resistance might only occur after prolonged incubation in liquid culture. In
397 contrast to these findings, regrowth was reported 5 to 8 h after significant reductions or briefly
398 after phage application in other studies (38, 56), and O'Flynn et al. detected bacteriophage
399 insensitive mutants after 99% reduction of an *S*. Typhimurium strain by the commercial phage
400 Felix 01 (56). Bai et al. concluded that the rapid occurrence of bacteriophage resistance in

Downloaded from https://journals.asm.org/journal/aem on 02 October 2021 by 188.159.185.175.

Accepted Manuscript Posted Online

Applied and Environmental Microbiology

AEM

Applied and Environmental Microbiology

401 bacterial populations is a significant limitation for the efficacy of phage application (6).
402 However, findings of other studies were in accordance with our results (7, 44).
403
404 In conclusion, the fast and significant reduction in foodborne *S*. Typhimurium isolates in
405 liquid medium for 10 h without considerable bacterial regrowth and under conditions
406 resembling the situation in the food production chain on chicken skin, demonstrates that
407 LmqsSP1 is a potential candidate for biocontrol of *Salmonella* in food. LmqsSP1 could be
408 used for the reduction of known *Salmonella* strains, in bacteriophage cocktails or in
409 combination with other techniques as a multi-hurdle approach. Based on the genome
410 sequence, the phage LmqsSP1 was assessed to be well suited for phage application, as no
411 genes with undesired effects were identified. Some gene products showed a structural and
412 functional relationship to homing endonucleases. These gene copies might represent target
413 sites for homologous recombination, potentially leading to genomic adaptions of the bacterial
414 host and/or the phage. Within our study, we found no indication of a genetic instability of the
415 phage genome that might be associated with potential changes of either its lytic activity or the
416 spectrum of the infected bacterial hosts. However, further long-time analyses are necessary to
417 assess the genetic stability of the phage in detail. Based on the prevailing results from the
418 bioinformatics analysis, LmqsSP1 seemed to be suited for application issues.
419 Nonetheless, the presence of some mobile genes might influence the genetic stability of the
420 phage during successive or long-term usage. Up to now, there is no indication that the phage
421 sequence and/or the organization of the genes is significantly changed during *in vitro*
422 reduction tests. Further experiments are necessary to determine the genetic stability *in vivo*. In
423 general, members of the T5-like phages are broadly assigned as promising tools for biocontrol
424 or therapeutic applications against *E. coli*, *Salmonella*, and other bacteria (57-59).

425 **Materials and Methods**

Downloaded from https://journals.asm.org/journal/aem on 02 October 2021 by 188.159.185.175.

Accepted Manuscript Posted Online

Applied and Environmental Microbiology

AEM

Applied and Environmental Microbiology

426 **Bacterial strains and growth conditions**

427 The *S. enterica* subsp. *enterica* serovar Typhimurium strain LT2 was used for bacteriophage
428 detection and isolation. Host range analysis was conducted using 76 *Salmonella* isolates
429 originating from current food samples of different animal species (Table 2), *S.* Typhimurium
430 host strain LT2 and two *E. coli* O157:H7 (DSM 19206 and DSM 17076). Host bacteria were
431 cultivated on blood agar (Columbia Agar with 5 % sheep blood, Thermo Scientific Inc.,
432 Waltham, MA, USA) at 37 °C overnight for all experiments. Thereafter, one colony was used
433 for a subsequent culturing in lysogeny broth (LB) (Carl Roth GmbH, Karlsruhe, Germany)
434 supplemented with $CaCl_2$ (at a final concentration of 1 mM/L) at 37 °C for 3 h for an
435 approximate optical density (OD_{600}) of 0.5. This early logarithmic culture was then used for
436 phage infection assays.

437 **Bacteriophage isolation and propagation**

438 The soft-agar overlay technique was used to detect *Salmonella*-specific phages from different
439 sample matrices. Out of 52 samples originating from different farms in northern Germany, the
440 majority were chicken feces (n=34) or cecal content of poultry (chickens (n=6), turkeys (n=2)
441 and Peking ducks (n=1)), while only nine samples were nasal swabs (n=5) and manure (n=4)
442 from cattle.

443 For sample preparation, ~6 g of each matrix was suspended for 1 min in 30 mL sodium
444 chloride-magnesium sulfate (SM) buffer (50 mM Tris-HCl, 8 mM magnesium sulfate, 100
445 mM sodium chloride, and 0.01% gelatin, pH 7.5) using a Turrax homogenizer (IKA-Werke
446 GmbH & Co. KG, Staufen, Germany). After overnight shaking in the refrigerator, the
447 suspensions were centrifuged twice at 4 °C (4,000 x g for 20 min and 13,000 x g for 10 min).
448 The remaining supernatant was filtered through a 0.22 µm membrane filter (VWR
449 International GmbH, Darmstadt, Germany). For phage isolation and purification, the *S.*
450 Typhimurium strain LT2 was cultured with the sample using soft-agar overlay technique as

Downloaded from https://journals.asm.org/journal/aem on 02 October 2021 by 188.159.185.175.

Accepted Manuscript Posted Online

Applied and Environmental Microbiology

AEM

Applied and Environmental Microbiology

451 described previously (60). Briefly, 100 μL of the samples and 100 μL of exponentially
452 growing *S.* Typhimurium strain LT2 were added to 5 mL of LB soft agar (0.4% w/v agar
453 bacteriological, 2 mM $CaCl_2$), poured onto petri dishes containing LB agar (1.5% w/v agar
454 bacteriological, 2 mM $CaCl_2$) and incubated at 37 °C overnight. Phages were isolated and
455 purified by a successive three-fold picking and plating procedure of single plaques.
456 Subsequently, phages were propagated to obtain concentrations of 10^9 to 10^{10} PFU/mL and
457 stored at 4 °C for further use. The phage titer was determined by plating 100 μL of a 10-fold
458 serial dilution series of the phage suspension on *S*. Typhimurium LT2 using the soft-agar
459 overlay technique. For testing phage genome stability, 100 μL of filtrated phage culture was
460 serially incubated with 100 μL of *S*. Typhimurium strain LT2 overnight culture for 24-72 h at
461 37 °C in 5 mL Mueller-Hinton broth. The phage filtrate of one incubation was used for the
462 subsequent 90 consecutive incubation cycles. The final phage culture was used for phage
463 propagation and DNA extraction, sequencing and bioinformatics analysis as described below.

464 **Host range and efficiency of plating (EOP)**

465 Serial dilutions were spotted on soft-agar overlays to determine the relative efficiency of
466 plating as previously described by Kutter et al. (61). Briefly, the bacterial overlay agar was
467 prepared as described above using the food-derived *Salmonella* isolates, *S.* Typhimurium
468 strain LT2 and the *E. coli* strains without the addition of phages. Subsequently, 10 μL of 10-
469 fold serial dilutions of the purified phages were applied on these lawns. The plates were
470 incubated at 37 °C for 24 h after the phage suspensions had been absorbed by the medium.
471 Each test was performed in triplicate. The sensitivity of the tested bacteria to the phages was
472 determined by counting the number of plaques in the spots. The relative efficiency of plating
473 (EOP) was defined as the phage titer on a given bacterial lawn divided by the maximum titer
474 observed on the original host *S*. Typhimurium strain LT2.

475 **Negative-staining of phages**

Downloaded from https://journals.asm.org/journal/aem on 02 October 2021 by 188.159.185.175.

Accepted Manuscript Posted Online
Applied and Environmental Microbiology
AEM
Applied and Environmental Microbiology

476 Thin carbon support films were prepared by evaporating a carbon thread onto a freshly
477 cleaved mica surface (Bal-Tech SCD500). After cutting small pieces of mica (approximately
478 3 mm in length), the phages were negatively stained with 4% (w/v) aqueous uranyl acetate,
479 pH 5.0, in accordance with the method of Valentine et al. (62). In brief: Phages were adsorbed
480 for 15-30 sec onto the carbon film, washed in TE buffer (10 mM TRIS, 1 mM EDTA, pH 6.9)
481 and picked up with a 300-mesh nickel grid, blotted dry on a filter paper and subsequently air-
482 dried. Dried samples were examined in a TEM 910 transmission electron microscope (Carl
483 Zeiss Industrielle Messtechnik GmbH, Oberkochen, Germany) at an acceleration voltage of
484 80 kV. Images were taken at calibrated magnifications using a line replica. Images were
485 recorded digitally with a Slow-Scan CCD-Camera (ProScan, 1024x1024, Proscan
486 Elektronische Systeme GmbH, Scheuring, Germany) with ITEM-Software (Olympus Soft
487 Imaging Solutions GmbH, Münster, Germany).

488 The head diameter and tail length were determined using Image J software version 1.51q and
489 calculating the average size from a minimum of ten measurements.

490 **One-step growth experiments**

491 One-step growth experiments were performed as previously described by Hyman and Abedon
492 (63) with some modifications. For this purpose, LmqsSP1 was incubated at 37 °C with the
493 *Salmonella* LT2 and the food-derived isolates S1 and S2. Briefly, the cultures of the host
494 bacteria were grown to an OD_{600} of 0.5 and mixed with LmqsSP1 at a multiplicity of infection
495 (MOI) of 0.1. After allowing the phage to adsorb to the target bacteria for 10 min at room
496 temperature, samples were centrifuged at 1,300 x g for 4 min at 4 °C and the excess free
497 phages in the supernatant were discarded. The pelleted cells were suspended in fresh LB and
498 placed in a heating block at 37 °C for the duration of the experiment. Samples were
499 withdrawn and concentrations of phages in the samples were measured immediately at 5 or 10
500 min-intervals for up to 2 h. Experiments were performed in duplicate and were repeated three

Downloaded from https://journals.asm.org/journal/aem on 02 October 2021 by 188.159.185.175.

Accepted Manuscript Posted Online

Applied and Environmental Microbiology

AEM

Applied and Environmental Microbiology

501 times. The latent period and burst size were calculated as described previously (63).

502 **Bacterial challenge tests - *in vitro* experiments at 37 °C**

503 **Growth inhibition experiments**

504 Growth inhibition of *S*. Typhimurium LT2, and the food-derived isolates S1 and S2 was
505 tested using the method described by O'Flynn et al. (56), with some modifications. Briefly,
506 bacterial cells were grown to a McFarland of 2.4 and diluted to McFarland 0.5 in fresh LB
507 (approximately 10^8 CFU/mL). Three hundred microliters of the aforementioned cultures and
508 of LmqsSP1 at concentrations of 10^3 to 10^9 PFU/mL were mixed in a 48-well microplate, and
509 after 10 min at room temperature, incubated in a TECAN SPARK® microplate reader (Tecan
510 Group AG., Männedorf, Switzerland) at 37 °C. Bacterial cultures inoculated with LB instead
511 of phages served as negative controls. The absorbance at 595 nm was measured every 10 min
512 during a 10-h period.

513 **Bacterial challenge tests - *in vitro* experiments at 4 °C and on chicken skin**

514 **Reduction experiments *in vitro***

515 To determine the efficacy of LmqsSP1 for the reduction of salmonellae at 4 °C, phages were
516 added to bacterial cultures in LB at an MOI of 10^4 and 10^5 CFU/mL. Briefly, exponentially
517 growing salmonellae (strain LT2 and the food-derived isolates S1 and S2) were diluted in LB
518 to a final concentration of 10^4 and 10^3 CFU/mL and 100 µL of LmqsSP1 at a titer of 10^{10}
519 PFU/mL was added. The same amount of SM buffer (50 mM Tris-HCl, 8 mM magnesium
520 sulfate, 100 mM sodium chloride, and 0.01% gelatin, pH 7.5) was added instead of phage for
521 negative controls. The number of viable *Salmonella* cells was determined after incubation at 4
522 °C for 24 h by using the pour plate method in accordance with ISO 4833-1:2013 (64) with
523 slight modifications. In brief, LB agar (1.5 % agar, Carl Roth GmbH & Co. KG) was poured
524 into petri dishes containing 1 mL of serial dilutions of the samples. The agar plates were
525 prepared in duplicate and incubated at 37 °C, and colonies were counted after 24 h. Each test
526 was performed three times.

Downloaded from https://journals.asm.org/journal/aem on 02 October 2021 by 188.159.185.175.

Accepted Manuscript Posted Online

Applied and Environmental Microbiology

AEM

Applied and Environmental Microbiology

527 **Reduction experiments on chicken skin at 4 °C**

528 To determine the efficacy of LmqsSP1 for reduction of salmonellae on chicken skin, the
529 experiments were carried out following the method described by Guenther et al. (52), with
530 some modifications. Briefly, irradiated chicken skin pieces of 25 cm^2 (radiation in a Cobalt-60
531 Gamma irradiation facility 3000 at a dose of 13.77 kGy ± 1) were inoculated with 100 µL of
532 the respective *Salmonella* isolate or strain at a concentration of 10^5 CFU/mL on both sides,
533 aiming at an initial concentration of approximately 2 x10^3 CFU/cm^2. Skin pieces were
534 incubated at 4 °C for 1 h, allowing the bacteria to adapt to the low temperature. Subsequently,
535 100 µL of LmqsSP1 were applied to the skin pieces, aiming at a concentration of about 2.5 x
536 10^8 PFU/cm^2. On the negative controls, 100 µL SM buffer (50 mM Tris-HCl, 8 mM
537 magnesium sulfate, 100 mM sodium chloride, and 0.01% gelatin, pH 7.5) was applied instead
538 of phages, and positive controls were treated with phages without *Salmonella* contamination.
539 One skin piece was used for confirming the sterility after incubation. Samples were incubated
540 for one week at 4 °C in a refrigerator. An additional experiment with a phage concentration of
541 2.5 x 10^4 PFU/cm^2 was carried out for 48 h. The experiments were conducted in three
542 replicates for *S*. Typhimurium LT2 and repeated four times for the field isolates S1 and S2.

543 Concentrations of viable bacteria on the skin pieces were enumerated after 3 h, 24 h and 48 h
544 and after one week of storage, respectively. For this purpose, 20 mL SM buffer was added to
545 each skin sample in a sterile plastic bag and samples were shaken and squeezed for 2 min. A 1
546 mL aliquot of the suspension was serially diluted 1:10 and plated in duplicate on LB agar by
547 using the pour plate method. Plates were incubated for 48 h at 37 °C, and *Salmonella* counts
548 were enumerated after 24 h and 48 h.

549 Phage concentration on the skin pieces was determined after 24 h by using the soft-agar
550 overlay technique as described above. Briefly, samples derived from enumeration of bacteria

Downloaded from https://journals.asm.org/journal/aem on 02 October 2021 by 188.159.185.175.

Accepted Manuscript Posted Online
Applied and Environmental Microbiology
AEM
Applied and Environmental Microbiology

551 were filtrated through 0.22 µm pore-size filters (VWR International GmbH, Hannover,
552 Germany) and serially diluted (1:10) in SM buffer and plated in duplicate on LB agar.

553 **Bacterial resistance testing**

554 To assess the phage-susceptibility among bacteria that had survived treatment with LmqsSP1
555 in the experiments on chicken skin, soft-agar overlay technique was used to verify plaque
556 formation. *Salmonella* colonies were picked from LB agar and were plated with 100 µL of
557 serially diluted phage suspension containing up to 10^9 PFU/mL. Isolates were considered to
558 show reduced susceptibility when plaque formation was reduced by more than one
559 logarithmic unit compared to plaque formation on the original *Salmonella* isolate. Bacterial
560 resistance was tested after challenge tests and in a separate experiment after an extended
561 incubation period of 7 days in Mueller-Hinton broth with *Salmonella* LT2. After incubation,
562 dilutions of the culture were plated on LB-agar and 50 *Salmonella* isolates were picked and
563 examined for their susceptibility towards LmqsSP1 as described above for host range testing
564 but with only one replicate per isolate.

565 **Extraction of phage DNA, whole genome sequencing and bioinformatics analysis**

566 For DNA extraction, phage solution was prepared using a cesium chloride density gradient as
567 described previously (65), with some modifications. Subsequently, DNA was extracted by
568 using the Promega Wizard-Kit in accordance with the manufacturer's instructions (Promega
569 GmbH, Walldorf, Germany). Briefly, 300 mL of phage lysate from a liquid culture in LB
570 containing about 10^9 PFU/mL was centrifuged for sedimentation of phages at 24,000 x g for 2
571 h at 10 °C (Avanti J-26S XP, Beckmann Coulter Inc., Brea, CA, USA). After removing the
572 supernatant, phage sediment was suspended overnight in 1.6 mL of SM buffer (50 mM Tris-
573 HCl, 8 mM magnesium sulfate, 100 mM sodium chloride, and 0.01% gelatin, pH 7.5) on an
574 orbital shaker at 120 rpm and filtered through 0.22 µm pore size filters (Rotilabo syringe
575 filter; Carl Roth GmbH & Co KG). Subsequently, the gradient was prepared by pipetting 500

Downloaded from https://journals.asm.org/journal/aem on 02 October 2021 by 188.159.185.175.

Accepted Manuscript Posted Online

Applied and Environmental Microbiology

AEM

Applied and Environmental Microbiology

576 µL of four cesium chloride solutions at p = 1.6, 1.5, 1.4, and 1.3 sequentially into the bottom
577 of a thinwall tube. Two milliliters of the phage suspension was layered on the top of the CsCl
578 gradient and tubes were subjected to ultracentrifugation in an Optima XPN-100 with an SW
579 60 Ti Rotor (Beckmann Coulter Inc.) at 165,100 x g and 4 °C for 2 h. Phage bands were
580 collected and dialyzed against SM buffer overnight. The phage suspensions were treated with
581 10-fold reaction buffer (100 mM Tris-HCl (pH 7.5), 25 mM $MgCl_2$ und 1 mM $CaCl_2$, Thermo
582 Fisher Scientific Inc., Waltham, MA, USA), 0.2 mg/mL RNase A and 0.002 U/µL DNase I
583 (Thermo Fisher Scientific Inc. at 37 °C overnight, and DNA was extracted by using the
584 Promega Wizard-Kit in accordance with the manufacturer's instructions. Whole genome
585 sequencing of phage DNA was performed in-house at the German Federal Institute for Risk
586 Assessment, Berlin, Germany (BfR). DNA-sequencing libraries were generated using the
587 Nextera XT DNA Library Flex Preparation Kit (Illumina Inc., San Diego, CA, USA)
588 according to the recommendations of the manufacturer. Short read, paired-end sequencing
589 was conducted on an Illumina MiSeq benchtop device using the MiSeq Reagent v3 600-cycle
590 Kit (Illumina). Long-read WGS was conducted using phage DNA on a MinIon device
591 (Oxford Nanopore, Oxford, UK). The raw reads from both short- and long-read sequencing
592 platforms were subjected to a hybrid assembly (unicycler, v.0.44). Initial annotation was
593 performed using the Pathosystems Resource Integration Center (www.patricbrc.org). For final
594 prediction of specific gene product functions BLASTp (NCBI) was used. Further basic
595 sequence analyses and DNA alignments were carried out using DS Gene (Accelrys Inc., San
596 Diego, CA, USA). Prediction of genetic elements (i.e., ORFs, transcription terminators and
597 tRNAs) on the phage genome was conducted as described previously (66, 67).

598 **Nucleotide sequence accession number**

599 The complete nucleotide sequence of the *Salmonella* phage vB_StyS-LmqsSP1 was deposited
600 in GenBank under the accession number MT577844.

Downloaded from https://journals.asm.org/journal/aem on 02 October 2021 by 188.159.185.175.

Accepted Manuscript Posted Online
Applied and Environmental Microbiology
AEM
Applied and Environmental Microbiology

601 **Statistical analysis.** Bacterial concentrations of the groups were compared using paired t-test
602 with Bonferroni correction for detecting significant differences. Comparison of bacterial
603 numbers in treated groups and the control group was performed by using the Dunnet's test.
604 Analysis of trend in time was carried out using mixed models, considering time as the random
605 effect. All statistical analyses of bacterial challenge tests were performed using the R software
606 package version 3.5.3, and clustering (20) of bacteriophage host range was performed using
607 version 3.5.2.

608

609 **Funding**

610 WGS of the phage genome was financially supported by a grant (43-001) from the German
611 Federal Institute for Risk Assessment.

612

613 **References**

614 1. Ferrari RG, Rosario DKA, Cunha-Neto A, Mano SB, Figueiredo EES, Conte-Junior CA. 2019.
615 Worldwide epidemiology of *Salmonella* serovars in animal-based foods: a meta-analysis. Appl
616 Environ Microbiol 85:e00591-19.
617 2. EFSA, ECDC. 2019. The European Union summary report on trends and sources of zoonoses,
618 zoonotic agents and food-borne outbreaks in 2017. EFSA Journal 17:5926.
619 3. Marder Mph EP, Griffin PM, Cieslak PR, Dunn J, Hurd S, Jervis R, Lathrop S, Muse A, Ryan P,
620 Smith K, Tobin-D'Angelo M, Vugia DJ, Holt KG, Wolpert BJ, Tauxe R, Geissler AL. 2018.
621 Preliminary incidence and trends of infections with pathogens transmitted commonly
622 through food - Foodborne Diseases Active Surveillance Network, 10 U.S. Sites, 2006-2017.
623 MMWR Morb Mortal Wkly Rep 67:324-328.
624 4. Leverentz B, Conway WS, Alavidze Z, Janisiewicz WJ, Fuchs Y, Camp MJ, Chighladze E,
625 Sulakvelidze A. 2001. Examination of bacteriophage as a biocontrol method for *Salmonella*
626 on fresh-cut fruit: a model study. J Food Prot 64:1116-21.
627 5. Pawlowska AM, Zannini E, Coffey A, Arendt EK. 2012. "Green preservatives": combating fungi
628 in the food and feed industry by applying antifungal lactic acid bacteria. Adv Food Nutr Res
629 66:217-38.
630 6. Bai J, Jeon B, Ryu S. 2019. Effective inhibition of *Salmonella* Typhimurium in fresh produce by
631 a phage cocktail targeting multiple host receptors. Food microbiology 77:52-60.
632 7. Carlton RM, Noordman WH, Biswas B, de Meester ED, Loessner MJ. 2005. Bacteriophage
633 P100 for control of *Listeria monocytogenes* in foods: genome sequence, bioinformatic
634 analyses, oral toxicity study, and application. Regul Toxicol Pharmacol 43:301-12.
635 8. Lewis R, Hill C. 2019. Overcoming barriers to phage application in food and feed. Curr Opin
636 Biotechnol 61:38-44.

Downloaded from https://journals.asm.org/journal/aem on 02 October 2021 by 188.159.185.175.

Applied and Environmental Microbiology
AEM
Applied and Environmental Microbiology
Accepted Manuscript Posted Online

637 9. Moye ZD, Woolston J, Sulakvelidze A. 2018. Bacteriophage applications for food production
638 and processing. Viruses 10:205.
639 10. Pietracha D, Misievicz A. 2016. Use of products containing a phage in food industry as a new
640 method for *Listeria monocytogenes* elimination from food (*Listeria monocytogenes* phages in
641 food Industry) - A review. Czech J Food Sci 34:1-8.
642 11. USDA. 2019. Safe and suitable ingredients used in the production of meat, poultry, and egg
643 products. Related Documents for FSIS Directive 71201.
644 12. Debarbieux L, Pirnay JP, Verbeken G, De Vos D, Merabishvili M, Huys I, Patey O, Schoonjans
645 D, Vaneechoutte M, Zizi M, Rohde C. 2016. A bacteriophage journey at the European
646 Medicines Agency. FEMS Microbiol Lett 363:fnv225.
647 13. EFSA. 2009. Scientific Opinion on "The use and mode of action of bacteriophages in food
648 production". EFSA Jounal 2:1076.
649 14. Nagel TE, Chan BK, De Vos D, El-Shibiny A, Kang'ethe EK, Makumi A, Pirnay JP. 2016. The
650 developing world urgently needs phages to combat pathogenic bacteria. Front Microbiol
651 7:882.
652 15. Kassa T. 2021. Bacteriophages against pathogenic bacteria and possibilities for future
653 application in Africa. Infection and Drug Resistance 14:17-31.
654 16. Khalid A, Lin RCY, Iredell JR. 2020. A Phage Therapy guide for clinicians and basic scientists:
655 Background and highlighting applications for developing countries. Front Microbiol
656 11:599906.
657 17. Grant A, Parveen S, Schwarz J, Hashem F, Vimini B. 2017. Reduction of *Salmonella* in ground
658 chicken using a bacteriophage. Poult Sci 96:2845-2852.18. Augustine J, Bhat SG. 2015.
659 Biocontrol of *Salmonella* Enteritidis in spiked chicken cuts by lytic bacteriophages PhiSP-1
660 and PhiSP-3. J Basic Microbiol 55:500-3.
661 19. Chen Y, Sun E, Song J, Tong Y, Wu B. 2018. Three *Salmonella enterica* serovar Enteritidis
662 bacteriophages from the siphoviridae family are promising candidates for phage therapy. Can
663 J Microbiol 64:865-875.20. Kang HW, Kim JW, Jung TS, Woo GJ. 2013. wksl3, a New
664 biocontrol agent for *Salmonella enterica* serovars enteritidis and typhimurium in foods:
665 characterization, application, sequence analysis, and oral acute toxicity study. Appl Environ
666 Microbiol 79:1956-68.
667 21. Ross A, Ward S, Hyman P. 2016. More Is Better: Selecting for broad host range
668 bacteriophages. Front Microbiol 7:1352.
669 22. Gill JJ, Hyman P. 2010. Phage choice, isolation, and preparation for phage therapy. Current
670 pharmaceutical biotechnology 11:2-14.
671 23. Zampara A, Sorensen MC, Elsser-Gravensen A, Brondsted L. 2017. Significance of phage-host
672 interactions for biocontrol of *Campylobacter jejuni* in food. Food Control 73:1169-1175.
673 24. Chinivasagam HN, Estella W, Maddock L, Mayer DG, Weyand C, Connerton PL, Connerton IF.
674 2020. Bacteriophages to control *Campylobacter* in commercially farmed broiler chickens, in
675 Australia. Front Microbiol 11:632.
676 25. Weld RJ, Butts C, Heinemann JA. 2004. Models of phage growth and their applicability to
677 phage therapy. J Theor Biol 227:1-11.26. Cairns BJ, Timms AR, Jansen VA, Connerton
678 IF, Payne RJ. 2009. Quantitative models of in vitro bacteriophage-host dynamics and their
679 application to phage therapy. PLoS Pathogen 5:e1000253.27. Oechslin F. 2018. Resistance
680 development to bacteriophages occurring during bacteriophage therapy. Viruses 10:351.
681 28. Zinno P, Devirgiliis C, Ercolini D, Ongeng D, Mauriello G. 2014. Bacteriophage P22 to
682 challenge *Salmonella* in foods. Int J Food Microbiol 191:69-74.
683 29. WHO. 2018. *Salmonella* (non-typhoidal). Available from: URL: https://www.who.int/news-
684 room/fact-sheets/detail/salmonella-(non-typhoidal)(2021/06/03).
685 30. Scallan E, Griffin PM, Angulo FJ, Tauxe RV, Hoekstra RM. 2011. Foodborne illness acquired in
686 the United States--unspecified agents. Emerg Infect Dis 17:16-22.
687 31. Carey-Smith GV, Billington C, Cornelius AJ, Hudson JA, Heinemann JA. 2006. Isolation and
688 characterization of bacteriophages infecting *Salmonella* spp. FEMS Microbiol Lett 258:182-6.

Downloaded from https://journals.asm.org/journal/aem on 02 October 2021 by 188.159.185.175.

Accepted Manuscript Posted Online

689 32. Demczuk W, Ahmed R, Ackermann HW. 2004. Morphology of *Salmonella enterica* serovar
690 Heidelberg typing phages. Can J Microbiol 50:873-5.
691 33. Hyman P, Abedon ST. 2010. Bacteriophage host range and bacterial resistance. Adv Appl
692 Microbiol 70:217-48.
693 34. Bao H, Zhang P, Zhang H, Zhou Y, Zhang L, Wang R. 2015. Bio-Control of *Salmonella*
694 Enteritidis in foods using bacteriophages. Viruses 7:4836-53.
695 35. Chan BK, Abedon ST, Loc-Carrillo C. 2013. Phage cocktails and the future of phage therapy.
696 Fut Microbiol 8:769-83.
697 36. Hooton SP, Atterbury RJ, Connerton IF. 2011. Application of a bacteriophage cocktail to
698 reduce *Salmonella* Typhimurium U288 contamination on pig skin. Int J Food Microbiol
699 151:157-63.
700 37. Kittler S, Mengden R, Korf IHE, Bierbrodt A, Wittmann J, Plotz M, Jung A, Lehnherr T, Rohde
701 C, Lehnherr H, Klein G, Kehrenberg C. 2020. Impact of bacteriophage-supplemented drinking
702 water on the *E. coli* population in the chicken gut. Viruses 12:1470.
703 38. Park M, Lee JH, Shin H, Kim M, Choi J, Kang DH, Heu S, Ryu S. 2012. Characterization and
704 comparative genomic analysis of a novel bacteriophage, SFP10, simultaneously inhibiting
705 both *Salmonella enterica* and *Escherichia coli* O157:H7. Appl Environ Microbiol 78:58-69.
706 39. Phothaworn P, Dunne M, Supokaivanich R, Ong C, Lim J, Taharnklaew R, Vesaratchavest M,
707 Khumthong R, Pringsulaka O, Ajawatanawong P, Klumpp J, Brown N, Imam M, Clokie MRJ,
708 Galyov EE, Korbsrisate S. 2019. Characterization of flagellotropic, Chi-Like *Salmonella* phages
709 isolated from Thai poultry farms. Viruses 11:520.
710 40. Duc HM, Son HM, Yi HPS, Sato J, Ngan PH, Masuda Y, Honjoh KI, Miyamoto T. 2020. Isolation,
711 characterization and application of a polyvalent phage capable of controlling *Salmonella* and
712 *Escherichia coli* O157:H7 in different food matrices. Food Res Int 131:108977.
713 41. Zhang X, Peng Z, Li P, Mao Y, Shen R, Tao R, Diao X, Liu L, Zhao Y, Luo X. 2020. Complex
714 internal microstructure of feather follicles on chicken skin promotes the bacterial cross-
715 contamination of carcasses during the slaughtering process. Front Microbiol 11:571913.
716 42. Campten BRI. 2016. Systematic and critical review on the potential use of bacteriophage on
717 foods. Available from: URL: https://www.food.gov.uk/research/research-
718 projects/systematic-and-critical-review-on-the-potential-use-of-bacteriophages-on-foods
719 (2021/06/03).
720 43. Sharma CS, Dhakal J, Nannapaneni R. 2015. Efficacy of lytic bacteriophage preparation in
721 reducing *Salmonella* in vitro, on turkey breast cutlets, and on ground turkey. J Food Prot
722 78:1357-62.
723 44. El-Shibiny A, El-Sahhar S, Adel M. 2017. Phage applications for improving food safety and
724 infection control in Egypt. J Appl Microbiol 123:556-567.
725 45. Hungaro HM, Mendoça RCS, Gouvêa DM, Vanetti MCD, Oliveira Pinto CL. 2013. Use of
726 bacteriophages to reduce *Salmonella* in chicken skin in comparison with a chemical agents.
727 Food Res Int 52:75-81.
728 46. Goode D, Allen VM, Barrow PA. 2003. Reduction of experimental *Salmonella* and
729 *Campylobacter* contamination of chicken skin by application of lytic bacteriophages. Appl
730 Environ Microbiol 69:5032-6.
731 47. Phothaworn P, Supokaivanich R, Lim J, Klumpp J, Imam M, Kutter E, Galyov EE, Dunne M,
732 Korbsrisate S. 2020. Development of a broad-spectrum *Salmonella* phage cocktail containing
733 viunalike and jerseylike viruses isolated from Thailand. Food Microbiol 92:103586.
734 48. Islam MS, Zhou Y, Liang L, Nime I, Liu K, Yan T, Wang X, Li J. 2019. Application of a phage
735 cocktail for control of *Salmonella* in foods and reducing biofilms. Viruses 11:841.
736 49. Esmael A, Azab E, Gobouri AA, Nasr-Eldin MA, Moustafa MMA, Mohamed SA, Badr OAM,
737 Abdelatty AM. 2021. Isolation and characterization of two lytic bacteriophages infecting a
738 multi-drug resistant *Salmonella* Typhimurium and their efficacy to combat salmonellosis in
739 ready-to-use foods. Microorganisms 9:423.

Downloaded from https://journals.asm.org/journal/aem on 02 October 2021 by 188.159.185.175.

Applied and Environmental Microbiology
AEM
Accepted Manuscript Posted Online
Downloaded from https://journals.asm.org/journal/aem on 02 October 2021 by 188.159.185.175.

740 50. Petsong K, Benjakul S, Chaturongakul S, Switt AIM, Vongkamjan K. 2019. Lysis Profiles of
741 *Salmonella* phages on *Salmonella* isolates from various sources and efficiency of a phage
742 cocktail against *S*. Enteritidis and *S*. Typhimurium. Microorganisms 7:100.
743 51. Atterbury RJ, Connerton PL, Dodd CE, Rees CE, Connerton IF. 2003. Application of host-
744 specific bacteriophages to the surface of chicken skin leads to a reduction in recovery of
745 *Campylobacter jejuni*. Appl Environ Microbiol 69:6302-6.
746 52. Guenther S, Huwyler D, Richard S, Loessner MJ. 2009. Virulent bacteriophage for efficient
747 biocontrol of *Listeria monocytogenes* in ready-to-eat foods. Appl Environ Microbiol 75:93-
748 100.
749 53. Atterbury RJ, Gigante AM, Rubio Lozano MS, Mendez Medina RD, Robinson G, Alloush H,
750 Barrow PA, Allen VM. 2020. Reduction of *Salmonella* contamination on the surface of chicken
751 skin using bacteriophage. Virology J 17:98.
752 54. Sukumaran AT, Nannapaneni R, Kiess A, Sharma CS. 2015. Reduction of *Salmonella* on
753 chicken meat and chicken skin by combined or sequential application of lytic bacteriophage
754 with chemical antimicrobials. Int J Food Microbiol 207:8-15.
755 55. Yeh Y, Purushothaman P, Gupta N, Ragnone M, Verma SC, de Mello AS. 2017. Bacteriophage
756 application on red meats and poultry: Effects on *Salmonella* population in final ground
757 products. Meat Sci 127:30-34.
758 56. O'Flynn G, Coffey A, Fitzgerald GF, Ross RP. 2006. The newly isolated lytic bacteriophages
759 st104a and st104b are highly virulent against *Salmonella enterica*. J Appl Microbiol 101:251-
760 9.
761 57. Amarillas L, Rubi-Rangel L, Chaidez C, Gonzalez-Robles A, Lightbourn-Rojas L, Leon-Felix J.
762 2017. Isolation and characterization of phiLLS, a novel phage with potential biocontrol agent
763 against multidrug-resistant *Escherichia coli*. Front Microbiol 8:1355.
764 58. Niu YD, Stanford K, Kropinski AM, Ackermann HW, Johnson RP, She YM, Ahmed R, Villegas A,
765 McAllister TA. 2012. Genomic, proteomic and physiological characterization of a T5-like
766 bacteriophage for control of Shiga toxin-producing *Escherichia coli* O157:H7. PLoS One
767 7:e34585.
768 59. Hartman S, Zeng C, O'Leary C, Newkirk H, Kongari R, Gill J, Liu M. 2019. Complete genome
769 sequence of *Salmonella enterica* serovar enteritidis siphophage seafire. Microbiol Resour
770 Announc 8:e01167-19.
771 60. Adams MH. 1959. Bacteriophages, vol 1. Interscience Publishers, New York.
772 61. Kutter E. 2009. Phage host range and efficiency of plating. Methods Mol Biol 501:141-9.
773 62. Valentine RC, Shapiro BM, Stadtman ER. 1968. Regulation of glutamine synthetase. XII.
774 Electron microscopy of the enzyme from *Escherichia coli*. Biochem (Mosc) 7:2143-52.
775 63. Hyman P, Abedon ST. 2009. Practical methods for determining phage growth parameters.
776 Methods Mol Biol 501:175-202.
777 64. Standardization IOo. 2013. ISO 4833-1:2013 Microbiology of the food chain — Horizontal
778 method for the enumeration of microorganisms — Part 1: Colony count at 30 degrees C by
779 the pour plate technique. Geneva, Switzerland.
780 65. Bachrach U, Friedmann A. 1971. Practical procedures for the purification of bacterial viruses.
781 Appl Microbiol 22:706-15.
782 66. Jackel C, Hertwig S, Scholz HC, Nockler K, Reetz J, Hammerl JA. 2017. Prevalence, host range,
783 and comparative genomic analysis of temperate *Ochrobactrum* phages. Front Microbiol
784 8:1207.
785 67. Lesnik EA, Sampath R, Levene HB, Henderson TJ, McNeil JA, Ecker DJ. 2001. Prediction of rho-
786 independent transcriptional terminators in *Escherichia coli*. Nucleic Acids Res 29:3583-94.

787

Applied and Environmental Microbiology

Accepted Manuscript Posted Online

Applied and Environmental Microbiology

AEM

Applied and Environmental Microbiology

788 **TABLE 1** Origin and growth characteristics of isolated phages

Phage isolate	Sample type	Animal origin[a]	Propagation (PFU increase)
LmqsSP1	nasal swab	cow	fast
LmqsSP2	feces	chicken	fast
LmqsSP3	feces	chicken	fast
LmqsSP4	feces	chicken	fast
LmqsSP5	feces	chicken	low[b]
LmqsSP6	feces	chicken	low[b]
LmqsSP7	feces	chicken	low[b]
LmqsSP8	feces	chicken	low[b]
LmqsSP9	feces	chicken	low[b]
LmqsSP10	feces	chicken	low[b]

789 [a]Samples originated from different farms in northern Germany

790 [b]A sufficient concentration of >10^6 PFU/mL was not met after two production cycles

791

792 **TABLE 2** Origin and serotype of food-derived *Salmonella* isolates

Salmonella isolate	Food sample	Animal origin	Serovar or -group
S1	minced meat	Pig	Typhimurium
S2	minced meat (mixed)	Cattle, Pig	Typhimurium
S3	heart	Pig	Typhimurium
S4	meat	Pig	Typhimurium
S5	liver	Pig	Typhimurium
S6	minced meat (mixed)	Cattle, Pig	Serogroup B
S7	meat	Deer	Saintpaul
S8	crust	Pig	Typhimurium
S9	meat	Poultry	Typhimurium
S10	minced meat (mixed)	Cattle, Pig	Derby
S11	meat	Poultry	Serogroup C
S12	minced meat (mixed)	Cattle, Pig	Derby
S13	minced meat (mixed)	Cattle, Pig	Typhimurium

Downloaded from https://journals.asm.org/journal/aem on 02 October 2021 by 188.159.185.175.

Accepted Manuscript Posted Online
Applied and Environmental Microbiology
AEM
Applied and Environmental Microbiology

S14	minced meat	Pig	Typhimurium
S15	minced meat	Pig	Typhimurium
S16	meat	Turkey	Bovismorbificans
S17	minced meat	Pig	ssp. *enterica*. rough variant
S18	meat	Poultry	ssp. *enterica* rough variant
S19	minced meat	unknown	ssp. *enterica* rough variant
S20	meat	Poultry	ssp. *enterica* rough variant
S21	minced meat	Pig	Livingstone
S22	minced meat	unknown	Infantis
S23	meat	Poultry	Enteritidis
S24	meat	Poultry	Heidelberg
S25	meat	Poultry	Minnesota
S26	minced meat	Pig	Typhimurium
S27	meat	Poultry	ssp. *enterica* rough variant
S28	meat	Poultry	Kottbus
S29	meat	Pig	Derby
S30	meat	Poultry	Infantis
S31	meat	Poultry	Minnesota
S32	minced meat	unknown	Typhimurium
S33	meat	Poultry	ssp. *enterica* rough variant
S34	meat	Poultry	Paratyphi B
S35	meat	Poultry	Enteritidis
S36	meat	Poultry	Montevideo
S37	meat	Poultry	Paratyphi B
S38	meat	Poultry	Heidelberg
S39	meat	Poultry	Heidelberg
S40	meat	Poultry	Heidelberg
S41	meat	Poultry	Paratyphi B

Downloaded from https://journals.asm.org/journal/aem on 02 October 2021 by 188.159.185.175.

Accepted Manuscript Posted Online

Applied and Environmental Microbiology

AEM

Applied and Environmental Microbiology

S42	meat	Poultry	Enteritidis
S43	meat	Poultry	Derby
S44	meat	Poultry	Newport
S45	minced meat	unknown	Typhimurium monophasic variant
S46	minced meat	Pig	Derby
S47	minced meat (mixed)	Cattle, Pig	Muenchen
S48	minced meat	Cattle	Infantis
S49	minced meat	Pig	London
S50	meat	Poultry	Minnesota
S51	minced meat	Cattle	Coeln
S52	meat	Poultry	Hadar
S53	meat	Poultry	Paratyphi B
S54	meat	Poultry	Infantis
S55	meat	Poultry	Livingstone
S56	minced meat	Pig	Typhimurium
S57	meat	Poultry	Braenterup
S58	meat	Poultry	Schwarzengrund
S59	meat	Poultry	Newport
S60	meat	Poultry	Newport
S61	ground pork	Pig	Infantis
S62	ground pork	Pig	Typhimurium monophasic variant
S63	meat	Poultry	Minnesota
S64	meat	Poultry	Anatum
S65	meat	Poultry	Indiana
S66	meat	Poultry	Enteritidis
S67	meat	Poultry	Indiana
S68	meat	Poultry	Infantis
S69	ground pork	Pig	Typhimurium
S70	meat	Poultry	ssp. *indica*
S71	meat	Poultry	ssp. *indica*
S72	meat	Poultry	Infantis

Downloaded from https://journals.asm.org/journal/aem on 02 October 2021 by 188.159.185.175.

Accepted Manuscript Posted Online
Applied and Environmental Microbiology
AEM
Applied and Environmental Microbiology

S73	minced meat	unknown	Paratyphi B
S74	meat	Poultry	Saintpaul
S75	minced meat	Pig	Brandenburg
S76	ground pork	Pig	Derby

793
794
795
796 **FIG. 1.** Heatmap of phage host range; phages are displayed on the x-axis and bacterial isolates and strains on the
797 y-axis; no lysis, at highest phage titer EOP < 0.001 with turbid plaques or inhibitory zone,
798 EOP < 0.1, 0.1≤EOP< 1, 1≤EOP≤10, EOP>10.
799
800
801
802 **FIG. 2.** (A) Plaques of the examined bacteriophage vB_StyS-LmqsSP1; scale bar represents 3 mm; (B)
803 Transmission electron micrograph of negatively stained phage vB_StyS-LmqsSP1; scale bar represents 100 nm.
804
805
806
807
808 **FIG. 3.** Growth of vB_StyS-LmqsSP1. One-step growth experiments at a multiplicity of infection of 0.1 using
809 *Salmonella* LT2 (A) as well as the field isolates S1 (B) and S2 (C) as bacterial hosts. Each experiment was
810 performed in triplicate. The highest concentration (PFU/mL) indicates the burst size (S) and double-headed
811 arrows indicate the latent period (L). Error bars represent standard error of the mean.
812
813
814
815 **FIG. 4.** Efficacy of the bacteriophage vB_StyS-LmqsSP1 in inhibiting growth of *S.* Typhimurium LT2 (A) and
816 field isolates S1 (B) and S2 (C) at different MOIs in LB. Graphs represent mean values of optical density of
817 *Salmonella* cultures infected with bacteriophage vB_StyS-LmqsSP1 from three experiments.
818
819
820

Downloaded from https://journals.asm.org/journal/aem on 02 October 2021 by 188.159.185.175.

Accepted Manuscript Posted Online

Applied and Environmental Microbiology

AEM

Applied and Environmental Microbiology

821 **FIG. 5.** Efficacy of bacteriophage vB_StyS-LmqsSP1 in reducing *S.* Typhimurium LT2 (A), and field isolates
822 S1 (B) and S2 (C) in LB at 4 °C. Exponentially growing *Salmonella* cultures at concentrations of 10^3 and 10^4
823 CFU/mL were inoculated with 10^9 PFU/ml vB_StyS-LmqsSP1. Gray bars indicate mean concentrations of
824 *Salmonella* in the control and white bars in the experiment. Experiments were performed in triplicate. Error bars
825 indicate the standard error mean (SEM).

826

827

828

829 **FIG. 6.** Efficacy of vB_StyS-LmqsSP1 in reducing *S.* Typhimurium LT2 (A) and field isolates S1 (B) and S2
830 (C) on chicken skin. Skin pieces were treated with phages at an MOI of 10^5 and stored for one week at 4 °C.
831 Mean concentrations of *Salmonella* on treated skin pieces (triangles, solid lines) and controls (dots, dashed lines)
832 are presented as mean $\log_{10}$ CFU/mL ± SEM of three (A) or four ((B), (C)) experiments.

833

834

835

836 **FIG. 7** vB_StyS-LmqsSP1 genome analysis and similarities to other phages (A) Genetic map of the examined
837 bacteriophage LmqsSP1. Putative genes are colored according to the predicted functions of their products. (B)
838 Phylogenetic analysis of LmqsSP1 and the related *E. coli* phages slur09, EASG3 and HASG4. (C) Agreement of
839 the genome organization of LmqsSP1 and the T5 like phages, slur09 and EASG3.

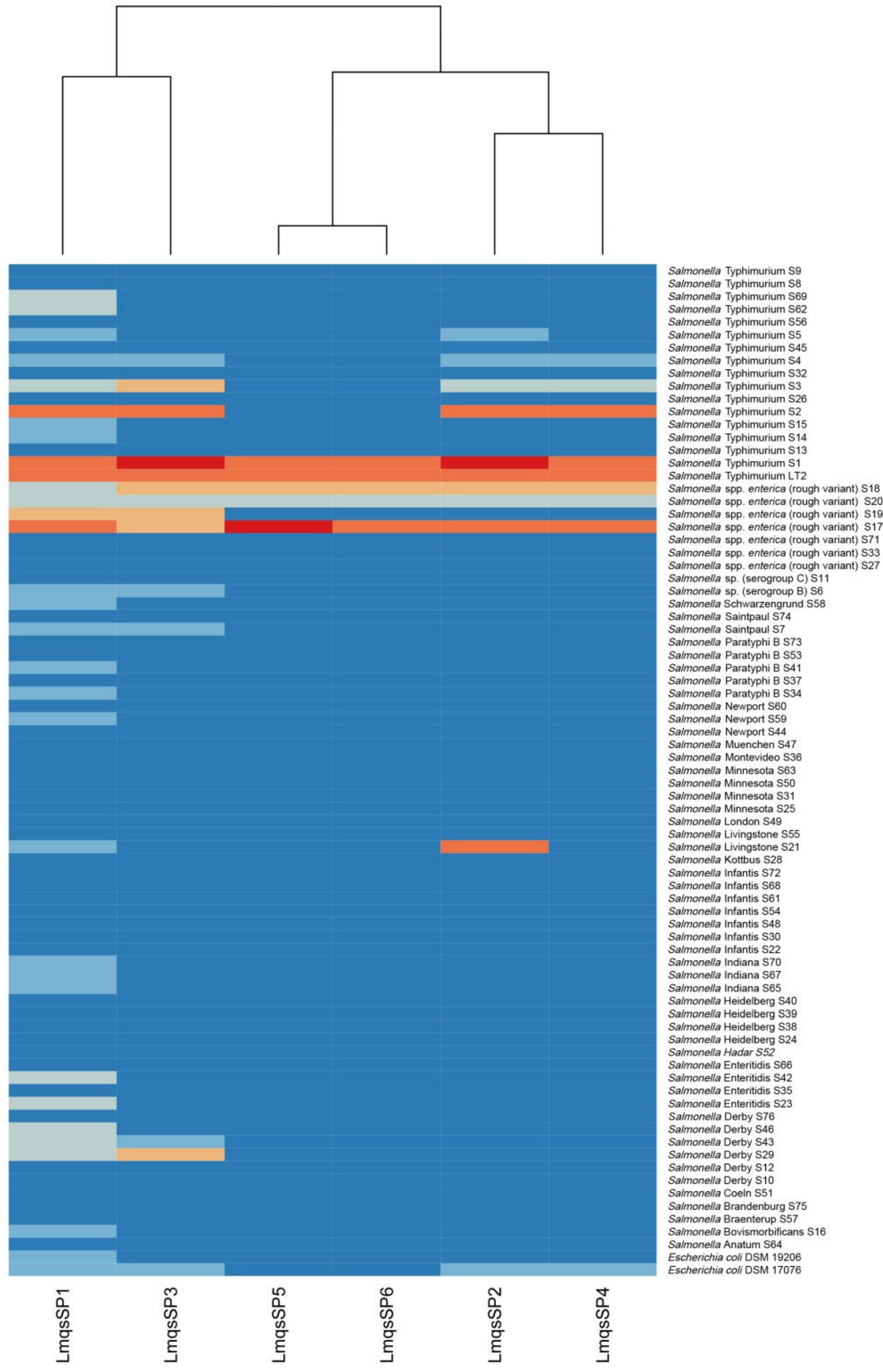
840

841

842

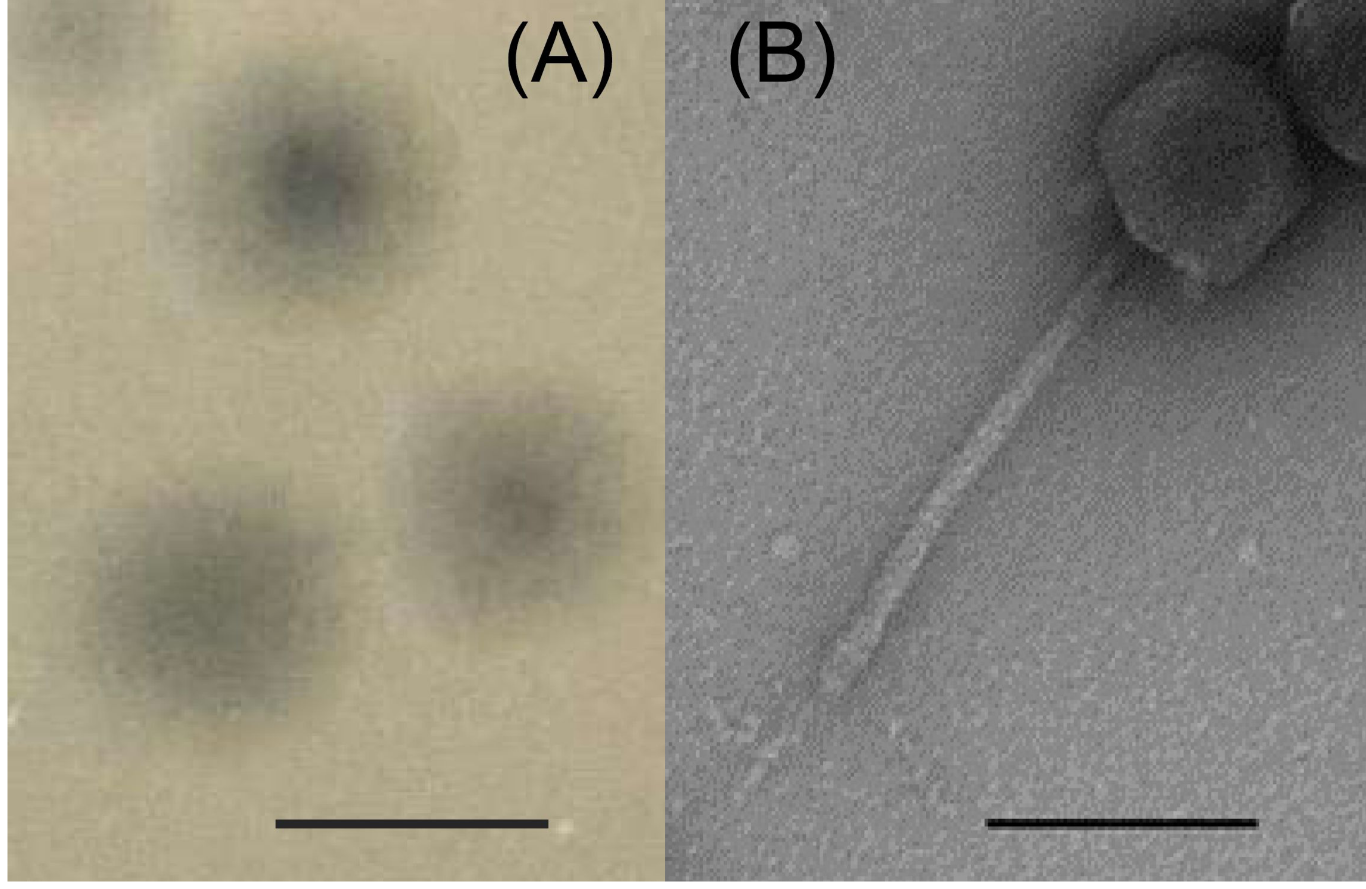
Downloaded from https://journals.asm.org/journal/aem on 02 October 2021 by 188.159.185.175.

Accepted Manuscript Posted Online
Applied and Environmental Microbiology
AEM
Applied and Environmental Microbiology



Downloaded from https://journals.asm.org/journal/aem on 02 October 2021 by 188.159.185.175.

Accepted Manuscript Posted Online
Applied and Environmental Microbiology
AEM
Applied and Environmental Microbiology



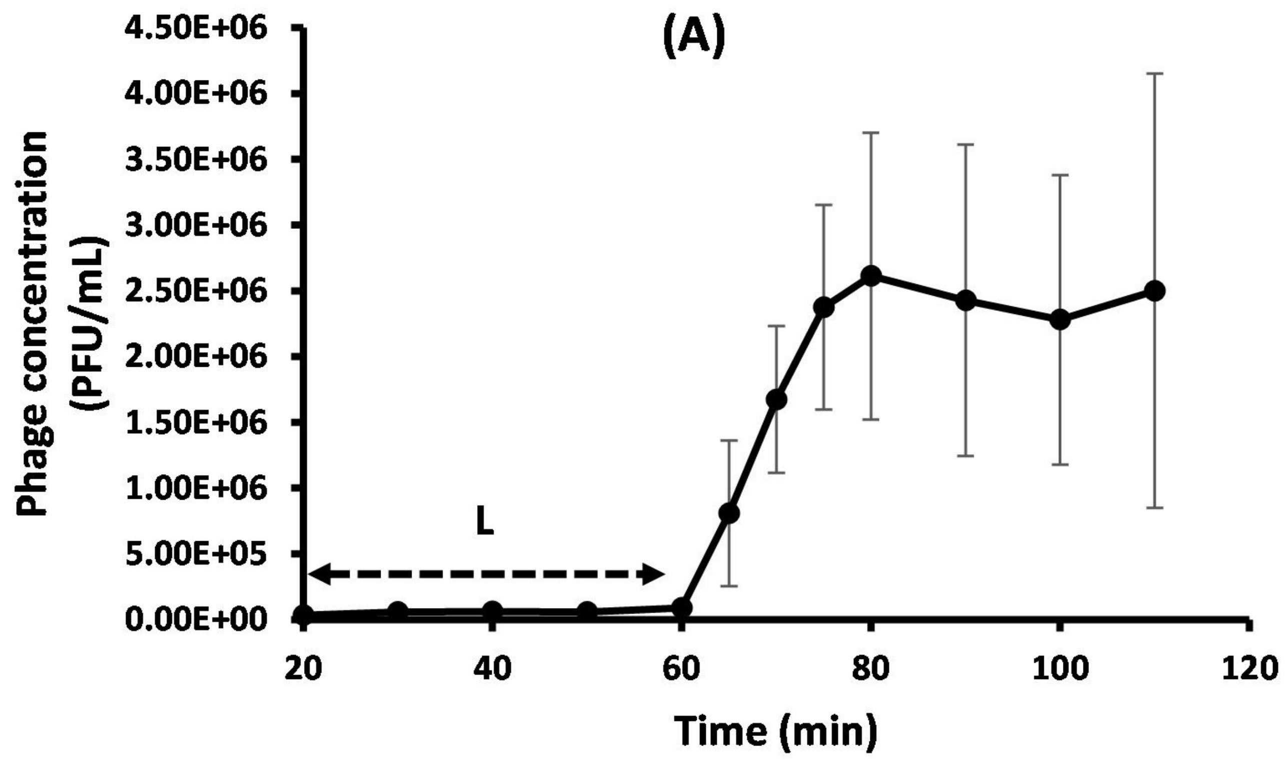
Downloaded from https://journals.asm.org/journal/aem on 02 October 2021 by 188.159.185.175.

Accepted Manuscript Posted Online

Applied and Environmental Microbiology

AEM

Applied and Environmental Microbiology



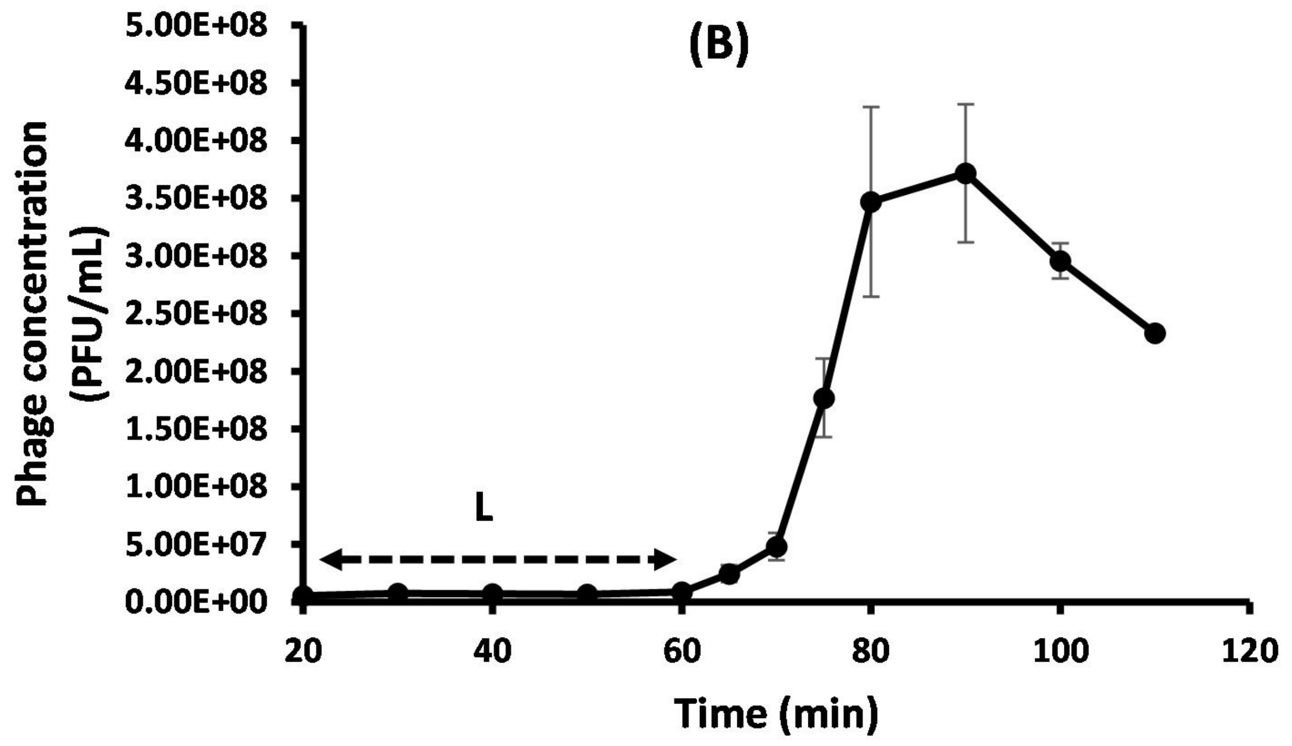
Downloaded from https://journals.asm.org/journal/aem on 02 October 2021 by 188.159.185.175.

Accepted Manuscript Posted Online

Applied and Environmental Microbiology

AEM

Applied and Environmental Microbiology



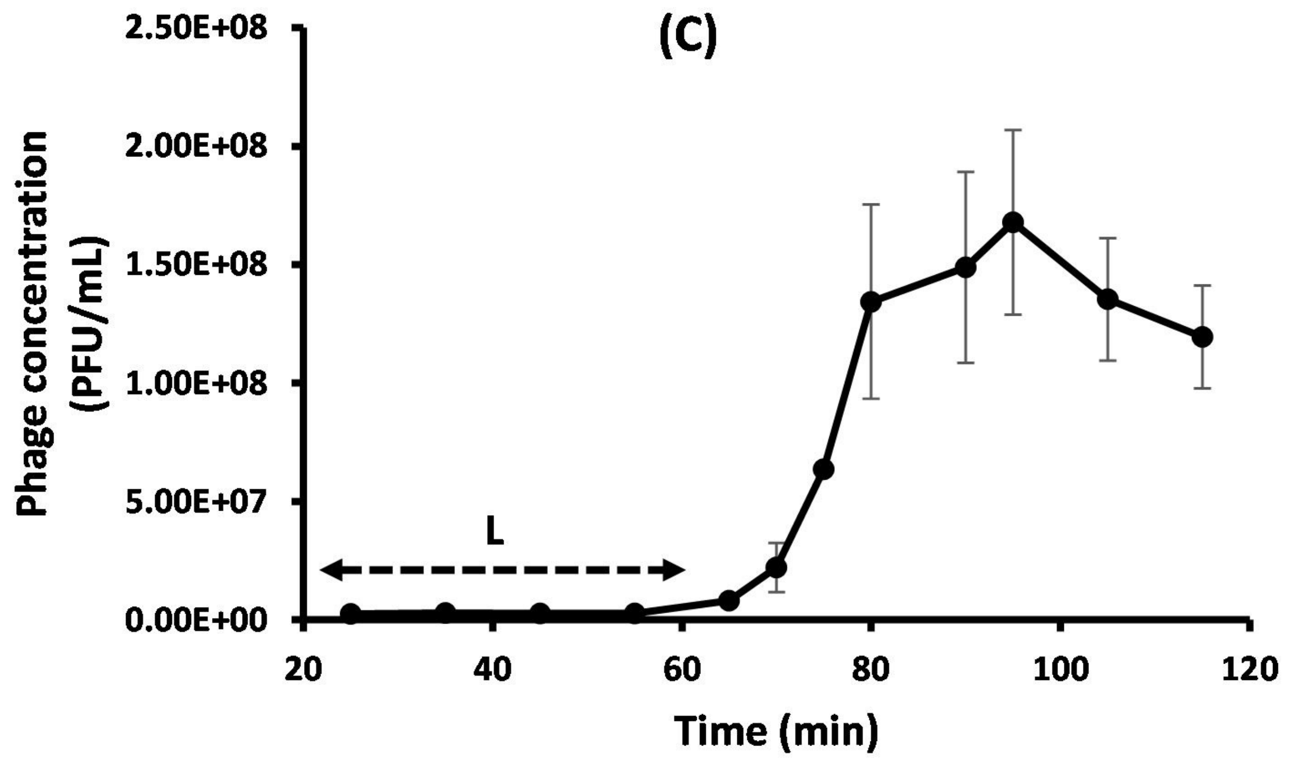
Downloaded from https://journals.asm.org/journal/aem on 02 October 2021 by 188.159.185.175.

Accepted Manuscript Posted Online

Applied and Environmental Microbiology

AEM

Applied and Environmental Microbiology



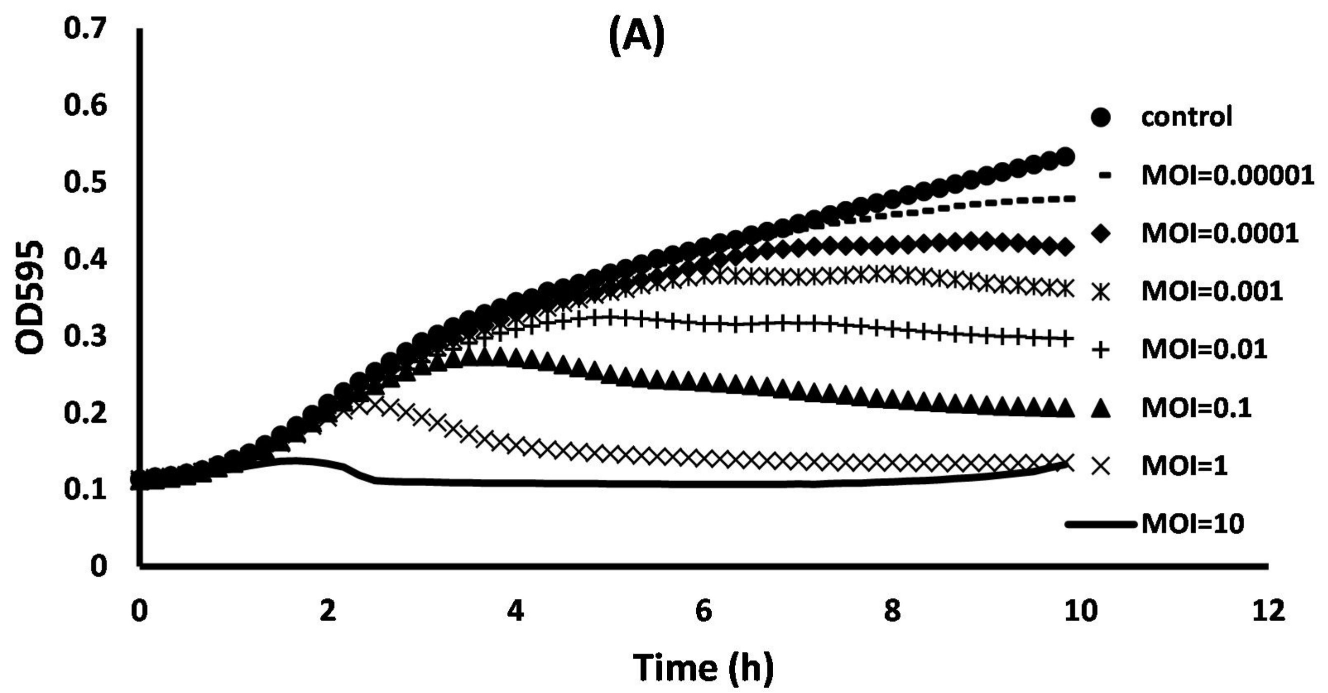
Downloaded from https://journals.asm.org/journal/aem on 02 October 2021 by 188.159.185.175.

Accepted Manuscript Posted Online

Applied and Environmental Microbiology

AEM

Applied and Environmental Microbiology



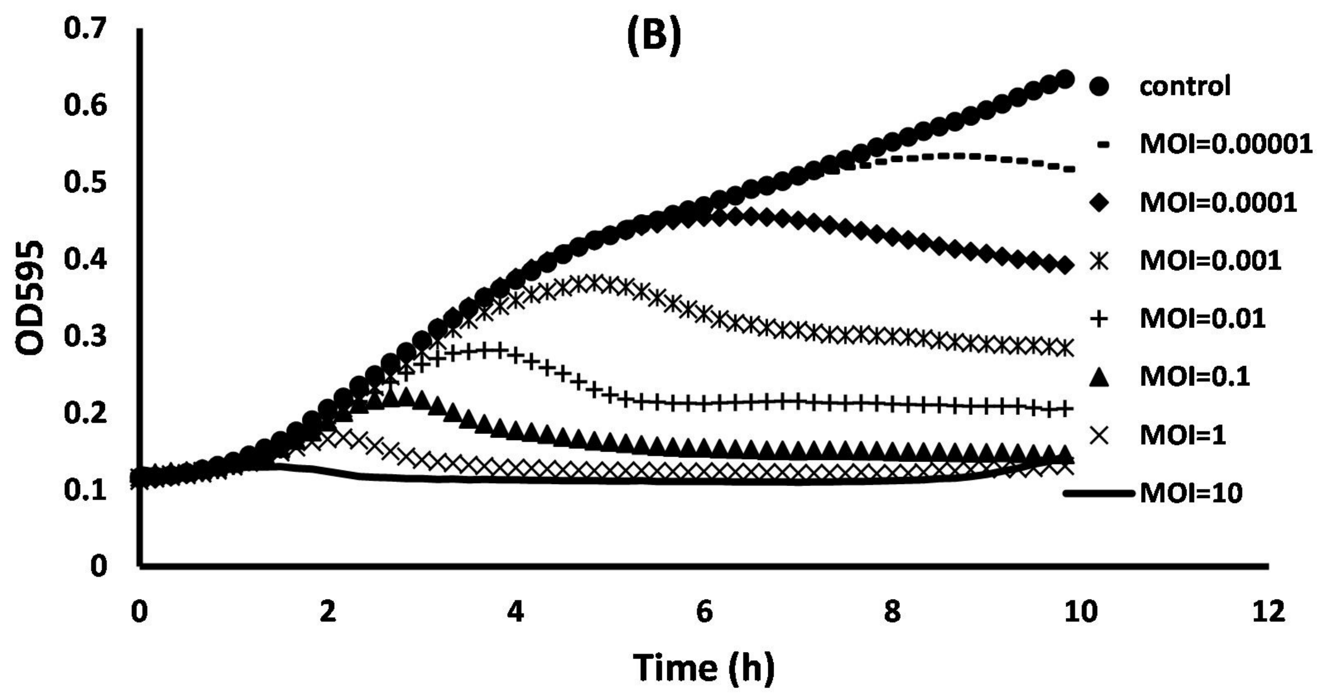
Downloaded from https://journals.asm.org/journal/aem on 02 October 2021 by 188.159.185.175.

Accepted Manuscript Posted Online

Applied and Environmental Microbiology

AEM

Applied and Environmental Microbiology



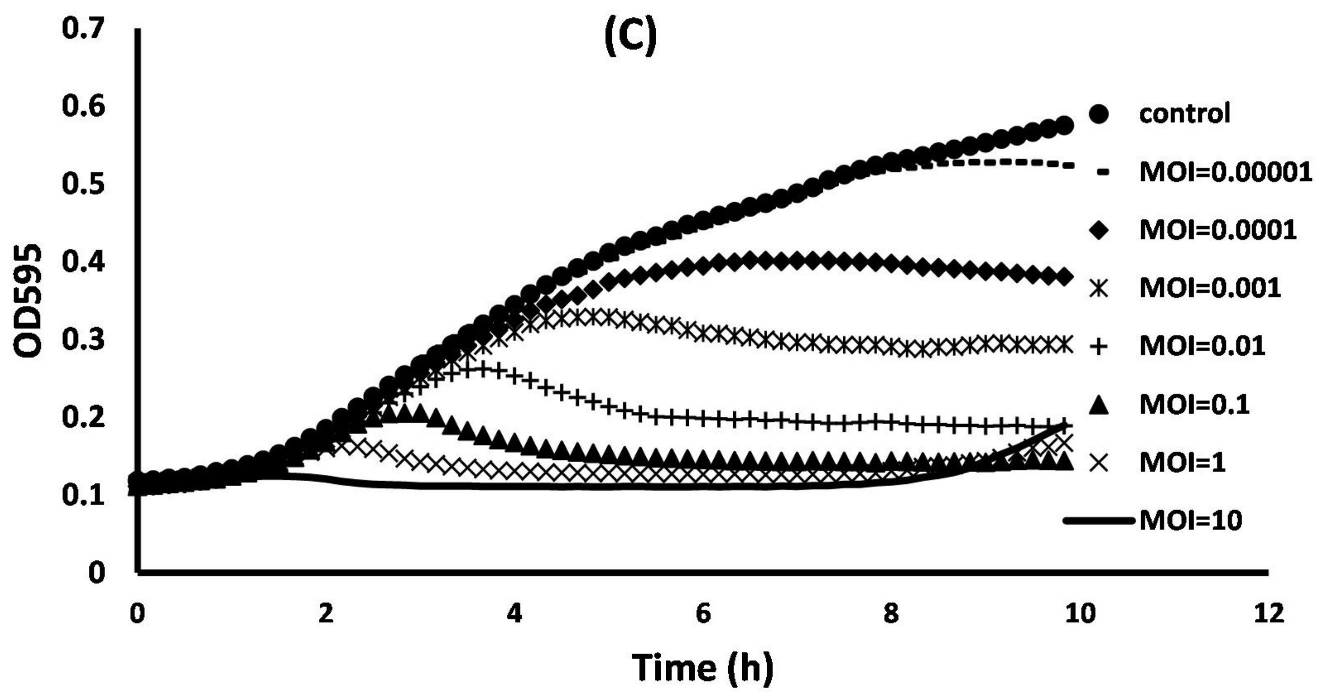
Downloaded from https://journals.asm.org/journal/aem on 02 October 2021 by 188.159.185.175.

Accepted Manuscript Posted Online

Applied and Environmental Microbiology

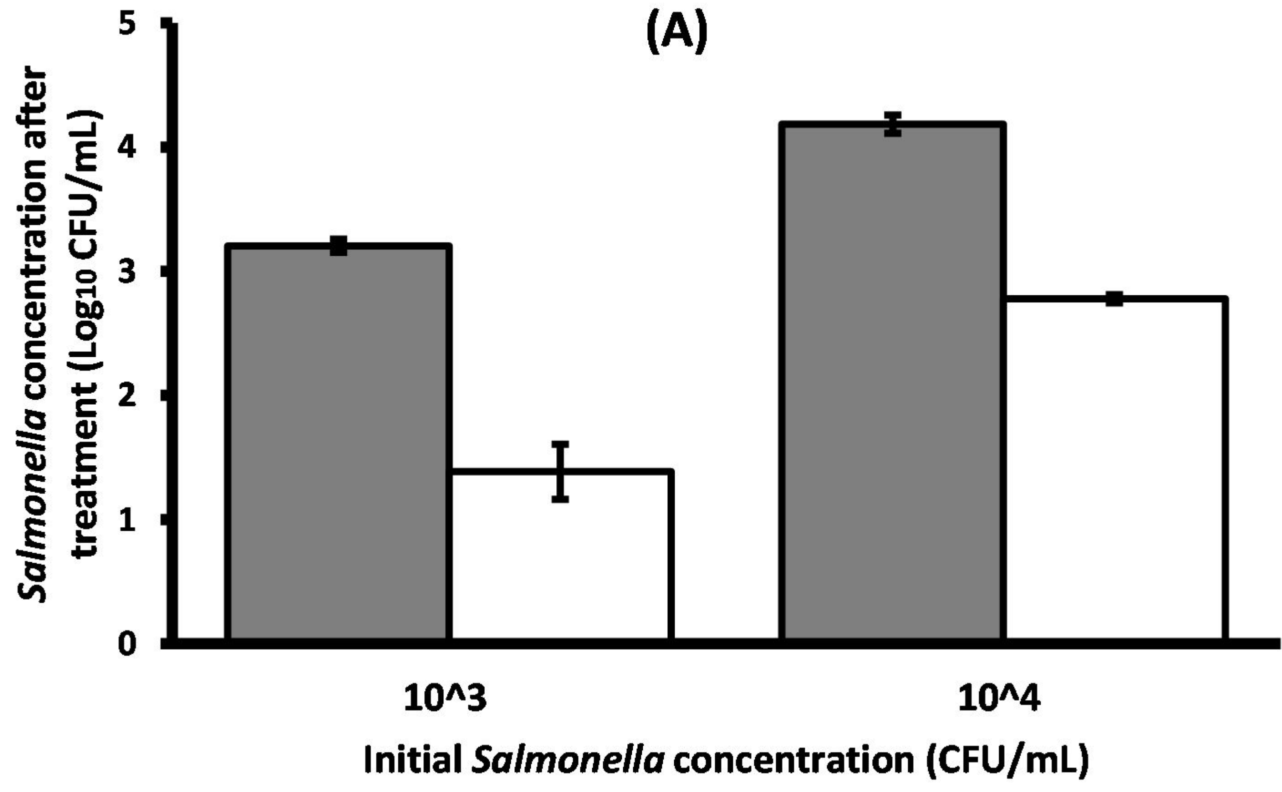
AEM

Applied and Environmental Microbiology



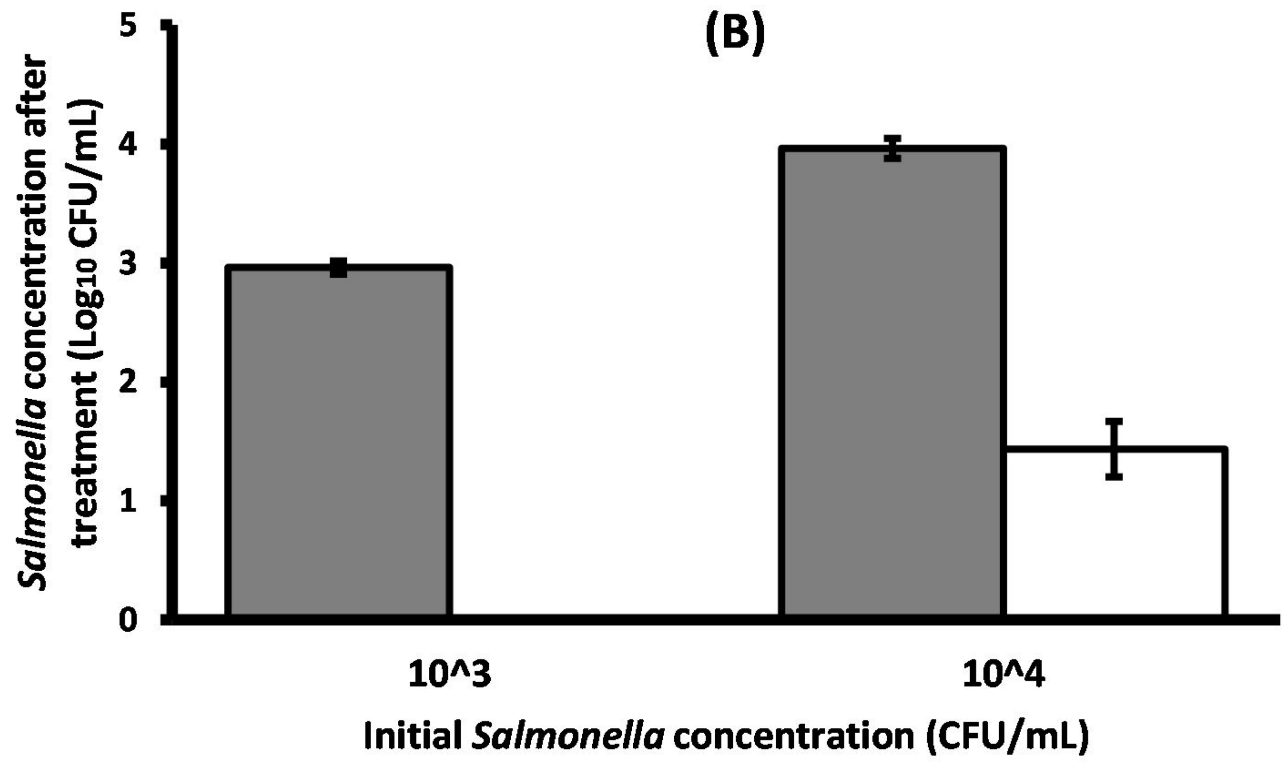
Downloaded from https://journals.asm.org/journal/aem on 02 October 2021 by 188.159.185.175.

Applied and Environmental Microbiology
AEM
Applied and Environmental Microbiology
Accepted Manuscript Posted Online



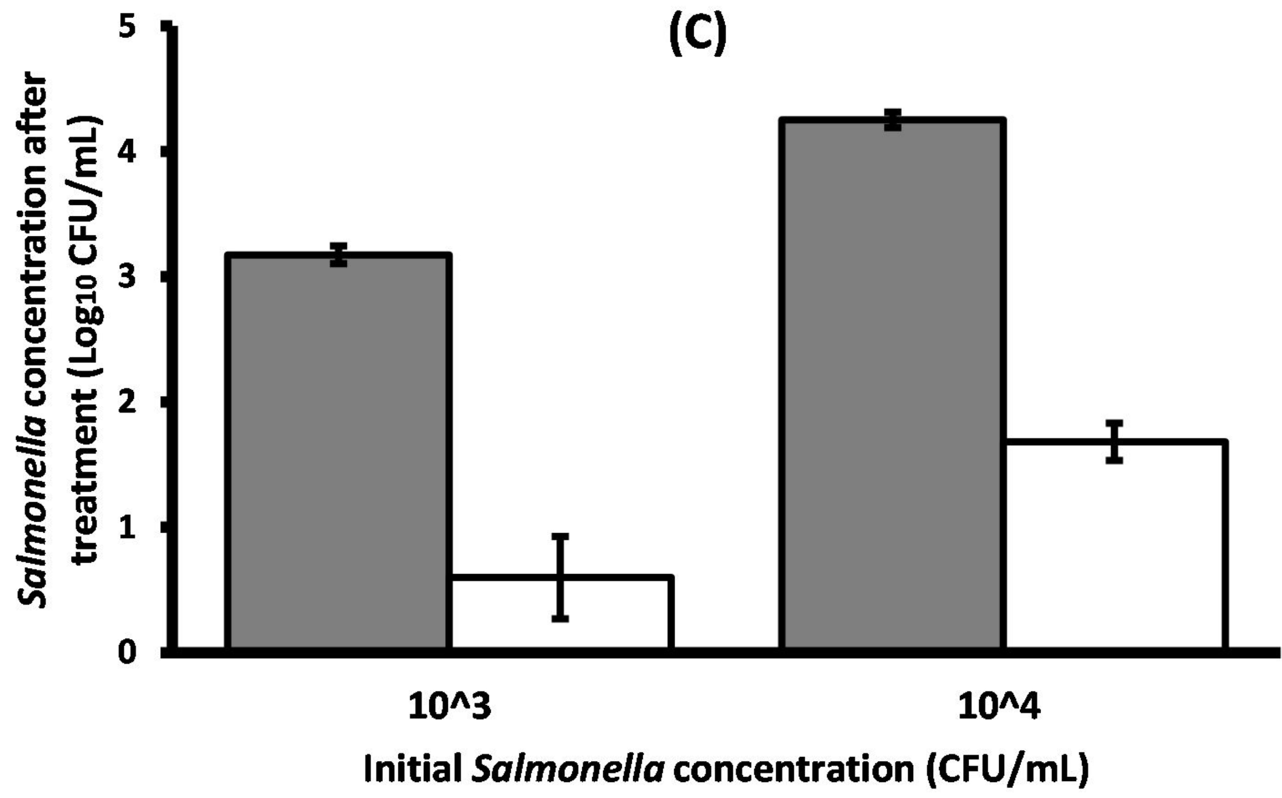
Downloaded from https://journals.asm.org/journal/aem on 02 October 2021 by 188.159.185.175.

Accepted Manuscript Posted Online
Applied and Environmental Microbiology
AEM
Applied and Environmental Microbiology



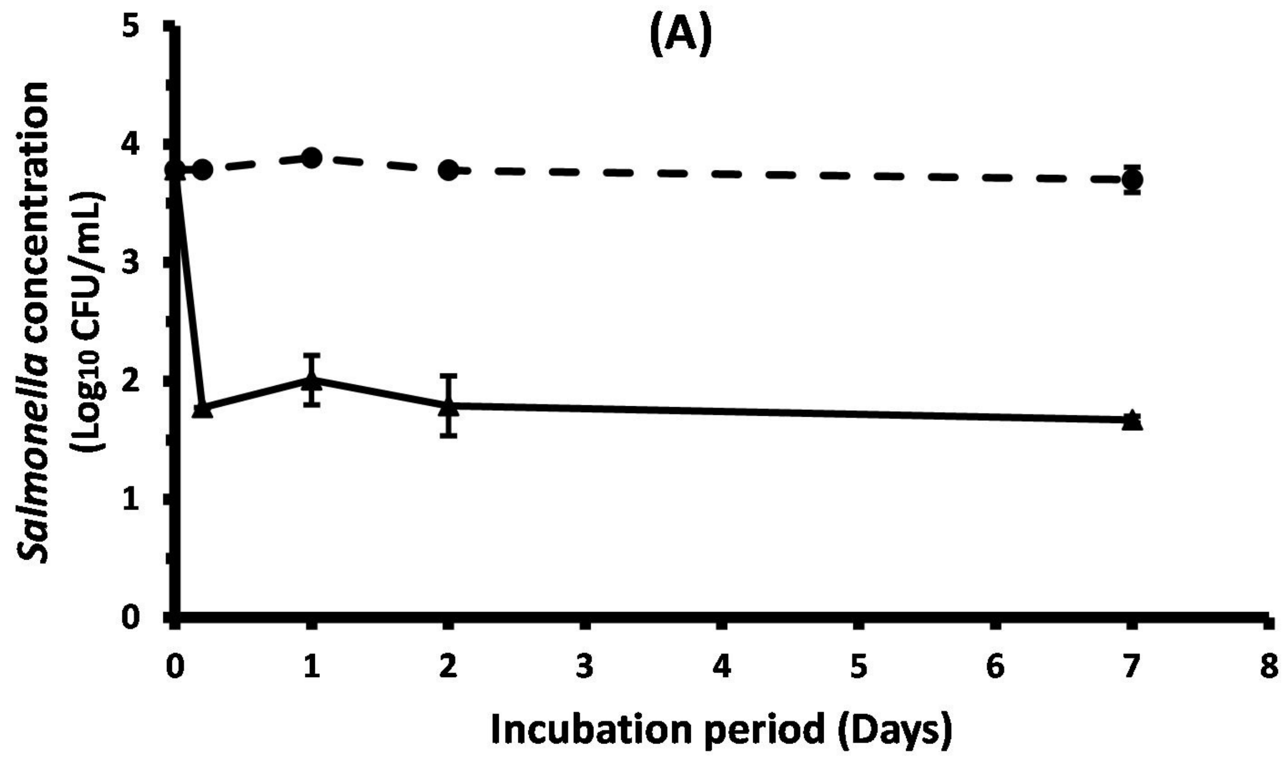
Downloaded from https://journals.asm.org/journal/aem on 02 October 2021 by 188.159.185.175.

Accepted Manuscript Posted Online
Applied and Environmental Microbiology
AEM
Applied and Environmental Microbiology



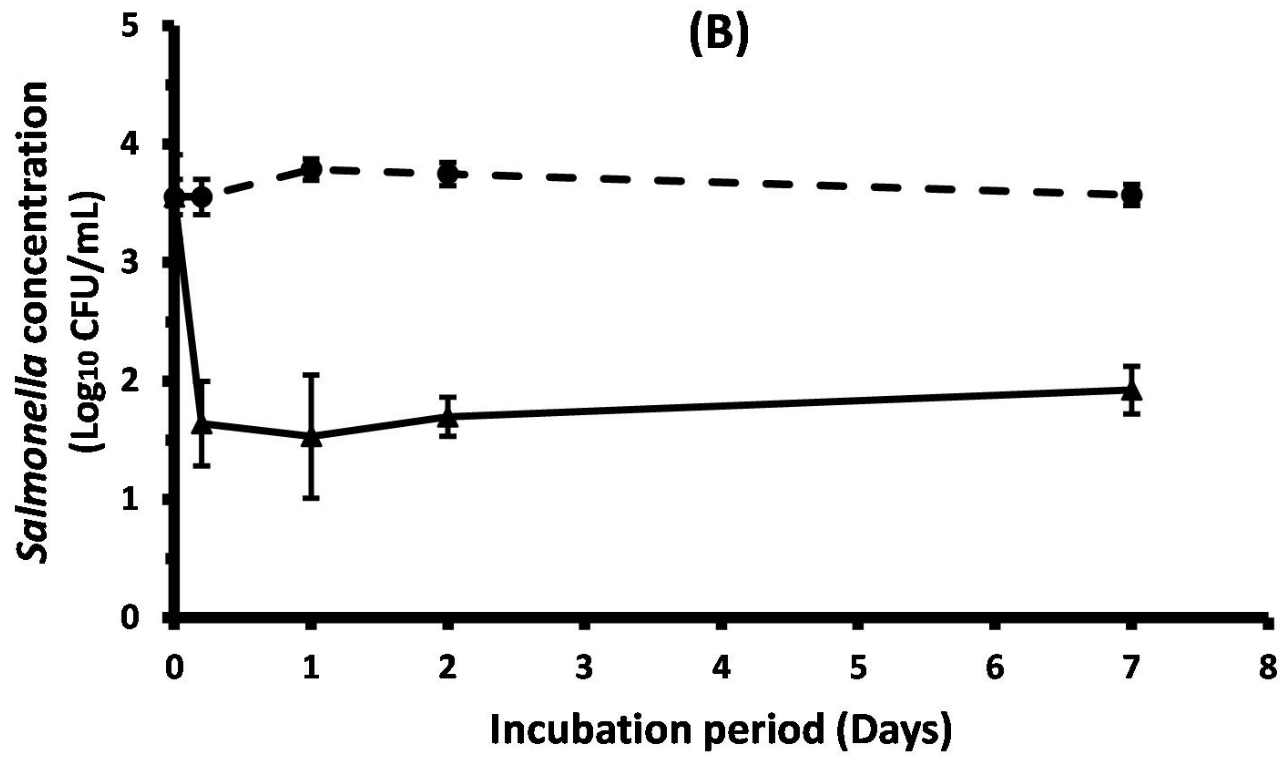
Downloaded from https://journals.asm.org/journal/aem on 02 October 2021 by 188.159.185.175.

Accepted Manuscript Posted Online
Applied and Environmental Microbiology
AEM
Applied and Environmental Microbiology



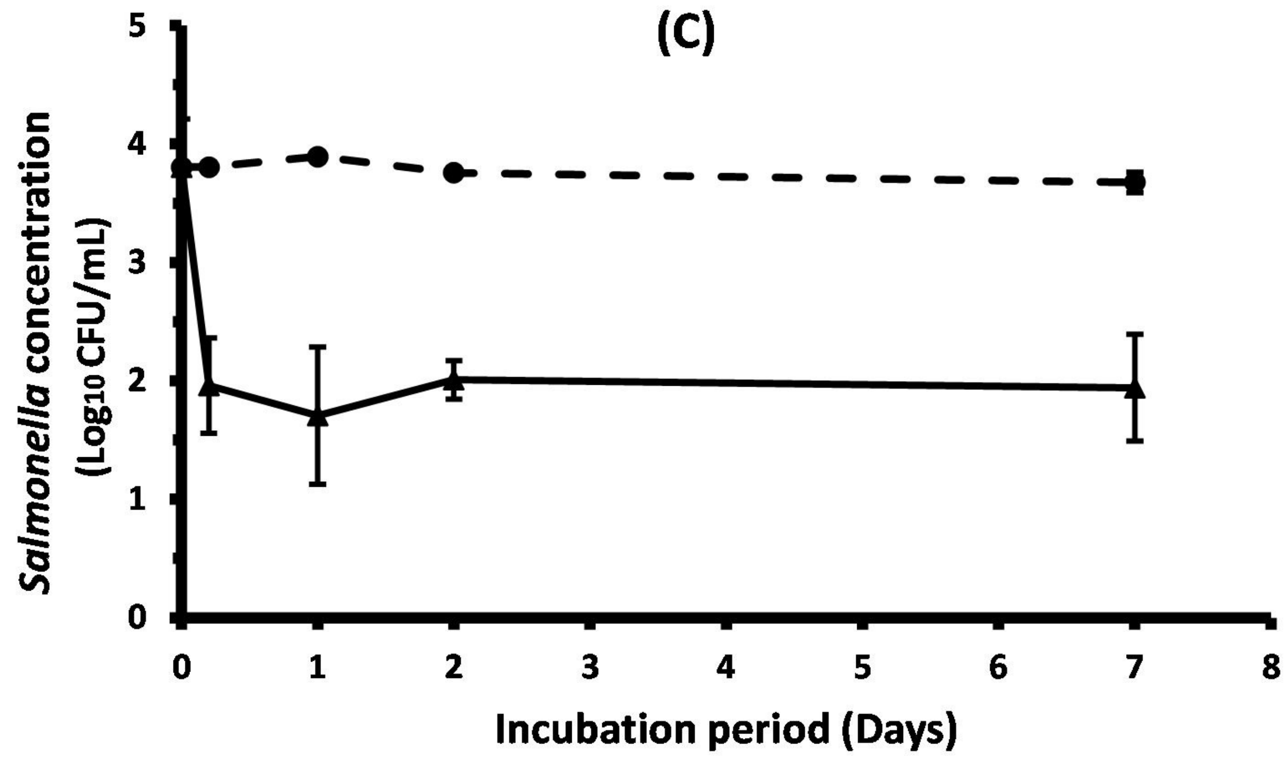
Downloaded from https://journals.asm.org/journal/aem on 02 October 2021 by 188.159.185.175.

Applied and Environmental Microbiology
AEM
Applied and Environmental Microbiology
Accepted Manuscript Posted Online



Downloaded from https://journals.asm.org/journal/aem on 02 October 2021 by 188.159.185.175.

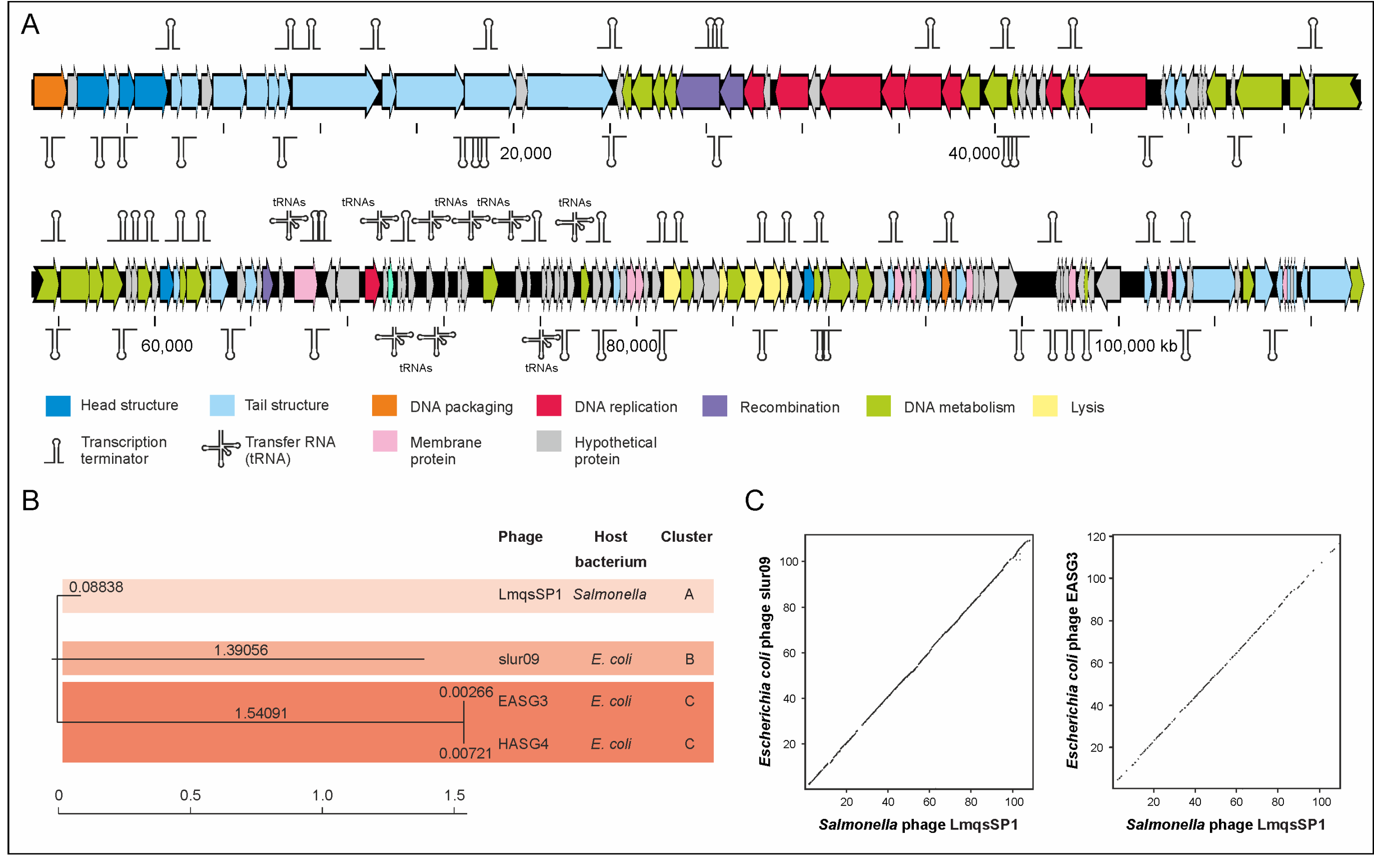
Accepted Manuscript Posted Online
Applied and Environmental Microbiology
AEM
Applied and Environmental Microbiology



Downloaded from https://journals.asm.org/journal/aem on 02 October 2021 by 188.159.185.175.

Accepted Manuscript Posted Online

Applied and Environmental Microbiology



AEM

Applied and Environmental Microbiology

Downloaded from https://journals.asm.org/journal/aem on 02 October 2021 by 188.159.185.175.