Identification and characterization of new broad host-range rV5-like coliphages C203 and P206 are directed against enterobacteria.Domonkos Sváb [1], Linda Falgenhauer [2], Manfred Rohde [3], Trinad Chakraborty [2] and István Tóth [1,*]

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Abstract

We isolated and characterized two novel rV5-like lytic bacteriophages from independently collected food samples. Nucleotide sequence analysis revealed that these phages have linear double-stranded DNA genomes comprising 138,073 bp with 213 CDS and 5 tRNA genes. The two genomes contain completely identical nucleotide sequence, albeit there is a 10,718 bp-long shift in the sequence. The GC content of the phage genomes was 43.7% and they showed high general homology to rV5-like phages. The new phages were termed C203 and P206. The genome of both phages contains a unique ORF that encodes for a putative phage homing endonuclease. The phage produced clear plaques with a burst size of approx. 1000 viral particles and a latent period of 60 minutes. Morphological investigation indicated that the new phages are members of the family Myoviridae with an approximate head length of 85 nm, tail length of 75 nm, and a head width of 96 nm. C203 and P206 exhibit a broad and uniform host range, which included enterohemorrhagic *Escherichia coli* strains of serogroup O157, multi drug resistant (MDR) *E. coli* strains of various sero- and pathotypes, and both *Shigella sonnei* and *S. dysenteriae* strains. C203 and P206 both effectively reduced the number of living EHEC O157:H7 Sakai in experimentally inoculated minced meat. The same broad host range, the lack of any virulence related genes, the stability and its short latent period suggest that these newly found phages could be suitable candidates as a bio-control agents against food-borne pathogenic Enterobacteria.

Keywords: bacteriophage, rV5-like phage, whole phage genome; phylogeny, host specificity; *E. coli* O157, food sample

1. Introduction

With the increasing resistance against antibiotics, there is now renewed interest in bacteriophages (shortly phages) capable of lysing important pathogenic bacteria (reviewed by Hagens and Loessner, 2010). Enterohemorrhagic *Escherichia coli* (EHEC) strains of the O157:H7 serotype are considered to be among the most dangerous foodborne pathogens (reviewed in (Croxen et al., 2013; Gyles, 2007). They can cause serious hemorrhagic colitis (HC), in some cases with the life-threatening complication of hemolytic-uremic syndrome (HUS) and thrombocytopenia (reviewed in (Bielaszewska and Karch, 2005; Croxen et al., 2013). Because of these features, *E. coli* O157:H7 strains have been prime targets in studies aiming to identify bacteriophages capable of eradicating them from its host or from food products. Several groups have isolated and characterized bacteriophages capable of lysis or *in vitro* growth inhibition of *E. coli* O157:H7 type strains. These include both T5- and T4-like phages (Lee et al., 2016; Liu et al., 2015; Raya et al., 2011).

rV5-like phages (V5virus genus) are a recently established genus of tailed bacteriophages belonging to the family Myoviridae with a large genome of over 100 kb long (Kropinski et al., 2013; Santos et al., 2011) and exhibiting a relatively wide host spectrum (Kropinski et al., 2013). Several members of the group are notable for their capacity to lyse *E. coli* O157:H7 strains (Kropinski et al., 2013; Truncaite et al., 2012). In the study which suggested the establishment of the rV5 genus, the authors highlighted the lack of restriction sites, the usage of inner core lipopolysaccharide receptors and the absence of lysogeny-associated genes that contribute to the phages' broad host range and make phage PVP-SE1 good candidate for biocontrol against *Salmonella* (Santos et al., 2011).

In an earlier study, we assessed the risk presented by food-borne pathogens present in foodstuff illegally imported into Europe, with a special emphasis on Shiga-toxin producing

E. coli (STEC; Nagy et al., 2015). We hypothesized that phages capable of lysing these pathogens may also be present in the same foodstuff.

In the current study, we characterized two new rV5-like phages designated C203 and P206 originating from foodstuff. The phages isolated are founding members of a new genotype, and apart from several *E. coli* O157:H7 strains, they are capable of lysing an unusually wide spectrum of pathogenic *E. coli* from other serotypes as well.

2. Materials and Methods

2.1. Bacteriophage isolation

Bacteriophages were isolated from two independent sources, from cottage cheese and from poultry liver confiscated on the Hungarian border, the samples of which were designated C203 and P206, respectively. The samples underwent the first steps of the ISO 16654:2001 method for isolating *E. coli* O157. Briefly, 5 g pieces of the food samples were homogenized at 1:10 weight to volume ratio of tryptic soy broth supplemented with bile salts, and incubated for 24 hours at 42 ^{o}C. After removing the bacteria by centrifugation the samples' supernatants were spread or spotted onto layered agar plates containing *E. coli* K-12 derivative strains C600 and MG1655. *E. coli* K-12 derivative strains C600 and MG1655. After overnight incubation at 37 ^{o}C, single plaques were picked up and purified by amplification on *E. coli* MG1655 at least three times, until high titer (at least 10^{11} PFU/ml) phage stocks were produced.

2.2. Bacterial strains

We used mainly the *E. coli* K-12 derivative strain MG1655 for propagation of the phages. The efficiency of plating (EOP) was tested on various enterobacterial strains listed

86 in Table 1 and 2. A rifampicin-resistant mutant of the EHEC O157:H7 Sakai strain was
87 used in the *in situ* bacterial challenge test (2.9.).
88
89 2.3. Phage DNA isolation
90 Phage DNA was isolated from phage stocks with a concentration of at least 10^{11}
91 PFU/ml. The phenol-chloroform method described by Sambrook et al (Sambrook et al.,
92 1987) was used for DNA isolation, with the modifications outlined as described before
93 (Tóth et al., 2016).
94
95 2.4. Genome sequence determination and analysis
96 Genomic DNA sequencing libraries were prepared using the Nextera XT kit (Illumina,
97 Eindhoven, NL). Sequencing was performed using Nextseq Mid-output reagent kit v2
98 (2×150 bp) on an Illumina NextSeq 500. Assembly was performed with CLC Genomic
99 Workbench 9.0. The genome was annotated using the RAST server (Overbeek et al., 2014).
100 Homology searches were conducted with the BLAST tools available at the NCBI website,
101 with PSI-BLAST results supplementing the annotation.
102 The genome sequences of phage C203 and P206 were deposited in GenBank under the
103 accession nos. MG022439 and MG022440, respectively.
104
105 2.5. Phylogenetic analysis
106 Whole-genome based phylogenetic analysis was conducted with VICTOR (Meier-
107 Kolthoff and Goeker, 2017). A progressive Mauve alignment (Darling et al., 2011)
108 including bacteriophage genomes 206, FFH2, FV3, Murica, slur16 and rV5 (GenBank nos.
109 MG022440, LN881727.1, KJ190158, NC_019517, KT001917, DQ832317) was also
110 conducted.
111

112 2.6. Host specificity and efficiency of plating

113 Host specificity and EOP was tested on a wide array of pathogenic *E. coli, Salmonella*
114 and *Shigella* strains, with an emphasis on *E. coli* O157 strains. Among *E. coli* pathotypes,
115 EHEC, enteropathogenic (EPEC), atypical *E. coli* O157 and multidrug resistant (MDR)
116 strains of human origin were also included. *E. coli* O157:H7 strain C83/00 representing
117 phage type 55 was kindly provided by Ivelina Damjanova (National Institute of Hygiene,
118 Budapest). The serotype, pathotype and other characteristics of these bacterial strains are
119 given in Table 1 and Table 2.

120 Host spectrum and EOP was determined by applying serial dilutions of the phage
121 suspension in spot assays on layered soft agar containing cultures of the test strains,
122 according to the protocol described by Strauch et al., (2001). The ratio of phage titer on the
123 test strain divided by the titer measured on *E. coli* MG1655 was considered the EOP of the
124 phage on the given strain.

125

126 2.7. One step growth experiments

127 One step growth experiment to determine burst size was performed on *E. coli* MG1655
128 according the protocol of Lee et al., (2016) with some modifications. Briefly, $2x10^8$
129 bacteria were mixed with $2x10^6$ phages, setting multiplicity of infection (MOI) to 0.01 in
130 Luria-Bertani broth (LB), and incubated for 20 minutes at room temperature. After
131 incubation the mixture was centrifuged at 6,000 x g for 10 minutes, the pellet was
132 resuspended in 50 ml of fresh LB, and incubated at 37 ◦C for 1 hour. Samples were taken
133 every 5 minutes, and plated on layered soft agar for counting. Three independent
134 experiments were run in two parallels. Burst size was determined as a ratio of the phage
135 count before and after the burst.

136

137 2.8. *In vitro* and *in situ* bacterial challenge tests

In order to assess the activity of the phages against EHEC O157 strains, a bacterial challenge test was performed. *E. coli* O157:H7 Sakai strain was grown overnight in liquid LB culture by shaking with 180 rpm at 37 ^{o}C, then diluted 100-fold and grown again to OD_{600}=1. Culture was diluted 10-fold and aliquoted into 48 separate cultures. Twenty-four of the cultures received P206 in MOI=10 and all cultures were grown again at 37 ^{o}C by shaking with 180 rpm for 12 hours. At 1-hour intervals the OD_{600} of two cultures each of the treated and non-treated sets was measured.

In order to isolate rifampicin resistant mutants of EHEC O157:H7 Sakai strain, two hundred microliters of overnight LB culture of *E. coli* O157:H7 Sakai strain was spread onto rifampicin (150 µg/µl) containing LB agar plate and incubated at 37°C for 16 hours. Single colonies were selected and further purified by several passages on selective plates. One of the resistant colonies was used for further experiments.

In situ biocontrol potential of P206 was investigated in the following way: One gram of minced meat, bought at a local supermarket was homogenized by using 1 ml LB and inoculated with high number ($2.5x10^{10}$) of the rifampicin resistant derivative of *E. coli* O157:H7 Sakai cells. We then applied $1.5x10^{9}$ and $1.5x10^{8}$ phage particles of P206 to the mixture, which corresponded to multiplicity of infection (MOI) 0.6 and 0.06, respectively. Control tubes with bacterial cells alone were also used. The experiment was conducted in triplicate. After two hours incubation at 37 °C samples were homogenized and serial dilutions were plated out on LB plates containing 150 µg/µl of rifampicin in duplicate. Number of surviving bacterial colonies was determined after 16 hours incubation at 37 °C.

2.9. Determination of phage morphology

Bacteriophages were investigated by transmission electron microscopy (TEM). Briefly, drops of high titer bacteriophage suspensions were placed on parafilm, absorbed onto carbon film, washed in TE buffer (10 mM TRIS, 1 mM EDTA, pH 6.9) and negatively-stained with 2% aqueous uranyl acetate, pH 5.0. Carbon film was collected with 300 mesh

copper grids and excess negative-stain was removed with filter paper and subsequently air-dried. Samples were examined in a TEM 910 transmission electron microscope (Carl Zeiss, Oberkochen) at an acceleration voltage of 80 kV. Images were recorded digitally at calibrated magnifications with a Slow-Scan CCD-Camera (ProScan, 1024x1024, Scheuring, Germany) with ITEM-Software (Olympus Soft Imaging Solutions, Münster, Germany). Contrast and brightness were adjusted with Adobe Photoshop CS3.

2.10. Heat stability tests

Heat stability of phages was tested as follows: 1 ml of LB containing 10^9 PFU/ml phage was incubated for 1 hour at 25, 37, 42 and 80 °C, respectively. After incubation, the titer of the treated stocks was determined with a spot assay on *E. coli* MG1655. Experiments were performed in two parallels on each temperature.

2.11. pH tolerance tests

Stability of the phages under different pH values was tested by incubating 10^9 PFU/ml phages for 1 hour at 37 °C in 1ml LB stocks pH-adjusted to 3, 5, 7, 9 and 10 with HCl and NaOH solutions. After incubation, the titer of the treated stocks was determined with spot assay on *E. coli* MG1655. Experiments were performed in two parallels on each pH value.

3. Results

3.1 Morphology

We isolated two bacteriophages from two independent food sources by using *E. coli* K12 derivatives as propagating strains. Both phages showed Myoviridae morphology, with head length around 85 nm and width around 75 nm. The characteristic contractile tail was around 96 nm long (Figure 1).

3.2 Genome characteristics

We found that from the two independent samples, We designated the new bacteriophages as C203 and P206 (with C and P referring to the cheese and poultry sources). The genome sequences of the phages, as determined on the Illumina platform, are 138,073 bp in length, and contain 213 ORFs and 5 tRNA genes. The GC content of the genomes is 43.7%. Average read length was 183 for phage C203 and 177 for P206, the average coverage was 1173.97 and 1453.33, respectively. Homology searches showed that genome sequence of the phages are identical, but the sequence start and endpoints are different. This causeesa 10,718 bp-long shift between the two phages, therefore ORFs 1-7 in the C203 genome correspond to ORFs 207-213 in the P206 genome. Therefore ORF1 in P206 is a truncated version of ORF8 in C203, the rest of the gene was transferred to the end of the genome in P206 as non-coding region. Similarly, ORF1 in C203 is a truncated version of ORF207 in P206. The RAST and PSI-BLAST homology searches enabled to assign a function to 36% (78/213) of the ORFs, with the rest encoding proteins with unknown functions. The complete list of ORFs for both phages is given in Supplementary Tables 1 and 2.

The overall genome structure of C203 and P206 regarding the order of genes is similar to the rV5-like bacteriophage genomes sequenced earlier, with sequence shifts as indicated by the MAUVE alignment (Figure 2). On the nucleotide level according to whole-genome BLAST search it showed the highest similarity to *Escherichia* phage vB_EcoM_FFH2 (GenBank KJ190158.1) with 97% coverage and 97% average sequence identity, albeit some genes from C203/P206 rather showed significant homology to those carried by other rV5-like phages, these are indicated in Supplementary Tables 1 and 2. A large region comprising of tail fiber protein CDSs AHN83624.1 and AHN83629.1 in vB_EcoM_FFH2, is absent in C203/P206. Individual sequence homology searches showed that ORF12 and 5

of C203 and P206 respectively, are highly homologous to the tail fiber genes carried by the *Salmonella* specific phages slur12 and slur16, and the phage EcoM_FV3 (GenBank nos. LN881735.1, LN881727.1, and NC_019517). Additionally, ORF16/9 in C203/P206 is homologous to the corresponding gene of the original rv5 phage (CDS ABI79112.1) and those of three O157-typing phages (TP4, TP 5 and TP14). Further differences between C203/P206 and vB_EcoM_FFH2 are the absence of genes encoding a putative NTP reductase small subunit (AHN83629.1) together with two putative HNH endonucleases. One of the endonuclease genes (AHN83797.1 in phage EcoM_FFH2) disrupts the phage terminase large subunit, which is intact in C203/P206 (ORF39/32), like in the case of the Murica phage (KT001917). The other endonuclease in EcoM_FFH2 (AHN83797.1) is situated between a DNA polymerase and a DNA primase (corresponding ORFs 181-182 in C203 and 174-175 in P206) similarly to the original rV5-like phage (GenBank DQ832317.1) as well as to EcoM_FV3. Table 3 summarizes the relations of ORFs annotated as tail fiber genes, comparing them on the amino acid level to other rV5-like phages.

3.3 Phylogenetic relations

Phylogenetic trees of rV5-like phages including bacteriophage C203 and P206 were constructed based with the VICTOR whole genome phylogenetic analysis It showed that throughout the rV-like phages which were included, whole genome sequences had a pairwise identity of at least 90%. The resulting tree suggests the slur12 and slur16 phages as closest relatives to the new phages (Figure 3.).

3.4 Host spectrum, efficiency of plating, burst size and stability

Phages C203 and P206 propagated on a wide array of commensal and pathogenic *E. coli* strains, most notably on the prototype strains of EHEC O157:H7 Sakai and EDL933. The lysis pattern of C203 and P206 was completely identical.Additionally to *E. coli* strains, phages C203 and P206 also propagated on four out of six *Shigella* strains representing three *Shigella* species, while at the same time none of six *Salmonella* strains belonging to different serovars was lysed by these phages. The full host spectrum of phages C203 and P206 together with categorized EOP values is listed in Table 1. In the one step growth experiments the burst size proved to be around 1000 with a latent period of approximately 60 minutes (Figure 4). The lysis pattern of C203/P206 on various O157:H7 strains paired with phage types is shown separately in Table 2. Note that the plaque morphology of the phages was cloudy on the O157 strains, hence only an approximate minimum EOP value is given, as only the endpoint could be determined without exactly countable plaque numbers. Differences between the six CDSs identified as tail fibers (Supplementary Tables 1 and 2) could account for the different lysis pattern between C203/P206 and the O157 typing phages. All these CDSs had at least one amino acid switch when compared to the corresponding CDSs of the typing phages, and in one case, ORF12/6 in C203/P206 shows only 78% identity to the corresponding CDS in typing phage 14 on the amino acid level.

A stock of P206 propagated on the *E. coli* O157:H7 Sakai strain was re-plated on all bacterial strains which proved to be sensitive to the phage earlier. All of them were still sensitive with roughly the same EOP values, but in the case of the EIEC O152 strain Bra2

261 26, a 10^4-fold EOP increase could be observed, while in the case of bovine EHEC O157:H7
262 strain 318 a 10^3-fold EOP decrease occurred.

263 Heat stability experiments showed that incubation at 25, 37 and 42 °C did not affect the
264 EOP of the phages. Incubation at 80 °C, however, caused 10^4-fold average decrease in the
265 phage titer. Throughout the experiments in general, the phage proved to be stable after
266 weeks of incubation at 4 °C.

267 At pH value of 3 the phages were completely inactivated, as no plaques were observable
268 even in the spots of the concentrated suspension. On the other hand, no significant decrease
269 in titer was observed at pH values 5, 7, 9 and 10.

270

271 3.5 In vitro and in situ bacterial challenges

272 In a MOI of 10, phage P206 could effectively inhibit the growth of *E. coli* O157:H7
273 Sakai in LB for 5 hours, and the treated samples showed lagged growth even 12 hours
274 following phage addition (Figure 5).

275 Since O157 contaminated meat is the leading source of human enterohemorrhagic
276 infections, we aimed to explore the elimination potential of phage C203/P206 in minced
277 meat experimentally contaminated with a rifampicin resistant derivative of *E. coli* O157:H7
278 Sakai strain. The *in situ* bacterial challenge experiments showed that phage P206
279 effectively reduced the extremely high number (2.5×10^{10}) of EHEC O157:H7 cells in the
280 contaminated meat samples. By using phages in MOI 0.6 and 0.06, after 2 hours of
281 infection, the number of surviving EHEC cells was reduced by 10^4 and 10^2 times,

respectively. The unincoulated meat did not contain rifampicin resistant bacteria (data not shown).

4. Discussion

In the current study we isolated and characterized two rV5-like bacteriophages designated C203 and P206 from independent food samples, carrying identical genome content. Interestingly the phage genomes have a 10,718 bp-long shift in their sequences. These phages are capable of lysing a broad spectrum of pathogenic *E. coli* strains, including most notably the reference EHEC O157:H7 strains (Hayashi et al., 2001; Perna et al., 2001). It is worth mentioning that while the phages lysed numerous *E. coli* O157 strains, strains of this serogroup were not detected in the original food samples from which it was isolated (Nagy et al., 2015). C203 and P206 effectively lysed a rifampicin resistant derivative of EHEC O157 Sakai strain *in situ.*

In the recent years, rV5-like phages have been established as a 'singleton' genus within the Myoviridae family, showing similar genomic organization and phenotypic characteristics to each other, but exhibiting significant differences to other coliphages (Smith et al., 2015; Truncaite et al., 2012). Based on their morphology, all of them are members of the Myoviridae family, and phage C203 and P206 are no exception from this notion.

The genomes of C203 and P206 have identical gene content except for truncated genes at the genome ends. Their closest homologue based on BLAST searches is phage vB_EcoM_FFH2, but there are striking differences in the tail fiber genes, and genes

303 encoding DNA modifying enzymes present in vB_EcoM_FFH2, are absent from
304 C203/P206. These genes, as shown by BLAST searches, are rather similar to those found in
305 the *Salmonella* phages slur12 and slur16 (Smith et al., 2015), the phage EcoM_FV3
306 (Truncaite et al., 2012) and Murica (Wilder et al., 2015). It has to be noted that the Mauve
307 alignment of rV5-like genomes indicated that the order of genes is generally uniform in rV5
308 phages, with sequence shifts responsible for different organizations (Figure 2).
309 It is also noteworthy that the genetic relationship suggested by BLAST, with
310 vB_EcoM_FFH2 showing closest general homology on the nucleotide level to C203/P206,
311 was not reflected by the whole-genome based phylogenetic analysis, which rather depicted
312 a closer relationship of C203/P206to slur16 and slur12 (Figure 3).
313 The host specificity of rV5-like phages is wide, but C203/P206 showed the broadest lysis
314 spectrum of those reported. EOP values of strains other than the K-12 derivative strain
315 MG1655 were generally orders of magnitude lower (Table 1). Strains lysed by C203/P206
316 include several major pathotypes of intestinal pathogenic *E. coli* besides EHEC O157:H7,
317 such as enteroinvasive (EIEC), avian pathogenic (APEC) as well as strains with atypical or
318 unknown pathotype, with MDR strains among the latter.
319 The rV5-like phages also include three of the typing phages used in the phage typing of *E.*
320 *coli* O157:H7 strains (Ahmed et al., 1987; Cowley et al., 2015) namely TP 4, 5 and 14,
321 whose whole genomes are also available (Cowley et al., 2015). For comparative reasons,
322 several *E. coli* O157:H7 strains (Tóth et al., 2009) of various phage types (PT) were also
323 included in testing the host spectrum of C203/P206, and we found that their lysis pattern

was different from those of the typing phages, viz., it produced lysis on two out of three PT21 strains and one PT50d strain (Table 2). Differences in the protein sequence of C203/P206 tail fibers and those of TPs could be responsible for the discrepancies. These differences are summarized in Table 3, with the ORFs of phage C203 as reference. It is noteworthy that ORF5 is identical to the corresponding ORFs of slur12 and slur16 phages, ORF7 to that carried by ApCEC02, and ORF8 to those in ApCEC02 and 2 JES-2013. On the other hand, the longer tail fiber ORFs, 12 and 16 show significant variability and in many cases only partial coverage. These data suggest that the host spectrum of rV5-like phages could be variable, but currently our study is the most comprehensive in this regard, as we aimed ot include most of the significant pathotypes and species of Enterobacterial pathogens.

The fact that C203 and P206, besides *E. coli*, were able to lyse *Shigella dysenteriae* and *S. sonnei* strains deserves attention. To our knowledge, no rV5-like bacteriophage has been reported to infect *Shigella* strains, which, especially *S. dysenteriae* even today remain the leading agents of bacillary dysentery (Anderson et al., 2016). As MDR *Shigella* strains emerge frequently (Doyle, 2015), any potential alternative antibacterial agent that could be used against them is an important finding. Noteworthy is the finding that bacteriophage P206 effectively reduced the number of living EHEC O157:H7 Sakai in experimentally inoculated minced meat. Throughout the experiments, we did not observe the appearance of potentially lysogenic colonies. The annotation did not show the presence of any integrase or recombinase genes, suggesting that phages C203 and P206 are incapable of lysogeny.

345 Schwarzer et al., (2012) characterized an rV5-like phage with a similarly broad host range,
346 capable of infecting several *Salmonella* serovars besides *E. coli* K-12 derivatives. They
347 suggested that multiple tail fiber genes could be responsible for the wide host spectrum of
348 phi92 (Schwarzer et al., 2012), which could also be the case with C203/P206, as the
349 genome harbors several ORFs annotated as tail fibers. It has to be noted however, that there
350 is very low nucleotide-level homology between phi92 and C203/P206. It is also worth
351 mentioning that the truncation of ORF1 annotated as "tail fibers" in P206 (ORF5 in C203)
352 seems to have no effect on the host specificity of the phage when compared to C203.
353 Lee et al (2016) have proposed that genes associated with host specificity should be cloned
354 into one bacteriophage genome, which then could be used as a universal agent against
355 several related pathogenic bacteria. Broad host spectrum bacteriophages such as C203/P206
356 could be a potential source of such host specificity associated genes, or alternatively, it
357 could be used in its native form as biocontrol agent either as a stand-alone or in phage
358 cocktails.
359 In conclusion, we isolated and characterized two new members of rV5-like bacteriophages,
360 which showed an unusually broad host spectrum spanning more pathotypes of pathogenic
361 *E. coli, Salmonella* and *Shigella* than any other similar phage isolated before. To our
362 knowledge, this is also the first rV5-like phage to be isolated from foodstuff. These
363 characteristics, together with its stability, strictly lytic lifestyle and the fact that they do not
364 carry any pathogenicity-associated genes, makes C203 and P206 suitable candidates to be

tested as a biocontrol agent against EHEC O157 strains and other foodborne pathogenic enterobacteria.

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Table 1. Host specificity and efficiency of plating of bacteriophages C203 and P206. EOP values are given relative to the titer of the phages on *E. coli* MG1655. Zero (0) values mean that no lysis was observable on the given strain.

Strain	Pathotype / serovar /species	Serogroup or serotype	EOP	Strain reference
MG1655	*E. coli* K-12	O16:H48	1	(Blattner et al., 1997)
Sakai	EHEC	O157:H7	$7x10^{-1}$	(Hayashi et al., 2001)
EDL933	EHEC	O157:H7	$2x10^{-5}$	(Perna et al., 2001)
E22	EHEC	O103:H2	0	(Marchès et al., 2003)
E2348/69	EPEC	O127:H6	$1.3x10^{-2}$	(Iguchi et al., 2009)
II95-36	EIEC	O121	$2x10^{-8}$	(Sváb et al., 2018)
20	EIEC	O124	$4x10^{-1}$	(Sváb et al., 2018)
Bra2 26	EIEC	O152	$2x10^{-7}$	(Sváb et al., 2018)
Saigon	EIEC	O164	$8x10^{-8}$	(Sváb et al., 2018)
T22	atypical	O157:H43	$2x10^{-6}$	(Tóth et al., 2009)
536	UPEC	O6:K15:H31	0	(Hochhut et al., 2006)
IHE3034	ExPEC	O18:K1:H7	0	(Moriel et al., 2010)
E250	APEC	O1:K1:H7	$2x10^{-6}$	(Tóth et al., 2003)
5871	MDR *E. coli*	O15	$2x10^{-6}$	(Sváb et al., 2018)
18531	MDR *E. coli*	O73	0	(Sváb et al., 2018)
29095	MDR *E. coli*	O90	$2x10^{-9}$	(Sváb et al., 2018)
ILC169	*Citrobacter rodentium*	N/A	0	(Sváb et al., 2018)
20080	*Shigella dysenteriae* 1A	N/A	10^{-1}	(Sváb et al., 2018)
M90T	*Shigella flexneri*	N/A	10^{-2}	(Sváb et al., 2018)
20038	*Shigella boydii*	N/A	0	(Sváb et al., 2018)
866-F	*Shigella sonnei*	N/A	$4x10^{-7}$	(Allué-Guardia et al., 2011)
20045	*Shigella sonnei*	N/A	0	(Sváb et al., 2018)
75/02	*Shigella sonnei*	N/A	$3.3x10^{-8}$	(Sváb et al., 2017)
1201	*Salmonella* Typhimurium	N/A	0	(Sváb et al., 2018)

	1			
1202	*Salmonella* Infantis	N/A	0	(Sváb et al., 2018)
1203	*Salmonella* Panama	N/A	0	(Sváb et al., 2018)
1199	*Salmonella* Typhi	N/A	0	(Sváb et al., 2018)
1198	*Salmonella* Gallinarum	N/A	0	(Sváb et al., 2018)
1200	*Salmonella* Enteritidis	N/A	0	(Sváb et al., 2018)

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525 **Table 2.** Lysis pattern and efficiency of plating of bacteriophage C203 and P206 on *E. coli*
526 strains of the O157 serogroup, with *E. coli* MG1655 as reference efficiency. As in the case
527 of Table 1, the lysis pattern of C203 and P206 was completely identical. Exclusive lysis by
528 C203/P206 is marked with gray. Note that the plaque morphology of the phages was cloudy
529 on the O157 strains, hence only an approximate minimum EOP value is given, as only the
530 endpoint could be determined without exactly countable plaque numbers.

Strain	Pathotpye	Serotype	Phage type[1]	EOP[2]	Lysed by typing phage[4]			Reference
					TP4	TP5	TP14	
MG1655	-	-	-	1				(Blattner et al., 1997)
Sakai	EHEC	O157:H7	PT14	$>10^{-4}$	+	+	+	(Hayashi et al., 2001)
EDL933	EHEC	O157:H7	PT21	$>10^{-4}$	-	-	-	(Perna et al., 2001)
34	EHEC	O157:H7	PT21	0	-	-	-	(Tóth et al., 2009)
52	EHEC	O157:H7	PT33	0	-	-	-	(Tóth et al., 2009)
318	EHEC	O157:NM	PT8	$>10^{-5}$	+	+	+	(Tóth et al., 2009)
65	EPEC	O157:H7	PT33	0	-	-	-	(Tóth et al., 2009)
68	EPEC	O157:H7	PT8	$>10^{-3}$	+	+	+	(Tóth et al., 2009)
103	EPEC	O157:H7	PT50d	$>10^{-5}$	-	-	-	(Tóth et al., 2009)
144	EPEC	O157:H7	NT-R	0	-	-	-	(Tóth et al., 2009)
B20	AT[3]	O157:H12	NT-R	$>10^{-5}$	-	-	-	(Tóth et al., 2009)
B47	AT[3]	O157:NM	NT-R	0	-	-	-	(Tóth et al., 2009)
T22	AT[3]	O157:H43	NC	$>10^{-5}$	no data	no data	no data	(Tóth et al., 2009)
T34	AT[3]	O157:H9	PT21	$>10^{-5}$	-	-	-	(Tóth et al., 2009)
T49	AT[3]	O157:H37	NC	$>10^{-5}$	no data	no data	no data	(Tóth et al., 2009)
C83/00	EHEC	O157:H7	PT55	$>10^{-4}$	+	+	+	(Sváb et al., 2018)

531 [1] NT: non-typeable, R: phage resistant, NC: non-characteristic phage type;

532 [2]efficiency of plating;

533 [3]atypical pathotype (*stx* and *eae* negative);

[4] + = lysis; - = no lysis

Supplementary Table 1. List of ORFs and their assigned functions and homologies in bacteriophage C203.

Supplementary Table 2. List of ORFs and their assigned functions and homologies in bacteriophage P206.

Figure legends

Figure 1. Transmission electronmicrograph of negatively-stained (2% uranyl acetate) bacteriophages C203 (A and B) and P206 (C and D). Both phages exhibit the typical Myoviridae morphology of non-bended and contractile tails. Bars represent 100 nm.

Figure 2. Progressive Mauve alignment of rV5-like bacteriophage genomes C203, P206, slur16, FFH2, FV3, Murica and rV5 (GenBank nos. MG022439, MG022440, LN881727.1, KJ190158, NC_019517, KT001917, DQ832317)

Figure 3. Whole-genome based phylogenetic relations of rV5-like bacteriophages made with VICTOR. The bacteriophages and their accession numbers are: Murica, KT001917.1; V18, KY683736.1; APCEc02, KR698074.1; O157 typing phage 14, KP869112.1; slur12, LN881735.1; slur16, LN881727.1; O157 typing phage 5, KP869103.1; rV5, DQ832317.1; O157 typing phage 4, KP869102.1; 2 JES-2013, KC690136.1; vB_EcoM_FFH2, KJ190158.1; vB_EcoM-FV3, JQ031132.1.

Figure 4. One step growth curve of phage P206 on *E. coli* K-12 MG1655.

Figure 5. Bacterial challenge assay of bacteriophage P206 against *E. coli* O157:H7 Sakai strain. A dilution of overnight bacterial culture was infected with phage P206 at a MOI of 10. Non-phage treated bacterial culture was used as a control. The graphs show OD at 600 nm for the cultures every hour. The error bars indicate standard deviations from the results of the experiment.

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