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Research paper

Identification and characterization of new broad host-range rV5-like coliphages C203 and P206 directed against enterobacteria

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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 min. 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.

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

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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 h at 42 °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. After overnight incubation at 37 °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

E. coli K-12 derivative strain MG1655 was mostly used for propagation of the phages. The efficiency of plating (EOP) was tested on various enterobacterial strains listed in Tables 1 and 2. A rifampicin-resistant mutant of the EHEC O157:H7 Sakai strain was used in the *in situ* bacterial challenge test (2.8).

2.3. Phage DNA isolation

Phage DNA was isolated from phage stocks with a concentration of at least 10^{11} PFU/ml. The phenol-chloroform method described by Sambrook et al. (1987) was used for DNA isolation, with the modifications outlined as described before (Tóth et al., 2016).

2.4. Genome sequence determination and analysis

Genomic DNA sequencing libraries were prepared using the Nextera XT kit (Illumina, Eindhoven, NL). Sequencing was performed using Nextseq Mid-output reagent kit v2 (2 × 150 bp) on an Illumina NextSeq 500. Assembly was performed with CLC Genomic Workbench 9.0. The genome was annotated using the RAST server (Overbeek et al., 2014). Homology searches were conducted with the BLAST tools available at the NCBI website, with PSI-BLAST results supplementing the annotation.

The genome sequences of phage C203 and P206 were deposited in GenBank under the accession nos. MG022439 and MG022440, respectively.

2.5. Phylogenetic analysis

Whole-genome based phylogenetic analysis was conducted with VICTOR (Meier-Kolthoff and Goeker, 2017). A progressive Mauve alignment (Darling et al., 2011) including bacteriophage genomes C203, P206, FFH2, FV3, Murica, slur16 and rV5 (GenBank nos. MG022440, LN881727.1, KJ190158, NC_019517, KT001917, DQ832317) was also conducted.

2.6. Host specificity and efficiency of plating

Host specificity and EOP was tested on a wide array of pathogenic *E. coli*, *Salmonella* and *Shigella* strains, with an emphasis on *E. coli* O157 strains. Among *E. coli* pathotypes, EHEC, enteropathogenic (EPEC), atypical *E. coli* O157 and multidrug resistant (MDR) strains of human origin were also included. *E. coli* O157:H7 strain C83/00 representing phage type 55 was kindly provided by Ivelina Damjanova (National Institute of Hygiene, Budapest). The serotype, pathotype and other

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	7×10^{-1}	(Hayashi et al., 2001)
EDL933	EHEC	O157:H7	2×10^{-5}	(Perna et al., 2001)
E22	EHEC	O103:H2	0	(Marchès et al., 2003)
E2348/69	EPEC	O127:H6	1.3×10^{-2}	(Iguchi et al., 2009)
II95–36	EIEC	O121	2×10^{-8}	(Sváb et al., 2018)
20	EIEC	O124	4×10^{-1}	(Sváb et al., 2018)
Bra2 26	EIEC	O152	2×10^{-7}	(Sváb et al., 2018)
Saigon	EIEC	O164	8×10^{-8}	(Sváb et al., 2018)
T22	atypical	O157:H43	2×10^{-6}	(Tóth et al., 2009b)
536	UPEC	O6:K15:H31	0	(Hochhut et al., 2006)
IHE3034	ExPEC	O18:K1:H7	0	(Moriel et al., 2010)
E250	APEC	O115	2×10^{-6}	(Tóth et al., 2009a)
5871	MDR *E. coli*	O15	2×10^{-6}	(Sváb et al., 2018)
18531	MDR *E. coli*	O73	0	(Sváb et al., 2018)
29095	MDR *E. coli*	O90	2×10^{-9}	(Sváb et al., 2018)
ILC169	*Citrobacter rodentium*	N/A	0	(Petty et al., 2011)
20080	*Shigella dysenteriae* 1A	N/A	10^{-1}	(Sváb et al., 2018)
M90 T	*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	4×10^{-7}	(Allué-Guardia et al., 2011)
20045	*Shigella sonnei*	N/A	0	(Sváb et al., 2018)
75/02	*Shigella sonnei*	N/A	3.3×10^{-8}	(Sváb et al., 2017)
1201	*Salmonella* Typhimurium 1	N/A	0	(Sváb et al., 2018)
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)