

Implications of interaction between human pathogen *Campylobacter jejuni* and its bacteriophages



Nika Janež¹, Andreja Kokošin¹, Eva Zaletel¹, Tomaž Accetto², Aleš Podgornik¹, Matjaž Peterka¹

¹ The Centre of Excellence for Biosensors, Instrumentation and Process Control - COBIK,
Velika pot 22, SI-5250 Solkan, Slovenia

² University of Ljubljana, Biotechnical Faculty, Animal sciences department, Slovenia

Introduction

Campylobacter jejuni is most commonly reported cause of zoonosis in EU. Poultry is considered to be the main source of infection, because *C. jejuni* colonizes their intestine to high level. Bacteriophages have been proven to reduce number of *C. jejuni* in broilers before slaughter for 1 - 4 log₁₀. However, for successful implementation of bacteriophages as antimicrobial agent, a deeper understanding of *Campylobacter* bacteriophage biology and ecology is needed.

A set of *Campylobacter* specific bacteriophages has been isolated from poultry and pig samples. They all show similar morphology and belong to *Myoviridae* family. They could be distinguished by their genome size, DNA restriction profiles and their ability to infect *C. jejuni* strains of human, poultry, pig and environmental origin. Molecular characterisation of interaction between *Campylobacter* bacteriophages and their hosts has shown that capsule is required to initiate infection of host bacteria. Active flagella are also important in infection process. *In vivo* trials have shown that resistance arises also due to rearrangement of prophage sequences in genomic DNA of *C. jejuni* (Scott et al., 2007). *C. jejuni* strains have been found to carry prophage related sequences in their genomes; however, prophages could not be isolated from these strains by mitomycin C induction.

Bacteriophage Isolation and Characterisation

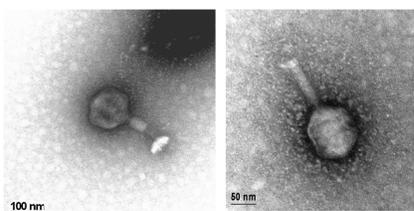
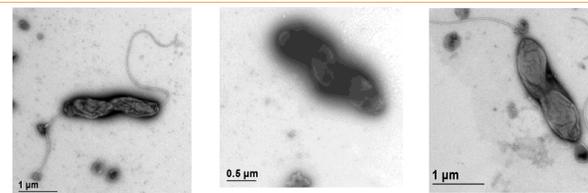


Figure 1. Nine *Campylobacter* phages were isolated and all of them show pin-point plaque morphology and belong to *Myoviridae* family.

Campylobacter jejuni strains were tested against isolated strains and screened for presence of prophage like sequences.

strain	source	prophage related sequence (CJIE)	susceptibility to isolated bacteriophages
NCTC 11168	human	-	+
LBA82	poultry	+	+
LBA67	poultry	-	+
LBA80	poultry	-	+
LBA66	poultry	-	+
LBA74	poultry	+	-
LBA46	poultry	-	-
LBA97	human	-	+
LBA51	meat	-	-
LBA106	human	-	+
BLA100	human	+	+
LBA75	poultry	+	+
LBA47	meat	-	+
LBA92	human	+	+
LBA95	human	-	+
LBA65	poultry	-	+
LBA24	human	+	-
LBA104	human	-	+
LBA105	human	-	+
LBA96	human	-	+
LBA109	human	-	+
LBA22	human	-	+
LBA45	meat	+	-
LBA103	human	-	+
LBA98	human	+	+
LBA101	human	-	+
LBA7	poultry	+	-
LBA9	poultry	+	-
LBA93	human	-	+
LBA94	human	+	+
LBA99	human	+	+
LBA107	human	-	-
BLA108	human	-	+
LBA10	poultry	-	-
LBA61	meat	+	-
LBA102	human	+	-
LBA11	poultry	+	-
LBA12	poultry	-	-
LBA62	meat	+	-
LBA54	meat	-	+
LBA55	meat	-	-
LBA40	meat	+	-
LBA60	meat	-	-
LBA21	human	-	-
LBA83	poultry skin	+	+

Interaction with Host *C. jejuni*



bacteriophage	wild type strain	flhA::kan mutant strain	kpsM::cat mutant strain
PC5	+	+	-
PC14	+	+	-
PC17	+	+	-
PC35	+	+	-
PK80	+	+	-
PK83	+	+	-
PM1	+	+	-
PM5	+	+	-
ATCC 335922	+	+	-

Figure 2. For molecular characterisation of interaction between bacteriophages and bacteria we used two *C. jejuni* indicator strains LBA 65 and NCTC 11168.

A gene involved in the transport of the capsule through the membrane (*kpsM*) and a gene encoding flagellin transport protein (*flhA*) were inactivated in *C. jejuni* LBA65 by marker rescue technique. Phenotypes of defined mutant strains were compared to wild type strains with regard to their ability to resist infection by isolated phages.

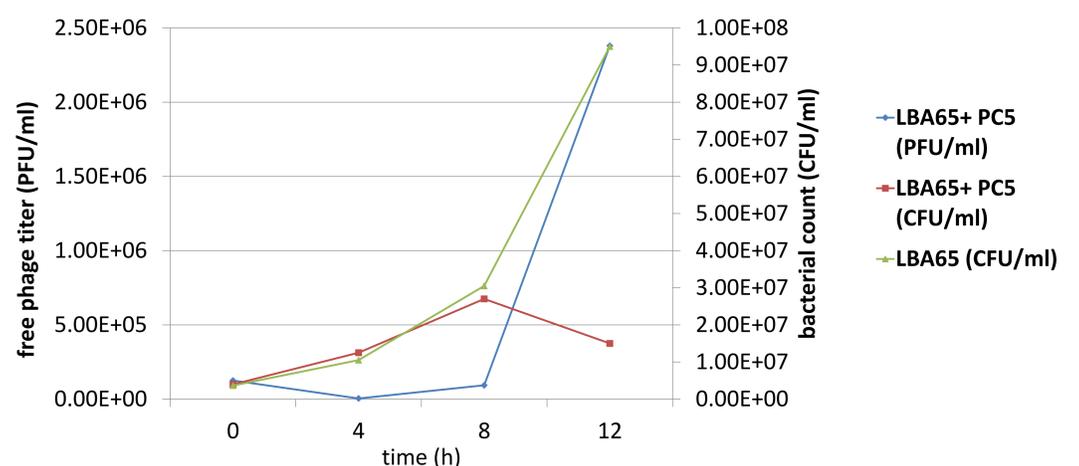


Figure 3. *Campylobacter jejuni* bacteriophage PC5 replication on host LBA 65. *C. jejuni* LBA65 was grown to exponential phase and was diluted in fresh MH medium and infected with PC5 at MOI=0,01. Cell number was determined on Abeyta-Hunt- Bark (AHB) agar and number of released phages was determined using plaque assay.

Conclusions

- Isolated bacteriophages belong to *Myoviridae* family and differ in their ability to lyse a set of human and poultry *C. jejuni* isolates
- Interaction between tested bacteriophages and *C. jejuni* indicator strains is associated with capsule and to less extent to motility
- Prophage associated sequences can be found in *C. jejuni* of poultry and human origin, but active prophages could not be induced by antibiotic mitomycin C

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