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EFFECTIVE BACTERIOLYSIS OF SHIGA TOXIN-PRODUCING *ESCHERICHIA COLI* O157: H7 CAUSED BY SPECIFIC BACTERIOPHAGE ISOLATED FROM PIG SLURRY

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Abstract. This study was aimed to in vitro evaluation of a bacteriophage isolated from pig slurry samples as a bacteriolytic agent to elimination Shiga toxin-producing *E. coli* (STEC) O157: H7. Collected STEC O157: H7 strains was susceptible for bacteriophage infection. The phage infection at the multiplicity of infection (MOI) of 1, 5 or 8 caused a rapid cell lysis resulting in lack of growth in the cell concentration. In conclusion, the data obtained from the present study shows that bacteriophage isolated from pig slurry is a lytic bacteriophage capable to killing strains of *E. coli* O157: H7. Our results demonstrate that specific bacteriophages have the potential to biocontrol STEC strains in environment.

Key words: Key words: STEC, *E. coli* O157: H7, bacteriophages, biocontrol, pig slurry

INTRODUCTION

Shiga toxin-producing *Escherichia coli* (STEC) O157: H7 was isolated in 1982 and has emerged as one of the major food-borne pathogen [Tuttle et al. 1999]. Cattle, sheep and goat, important domestic ruminants, are natural asymptomatic hosts for this human pathogen. In addition dogs, pigs, deer, cats, horses

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and birds can be host for these bacteria. Faeces from ruminants or from infected humans may contaminate food or water, this type of contamination increase a risk for human infection [Gyles 2007, Krüger and Lucchesi 2015]. However, recent outbreaks have been linked with leafy green vegetables, such as spinach and lettuce, from crops fertilized with ruminant manure [Vugia et al. 2008]. *E. coli* O157: H7 has a very low infectious dose as low as 10 cells [Tuttle et al. 1999]. STEC strains are also known to cause edema disease (ED) and post-weaning diarrhoea (PWD) in piglets [Niewerth et al. 2001].

The primary role in the pathogenesis of severe human diseases (e.g., haemorrhagic colitis and haemolytic uraemic syndrome) caused by STEC strains is played by Shiga toxins (Stx). The genes encoding Stx are located in genomes of lambdoid prophages (phage integrated into the host chromosome). Associated with prophage induction (caused by factors such as antibiotics) the effective production of Shiga toxin and their release after, caused by phage, lysis of bacterial cells. Some antibiotics (e.g., ciprofloxacin or trimethoprim-sulfamethoxazole) should not be administered to patients with *E. coli* O157: H7 infections [McGannon et al. 2010, Łoś and Węgrzyn 2011, Łoś et al. 2011].

Bacteriophages (commonly called phages) are very specific viruses of bacteria, although they are unable to infect human cell and other eukaryotes. Bacteriophages have unique ability to kill the host bacteria which they infected and in the same time increase phage numbers when they destroy target bacteria. Their antibacterial activity has been used not only by physicians treating bacterial infections in humans but also by researchers trying to control food-borne pathogens before they cause of major human diseases. The use of bacteriophages as antibacterial agents has several advantages over traditional antibacterial methods. Phages do not change food quality because they are unable to produce any substances that could modify the flavour, composition, smell or colour of foods. Bacteriophages specific for pathogenic bacteria do not disrupt normal microflora in humans [Kudva et al. 1999].

The aim of this study was in vitro evaluation of a bacteriophage isolated from pig slurry samples as a bacteriolytic agent to elimination of highly pathogenic strains of Shiga toxin-producing *E. coli* O157: H7.

MATERIAL AND METHODS

Two strains of *E. coli* O157: H7 (Stx-positive) from National Institute of Public Health – National Institute of Hygiene, Warsaw, Poland was used as a representative. One own isolate of non – O157: H7 wild strain of Shiga toxin-producing *E. coli* was used as the growth control of phage-insensitive strain. All strains used in study are presented in Table 1. Lytic phages infecting STEC O157:

H7 strains were isolated from pig slurry and characterized for specificity with respect to *E. coli* O157: H7 strains (data not shown). Propagated phage particles was suspended in TM–buffer and stored at 4°C. Host range of bacteriophage was examined by modified spot-test method. Sensitivities of bacterial strains to bacteriophage infection was evaluated on the basis of multiplicity of infection in modified microplate phage virulence assay described by Niu et al. [2009]. To estimate multiplicity of infection (MOI), high titre phage lysate (10^9–10^{10} PFU · ml^{-1}) were serially diluted and incubated at 37°C for 5 h with 10 fold diluted overnight cultures of *E. coli* O157: H7 in a 96-well microplate. After incubation, microplate were examined using microplate reader NanoQuant infinite M200Pro (Tecan), the OD was measured at wavelength of 600 nm and the highest dilution that resulted in no discernable turbidity of bacteria was recorded. To evaluation of lytic capability bacteria strain were grown at 37°C in Lysogeny Broth (LB). The sample taken from bacterial culture in OD = 0.2 was split into five parts. Four samples were inoculated with various concentrations of a phage to reach different MOI (multiplicity of infection – the ratio of the number of bacteriophages particles to the number of bacterial cells present in a defined space) and were incubated at 37°C. One sample which was used as a growth control of bacteria was not exposed to analysed O157-specific bacteriophage, to this sample phage-free buffer was added. The OD indicating bacterial cells concentration was measured at the wavelength of 600 nm in 96-well plates with 200 mL of each sample of bacterial cultures. Measurement was performed every 30 min for further 420 min. The incubation was continued for 24 h and the OD of bacterial cells concentration was measured again in 18 and 24 h.

Results shown in this study originated from more than three independent experiments in which duplicate samples with triple measurements were performed at each time interval. Statistical analysis of bacterial survival as optical density between each multiplicity of infection values and incubation time were carried out by ANOVA, and the least squares method was used to determine significant differences ($P < 0.05$). analysis was carried out by software Statistica 10.

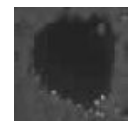
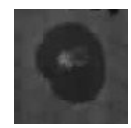
RESULTS AND DISCUSSION

Bacterial strain No. 2 was extremely susceptible for bacteriophage infection, strain No. 1 was highly susceptible, control strain No. 3 was non-susceptible for phage infection (Table 1). The lytic capability of phage against its host *E. coli* O157: H7 was investigated at four different MOIs. The phage exhibited strong lytic capability against STEC O157: H7.

The infection of *E. coli* O157: H7 strains demonstrated that addition of the phage at the MOI of 0.1 reduced growth of these bacteria. The phage infection at

Table 1. Strains used in study and their sensitivity for phage infection

Tabela 1. Szczepy użyte w doświadczeniu oraz ich wrażliwość na zakażenie bakteriofagiem

No. Nr	Strain Szczep	Reference Źródło	Source of isolation Źródło izolacji	Shiga toxin Toksyna Shiga	Sensitivity[A] Podatność[A]	Host range Organizmy
1	STEC O157: H7	National Institute of Public Health – National Institute of Hygiene Narodowy Instytut Zdrowia Publicznego – Państwowy Zakład Higieny	Human Człowiek	Stx1	++	
2	STEC O157: H7	National Institute of Public Health - National Institute of Hygiene Narodowy Instytut Zdrowia Publicznego – Państwowy Zakład Higieny	Milk Mleko	Stx2	+++	
3	Non – O157: H7 wild strain of *E. coli* dziki szczep *E.coli*	Own isolate Izolacja własna	Calf Cielę	Stx1	–	

[A] Sensitivity for phage infection – Wrażliwość na zakażenie fagiem (podatność)
+++ Extremely susceptible (MOI < 0.01) – Wyjątkowa podatność (MOI < 0,01).
++ Highly susceptible (0.01 ≤ MOI < 1) – Duża podatność (0,01 ≤ MOI < 1).
+ Moderately susceptible (1 ≤ MOI < 10) – Umiarkowana podatność (1 ≤ MOI < 10).
– Non-susceptible (i.e. no lysis observed) – Brak podatności (tzn. brak widocznej lizy).

the MOI of 1, 5 or 8 caused a rapid cell lysis resulting in lack of growth in the cell concentration (Figs. 1 and 2).

We observed that the cultures with an initial MOI of 1 or 5 started to grow after 18 h, whereas the cultures with an initial MOI of 8 did not start to increase cell concentration after 18 h (Fig. 3). The phage led to significant reduction in the number of cells at 37°C. After 24 h cultures with an initial MOI of 8 did not increase cell concentration.

Other authors showed similar lytic activity of phage in relation to *E. coli* O157: H7, but the MOI values obtained by them were significantly higher [Viazis et al. 2011]. It must be emphasized that some of the temperate bacteriophages (a dormant phage, integrated into the host chromosome), including phages harbouring *stx* genes, are able to carrying virulence determinant. Excision of prophage DNA is often imprecise and bacterial genes may be incorporated into the infectious phage DNA and then transferred to next host cells [Krüger and Lucchesi 2015]. This process is commonly known as transduction and is responsible for the horizontal gene transfer between the bacterial cells. It has long been recognized

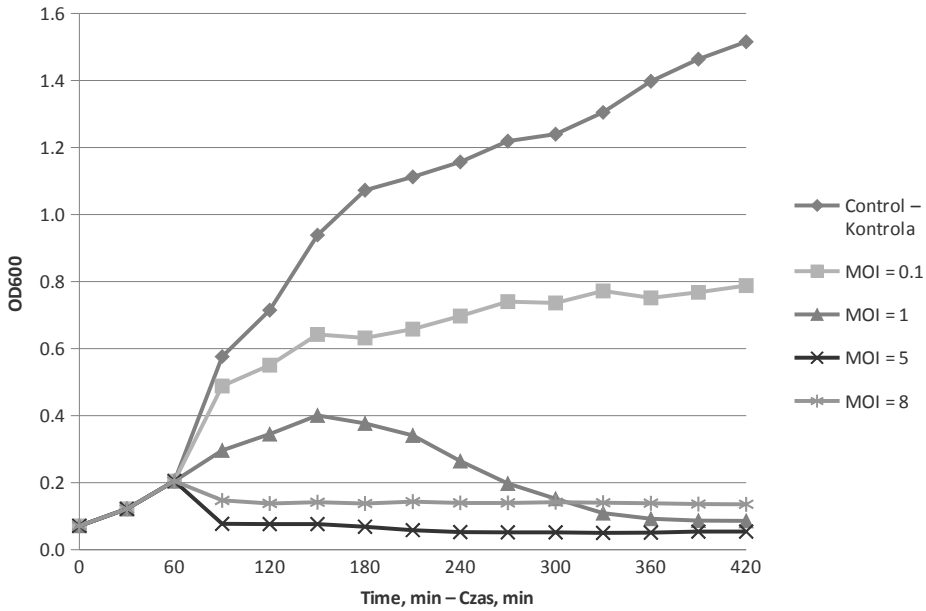


Fig. 1. Growth reduction efficiency of the strain No. 1 after phage infection

Rys. 1. Efektywność redukcji wzrostu szczepu nr 1 spowodowana infekcją fagową

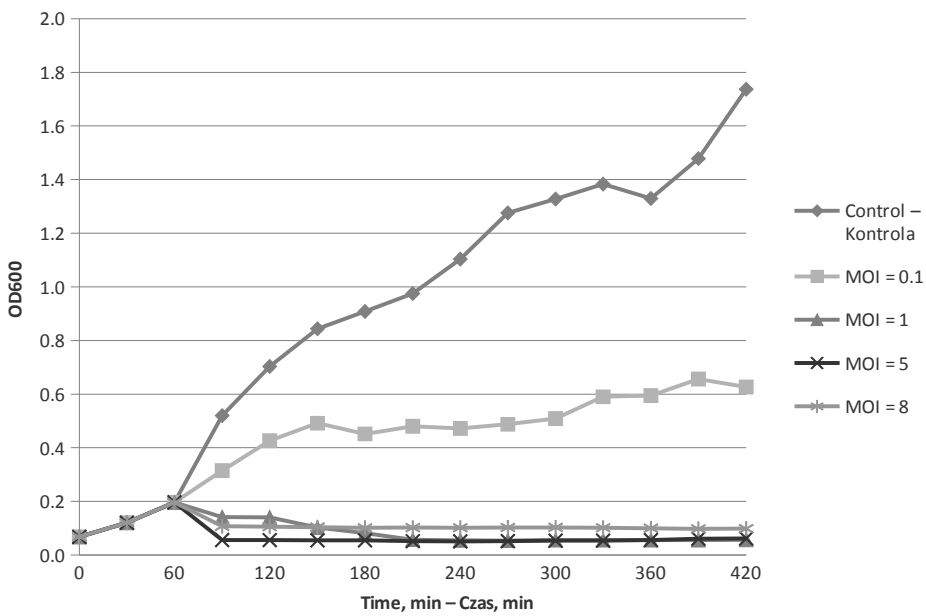


Fig. 2. Growth reduction efficiency of the strain No. 2 after phage infection

Rys. 2. Efektywność redukcji wzrostu szczepu nr 2 spowodowana infekcją fagową

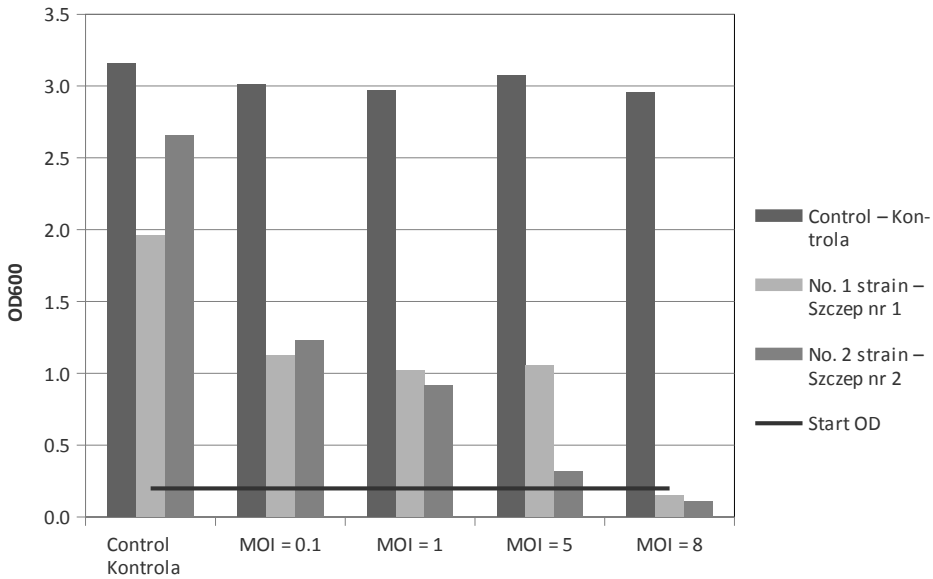


Fig. 3. Growth reduction efficiency of the strains used in this study after 18-h incubation post phage infection

Rys. 3. Efektywność redukcji wzrostu szczepów użytych w doświadczeniu po 18 godz. inkubacji spowodowana infekcją fagową

that non-transducing lytic phages are less likely to transfer virulence genes, which may constitute a lower risk [Hanlon 2007].

Furthermore, they can be used as a valuable tool for safeguarding food safety, animal and public health. Results shows that tested bacteriophage may be useful for the development of biocontrol agent against Shiga toxin-producing *E. coli*, especially O157: H7 serotype. The usage of bacteriophage as a biocontrol agent of *E. coli* strains in the pigsty could limit the morbidity of diseases (eg., edema disease or post-weaning diarrhoea) on piglets caused by selected strains of *E. coli*.

CONCLUSIONS

In conclusion, the data obtained in this study successfully demonstrated that bacteriophage isolated from pig slurry at different MOI values reduced inoculated STEC O157: H7 strains. Data shows that bacteriophage isolated from pig slurry is a lytic bacteriophage capable to killing strains of *E. coli* O157: H7. In the last few years, several authors have evaluated the potential of using phages in biocontrol of pathogenic bacterial strains, especially of human food-borne pathogens. Importance of bacteriophages is not limited only to biocontrol of pathogenic bac-

terial strains. Both the positive (e.g., biocontrol, phage therapy) and negative (e.g., contamination of industrial fermentations) roles of bacteriophages exhibit of their importance for humanity. Popularization of this knowledge is no less meaningful than concentration on research and has to be done for wider bacteriophage usage.

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EFEKTYWNA BAKTERIOLIZA SHIGATOKSYCZNYCH SZCZEPÓW *ESCHERICHIA COLI* O157: H7 SPOWODOWANA PRZEZ SPECYFICZNEGO BAKTERIOFAGA WYIZOLOWANEGO ZE ŚWIŃSKIEJ GNOJOWICY

Streszczenie. Badanie miało na celu ocenę in vitro efektywności bakteriolitycznej bakteriofaga wyizolowanego z próbek świńskiej gnojowicy, jako czynnika do eliminacji Shigatoksycznych szczepów *E. coli* (STEC) O157: H7. Wykorzystane szczepy STEC O157: H7 wykazywały wrażliwość na zakażenie analizowanym bakteriofagiem. Infekcja fagowa przy wielokrotności infekcji (MOI) wynoszącej 1, 5 oraz 8 spowodowała szybką lizę komórek i w efekcie brak wzrostu ich liczby. Podsumowując, otrzymane dane potwierdzają, że bakteriofag wyizolowany ze świńskiej gnojowicy jest lityczny i zdolny do zabijania szczepów *E. coli* O157: H7. Nasze wyniki wskazują na potencjał specyficznych bakteriofagów do zwalczania biologicznego szczepów STEC w środowisku.

Słowa kluczowe: Słowa kluczowe: STEC, *E. coli* O157: H7, bakteriofagi, biokontrola, gnojowica

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