Polish Journal of Environmental Studies Vol. 14, No. 3 (2005), 371-374

Letter to the Editor

The Influence of Environmental Factors on Survival of *Yersinia entrocolitica* O:3

B. Kot[1]*, M. Błaszczyk[2], U. Kulawiec[1]

[1]Department of Microbiology, Institute of Biology, University of Podlasie, 12 B. Prusa Street, 08-110 Siedlce, Poland
[2]Institute of Microbiology, Warsaw University, 1 Miecznikowa Street, 02-096 Warsaw, Poland

Received: 10 August, 2004
Accepted: 10 December, 2004

Abstract

The influence of the presence of bacteriophage ZD5, temperature, pH and the interaction of these factors on survival of *Y. enterocolitica* cells in pond water and buffers was examined. The presence of bacteriophage ZD5 and temperature have substantial influence on survival of *Y. enterocolitica* cells in buffers with different pHs.

The statistical analysis showed a significant influence of the presence of phage ZD5 on the decrease in the number of *Y. enterocolitica* cells incubated in buffers with pHs 6, 7, and 8. In the presence of phage ZD5, pH itself turned out to be of no significance because the average numbers of *Y. enterocolitica* cells obtained in pHs 6, 7, and 8 did not differ significantly at $p \leq 0.05$. Statistical analysis confirms significant influence of the presence of phage ZD5 on the reduction of the number of *Y. enterocolitica* cells in pond water at both 4°C and 20°C. The lowest average value of the number of cells was obtained at 4°C in the presence of phage ZD5, both in the tested buffers and in the pond water.

Keywords: *Yersinia entrocolitica*, survivability, bacteriophage

Introduction

Yersinia enterocolitica is a widespread Gram-negative species which contains several serotypes, some of which are pathogenic to humans. The major pathogens in Europe, Canada, Japan, and South Africa belong to serotypes O:3 and O:9, and those in the United States belong to serotype O:8 [1]. Domestic and wild animals represent a natural reservoir of *Y. enterocolitica*. Pathogenic and nonpathogenic strains are frequently isolated from various animals (birds, mammals, and reptiles) as well as from the environment. Pigs are considered as the basic hosts for these bacteria, in particular for the serotype *Y. enterocolitica* O:3 [2-5]. This microorganism was isolated from soil and water of different purity: well water, spring water bacteriologically stated as clean, surface waters classified into the first and second class of purity, heavily contaminated water [6]. They were also found in food (meat, milk, eggs, vegetables) contaminated with faeces of infected animals or secondarily during the technical process [7, 8, 9]. The ability of *Y. enterocolitica* to multiply at low temperatures can account for the increase in foodborne infections caused by these organisms as a result of the wide diffusion of refrigerated products. *Y. enterocolitica* multiplies and produces enterotoxin at 4°C. This enables long survival of these bacteria in cold water or refrigerated food. It has been observed that they can survive in cooled water for six months [6]. Bacteriophages are ubiquitous in the environment [10]. Phages are detected where bacteria are present: mainly in sewage water, rivers and other waters as well as in the alimentary tract of people and animals and in food products. The spread of bacteriophages causes their significant biological role resulting from lytic and lysogenic cycles.

The aim of the study was to evaluate the influence of bacteriophage ZD5, temperature, pH and the interaction of these factors on the survival of *Y. enterocolitica* cells in pond water and buffers.

*Corresponding author; e-mail: bkot@ap.siedlce.pl

Table 1. Influence of phage ZD5 and pH on number (ln) of *Y. enterocolitica* cells.

Phage ZD5	The number of *Y. enterocolitica* cells			Average
	pH			
	6	7	8	
Present	$8.4^{1(A)}$	$9.1^{1(A)}$	$8.6^{1(A)}$	8.7^{1}
Absent	$12.6^{2(A)}$	$15.5^{2(B)}$	$14.1^{2©}$	14.1^{2}
Average	10.7^{A}	12.3^{B}	11.3^{A}	11.4

Explanation: average values in columns designated with different numbers differ significantly at $p \leq 0.05$; average values in lines designated with different letters differ significantly at $p \leq 0.05$

Table 2. Influence of phage ZD5 and temperature on number (ln) of *Y. enterocolitica* cells in buffers with different pHs.

Temperature	The number of *Y. enterocolitica* cells	
	Phage ZD5	
	Present	Absent
4°C	$5.5^{1(A)}$	$12.2^{1(B)}$
20°C	$11.6^{2(A)}$	$15.9^{2(B)}$
Average	8.7^{A}	14.1^{B}

Explanation: average values in columns designated with different numbers differ significantly at $p \leq 0.05$; average values in lines designated with different letters differ significantly at $p \leq 0.05$

Materials and Methods

Microorganisms

The four strains of *Y. enterocolitica* O:3 including two isolated from human faeces and two isolated from pigs were used in the study. (These strains were previously described [11]). Serotyping was performed by slide agglutination of live cultures grown overnight on horse blood agar using antisera for slide agglutination (ITEST plus s.r.o. – Czech Republic). The bacteria were grown in LB broth at 25°C for 24 h. 0.2 ml of this culture was transferred to 5 ml the LB broth and was cultivated with shaking for 4 h at 25°C. Then the culture was centrifuged at 3000g for 20 min and the cells were resuspended in phosphate-buffered saline (PBS) at approximately 10^7CFU/ml.

Bacteriophage ZD5 was obtained thanks to Prof. C. Calvo from the University in Granada, Spain. The phage ZD5 was isolated from surface water sample collected in Granada. Phage ZD5 was grown in bulk by the standard agar layer technique [12] on *Y. enterocolitica* serotype O:3 strain 40.

Assays Conditions

The investigation was performed at 4°C and 20°C in presence or absence of bacteriophage ZD5. The survival of the tested *Y. enterocolitica* strains was investigated in pond water (previously filtered through the 0.2 µm cellulose nitrate filter - Sartorius) and in phosphate buffers (pH 6; 7; 8). One ml of *Y. enterocolitica* cell suspension was transferred to 99 ml of the tested water and buffers. If necessary 0.2 ml dilution of the bacteriophage ZD5 suspension was added to samples at approximately 10^6 - 10^7 PFU/ml (plaque-forming units). After 24 h, 48 h, 72 h and 96 h the densities of *Y. enterocolitica* cells were determined by plating the dilutions of culture on TSA plates. Statistical analysis was performed with the GLM Procedure of SAS programme. The significance of differences was defined at $p \leq 0.05$.

Results and Discussion

Influence of the presence of bacteriophage ZD5, temperature, pH and the interaction of these factors on survival of *Y. enterocolitica* cells in pond water and buffers was examined. All the tested *Y. enterocolitica* strains were sensitive to the phage ZD5 [13]. The presence of bacteriophage ZD5 and temperature have substantial influence on the survival of *Y. enterocolitica* cells in buffers with different pHs.

Statistical analysis showed a significant influence of the presence of phage ZD5 on the decrease in the number of *Y. enterocolitica* cells incubated in buffers with pHs 6, 7, and 8 (Table 1).

Comparing average numbers of *Y. enterocolitica* cells obtained in the tested buffers containing phage ZD5 with average numbers obtained without phage ZD5, significant differences were observed in each buffer. In the presence of phage ZD5, pH itself turned out to be of no significance because the average numbers of *Y. enterocolitica* cells obtained in pHs 6, 7, and 8 did not differ significantly at $p \leq 0.05$; the significant difference was obtained only at pH 9 (data not shown). However, the incubation of the tested strains in buffers with different pHs without phage ZD5 caused the obtained average numbers of *Y. enterocolitica* cells to differ significantly at $p \leq 0.05$. The highest average value were obtained at pH 7 (Table 1). A significant factor influencing the number of *Y. enterocolitica* cells was also temperature both in the presence and absence of phage ZD5 (Table 2). The lowest average value of the number

Table 3. Influence of pH and temperature on number (ln) of *Y. enterocolitica* cells.

Temperature	Number of *Y. enterocolitica* cells			Average
	pH			
	6	7	8	
4°C	$8.0^{1(A)}$	$9.48^{1(B)}$	$9.45^{1(B)}$	9.0^{1}
20°C	$12.9^{2(A)}$	$15.1^{2(B)}$	$13.2^{2(A)}$	13.7^{2}
Average	10.7^{A}	12.3^{B}	11.3^{A}	11.4

Explanation: average values in columns designated with different numbers differ significantly at $p \leq 0.05$; average values in lines designated with different letters differ significantly at $p \leq 0.05$.

Table 4. Influence of phage ZD5 and temperature on number (ln) of *Y. enterocolitica* cells in pond water.

Temperature	Number of *Y. enterocolitica* cells	
	Phage ZD5	
	Present	Absent
4°C	$4.9^{1(A)}$	$10.32^{1(B)}$
20°C	$11.3^{2(A)}$	$13.4^{2(B)}$
Average	8.2^{A}	11.9^{B}

Explanation: average values in columns designated with different numbers differ significantly at $p \leq 0.05$; average values in lines designated with different letters differ significantly at $p \leq 0.05$.

of *Y. enterocolitica* cells was obtained at 4°C in the presence of phage ZD5. The dependence of the number of *Y. enterocolitica* cells on the incubation temperature and pH is shown in Table 3. At 4°C no significant difference was obtained between the average values at pHs 7 and 8; these average values were the highest. At 20°C the average numbers of cells did not differ significantly at pHs 6 and 8, and the highest average number of cells was obtained at pH 7. Statistical analysis confirms significant influence of the presence of phage ZD5 on the reduction of the number of *Y. enterocolitica* cells in the pond water both at 4°C and 20°C. The lowest average value of the number of cells was obtained at 4°C in the presence of phage ZD5 (Table 4).

Y. enterocolitica is, among *Yersinia*, the single species in which lysogeny has been shown [14]. Bacteriophages isolated from lysogenic strains of *Y. enterocolitica* and active on other strains are used for classification of *Y. enterocolitica* in various phage types [14, 15]. The process of phages binding to the surface of bacteria, thus causing the lytic cycle in them depends on temperature, pH, medium, microorganisms motility and the presence of the specific receptors for a particular bacteriophage. The ZD5 phage is a lytic one and shows the lytic activity only on *Y. enterocolitica* 03 strains [13, 15]. Our investigation aiming at the determination of sensitivity of *Y. enterocolitica* to phage ZD5 at 4°C and 20°C showed that the phage was a major factor reducing cell number at both low and high temperatures. The observed greater activity of phage ZD5 at 4°C may be due to the limited increase in cell number of the tested strains at this temperature. *Y. enterocolitica* rods are sensitive to pH of the environment. They grow in medium with pH ranging from 4.4 to 9 [16]. Dominowska [17] obtained different results investigating rods of the same genus. She found that *Y. pseudotuberculosis* survives for over 20 days in NaCl with pH 5-9, but in pH 3 and 12 it disappears after 1-30 hours. In our investigation, analyzing pH and incubation temperature it turned out that in 6-8 pH without phage ZD5, a significant factor influencing the inhibition of *Y. enterocolitica* growth, was the temperature 4°C, while in pH 9 – the pH itself (data not shown). *Y. enterocolitica* shows the ability to survive and proliferate in distilled, drinking and chlorinated water. The obtained results confirm the ability of *Y. enterocolitica* to survive and proliferate in water. The time of survival of *Yersinia* rods in water depends closely on temperature. These bacteria were detected in water at 4°C for 36 days, at room temperature – for 11 days, and at 30°C – for 7 days [6]. The presence of *Yersinia* rods in well water was investigated by Malaszewska et al. [18]. They isolated these rods from water samples conforming to the sanitary regulations and from samples of water contaminated with *E. coli* and coliforms. Among the isolated strains *Y. enterocolitica* species was predominant. Isolating *Y. enterocolitica* rods not only from people and animals, but also from food, water and soil indicates their spread in nature. These bacteria are resistant to unfavourable environmental conditions. The wide spread of bacteriophages is, on the other hand, a significant factor limiting the number of bacteria in the environment, thus resulting in biological purification.

References

1. BOTTONE E. J. *Yersinia enterocolitica*: the charisma continues. Clin. Microbiol. Rev., **10**, 257, **1997**.
2. CHRISTENSEN S.G. *Yersinia enterocolitica* in Danish pigs. J. Appl. Bacteriol., **48**, 377, **1980**.
3. KAPPERUD G. *Yersinia enterocolitica* in food hygiene. Int. J. Food Microbiol, **12**, 53, **1991**.
4. KAPPERUD G., NESBAKKEN T., ALEKSIC S., MOLLARET H. Comparison of restriction endonuclease analysis

and phenotypic methods for differentiation of *Yersinia enterocolitica* isolates. J. Clin. Microbiol., **28**, 1125, **1990**.
5. THOERNER P., BIN KINGOMBE C., BÖGLI-STUBER K., BISSING-CHOISAT B., WASSENAAR T., FREY J., JEMMI T. PCR detection of virulence genes in *Yersinia enterocolitica* and *Yersinia pseudotuberculosis* and investigation of virulence gene distribution. Appl. Environ. Microbiol. **69**, 1810, **2003**.
6. KROGULSKA B., MALASZEWSKA J. Survival of *Yersinia enterocolitica* in water (in Polish). Rocz. PZH, **2**, 160, **1987**.
7. ANDERSEN J.K., SORENSEN R., GLENSBJERG M. Aspects of the epidemiology of *Yersinia enterocolitica*: a review. Int. J. Food Microbiol., **13,** 231, **1991**.
8. D′AMARIO C., FISCHETTI M., MELINO C. *Yersiniosis*. Clin. Ter., **141,** 51, **1992**.
9. HARTUNG M., GERIGK K. *Yersinia* in effluents from the foodprocessing industry. Rev. Sci. Tech., **10**, 799, **1991**.
10. PAJUNEN M., KILJUNEN S., SKURNIK M. Bacteriophage φYeO3-12, specific for *Yersinia enterocolitica* serotype O:3, is related to coliphages T3 and T7. J. Bacteriol., **182,** 5114, **2000**.
11. KOT B., WOŹNIAK-KOSEK A., KAWIAK J., BUKOWSKI K. Application of PCR for identification of pathogenic plasmid markers of *Yersinia enterocolitica* strains isolated from humans and pigs (in Polish). Medycyna Wet. **57,** 727, **2001**.
12. ADAMS M. H. Bacteriophages. Wiley-Intersciences, New York, **1959**.
13. KOT B., BUKOWSKI K., JAKUBCZAK A., KACZOREK I. The activity of chosen bacteriophages on *Yersinia enterocolitica* strains. Pol. J. Vet. Sci., **5**, 47, **2002**.
14. NICOLLE P., MOLLARET H., HAMON Y., VIEU J.F. Etude lysogenique, bacteriocinogenique et lysotypique de l′espece *Yersinia enterocolitica*. Ann. Inst. Pasteur, **112**, 86, **1967**.
15. CALVO C., BRAULT J., ALONSO J., MOLLARET H. New wateborne bacteriophages active on *Yersinia enterocolitica*. Appl. Environ. Microbiol., **42**, 35, **1981**.
16. EMIRSAJŁOW-ZALEWSKA W. Resistance of *Yersinia spp*. on natural environment factors and disinfectants (in Polish). Życie Wet., **4**, 113, **1996**.
17. DOMINOWKA C. The influence of some physical and chemical agents on survival of *Yersinia pseudotuberculosis*. Bull. Inst. Mar. Med., **22**, 63, **1971**.
18. MALASZEWSKA J., KROGULSKA B., BIELECKA Z., STYPUŁKOWSKA-MISIUREWICZ H. Presence of *Yersinia* organisms in the water from private wells (in Polish). Rocz. PZH, **5,** 396, **1988**.