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Phage FR38 Treatment on Sprague Dawley Rat Inferred from Blood Parameters and Organ Systems

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The ability of phage FR38 to lysis indigenous *Salmonella* P38 from feces of diarrheal patient has been studied. However, effects of phage FR38 on organ system were not revealed as yet. This study was conducted to observe the effect of phage FR38 on blood chemistry, kidney functions, and liver functions. Twelve Sprague-Dawley rats were used as a model for this study that were divided into two groups; (i) control and (ii) treated group with phage FR38. For treated phage group, each rat was administered by 5 ml/kg bw of $1.59 \cdot 10^7$ pfu/ml of phage intragastric. The blood parameters were analysed on day 16. The results revealed that body and organs weight, erythrocyte, hematocrit, hemoglobin, leukocyte, total protein, creatinine, SGOT, and SGPT of phage treatment rats were not significantly different with the control rats on day 16 ($P > 0.05$). Therefore, this study showed was no effect of phage FR38 on body weight, blood chemistry, kidney and liver functions of the rat ($P > 0.05$).

Key words: phage FR38, blood chemistry, kidney functions, liver functions

INTRODUCTION

Salmonella is a food borne pathogenic bacteria that cause food borne and water borne disease (Delibato 2006). *Salmonella* were used as an indicator of food hygiene and food safety (Abedon 2008). *Salmonella* P38 that performed antibiotic resistant had been isolated from feces of diarrheal patient.

Contamination of *Salmonella* on food had been reported in orange juice and fresh orange (Castillo *et al*. 2006), apple cider product (Zhuang *et al*. 2005), beverage product (Li & Mustapha 2005), milk (Tadesse *et al*. 2005), apple juice (Izzo & House 2011), and fresh shrimp (Ray (2001). In Indonesia, chemical preservatives mostly were used to decrease microbe, however the chemical preservatives showed toxic effect. Food producers currently used illegal preservative such as, formaldehyde, aluminate and hydrogen peroxyde due to the high price of the legal preservatives. Illegal preservative, such formaldehyde, also cause a negative effect on organ and body cell. Base on presentation upon, other alternative to decrease microbe on food is needed.

Phage lytic is a preservative alternative on food processing (Rode *et al*. 2011), have an environmentally-friendly characteristic (Castro *et al*. 1991), non toxic and is easy to be isolated, such as from humans, cattle, pigs, and chickens (Duijkeren *et al*. 2002) and can be produced (Brenner *et al*. 1991; Maura & Debarbieux 2011). Phage lytic can be isolated from the environment as well such as soil, water, human body, fermented food (Lu *et al*. 2003a), vegetable fermentation (Lu *et al*. 2003b) and food products. Isolate of phage lytic can be taken from various food products e.g. cheese and yoghourt (Binneti & Reinheimer 2000), salad, crisp and lettuce (Kennedy 1986).

Phage application as a biocontrol food had been used to decrease a microbe contaminant on food, such as, *Bacillus cereus* phage in outbreaks of food poisoning (Ahmed *et al*. 1995), psychrotrophic phage to prevent spoilage process on food (Greer 2005), *Xanthomonas* phage to prevent a spot on tomato (Flaherty 2000), *Listeria* phage (Leverentz *et al*. 2004) and *Salmonella enteriditis* phage on melon and apple slices (Leverentz *et al*. 2001). *Staphylococcus aureus* phage also had been applied on milk as well as *Salmonella enteritidis* phage on cheese (Greer 2005), *E. coli* phage on beef steak (O'Neill *et al*. 2001) and on food processing (Rode *et al*. 2011), *Flavobacterium columnare* phage on fish (Laanto *et al*. 2011), *Listeria* and *E. coli* phage on meat (Anani *et al*. 2011), and on milk (ellis *et al*. 1973).

The other application of phage was as a microbe therapy (Chairns & Payne 2009), such as, by using *Salmonella enterica* phage (Pang *et al*. 2011), *Yersinia pestis* (Schofield *et al*. 2009), cancer cell (Browska *et al*. 2010), *Mycobacterium* phage (Foddai *et al*. 2011), *Vibrio cholerae* phage (Chakrabarti *et al*. 2000), *Actinomycetes* phage (Nerney *et al*. 2004), phage of methicillin resistant *S. Aureus* (O'Neill *et al*. 2001; Murchan *et al*. 2004),

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Bacillus antrachis phage (Abshire *et al*. 2005), *Listeria monocytogenes* phage (Kim *et al*. 2012), phage of bacterial resistance to antibiotic (Edgar *et al*. 2011), and *E. coli* O18:K1:H7 phage (Bull *et al*. 2011). Phage therapy on poultry had been done by using of *Salmonella enteriditis* phage Sillankorva *et al*. (2010). The result of Budynek *et al*. (2010) points out that phage therapy on cancer patient can decrease the incident of microbe infection significantly. Ghaemi *et al*. (2010) reported that phage therapy on tumor can be done by use of λ-phage. Budiarti *et al*. (2011) reported that EPEC (Enteropathogenic *Escherichia coli*) can be degraded by phage isolated from the environment.

On preliminary study, phage FR38 had been used to decrease of *Salmonella* P38, an indigenous contaminant, on fresh milk and sausage. Nevertheless, the effect of phage FR38 on body damage was still unrevealed. Therefore, the aim of this study was to observe the effect of *Salmonella* P38 phage (phage FR38) on organs system by use Sprague Dawley's rat as the animal model.

MATERIALS AND METHODS

Phage Production. Palette of *Salmonella* P38 indigenous culture (OD=1) 10^8 cfu/ml were dropped by phage FR38 (1 ml) (collection of the second author), and were incubated at 37 °C for 30 minutes. The cocktail of *Salmonella* P38 phage were cultivated in 49 ml of nutrient broth (NB) medium, and incubated at 37 °C for 24 hours. After 24 hours incubation, bacteria-phage cocktail were centrifugated with 2800 rpm speed (Backman GPR Centrifuge), at 4 °C for 20 minutes. Supernatant (3 ml) were taken by using a 5 ml syringe and filtered by using Millipore membrane 0.22 µm (Whatmann). The supernatant from filtration process were transferred into sterile tube (Clokie & Kropinski 2009). After double overlay process, the phage were counted by use Clokie and Kropinski formula, i.e., phage total = $1.59 \cdot 10^7 \pm 2.449 \cdot 10^7$ pfu/ml (Figure 1).

Experimental Design. A total of 12 *Sprague Dawley* rats, all were in the same two months age rats were obtained from Veterinary Medicine Faculty, Bogor Agricultural University. Experimental rats were aclimitated at rat cage for 15 days, and then divided into two groups. The first group was rats as control and the other group was given by phage treatment. During adaptation, all of rat was fed with Japfa animal feed with standard drink (Table 1).

Research designs were the randomized control group post test design. The treatments of this research were control and phage treatment (5 ml phage FR38/kg bw; 1 ml = 1.59 x 10^7 pfu). Layout of experiment was arranged by coding of the sample, such as, control treatment code (K1, K2, K3, K4, K5, and K6) and phage treatment code (P1, P2, P3, P4, P5, and P6). After treatment coding, rat code were placed in random position (Table 2).

Phage Treatment. All rats were weighed and were labeled with treatment codes. Body weights of rats were measured every two days for 15 days. The doses of treatment were (i) control group and (ii) Phage FR38 group. Each group was administered (5 ml kg^{-1} bw) by phage FR38 every day for 15 days.

Intragastric Administration. Treatment on rat (control group and Phage FR38 group) was carried out using 16 G intra-gastric syringe. For safety intra-gastric administration, the syringes were manipulated and added a bulbed needle.

Data Administration. After given the treatment for 15 days, data collected on day 16 by surgical technique on rat's body. The euthanasia processes of rat were used ether. The blood was taken from the posterior vena cava. The blood chemistry was analyzed for red cell (erythrocyte) and white cell number, hemoglobin, hematocrit, leukocyte differentiation (lymphocyte, neutrophil, eosinophil, and basophil), Serum Glutamic

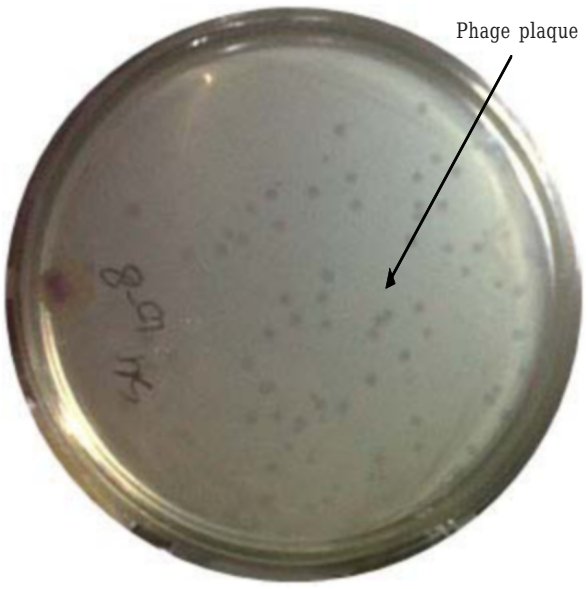

Figure 1. The appearance of plaque phage FR38.

Table 2. Treatment and lay out design

Lay out design			
Location code	Control lay out	Location code	Treatment lay out
1	K4	7	P2
2	K5	8	P1
3	K1	9	P6
4	K6	10	P5
5	K3	11	P3
6	K2	12	P4

Table 1. Feed and treatment given to the rats

Treatment	Adaptation period (for 14 days)	Treatment Period (for 15 days)	
		Feed	Treatment
Control	Feed	Feed	Drink
Phage	Platelet from Jafpa	Platelet from Jafpa	phage = 1 ml/200g bw