SAFETY STUDY OF USING *SALMONELLA sp.* BACTERIOPHAGE AS A NATURAL ANTI MICROBE TO DECREASE *SALMONELLA* CONTAMINANT ON FOOD AND ENVIRONMENTAL

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ABSTRACT

Bacteriophage can be used as an alternative to decrease of microbial content on food or environmental. Bacteriophage can lyse *Salmonella* bacteria in food significantly. This study was to determine the safety effect of using Salmonella bacteriophage on food when it was consumed in vivo. Bacteriophage safety test was done with using by mice. The Mice were Sprague Dawley strain. Parameters that was observed were organs of the heart, lung, and colon. The study conclusion was the Effect of bacteriophage treatment 10^7 ml /day for 16 days on Sprague Dawley Rat was not give an effect on organ, such as, 1) heart weight (0.813 ± 0065), colors, and the overall shape; 2) lungs weight (2.002 ± 0126), colors, and the overall shape; 3) colon weight (22.869 ± 0136), color, and overall shape. The rat that was treat by bacteriophage is not different than control significantly. So, It indicates that using the bacteriophage as a natural anti microbe to Decrease salmonella on food and environmental was safety.

Keyword: Bacteriophage, safety, *in-vivo.*

I. INTRODUCTION

Contamination of *Salmonella sp.* on the environment, such as, soil and water, increased in both developed and developing countries. Water and soil was contaminated with Salmonella, in the other case, a food and beverage was agent pollutants too, that trigger outbreaks of salmonellosis on the environment. More than 50% of diarrhea case in the world was caused of food that was contaminated by Salmonella (Miliotis & Bier 2003).

Salmonella sp. Contaminant on food was causing food borne and water borne disease (Bell & Kyriakides 2002); food poisoning (Zhuang and Mustapha, 2005). That was caused by *Salmonella sp.* So, it had needed something that could decrease of *Salmonella sp.* contaminant. Bacteriophage can be used as an

alternative as a antimicrobe on the food processing, because it was a natural, and there are many on the environment (Abedon 2008); can be isolated from a shellfish (Albert et al. 1994); on carrots (Endley et al. 2003); on cheese (Gautier et al. 1995); on meat (Atterburry et al. 2001); on yogurt (Kilic et al. 1996).

In Indonesia, bacteriophage isolation has been done, such as, Xanthomonas campestris bacteriophage isolation (Triana, 1996); enteropathogenic E. coli bacteriophage (Budiarti et al. 2011); and FR38 Salmonella bacteriophage (Budiarti & Rusmana 2010). Sartika (2012) reported that the use of bacteriophage to decrease of Salmonella contamination on milk, sausages, and water was a effective significantly. The result of research on Bacteriophage safety showed that the liver, stomach, spleen and small intestine of animal test that be given bacteriophage 10^7/day for 15 days was normal (Sartika et al. 2012), however, the security of in-vivo studies on the heart, lung, and colon has not been done. In this research, will be study of bacteriophage safety when used as a natural anti-microbial on food and environment, with observe through the heart, lung, and colon of Sprague Dawley. Rats.

2. MATERIALS METHODS

2.1 Phage Production

After 24 hours incubation, bacteria-phage cocktail were centrifugated with 2800rpm speed (Backman GPR Centrifuge), at 4^oC for 20 minutes (The cocktail of *Salmonella* P38 phage were cultivated in 49 ml of NB (Nutrient Broth) medium, were incubated at 37° C for 24 hours). Supernatan (3 ml) were took by use a syringe (vol. 5ml) and be done the filtration process by use a milipore's membrane 0,22 □m (Whatmann). The result from filtration process as a

supernatant were moved into sterile tube (Clokie & Kropinski, 2009). The end ptocess were done the double overlay process, the phage were counted by use Clokie And Kropinski formula, (Figure 1).

Figure 1. The plaque *Salmonella sp*. Bacteriophage Appearance

2.2 Experimental Design

Sprague Dawley rat were used on this research.. The rate age of rats were 2 months old, as much as 12 rats. The aclimitated of experimental rat were at rat cage for 15 days, 2 groups. The first group were the rat that given the bacteriophage treatament and the others group were control. The adaptation process, all of rat were given drink with a standard drink and given feed with Japfa animal feed.

The treatments of this research are bacteriophage treatment (5 ml/kg bw; 1 ml = 1.59 x 10^7 pfu). The layout of experiment was arranged by coding of the sample, such as, phage treatment code (P1, P2, P3, P4, P5, and P6). and the control's treatment code (K1, K2, K3, K4, K5, and K6). After The coding process, were done a randomization. Experimental design were randomized group design, with model design as a follows:

$$Y_{ij} = u + A_i + E_j.$$

2.3 Bacteriophage Treatments.

All of rat were done the body weights measurement and labelled with

treatment code. The measurement of body weight rats were done every 2 days for 15 days. The treatment doses were (i) Bacteriophage FR38's group and (ii) control group. Each group was given treatment (5 ml kg^{-1} bw) by bacteriophage every day for 15 days..

2.4 Intragastric Administration.

The rat treatment on (control group and bacteriohage group) was feeded using by 16 G intra-gastric syringe. For intra-gastric safety administration, the treatment syringe were manipulated and added a needle of bulbed (Sartika (2012).

2.5 The Administration of Data

The collected of Data were by technique of surgical on body rat (16th day). After was given the treatment for 15 days, the collecting of data was done on 16th day. The euthanasia process of rat were ether. The blood collected used by bleeding method from the posterior vena cava. The chemistry blood was analyzed for white cell count, haemoglobin, red cell count (erythrocyte), hematocrit, leukocyte differentiation. The performances (shape and color) of rat feces also was collected for 16 days.

2.6 Statistical Analysis

Statistical analysis of this research was carried out using student's t-test. The results are presented as the differences mean between individual groups with P (less than or equal to) 0.05 considered significant of statistically.

III. RESULTS AND DISCUSSION

3.1 The Bacteriophage Effect on faeces And Urine Appearance

The Bacteriophage treatment for 15 days on rat did not affect either the form of faeces characteristics. The appearance of faeces was normal, black-grey color, and solid integrity. The research result was summarized in Table 1 as a follows.

Table 1. Effect Of Bacteriophage Treatment On Shapes And Colors Faeces

No	Faeces Characteristic	Bacteriophage Treatment for 15 days	Control
1	Shapes	Normal	Normal
2	Color	Black-grey	Black-grey
3	Integrity	solid	solid
4	The overall appearance	Normal	Normal
5	Figure		

The rats display on the 16th day, both bacteriophage treatment and control, are presented on the following figure:

Figure 2. The appearance of the bacteriophage treatment rat on 15th day

This case was in line with the observation of the bacteriophage treatment effect with administration for 15 day on Sprague Dawley rats. When it was observed at 16th days, did not different effect on the Urine characteristics, such as, normal odor, yellow color, and the normal whole-appearance. The research result was summarized in table 2 as a follows:
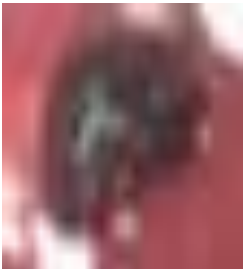
Table 2. Effect of Bacteriophage Treatment on Appearance And Color of Urines

No	Urines Characteristic	Bacteriophage Treatment for 15 days	Control
1	Shapes	Normal	Normal
2	Color	Yellow	Yellow
3	Overrall Appearances	Normal	Normal

3.2 The Bacteriophage Effect on Heart Organ

Observations on the cardiac showed that the treatment of bacteriophage for 15 days on Sprague Dawley rat did not gave an bad characteristics effect. The heart of treatment rat had normal characteristic, such as, heavy (Normal not different significantly compared with controls), color (bright red). The appearance of the whole was summarized in Table 3 as a follows:

Table 3. The Bacteriophage Effect on Heart Organ

No	Heart Organ Characteristic	Bacteriophage Treatment for 15 days	Control
1.	Weight	0.813 ± 0.065g [a]	0.807 ± 0.070g [a]
2.	Color	Merah Cerah	Merah Cerah
3.	Overrall Appearances	Normal	Normal
4..	figure		

Undifferent letter(s) in each column indicated unsignificant difference on $P > 0.05$

3.3 The Bacteriophage Effect on Lung Organ

Observations of Bacteriophage effects on the lung showed that the treatment of bacteriophage for 15 days on Sprague Dawley rat did not gave an bad characteristics effect. The lung of treatment rat had normal characteristic, such as,

heavy (Normal not different significantly compared with controls), color (bright red). The appearance of the whole was summarized in Table 4 as a follows:

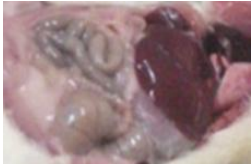Table 4. The Bacteriophage Effect on Lung Organ

No	Lung Organ Characteristic	Bacteriophage Treatment for 15 days	Control
1.	Weight	$2.002\pm0.126g^a$	$2.011\pm0.111g^a$
2.	Color	Bright red	Bright red
3.	Overrall Appearances	Normal	Normal
4.	figure		

Undifferent letter(s) in each column indicated unsignificant difference on P > 0.05

3.4 The Bacteriophage Effect on Colon

Observations of Bacteriophage effects on the colon showed that the treatment of bacteriophage for 15 days on Sprague Dawley rat did not gave an bad characteristics effect. The colon of treatment rat had normal characteristic, such as, heavy (Normal not different significantly compared with controls), color (normal). The appearance of the whole was summarized in Table 5 as a follows:

Table 5. The Bacteriophage Effect on Colon

No	Heart Organ Characteristic	Bacteriophage Treatment for 15 days	Control
1.	Weight	$22.869\pm0.136a$	$22.707\pm0.627a$
2.	Color	Normal	Normal
3.	Overrall Appearances	Normal	Normal
4.	figure		

Undifferent letter(s) in each column indicated unsignificant difference on P > 0.05

IV. CONCLUSION

The conclusion from this study was (1) Effect of bacteriophage treatment on Sprague Dawley rats for 15 days did not give unnormal affect; the characteristics of weight (0.813 ± 0065g); color, and apperance of heart was not different significantly than controls, (2) The characteristics of weight (2.002 ± 0126g) color, and apperance of lung was not different significantly than controls, (3) The characteristics of weight (22.869 ± 0136g) color, and apperance of colon was not different significantly than controls. So, The conclusion from this study was the bacteriophage was safety on rat organ, such as, lung, heart and colon. It indicates that using the bacteriophage as a natural anti microbe to Decrease salmonella on food and environmental was safety.

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