**THE EFFECTIVITY OF LYTIC BACTERIOPHAGE FR 38 TO DECREASE *SALMONELLA* P38 INDIGENOUS ON MILK AND CHICKEN SAUSAGE**

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**Abstract**

The ability of bacteriophage FR38 to lysis an indigenous Salmonella P38 from faeces of diarrhea patient has been studied, however its effects on food is not studied yet. This study was conducted to observe the effects of bacteriophage FR38 on milk.Lysis efectivity of bacteriophage FR38 on food were measured on milk. The total colony of Salmonella P38 was counted by surface plate method. The result showed that indigenous bacteriophage FR38 had been able to decrease of indigenous Salmonella P38 on fresh milk (alpha0,01). Bacteriophage FR38 was effective to decrease of *Salmonella*P38 on milk during 24 hours (940cfu/ml), 48 hours (1200cfu/ml) significantly than untreatment (alpha0,01). Bacteriophage FR38 was effective to decrease of*Salmonella* P38 on sausages during 24 hours, 48 hours significantly than untreatment (alpha0,01).

Keyword: Bacteriophage FR38,*Salmonella* P38, effectivity,Milk, Sausages

**INTRODUCTION**

*Salmonella*is a foodborne pathogenic bacteria that cause food borne diseases and water borne disease(Delibato 2006).*Salmonella*were used as an indicator of food hygiene and food safety (Abedon 2008).Contaminant of *Salmonella* on food had been analyzed on orange juice,fresh orange,apple cider product, beverage's product, milk, apple juice and fresh shrimp (Castillo *et al*. (2006), Zhuang& Mustapha (2005), Li & Mustapha (2004), Tadesse*et al.* (2005), Izzo(2011), Ray (2001)). In Indonesia, decreasing microbe had been done with a chemicalpreservative.In the fact, the chemical preservatives not only expensive prices, but have a toxic effect

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The high prices of the legal preservative, apparently a food producer was using un-legal preservative, such as, formaldehyde, aluminate and hydrogen peroxyde. Un-legal preservative, such formaldehyde, also cause a negative effect on organ and body cell. Base on presentation upon, it's needs the other alternative to decrease microbe on food.

Bacteriophagelytic is a preservative alternative on food processing (Rode *et al*. 2011); have an environmentally-friendly characteristic (Castro *et al*. 1991);non toxicand is easily 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).Bacteriophage lytic can be found on environment, earth, water, body, fermented food (Lu *et al*. 2003a); vegetable fermentation (Lu *et al*. 2003b); and food product. Isolate bacteriophage lytic can be taken from various food kind e.g. cheese, yoghourt (Binneti&Reinheimer 2000); salad, crispy, and letucce(Kennedy 1986).

Bacteriophage application as a biocontrol food, had been used to decrease a microbe contaminant on food, such as, *Bacilluscereus*bacteriophage in outbreaks of food poisoning (Ahmed *et al*. 1995);psychrotrophicbacteriophageto prevent spoilage processon food (Greer 2005); *Xanthomonas*bacteriophage to prevent a spot on tomato (Flannerty 2005); *Listeria* bacteriophage (Leverentz*et al*. 2004) and *Salmonellaenteriditis*bacteriophageon melon and apple slices (Leverentz, Conway, Alavidze 2001). According Greer (2005) that*Staphylococcus aureus*bacteriophagealsobe applied on milkand *Salmonellaenteritidis*bacteriophage on cheese. *E. coli*bacteriophageon beef steak (O’Neill, Murchan, Setas 2004); *E. coli*bacteriophageon food processing (Rode, Axelsson, Granum2011)*Flavobacteriumcolumnare*bacteriophage on fish (Laanto, Sundberg, Bamford2011); *Listeria* and *Ecoli*bacteriophage on meat (Anani, Chen, Pelton2011).

The others application of bacteriophage was as a microbe therapy, such as, by using *Salmonellaenterica*bacteriophage (Pang *et al*. 2011);*Yersiniapestis* (Schofield *et al*. 2009); Mycobacterium bacteriophage (Foddai*et al,* 2011); *vibrio cholerae*bacteriophage (Chakrabarti *et al*. 2000);*Actinomycetes*bacteriophage (Nerney *et al*. 2004); bacteriophage of methicillin resistant *S.Aureus* (Murchan, *et al*. 2004&O'Neill *et al*. 2001); *Bacillusantrachis*bacteriophage (Abshire *et al*. 2005); *Listeriamonocytogenes*bacteriophage (Kim *et al*. 2012); bacteriophage of bacterial resistance to antibiotic (Edgar *et al*. 2011); and *Ecoli* O18:K1:H7 bacteriophage (Bull *et al*. 2011). According to Sillankorva *et al*. (2010), bacteriophage therapy on poultry hadbeen done by using of *Salmonellaenteriditis*bacteriophage*.* The resultresearch ofBudynek*et al.*(2010), point out that bacteriophage therapy on cancer patient can decrease the incident of microbe infect significantly. Ghaemi*et al* (2010) reported that bacteriophage therapy on tumor can be done by use of -bacteriophage.Budiarti*,* Pratiwi, Rusmana(2011) reported that EPEC (Enteropathogenic*Escherichia coli*) can be degraded of bacteriophage isolated from environment.

On pre-study, Bacteriophage FR38 had been used to decrease of *Salmonella*P38 indigenous on nutrient broth media. The result of study to point out that Bacteriophage FR38 indigenoushad been able to decrease *of Salmonella*P38 indigenous on nutrient broth media. Furthermore, the effectivity of lytic bacteriophage FR38 to decrease *of Salmonella* P38 on milk was unknown. The aim of this studywas to observesthe effectivityoflytic bacteriophage FR38 to decrease*Salmonella*P38 indigenous onmilk, sausage, and water.

**MATERIALS METHODS**

**BacteriophageProduction.**Palette of *Salmonella*P38 indigenous culture (OD=1) are 10 8 cfu/ml were dropped by bacteriophageFR38(1 ml) (Sri Budiarti collection), then bedone *vortex* and were incubated at 37oC for 30 minutes. The cocktail of *Salmonella* P38bacteriophage were cultivated in 49 ml of NB (Nutrient Broth) medium,were incubated at 37o C for 24 hours. After 24 hours incubation, bacteria-bacteriophagecocktail were centrifugated with 2800rpm speed (Backman GPR Centrifuge), at 4oC for 20 minutes. Supernatan (3 ml) were took by use a syringe (vol. 5ml)and be done the filtration process by use amilipore's membrane 0,22m (Whatmann). The supernatantresult from filtration process were moved into sterile tube (Clokie&Kropinski, 2009). After done the double overlay process, the bacteriophagewere counted by use ClokieAndKropinskiformula, which is,bacteriophagetotal = 1.59. 107± 2.449.107pfu/ml.

**Experimental Design**.The milk processing with bacteriophageFR38 treatment was designed in figure 1. The sausages processing with bacteriophage FR38 treatment was designed in figure 2. The milk and sausages sample of treatment (control and bacteriophage fFR38 treatment) were contaminated by indigenous *Salmonella*P38(4.3 x 104cfu). The bacteriophage treatment was added 3.8 x 104cfu of bacteriophage FR38.The research design were the randomized design. Experimental design for this research wererandomized group design, with model design as follows:

Yij = u + Ai + Ej.

**Data Administration**.After given the treatment for0, 24, 48 hours, the total of Salmonella P38 and nutrient content of the milk was counted.

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

**Pasteurization (85-950c) during 1-2 minute**

**Contol**

**Bacteriophage Treatment**

Figure 1. Application procedure of bacteriophage FR38 on milk

**Cutting**

**Curing**

**Storage For 24 hours**

**Grinding**

**Water 300g**

**Mixing**

**Sausage Forming**

**Heating T: 70OC; t: 20 Minute**

**Cooling, Room Temperature**

**Control**

**Bacteriophage Treatment**

Figure 2. Application procedure of bacteriophage FR38 on sausage

**RESULTS**

**A. Effectivity of Bacteriophage FR 38on Milk  
*1. Nutrition***

The content of ash, protein and fat on milk that be given treat bacteriophage were not different than control significantly (0.01) when milk was storage for 48 hours significantly (Table 1).It was s suspected that BacteriophageFR38 could inhibit Salmonella P38 action in denaturation of proteins and fats. The free Bacteriophage treatment (control) showed that the fat (1.76%), Abu (12:18%) and protein (1:09%) milk contentt was lower thanBacteriophage FR38treatment significantly. The milk with Bacteriophage FR38treatment had content characteristic was better than control, such as, of fat (3:32%), ash (0.25%), protein (2:20%) when milk was storage for 48 hours (99% confidenceinterval).

The composition of protein (mean = 2.58%) and fat (mean = 4:49%) in the milk sample is high. According Kluwer (2005), the food that containing high fat and protein is a good growth medium for Salmonella. This case was same with this research, the control treatment was containing high s*almonella* that could decrease on fat (1.76%) and protein content (1:09%) for 48 hoursstorages significantly. It was assummed that *Salmonella* has lipase and protease enzymes content that can break down fats and proteins (Figure 3).. Bacteriophage treatment was found to inhibit a break down process to content of fat, protein, moisture content, ash content and crude fiber of milk (0.01)by *salmonella* activity. This researchs proves that when applied to food eg milk, the Bacteriophage will not affect to the nutritional content.

Table1 The effect of bacteriophage FR38 treatment andincubation timetomilk nutrition content

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Treatment | Storage time (hour) | Water Content | Ash | Fat | Protein |  |
|  | (%) |  |  |  |
| Negativecontrol  Postive control (NB)  Postive control(NB + SM) | 0 | 62.92a  62.96a  62.96a | 0.64a  0.68a  0.67a | 4.57a  4.56a  4.51a | 2.57a  2.59a  2.57a |  |
| *Salmonella* P38 | 62.95a | 0.68a | 4.47a | 2.59a |  |
| *Salmonella* P38 danBacteriophageFR38 | 62.96a | 0.64a | 4.44a | 2.58a |  |
| Negativecontrol  Postive control (NB)  Postive control (NB + SM) | 24 | 62.33b  62.38b  62.39b | 0.40b  0.49b  0.41b | 3.86b  3.89b  3.88b | 2.47b  2.40b  2.46b |  |
| *Salmonella* P38 | 62.55c | 0.29c | 3.68c | 2.30c |  |
| *Salmonella* P38 danBacteriophageFR38 | 62.44d | 0.36d | 3.81d | 2.40d |  |
| Negativecontrol  Postive control (NB)  Postive control (NB + SM) | 48 | 87.11e  87.13e  87.12e | 0.30e  0.32e  0.31e | 3.47e  3.44e  3.45e | 2.46e  2.41e  2.47e |  |
| *Salmonella* P38 |  | 87.47f | 0.18f | 1.76f | 1.09f |  |
| *Salmonella* P38 danBacteriophageFR38 |  | 87.23g | 0.25g | 3.32g | 2.20g |  |

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

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| C:\Users\User\AppData\Local\Microsoft\Windows\Temporary Internet Files\Content.Word\DSCF1204.jpg | C:\Users\User\AppData\Local\Microsoft\Windows\Temporary Internet Files\Content.Word\DSCF1203.jpg | C:\Users\User\AppData\Local\Microsoft\Windows\Temporary Internet Files\Content.Word\DSCF1202.jpg | C:\Users\User\Documents\blackberry\pictures\IMG00635-20120409-0830.jpg | C:\Users\User\Documents\blackberry\pictures\IMG00629-20120409-0825.jpg |
| (A) | (B) | (C) | (D) | (E) |

Figure3. The Treatnent effect for 48 hour storage: (A) bacteriophage FR 38 and*Salmonella* P 38*;* (B) *Salmonella* P 38; (C) Control; (D) Buffer SM and (E) Nutrient Broth

**3. pH of milk**

Different treatment also affected to the pH of the milk during 24 hours, and 48 hours storage (Figure 4). According to Winarno (2008) Decomposition of fats into fatty acids will release of a H + atom. The release of atom H + causesof a decrease processof the milk pH during storage. The addition of Bacteriophage turned will inhibit the microorganisms action in the fatrancidity, so, a pH of milk that was stored for 48 hours with Bacteriophage treatment was better (6.00) than with no bacteriophagetreatment P38 (5:52) (α0,01) significantly with 99% confidence interval. It can be concluded that thebacteriophage addition can inhibit the growth of Salmonella destruction of milk by salmonella.

|  |  |
| --- | --- |
|  | % Bacteriophage |

Figure 4. The Bacteriophage effect on pH milk

**B. EffectivityBacteriophage FR38 on Sausages**

The addition of Bacteriophage FR38 on the sausage also affect to decrease of Salmonella P38 growth during 0, 24 and 48 hours storage, at room temperature (Table 2). Bacteriophage FR38 able to reduce the Salmonella P38 number for 24 hours storage (6.9 x 101cfu /ml) and 48 hours (7.8 x 102cfu /ml) significantly than nobacteriophage treatment, at the 99% confidence level (0,01). Different with unbacteriophage treatment, which increased the number of Salmonella on sausage,they are7.5 x 106cfu ml (24 hours storage) and 8.4 x 109cfu / ml (48 hours storage).

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Table2. Theeffect of bacteriophage FR38 treatment and incubation timet osausage nutrition content   |  |  |  |  |  |  |  | | --- | --- | --- | --- | --- | --- | --- | | Perlakuan | Storage time (hour) | Water Content | Ash | Fat | Protein |  | | (%) | | | | | | Negativecontrol  Postive control (NB)  Postive control (NB + SM) | 0 | 51.56a | 2.68a | 6.86a | 14.12a | 0.74a | | *Salmonella* P38 | 51.55a | 2.67a | 6.85a | 14.13a | 0.75a | | *Salmonella* P38 danBacteriophageFR38 | 51.57a | 2.68a | 6.86a | 14.12a | 0.73a | | Negativecontrol  Postive control (NB)  Postive control (NB + SM) | 51.56a | 2.68a | 6.84a | 14.12a | 0.74a | | *Salmonella* P38 | 51.55a | 2.68a | 6.85a | 14.13a | 0.75a | | *Salmonella* P38 danBacteriophageFR38 | 24 | 53.49b | 2.65b | 6.63b | 13.90b | 0.71b | | Negativecontrol  Postive control (NB)  Postive control (NB + SM) | 53.48b | 2.65b | 6.64b | 13.90b | 0.71b | | *Salmonella* P38 | 53.49b | 2.64b | 6.63b | 13.91b | 0.71b | | *Salmonella* P38 danBacteriophageFR38 | 55.11c | 2. 43c | 6.45c | 12.79c | 0.68c | | Negativecontrol  Postive control (NB)  Postive control (NB + SM) | 53.70d | 2. 55d | 6.59d | 13.85d | 0.70d | | *Salmonella* P38 | 48 | 55.39 e | 2.60e | 6.51e | 13.87e | 0.68e | | *Salmonella* P38 danBacteriophageFR38 | 55.38e | 2.61e | 6.53e | 13.88e | 0.66e | | Negativecontrol  Postive control (NB)  Postive control (NB + SM) | 55.38e | 2.61e | 6.52e | 13.87e | 0.67e | | *Salmonella* P38 | 60.19f | 2.49f | 6.21f | 11.06f | 0.60f | | *Salmonella* P38 danBacteriophageFR38 | 57.61g | 2.58g | 6.49g | 13.09g | 0.63g | |

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

**DISCUSSION**

The milk samples with Salmonella treatment showed that asample had an unlike performance, which was marked by the separation of dissolved solids and water during 24 hours storage. Bacteriophage are infectious only to target/specific host, example Salmonella (Abedon, 2008).According to Winarno (2008), denaturation of the protein was caused by the disintegration of the hydrogen bonds by external factors (such as, microbial). The disintegration of hydrogen bonds in a protein causes the protein denaturation. Denatured protein cause of solubility reduced, that give a bad effect, such as, the outside of proteins that have a hydrophilic characteristic will folded to inside part and hydrophobic parts will be folded out, so,it result a solids and liquids milk separated. The bad odorfrom the Salmonella P38 treatment was due to the decomposition processon fat components in milk, as a result of work by microorganisms. According to Winarno (2008),the molecules that wasbroken down from fats will beoxidized, that result a hydroperoxide compoundform, aldehydescomponent, and ketones, these reactions cause a bad odor (off-odor). This is in line with this observations.

The Sausage has makro component, such as, protein and fat. Decreasing macro component on sausage showed that quality level of sausage. Low protein and fat content was a low quality sausage performance.The addition of Bacteriophage FR38 on the sausage inhibitgrowthSalmonella P38, during storage, at room temperature. Bacteriophage FR38 able to reduce thetotal ofSalmonella P38 for 24 hours storage (6.9 x 101cfu /ml) and 48 hours (7.8 x 102cfu /ml) significantly.The result research showed that a bacteriophage FR38 able to decrease salmonella P38.

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